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Investigations of the Non-Covalent CH-Π Interactions Using Molecular Torsional Balances

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INVESTIGATIONS OF THE NON-COVALENT CH–π INTERACTIONS USING MOLECULAR TORSIONAL BALANCES

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ABSTRACT

Several bicycle $N$-arylimide based molecular balances were designed to study aliphatic CH–π interactions and aromatic CH–π interactions (edge-to-face arene-arene interactions). In each case, the geometries of the interactions were characterized in the solid-state via X-ray analysis, and the strengths of interactions were characterized in solution by their folded/unfolded ratios, as measured by integration of their $^1$H NMR spectra.

The balances are very sensitive to variations in the strengths of weak non-covalent interactions. Several different aspects of the CH–π interactions were studied, such as steric effects, conformational entropy, cooperativity, deuterium isotope effect, substitution effects, and solvent effects. It showed that due to the weak nature of CH–π interactions, many forces can contribute on determining their interacting energies with similar magnitudes. Approaches using “double-mutant cycles” to isolate the interactions of interest from secondary effects were presented. The balances can also be used to the study of other non-covalent interaction, and the investigations were included in the last chapter.
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CHAPTER 1
INTRODUCTION TO NON-COVALENT INTERACTIONS AND MOLECULAR BALANCES

Non-covalent interactions are ubiquitous in biomolecular systems and play a key role in their functions. They determine the secondary and tertiary structures of proteins, and are the main forces that drive enzyme-ligand binding and base-pairing in nucleic acids. They also play important roles in many chemical processes such as template-directed synthesis, transmission of stereochemical information, and determination of structures and properties of materials. Thus, systematic studies of these interactions are important to gain a better understanding of their natures and to build better predictive models for their applications.

Thus, the topic of this thesis is the application of a series of molecular balances to study weak non-covalent interactions, specifically, CH–π interactions. Before describing the experimental design and results, an introduction to the general types and properties of non-covalent interaction and the development of molecular balances will be provided.

1.1 NON-COVALENT INTERACTIONS

The term of “non-covalent interactions” describes the weak attractive forces between two adjacent atoms. Non-covalent interactions do not involve the sharing of electrons, and thus, are differentiated from covalent interactions. As a result, non-covalent interactions are usually weaker than covalent bonds (0.5–5 kcal/mol versus 50–150 kcal/mol). However, the cooperativity of multiple non-covalent interactions can
provide sufficient attraction to hold interacting functional groups together, such as in large biomolecules such as proteins and nucleic acids. On the other hand, the instability of these interactions can lead to greater flexibility and reversibility, and thus, they can provide dynamic properties such as stimuli-response, allosteric effect, and switching.

There are several general types of non-covalent interactions. These include ionic bonds, hydrogen bonds, dipole interactions, and solvophobic effects. One of the most common types of non-covalent interactions is ionic bonds. The ionic bonds are strong electrostatic attractions between oppositely charged ions. They are the strongest type of non-covalent interaction. The binding energy between a cation and an anion can be over 100 kcal/mol in the gas phase.

The non-covalent interactions of dipoles are also based on electrostatics. These include the attraction between an ion and a polar molecule with a dipole moment (ion–dipole interaction), and the interaction between two polar molecules (dipole–dipole interaction). Finally, dipoles can be induced by a nearby ion (ion–induced dipole interaction) or another dipole (dipole–induced dipole interaction) (Figure 1.1). The strength of a dipole interaction is typically between 0.5–2 kcal/mol.

![Figure 1.1: Examples of a) an ion–induced dipole interaction between a sodium cation and a water molecule, b) a dipole–induced dipole interaction between a water molecule and an oxygen molecule.](image)

Attractive non-covalent interactions can also arise between two dipoles that are instantaneously generated from the random motions of valence electrons on the surface of molecules. These are known as van der Waals interactions or London dispersion forces.
The CH–π interactions, which are the major interactions of interest in this thesis, can be classified as dispersion interactions. Although dispersion forces are relatively weak (< 1 kcal/mol) compared with the interaction of ions and dipoles, their contribution and influence can be significant, especially when there is a large contact area between the two molecules. For instance, they are the cause of the high boiling point of linear alkanes versus branched alkanes (Figure 1.2). However, because the interactions are a consequence of electron correlation, they cannot be quantitatively modeled with computational studies. Thus, experimental approaches on measuring dispersion forces, which is the major objective of this thesis, are of great significance.

Figure 1.2: Comparison of the interacting surface areas and boiling points of ethane, n-pentane, and neopentane.

Another type of non-covalent interactions that have been extensively studied is the hydrogen bond. The strength of hydrogen bonds can range from about 0.1 to 60 kcal/mol. Early definitions of hydrogen bonds were limited to the attraction between a hydrogen atom from a polar proton donor (X–H bond, X = O, N, S, halogen) and an electronegative atom having a lone-pair of electrons (e.g., O, N, S, or halogen) (Figure 1.3). These classical hydrogen bonds are highly directional, and are primarily electrostatic interactions. More recently, the definition of hydrogen bonding has been broadened to include a wider range of donor and acceptor functional groups. For example, the hydrogen donor can be a weakly polarized C–H bond, and the acceptor can
be a group with a region with high electron-density such as the $\pi$-face of an aromatic system. Using this broader definition, many of the non-covalent interactions of arenes can also be considered as weak hydrogen bonds. The driving forces for formation of these weak hydrogen bonds still contain electrostatic component but are dominated by van der Waals interactions.$^{14}$

![Figure 1.3: Illustration of a hydrogen bond between two alcohol molecules.](image)

In contrast to the interactions introduced above, the solvophobic effect is a non-covalent interaction that does not have electrostatic attraction as its major component. These solutes are not held together because of mutual attraction. Instead, the binding interaction is driven by the release of solvent molecules from the surfaces of each solute and the formation of stronger solvent-solvent interactions. The precise physical origin of solvophobic effect is still being debated. One of the most common solvophobic effect is the hydrophobic effect$^{15-17}$ The hydrophobic effect is an important component in controlling biological molecular recognition, and the strongest contributor to protein folding and membrane formation.$^{18,19}$

![Figure 1.4: Illustration of the aggregation of two solute molecules in solution caused by the solvophobic effect.](image)

1.2 NON-COVALENT INTERACTIONS OF ARENES

Non-covalent interactions involving aromatic rings, including CH–$\pi$ interactions, are important in molecular biology.$^{20-22}$ They can also be the major driving force for the
assembly of molecules\textsuperscript{23,24} and the selectivity of asymmetric organic reactions.\textsuperscript{25-27} The experimentally measured strength of a non-covalent interactions with arene rings is typically 1–5 kcal/mol.\textsuperscript{22} Based on the structures of the complementary functional groups that interact with the arene rings, the non-covalent interactions of aromatic surfaces can be classified into several subtypes, which include $\pi-\pi$ interactions, $XH-\pi$ interactions, and ion–$\pi$ interactions (Figure 1.5).

![Figure 1.5: Examples for different types of non-covalent interactions of arenes: a) $\pi-\pi$ stacking interactions, b) perpendicular arene–arene interactions, c) $XH-\pi$ (X = C, N, or O) interactions, and d) ion–$\pi$ interactions.

While each of these interactions has different contributing terms, many of these interactions can be treated as electrostatic hydrogen-bond-type interactions. Although benzene has no dipole moment, the six polarized $C^\delta^-H^\delta^+$ bonds leads to a large, permanent quadrupole moment (Figure 1.6). An electrostatic model of a benzene ring can be shown as a sandwich-like structure, with partial negative charges on the two $\pi$ electron-clouds above and below the faces of the ring, and partial positive charges on the edges of the ring.\textsuperscript{28} This model shows how an aromatic ring can act as a hydrogen-bond accepter. Brief descriptions for some of these interactions will be discussed as below.
1.2.1 Arene–Arene Interactions

The attractive interactions between two aromatic rings can be classified into two types: 1) parallel, including the aligned face-to-face and offset (parallel-displaced) stacking interactions (Figure 1.5, a), 2) perpendicular, including edge-to-face and edge-on (T-shape) interactions (Figure 1.5, b).

Figure 1.6: Depiction of the quadrupole of a benzene molecule: top view (left) and side view (right).

Figure 1.7: Relationship between the π–π interaction and the orientation of dimers based on Hunter’s electrostatic model. Adapted with permission from Hunter, C. A.; Sanders, J. K. M. J. Am. Chem. Soc. 1990, 112, 5525-5534. Copyright © 1990, American Chemical Society.

Hunter et al. developed an electrostatic model that describes and predicts the relationship between the interaction strength and the geometry of arene-arene interactions (Figure 1.7). The black areas in the Figure show arene–arene geometries where the interaction is attractive, which includes the offset face-to-face and edge-to-face...
geometries. The aligned face-to-face conformation will be a repulsive geometry due to the proximity of the two electronegative π-clouds.

1.2.2 CH–π Interactions

CH–π interactions are generally defined as the interactions between aliphatic CH’s and aromatic rings. Sometimes edge-to-face or edge-on arene–arene interactions are also considered as CH–π interactions. Both types of the interactions play significant role in conformations of marcomolecules, crystal packing, host-guest chemistry, determining reaction selectivities, and biochemical phenomena.

CH–π interactions can be classified as non-classical weak hydrogen bonds (1.5–2.5 kcal/mol). The interactions are primarily stabilized by dispersion forces, with the electrostatic forces as of only minor importance. Studies of the electronic substituent effects, solvent effects and thermodynamic properties of CH–π interactions have provided support for the weak hydrogen-bonding nature of these interactions. Exceptions are that in which the CH’s show strong proton-donating properties, such as Cl₃CH–π or C≡CH–π interactions.

1.2.3 Cation–π Interactions

The cation–π interactions are strong attractive interactions between positive charges and the π-clouds of aromatic rings. The strength of these non-covalent interactions are due to their strong electrostatic component. This is confirmed by the ability of simple electrostatic models to accurately describe the stability trends. There are a wide range of structural types, such as those found in proteins and artificial supramolecular receptors. Similar interactions have also been observed between cations and π-electrons of isolated alkenes and alkynes.
1.3 MOLECULAR BALANCES FOR MEASURING THE NON-COVALENT INTERACTIONS

Molecular balances are synthetic molecules designed to measure the strength of intramolecular non-covalent interactions. Due to their centrally located rotatable bonds, these structures are able to adopt two or more different conformations, one of which forms an intramolecular non-covalent interaction (Figure 1.8). Thus, the conformational equilibrium ratios are governed by the strength of the intramolecular interaction in the “folded” conformation and the strength of the solvent interactions.

\( \Delta G_{\text{fold}} \)

The difference in the free energy (\( \Delta G_{\text{fold}} \)) between folded and unfolded conformers of the balance provides a measurement of the strength of the intramolecular non-covalent interaction. To facilitate the measurement of the equilibrium ratios, the rate of the exchange of conformers should be slow enough to show distinct signals for each conformation in the NMR spectra, but rapid enough to allow conformational equilibrium to be reached within a reasonable timescale at room temperature. For room-temperature analysis using \(^1\text{H} \) NMR, this typically requires a rotational barrier that is larger than 16 kcal/mol. Ideally, the intramolecular interactions in the folded conformation can also be observed and characterized directly in the solid-state using X-ray crystallography.

There are several advantages in using molecular balances for the study of non-
covalent interactions versus biomolecular systems or supramolecular complexes. First, molecular balances are minimal single-molecule systems, which provide better control over the geometries of the interactions. Second, the interaction of interest can be more easily isolated from other intramolecular or intermolecular interactions in these minimal model systems. Thus, the observed behaviors of the molecular balances provide a more accurate measure of the interaction of interest. Finally, modifications of these structures and solvent environment are easier. This makes it easier to systematically study the variables that influence the strength of the interaction, such as substituent and solvent effects. A number of successful molecular balances have been developed, and several examples will be presented in the next section.

1.3.1 Triptycene-Based Torsional Balances

Figure 1.9: Equilibrium between different conformers of 1,9-disubstituted triptycenes used to study intramolecular interactions between the Y and Z groups.45

In 1970s, Oki et al. measured the rotational barriers of a series of bridge-head-substituted triptycene molecules (Figure 1.9).47 They found that by increasing the size of substituents at 1– and 9– position, they are able to raise the rotational barrier of the C–C bond, so that distinct signals for different conformers were observed in $^1$H NMR at low temperatures.48 Intramolecular interactions were able to be formed between the 1– and
9– substituents in the +/- syn conformations, and were broken in the anti conformation. By variation of the Y and Z groups, triptycene balances have been applied to the study of a broad range of non-covalent interactions, including CH–O, CH–π and oxygen/halogen–π, methoxymethyl–π, and π–π stacking interactions. 1.3.2 Wilcox’s Molecular Balances Wilcox et al. were the first to coin the term “molecular torsion balance” to define these functional model systems in 1994. Wilcox’s molecular balances adopted distinct folded and unfolded conformers (Figure 1.10) due to the restricted rotation of the aryl–aryl single bond, and the two conformers showed distinct signals in the 1H NMR spectra at room temperature. The folding energies were then used to quantify the stabilities of intramolecular interactions in the folded conformers.


The balances were originally applied to measure edge-to-face arene–arene interactions. By varying the substituents on the two interacting phenyl rings, the electrostatic nature of the interaction was systematically probed. The results showed that the variation of the edge-ring (ring b in Figure 1.10) had a strong influence on the folding behavior of the balances, while variation of the face-ring (ring c in Figure 1.10) only led to a slight change. This second observation was originally used to support the hypothesis
that dispersion forces play a more important role than electrostatic forces in edge-to-face interactions.\textsuperscript{63} However, further studies found that the lack of electrostatic trends for the face-ring is due to the solvent molecules screening electrostatic attraction between two phenyl rings.\textsuperscript{64-66}

Wilcox’s balance system is one of the most extensively studied molecular balances. These molecules were also modified to study aliphatic CH–π interactions,\textsuperscript{62,67} halogen–π interactions,\textsuperscript{68} and solvent effects.\textsuperscript{66,69} This system also inspired a number of computational studies.\textsuperscript{44,70}

1.3.3 Dibenzobicyclo[3, 2, 2]-Nonane Derivatives

A series of dibenzobicyclo[3, 2, 2]-nonane-based balances were developed by Motherwell \textit{et al.}\textsuperscript{71,72} for the study of the non-covalent interactions of aromatic rings (Figure 1.11). Each of these molecules exist in two conformations, in which either the Y or Z group interacts with the face of an aromatic ring (X = OH or OMe; Y = H, Me, n-Bu, CN, or C≡CH). The barrier for the conformational change is relatively low, and the two conformations are in rapid equilibrium on the NMR time-scale. However, accurate ratios of the two conformers could be measured from the \textsuperscript{1}H NMR $J$-couplings.\textsuperscript{71}

**Figure 1.11:** Motherwell’s balances for quantifying functional group–π interactions in organic solvent.\textsuperscript{71,72}

The solvent effects were studied in a balance with Y = CH$_3$ and Z = OH. In solvents with low polarity (cyclohexane, CCl$_4$, and benzene), the conformation that forms OH–π interactions dominated. In polar solvents that can act as H-bond acceptors
(pyridine, methanol and DMSO), the equilibrium shifts towards the one that forms the weaker CH–π interactions, allowing the OH group to form hydrogen-bonding interactions with solvent. The balances with Z = NH₂ were also studied, and the NH–π interaction was found to be weaker than the OH–π interaction under the same conditions.⁷²

1.3.4 Early Model System from Our Group

A series of naphthalene diimide balances were designed in our group to study parallel face-to-face aromatic stacking interactions (Figure 1.12).⁷³ The rotational barrier between the syn and anti conformations was sufficiently high (27 kcal/mol) that the two conformers could be isolated at room temperature. X-ray analysis of the anti conformer indicated the formation of two stacking interactions with the central naphthalene diimide surface. Upon heating, the two conformers reached equilibrium in period of (**minutes or hours), and the folding energy could be quantified by the ratios of the two conformers. Different sized arene groups were linked to the “arm” (*label on figure) position, and the folding energies were found to increase with the size of the arene groups. One explanation for this folding trend is that the dispersion forces are stronger for larger aromatic surfaces. Another explanation is solvophobic effects which scales with **. However, no solvent effect was observed for this series of balances.

![Figure 1.12:](image)

**Figure 1.12:** The syn and anti conformers for the naphthalene diimide molecular balances for measuring π–stacking interactions.
More recently, a new series of balances for measuring face-to-face $\pi$-stacking interactions were designed based on an $N$-arylsuccinimide phencyclone framework (Figure 1.13). Control balances were made with different sized shelves (**label on Fig). The balances with large shelves (phenanthrene, and pyrene) were found to have higher $folded/unfolded$ ratios than the ones with smaller benzene shelves which cannot form a $\pi-\pi$ interaction with the phenyl ring of the arm, and only forms a repulsive lone pair-$\pi$ interaction with the oxygen linker. The folding energies for the balances were measured in a series of solvents. The balances were more folded in more polar solvents, which is consistent with the theory that solvophobic effects drive the folding of balances.

![Diagram](image)

**Figure 1.13:** The equilibrium between unfolded and folded bicyclic molecular balances for measuring $\pi-\pi$ stacking interactions.

1.4 CONCLUSION

In this chapter, the description and nature of non-covalent interactions with a specific focus on interactions of arenes were introduced. Several examples of recent molecular systems for the study of non-covalent interactions of arenes were presented. The purpose of this chapter was to show how important these weak interactions are, and what a challenge to understand and predict their nature. Thus, designing new molecular balances for the further study of the non-covalent interactions is of great significance and importance.
In the following chapters of this thesis, the study of non-covalent CH–π interactions using the molecular balances developed in our group will be presented. Different aspects of the CH–π interactions were studied, such as steric, conformational entropy, cooperativity, deuterium isotope effect, substitution effects, and solvent effects.
CHAPTER 2

GENERAL EXPERIMENTAL DESIGN FOR THE MEASUREMENT OF CH–π INTERACTIONS WITH MOLECULAR BALANCES

The new bicyclic $N$–arylimide molecular balances introduced at the end of Chapter 1 were shown to be effective on measuring face-to-face $\pi$–$\pi$ stacking interactions (Figure 2.1).\textsuperscript{74} Compared with the other systems described in Chapter 1, the new balance system possesses several advantages. First, the balances have suitably high rotational barriers, so that the two confirmations show distinct peaks in the $^1$H NMR spectrum at room temperature, which simplified the measurement of the folded/unfolded rations in solution. Second, the balances were easier to synthesize, which made it more convenient to switch the interacting groups and study different non-covalent interactions. Finally, the balances showed good solubility in a wide range of solvents, which enabled the study of the solvent effects on the interactions.

Figure 2.1: The equilibrium between folded and unfolded conformers of the bicyclic $N$-arylimide molecular balance for study the face-to-face $\pi$–$\pi$ interaction.
The structures of the balances were modified to extend our study to other non-covalent interactions, but the experimental designs stayed similar regardless of the changes. In this chapter, general methods for the experimental measurement of CH–π interactions using molecular balances developed in our group will be introduced. Details of the introduction include the synthesis and characterization of balance molecules, the quantification of folded and unfolded conformations of the molecular balances, and the calculation of interacting energies, entropy values, and enthalpy values of each interaction.

2.1 STRUCTURES OF BALANCES

![Figure 2.2: Molecular balances A and B designed to measure aliphatic CH–π interactions and balances C to measure aromatic CH–π interactions (or edge-to-face arene–arene interactions). All structures were shown in folded conformations.](image-url)

The design of the balance system to measure CH–π interactions and edge-to-face arene–arene interactions (Figure 2.2) is based on an atropisomeric bicyclic N–arylimide framework that we have previously utilized to study face-to-face π–π interactions (Figure 2.1). Due to restricted rotation about the Caryl–Nimide bond, the molecular balances adopt two distinct conformers. In the folded conformation, the arm group (phenyl ether) is positioned over the arene shelf forming an intramolecular interaction. In the unfolded conformation, the arm group points away from the arene shelf and cannot form an intramolecular interaction. The two conformations are in slow exchange in solution at
room temperature on the $^1$H NMR timescale, which enables the easy characterization of *folded* and *unfolded* conformers by the distinct peaks on the $^1$H NMR spectrums.

First, the phenyl ether on the arm position of the balance was replaced with alkyl ether groups to study aliphatic CH–π interactions (balances A, Figure 2.2). The characterization in both solid-state and in solution proved the formation of desired interactions. However, due to the oxygen linker, the interacting surface area was limited, and only the interactions formed by methyl and ethyl groups could be effectively studied. The balances were then made with the alkyl groups directly linked to the phenyl rotor (balances B, Figure 2.2). Without the oxygen linker, we were able to study multiple CH–π interactions formed by various sized alkyl groups. The phenyl rotor was also replaced with 1-napthyl rings to form an edge-to-face arene–arene interaction with the arene shelves in the *folded* conformers (balances C, Figure 2.2). The formation of the intramolecular interactions was confirmed by modeling studies and the characterization data.

Figure 2.3: One-armed (1a–e), two-armed (1f, 1g, and 2c), and control (2a–b, 3a) molecular balances designed to measure CH–π interaction.

For each new series of balances, control balances with smaller or no arene shelves (benzene, ethylene) were made (balances 2a, 2b and 3a, Figure 2.3). They can help to measure the internal biases and secondary interactions that exist in each new balance
arisen from the framework and the central aromatic ring. “Two-armed” balances with two identical arms (1f, 1g, and 2c) were also made to force the balances to adopt the folded conformation. This allowed us to characterize the interactions in solid-state via X-ray crystallography when the unfolded conformation was more stable.

The balances with –CH₃ or –OCH₃ arm can also be applied to the study of deuterium isotope effect by comparisons of corresponding balances with –CD₃ or –OCD₃ arms. These balances form CH–π interactions within relatively open and unconfined environments. Therefore, these model systems were less susceptible to steric effects arisen from the small difference in the size of –CH₃ and –CD₃ groups.

2.2 GENERAL SYNTHETIC ROUTE

![Diagram]

**Figure 2.4:** General route for the synthesis of balances 1–3 (X = CO, O, or CH₂; Y = H or Ph).

The balances were quickly assembled in modular fashion (Figure 2.4). First, the Diels-Alder reaction between a cyclic diene and maleic anhydride yielded the endo-bicyclic anhydride containing the arene-shelf. Then, the thermal condensation of the crude anhydride with an ortho-substituted aniline formed the N–arylimide linkage of the molecular balance. Both reactions proceeded in high yields of >80% in all cases. The efficiency of this synthesis is one of the most attractive features of the N–arylimide
framework, and facilitated the rapid variation of the size and structure of the arm group and the arene shelf.

2.3 QUANTIFICATION OF FOLDED AND UNFOLDED CONFORMERS

Figure 2.5: $^1$H NMR spectra of balance 1a in CDCl$_3$ allowed for quantification of folded/unfolded ratios.

Measurement of the concentrations of the two conformers in solution for most balances was based on the upfield shifted ortho proton on phenyl rotor (H$_a$, Figure 2.5) of the unfolded conformer in the $^1$H NMR spectra. Due to its proximity to the arene shelf, the doublet-doublets of H$_a$ is shift dramatically upfield from its normal position (~7 ppm) to a clear region of the $^1$H NMR spectra. For balances with aromatic shelf (phenanthrene, pyrene, or benzene), the H$_a$ peak will be shifted to the region between 4.0 and 5.0 ppm. The two conformers also showed separate signals for the two succinimide protons, usually between 4.5 and 5.0 ppm (H$_b$, Figure 2.5). One of the two H$_b$ singlets, which had an area consistent with two times the area of unfolded H$_a$, was assigned as H$_b$ unfolded. The other singlet was thus signed as H$_b$ folded. Distinct peaks were also shown for the protons on the alkyl arm of the two conformers. The peaks for folded conformation were shifted upfield because of the shielding form the aromatic shelf.
For balances without an aromatic shelf, the signal for H<sub>a</sub> in the *unfolded* conformation also showed an upfield shift form the *folded* H<sub>a</sub> (Figure 2.6), but the difference was much smaller than the balances with aromatic shelves. Sometimes both H<sub>a</sub> peaks overlapped with the other aromatic peaks and could not be clearly identified. In these cases, the conformers were assigned using NOEs between the alkyl peak and vinyl protons in the *folded* conformation. The vinyl protons also showed split signals for the two conformers some times, but the relative position of for the two set of peaks was variable with the NMR solvents.

![Figure 2.6](image)

**Figure 2.6:** ¹H NMR spectra of a balance with ethylene shelf in CDCl₃ allowed for quantification of *folded/unfolded* conformations.

### 2.4 CALCULATION OF THE INTERACTING ENERGIES

The ratio of the *folded* and *unfolded* conformers provides a direct and accurate measure of the strength of the intramolecular interaction. The equation for the calculation of folding energy (ΔG<sub>fold</sub>) of each balance is shown as Equation 2.1:

\[
\Delta G_{\text{fold}} = -RT\ln(\text{folded/unfolded})
\]

(Equation 2.1)

The *folded/unfolded* ratio for each balance was measured after the conformers were allowed to reach equilibrium. The equilibration time should be at least 10 half-lives,
and was calculated based on the rotational barrier of the C–N linkage. The barrier was primarily determined by the size of the ortho arm group on the phenyl rotor. For balances with oxygen atoms in the ortho position, the rotational barrier was 20–21 kcal/mol based on the kinetic studies,\textsuperscript{74,75} which equates to a half-life of less than two minutes. Thus, the \textit{folded/unfolded} ratios were usually measured after allowing the dissolved balances to stand at room temperature for two hours.

For most of the balances with aromatic shelves, the calculations of \textit{folded/unfolded} ratios were based on the integrations of the H\textsubscript{a} peaks on \textsuperscript{1}H NMR spectra, because those peaks are usually in an unobscured region and were singlets, which allowed for easier integration and higher accuracy. The peaks for the protons on the alkyl arm groups can also be used for the calculation, and the ratios were almost identical with the results from the peaks of H\textsubscript{a}. However, in order to be consistent, unless the two peaks were not well resolved, which was obscured in the spectrums of balances without aromatic shelves, the folding energies were still calculated based on the H\textsubscript{a} peaks. The \textit{folded/unfolded} ratios of the ethylene balances were based on the ratios of the two protons of the ethylene shelf. In cases of poor separation of these signals, the peaks for alkyl group were used to measure the \textit{folded/unfolded} ratios.

The error of the analysis was calculated based on a conservative estimate of ±5\% for the \textsuperscript{1}H NMR integration error for each peak,\textsuperscript{77-79} which means a ±0.03 kcal/mol error when transferred into folding energy. Spectral deconvolution method using VNMRJ software “fitspec” command at corresponding areas was applied when analyzing the spectrums to reduce the error.

2.5 THERMODYNAMIC STUDIES

Variable-temperature \textsuperscript{1}H NMR (VT NMR) study of the balances enabled the
measurement of rotational barrier. For balances that showed distinct signals in the $^1H$ NMR spectrum for the two conformations at room temperature, the peaks corresponding to the same proton in the two conformers will shift closer and coalesce on heating. The coalescence temperature ($T_c$) can be used for the estimation of the rotational barrier ($\Delta G^\ddagger$) using Equation 2.2:\textsuperscript{80,81}

$$\Delta G^\ddagger = aT [9.972+\log(T_c/\Delta \nu)]$$

(Equation 2.2)

Where $\Delta \nu$ stands for the maximum peak separation of the low-temperature limit (in Hz), and $a = 4.575 \times 10^{-3}$ kcal/mol.\textsuperscript{80} For balance 1a, the $T_c$ was measured to be 135 °C in TCE–$d_2$. This equated to a rotational barrier of 20.5 kcal/mol.\textsuperscript{75} For balances with an ortho methyl group, the barrier was measured to be 20.6 kcal/mol.\textsuperscript{81}

![Figure 2.7: The van’t Hoff plots of the molecular balance 1b in CDCl$_3$ (25°C–55°C).](image)

The VT NMR experiments were also used to measure the differences in enthalpy ($\Delta H$) and entropy ($\Delta S$) between the two conformers. The full $^1H$ NMR spectras were acquired at 10 °C intervals, and the van’t Hoff plots were drawn with the $\ln$($\text{folded/unfolded}$) on the y-axis and the reciprocal of the temperatures on the x-axis. A typical van’t Hoff plot is shown as Figure 2.7. Live fitting of the lines gave slopes
corresponding to $-\Delta H/R$ and $y$-intercepts to $\Delta S/R$. The $\Delta H$ and $\Delta S$ values were then calculated using Equations 2.3 and 2.4:

\[
\Delta H = -\text{slope} \times R \quad \text{(Equation 2.3)}
\]
\[
\Delta S = y_{\text{int}} \times R \quad \text{(Equation 2.4)}
\]

The errors for slopes and intercepts are measured by the regression add-in in excel.

The folding energies could also be calculated using the measured entropy and enthalpy values based on equation 2.5:

\[
\Delta G_{\text{fold}} = \Delta H - T\Delta S \quad \text{(Equation 2.5)}
\]

The calculated $\Delta G$ values using the equation above were generally very close as the result calculated directly from the $\text{folded/unfolded}$ ratios. These multiple point $\Delta G_{\text{fold}}$ values were used as reference measurements to verify the certainty of the data from single-point experiments.

2.6 CONCLUSION

A series of molecular balances were designed and prepared based on previous designed bicyclic phencyclone framework to measure non-covalent CH–π interactions. General procedures for the synthesis of the balances were described, and the methods for the characterization of the formed interactions in solution were illustrated. Also, by conducting VT NMR experiments, we were also able to estimate the rotational barriers and to measure the enthalpy and enthalpy changes between the two conformers of the balances. Studies using our molecular balances on measuring different non-covalent interactions will be presented with details in the following chapters.
CHAPTER 3

Molecular Balances for Measuring Aliphatic CH–π Interactions with the Existence of Lone Pair–π Interactions

As introduced in Chapter 1, CH–π interactions are a series of important interactions with weak and non-directional nature. The direct and accurate measurement of CH–π interactions is thus difficult. The objective of this chapter is to introduce our first approach on measuring aliphatic CH–π interactions using molecular balances with O–Alkyl arms (Figure 3.1). As introduced in Chapter 1 and 2, all these balances were designed based on the same conformational dynamic framework previously developed in our group.

Figure 3.1: One-armed (1a–e), two-armed (1f, 1g, and 2c), and control (2a–b, 3a) molecular balances designed with alkoxy arm groups to measure CH–π interaction.

This work benefited from the effort of a previously group member, William Carroll, who initiated this project, developed the synthesis route, and helped on synthesizing balances 1a, 1b, and 2a. Major results presented in this chapter have been published in 201175 and were reprinted with permission (Copyright © 2011, American Chemical Society).
3.1 DESIGNS OF THE STRUCTURES OF MOLECULAR BALANCES

Three types of balances were studied for this study. Balances 1a–e have alkoxy groups of varying sizes (R₁ = Me, Et, i-Pr, n-Bu, c-Hex) that can interact with a large phenanthrene surface. Control balances 2a–b and 3a have smaller benzene or ethylene surfaces. Finally, ‘two-armed’ balances 1f, 1g, and 2c that have two identical ortho-alkoxy arms were made to force one of the alkoxy groups to position over the arene shelf (Figure 3.1). The purpose for have the oxygen linker in each of the balances is to 1) enable the systematic variation of the size of the alkyl group, and 2) compare with the balances with phenyl ether arm for studying face-to-face π–π interactions in previous study.⁷⁴

3.2 CHARACTERIZATION OF CH–Π INTERACTIONS IN SOLID STATE

![Figure 3.2: X-ray structures of (a) balance 1d, and (b) balance 3a, both shown in unfolded conformation. The bridge phenyl atoms of 1d were hidden for better viewing clarity.](image)

To verify the formation of an intramolecular CH–π interaction in the folded conformers, the solid-state structures of the balances were analyzed by X-ray crystallography. Unfortunately, the one-armed balances preferred to crystallize in the unfolded conformation (e.g. balance 1d and balance 3, Figure 3.2), due to the repulsive interaction between the ether oxygen linker and the arene shelf.
To force the molecule to crystallize in the *folded* conformation, two-armed balances 1f, 1g, and 2b were synthesized that have identical alkyl-ether substituents at both *ortho*-aryl positions. Therefore, one of the two arms would always be in the *folded* configuration. The X-ray crystal structures of the two-arm balances 1f, 1g, and 2b were then obtained (Figure 3.3).

![Figure 3.3: X-ray structures of the two-armed balances (a) 1f, (b) 1g, and (c) 2b. The solvent molecules and the bridgehead phenyl groups are hidden for viewing clarity. The inset boxes show top-views of the interacting alkoxy and arene surfaces in each balance.](image)

Geometries of the CH–π interactions obtained in the two-armed balances were analyzed. The OMe and OEt groups in 1f and 1g each form one well-defined CH–π interaction. A proton on the carbon bonded to the ether oxygen points down into the center of the outer ring of the phenanthrene shelf with atom to plane distances of 2.57 and 2.76 Å, respectively. These distances are less than the sum of the van der Waal's radii of the interacting H and C atoms (2.90 Å) and are also within the commonly used distance cut-off of 3.05 Å for the CH–π interaction. In the solid-state, the terminal carbon of the
OEt group in 1g does not form an additional CH–π interaction as it extends beyond the phenanthrene surface and is positioned over the central bay region (Figure 3.3, b). Similarly, control balance 2b with the shorter benzene surface (Figure 3.3, c) does not form a CH–π interaction, as the methyl group extends beyond the benzene shelf. However, 2b retains the repulsive lone pair–π interaction between the oxygen of the ether linker and the arene shelf that is also present in balance 1. The oxygen-to-aromatic plane distance in 2b is 3.37 Å, which is similar to distances for 1f and 1g (3.519 Å for OEt, 3.374 Å for OMe). Thus, comparison of the folding propensities of balances 1 and 2 provides a direct measure of strength of the intramolecular CH–π interaction.

3.3 QUANTIFICATION OF CH–π INTERACTIONS IN SOLUTION

As introduced in Chapter 2, the strengths of the CH–π interactions were measured by monitoring the folded/unfolded conformational equilibrium by 1H NMR. Due to restricted rotation around the Caryl–Nimide single bonds, the folded and unfolded conformations were in slow exchange at room temperature. The rotational barrier about the Caryl–Nimide bond in balance 1a was measured to be 20.5 kcal/mol by VT NMR method (with a coalescence temperature of 135 °C in TCE-d2), which equates to a half-life of 1.4 min at 23°C.

Separate peaks for the alkoxy-groups in different conformations were observed in the 1H NMR spectra. For the phenanthrene balances 1a–g that form intramolecular CH–π interactions, large upfield shifts of 1.4 to 1.6 ppm were observed for the alkoxy protons in the folded conformers due to the proximity of the arene shelf. In contrast, only small upfield shifts were observed for the folded conformers of control balances 2 (0.1 to 0.3 ppm) and 3 (0.01 ppm).

The folding propensities of the balances 1a–e and control balances 2a–b and 3a
were measured. Integration of the peaks for the respective conformers yielded the *folded/unfolded* ratios \( K_{eq} \) and \( \Delta G_{fold} \) values (Table 3.1). The singlets corresponding to two *syn*-protons on the succinimide rings of the balances provided the most accurate *folded/unfolded* ratios as they fell in a clear region of the \(^1\text{H} \) NMR spectra (4.2–4.8 ppm) and were well differentiated in most solvents. The conformers with aromatic shelves (1a–e and 2a–b) were assigned by the upfield shifts of the alkoxy protons in the *folded* conformers. For balance 3a, the conformers were assigned by NOEs between the methyl ether and vinyl protons in the *folded* conformer. Also, in order to verify that aggregation did not also attenuate the *folded/unfolded* ratio, the \( K_{eq} \) of balance 1a was measured over a wide concentration range. The *folded/unfolded* ratio remained constant from 1.9 mM to 17 mM in CDCl\(_3\), confirming that aggregation did not affect the *folded/unfolded* ratio.

**Table 3.1**: Comparison of *folded/unfolded* ratios and \( \Delta G_{fold} \) values for one-armed balances as measured by \(^1\text{H} \) NMR integrations, in CDCl\(_3\) at 23 °C.

<table>
<thead>
<tr>
<th>balances</th>
<th>alkoxy–arm</th>
<th>arene–shelf</th>
<th>( K_{eq} )</th>
<th>( \Delta G_{fold} ) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>OMe</td>
<td>phenanthrene</td>
<td>0.46</td>
<td>0.45</td>
</tr>
<tr>
<td>1b</td>
<td>OEt</td>
<td>phenanthrene</td>
<td>0.20</td>
<td>0.94</td>
</tr>
<tr>
<td>1c</td>
<td>Oi–Pr</td>
<td>phenanthrene</td>
<td>&lt; 0.05</td>
<td>&gt; 1.8</td>
</tr>
<tr>
<td>1d</td>
<td>On–Bu</td>
<td>phenanthrene</td>
<td>0.13</td>
<td>1.2</td>
</tr>
<tr>
<td>1e</td>
<td>Oc–Hex</td>
<td>phenanthrene</td>
<td>&lt; 0.05</td>
<td>&gt; 1.8</td>
</tr>
<tr>
<td>2a</td>
<td>OMe</td>
<td>benzene</td>
<td>0.09</td>
<td>1.40</td>
</tr>
<tr>
<td>2b</td>
<td>OEt</td>
<td>benzene</td>
<td>0.036</td>
<td>1.96</td>
</tr>
<tr>
<td>3a</td>
<td>OMe</td>
<td>Ethane</td>
<td>0.73</td>
<td>0.18</td>
</tr>
</tbody>
</table>

3.3.1 Comparison Between Methoxy andEthoxy Balances

The differences in the folding energies (\( \Delta \Delta G \)) of balances 1 and 2, that can and cannot form CH–π interactions respectively, provides a measure of the CH–π interactions. Therefore, the \( \Delta \Delta G \) for the methoxy balances 1a and 2a yields an estimate of −0.95 kcal/mol for the CH–π interaction in CDCl\(_3\) (\( \Delta \Delta G = 0.45–1.40 \) kcal/mol). An analogous analysis with ethoxy balances 1b and 2b yielded a value of −1.04 kcal/mol. The similar
magnitudes of the CH–π interactions for the methoxy and ethoxy balances were in accord with the crystal structure analyses that found a single CH–π interaction in both balances. The magnitude of the CH–π interaction also compares favorably to previous measurements by Wilcox of −0.44 kcal/mol for an intramolecular alkyl CH–π interaction in CDCl₃.⁶²

Although the CH–π interactions in 1a and 1b were attractive, the folded conformers were still not the major conformers. We hypothesized that this was due to an opposing repulsive interaction between the ether oxygen linkers and the arene surfaces. To measure the strength of the repulsive interaction, control balance 3a was prepared, which lacked an aromatic surface. Therefore, the $K_{eq}$ of 3a provided a measure of the intrinsic conformational bias of the N–arylimide framework in the absence of the attractive CH–π and the repulsive oxygen–arene interaction. As expected, $K_{eq}$ of 3a was close to unity (0.73). The slight bias for the unfolded conformer was attributed to differences in dipole and solvation energy of the conformers. The $\Delta \Delta G_{fold}$ for 2a and 3a was +1.22 kcal/mol. This repulsive oxygen–π interaction was slightly larger than the attractive CH–π interactions in 1a and 1b, providing and explanation for the overall bias for the unfolded conformers in both balances.

3.3.2 Balances with Large Alkoxy Groups

Folding energies of balances 1a–e with alkoxy arms of varying lengths and widths (OMe, OEt, Oi-Pr, On-Bu, and Oc-Hex) were also compared (Table 3.1). In general, larger alkoxy arms appeared to weaken the intramolecular CH–π interactions. For balances 1c and 1e, only the unfolded conformer was observed, and thus a maximum $folded/unfolded$ ratio of 0.05 in Table 3.1 was estimated based on a $^1$H NMR integration accuracy of ±2%.
This trend could be explained for the branched O\textsubscript{i}-Pr and O\textsubscript{c}-Hex groups in 1\textit{c} and 1\textit{d}. Modeling showed that these secondary alkoxy groups create significant steric strain in the \textit{folded} conformation. One of the two alkyl groups attached to the branch point was always pressed into the arene shelf. The destabilization of the balances with the longer linear alkoxy arms 1\textit{b} (OEt) and 1\textit{d} (On-Bu) was more difficult to explain. X-ray and molecular modeling studies predicted that 1\textit{a}, 1\textit{b}, and 1\textit{d} should have similar folding energies because: 1) they all form only a single CH–π interaction between the protons on the carbon attached to the ether oxygen and the phenanthrene surface, and 2) the more flexible linear alkoxy groups can adopt conformations that minimize any destabilizing steric interactions. A possible explanation was that the observed differences in $\Delta G_{\text{fold}}$ were due to differences in conformational entropy ($\Delta S$) of the alkoxy arms.

3.3.3 Comparison of Entropy and Enthalpy Values

![Figure 3.4:](image)

\textbf{Figure 3.4:} Folding energy ($\Delta G$), enthalpy ($\Delta H$) and entropy ($T\Delta S$) values with error bars in CDCl\textsubscript{3} for balance 1\textit{a}, 2\textit{a}, 1\textit{b}, 2\textit{b}, 3\textit{a} measured from van’t Hoff plots (25–55 °C).

To test the theory above, the entropic and enthalpic terms of the folding equilibriums were measured for balances 1\textit{a}, 1\textit{b}, 2\textit{a}, 2\textit{b}, and 3\textit{a} (Table 3.6, Figure 3.4). The van’t Hoff analysis for balances 1\textit{c}, 1\textit{d}, and 1\textit{e} were not performed because of the large errors in the analysis for balances with folded/unfolded ratios $< 0.1$ or $> 10$. 
The analysis confirmed that the apparent differences between the CH–π interactions formed in balances with OMe and OEt arms were due to differences in conformational entropy. For example, the ΔΔG of 0.47 kcal/mol for OEt and OMe balances, 1b and 1a, was due primarily to the differences in the entropic term, as –TΔΔS > ΔΔH (Table 3.2, entry 1). The additional methylene group of the OEt arm of 1b forms only a slightly stronger CH–π interaction, as ΔΔH was small. The larger change was in the entropic term (0.61 kcal/mol), which can be attributed the loss of rotational freedom in the OEt arm when it is held against the phenanthrene shelf in the folded conformation. The same entropic penalty was observed for the smaller benzene-shelf balances 2b and 2a (Table 3.2, entry 2) that cannot form CH–π interactions, confirming that the entropic penalty in the OEt group was due to rotational isomerism around the O–CH₂ bond and not the CH₂–CH₃. The magnitude of the entropic penalty was also consistent with estimates of loss of rotational freedom around the O–C bond of an ethoxy group (–TΔΔS = 0.43 kcal/mol).82

Table 3.2: Comparison of ΔΔG, ΔΔH and –TΔΔS values for balances for selected pairs of balances (in CDCl₃, 25°C).

<table>
<thead>
<tr>
<th>entry</th>
<th>comparison</th>
<th>ΔΔG (kcal·mol⁻¹)</th>
<th>ΔΔH (kcal·mol⁻¹)</th>
<th>–TΔΔS (kcal·mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1b–1a</td>
<td>0.47</td>
<td>−0.14</td>
<td>0.61</td>
</tr>
<tr>
<td>2</td>
<td>2b–2a</td>
<td>0.62</td>
<td>−0.03</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
<td>1a–2a</td>
<td>−0.95</td>
<td>−0.79</td>
<td>−0.15</td>
</tr>
<tr>
<td>4</td>
<td>1b–2b</td>
<td>−1.10</td>
<td>−0.96</td>
<td>−0.14</td>
</tr>
<tr>
<td>5</td>
<td>2a–3a</td>
<td>1.23</td>
<td>0.42</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The analyses in Table 3.2 also confirmed the validity of measuring the CH–π interaction via the difference in folding energies of the phenanthrene and benzene-shelved balances 1 and 2 (Table 3.2, entries 3 and 4). This comparison effectively removes the differences in conformational entropy in the folded and unfolded conformers,
isolating the enthalpic differences associated with the CH–π interaction. This can be seen by the dominant enthalpic terms (|ΔΔH| > |TΔΔS|). Also, the ΔΔH terms for 1a–2a and 1b–2b were very similar (−0.79 and −0.96 kcal/mol), which is consistent with both OMe and OEt arms forming a single CH–π interaction.

3.3.4 Solvent Study

The excellent solubility of this balance system enables the study on solvent effect. In previous study on face-to-face arene–arene interactions, solvents were observed to have a great influence on the strength of the interactions due to solvophobic effect. In a solvent with higher polarity, the solvophobic effect would be stronger, and the non-covalent interaction would be stabilized.

![Figure 3.5: Measured −ΔG of (a) balance 1a and 1b and (b) balances 1a, 2a and 3a in a series of solvents versus the E_T(30) for each solvent. Solvent from left to right are deuterated benzene, THF, chloroform, TCE, acetone, DMSO, and acetonitrile at 23 °C.](image)

In order to identify the magnitude of solvophobic effect on CH–π interactions, we attempt to study how polarity of solvent influent the strength of these interactions. Balances 1a and 1b was dissolved in a series of deuterated solvent (benzene, THF, chloroform, TCE, acetone, DMSO, acetonitrile), and the calculated −ΔG values were
plotted versus the $E_T(30)$ values of the solvents (Figure 3.5).

A linear correlation between the $-\Delta G$ and $E_T(30)$ values was observed. The polar solvents drive the balances into a higher folding degree, and trends of balances with different shelves or arms were close to parallel. The observation is consistent with Hunter’s hypothesis that other than the attractive interaction formed in the *folded* conformers, the solvophobic effect is also important factor that determines the folding ratio. The molecules of solvent with high polarity are more intended to interact with each other rather than with the arm or shelf of the balance molecules, and thus stabilized the *folded* conformer by forcing the intramolecular interactions to happen. Thus, even though the balances form different interactions, the trends of the folding energies in different solvents were similar. The observation also matched up with the previous study on face-to-face arene–arene interactions.

### 3.4 CONCLUSION

In conclusion, a series of molecular balances based on the versatile bicyclic $N$–arylimide framework were designed, which can accurately measure intramolecular CH–π interactions. Due to the weak nature of the CH–π interaction (~1.0 kcal/mol) and the sensitivity of the balances, stability trends were easily masked by other weak forces such as rotational entropy and repulsive lone pair–π interactions. However, through comparison with carefully designed control balances, we can isolate the relative contribution of the CH–π interaction to the $\Delta G_{\text{fold}}$. For example, the 0.45 kcal/mol $\Delta G_{\text{fold}}$ measured for balance 1a is the sum of three terms: (1) the attractive CH–π interaction between the methyl and phenanthrene surfaces (~0.95 kcal/mol), (2) the repulsive oxygen–π interaction (1.23 kcal/mol), and (3) the slight conformational bias of the balances for the *unfolded* conformer (0.17 kcal/mol), which was estimated based on the
folded/unfolded ratio for control balance 3a without form a CH–π interaction. The solvent effect on CH–π interactions was also studied, and the solvophobic effect was proved to be the main reason for changing folding energies in different solvents.

3.5 EXPERIMENTAL SECTION

NMR spectra were recorded on Varian 300 MHz and 400 MHz spectrometers. Chemical shifts are reported in ppm (δ) referenced to TMS. All chemicals were purchased from commercial suppliers and used as received unless otherwise specified. Flash chromatography was carried out using silica gel from Sorbent Technologies (60 Å, 200–400 mesh). Thin layer chromatography (TLC) was performed using pre-coated TLC plates (Merck pre-coated 0.25 mm silica gel 60 F254 plates).

3.5.1 Synthesis

The general synthetic route for balances 1–3 (Figure 3.1) was as shown in Figure 3.6. All balances were synthesized via the condensation between anilines 4 and anhydrides 5 made via Diels-Alder reaction. The detailed synthesis of each of these compounds and the characterization data are shown as follows.
Procedures for Preparing Nitrophenylethers 6c–6e

Compounds 6c–6e are known molecules and were prepared via modified procedure from existing synthetic route. To the mixture of potassium hydroxide or sodium hydride and alcohol, 1-fluoro-2-nitrobenzene was added drop-wise while stirring under nitrogen. After reacted for 24 h, the solvent was removed under vacuum. The residue was then diluted with 30 mL ethyl acetate and washed with 50 mL water for 3 times. The ethyl acetate was then removed under reduced pressure to afford accordingly substituted nitrobenzene.

Preparation of 1-iso-Propoxy-2-Nitrobenzene (6c)

Potassium hydroxide (0.27 g, 4.8 mmol), 1-fluoro-2-nitrobenzene (0.33 g, 2.4 mmol), and iso-propanol (5.0 mL) were used as reactants. 0.40 g product was obtained as yellow solid (94% yield). The spectra data were in agreement with reported. 1H NMR (400 MHz, CDCl₃) δ 7.77 (dd, J = 8.0 Hz, J = 1.4 Hz, 1 H), 7.47 (dt, J = 7.9 Hz, J = 1.4 Hz, 1 H), 7.07 (d, J = 8.4 Hz, 1 H), 7.00 (t, J = 7.8 Hz, 1 H), 4.65 (m, 1 H), 1.36 (d, J = 6.1 Hz, 6 H).

Preparation of 1-n-Butoxy-2-Nitrobenzene (6d)

Potassium hydroxide (0.23 g, 4.1 mmol), 1-fluoro-2-nitrobenzene (0.27 g, 1.9 mmol), and n-butanol (4.0 mL) were used as reactants. 0.33 g product was obtained as yellow oil, 90% yield. The spectra data were in agreement with reported. 1H NMR (300 MHz CDCl₃) δ 7.82 (dd, J = 8.0 Hz, J = 1.9 Hz, 1 H), 7.51 (dt, J = 8.0 Hz, J = 1.9 Hz, 1 H), 7.07 (d, J = 8.0 Hz, 1 H), 7.00 (t, J = 8.0 Hz, 1 H), 4.11 (t, J = 6.5 Hz, 2 H), 1.22 (d, J = 6.5 Hz, 6 H).
1.83 (m, 2 H), 1.46–1.59 (m, 2 H), 0.97 (t, \( J = 7.5 \) Hz, 3 H).

**Preparation of 1-Cyclohexyloxy-2-Nitrobenzene (6e)**

Sodium hydride (0.04 g, 1.0 mmol), 1-fluoro-2-nitrobenzene (0.13 g, 0.94 mmol) and cyclohexanol (3.0 mL) were used as reactants. 0.19 g product was obtained as yellow oil, 92% yield. The spectra data were in agreement with reported.\(^6\) \( ^1\)H NMR (300 MHz CDCl\(_3\)) \( \delta 7.75 (d, \( J = 7.5 \) Hz, 1 H), 7.47 (t, \( J = 7.5 \) Hz, 1 H), 7.08 (d, \( J = 7.5 \) Hz, 1 H), 6.96 (t, \( J = 7.5 \) Hz, 1 H), 4.44 (m, 1 H), 1.25–1.95 (m, 10 H).

**Procedures for Preparing Nitrophenylethers 6f, 6g**

![Reaction Scheme]

Compounds 6f and 6g are both known substances.\(^7,8\) They were prepared via modified procedure from existing synthetic route of similar condensation reaction.\(^8\) Iodoalkane was added drop wise to the mixture of potassium carbonate, 2-nitroresorcin and DMF while stirring under nitrogen. After stirred for 24 h, the reaction was poured into ice water mixture. The precipitate was then separated by filtration, washed with ice-cold water and dried under vacuum to give the product.

**Preparation of 2, 6-Dimethoxynitrobenzene (6f)**

Iodomethane (0.19 g, 1.35 mmol), 2-nitroresorcin (0.10 g, 0.65 mmol), potassium carbonate (0.18 g, 1.29 mmol) were used as reactants. Product was obtained as 0.085 g white powder, 71% yield. \( ^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta 7.24 (t, \( J = 8.5 \) Hz, 1 H), 6.57 (d, \( J = 8.5 \) Hz, 2 H), 3.81 (s, 6 H).

**Preparation of 2, 6-Diethoxynitrobenzene (6g)**

Ethyl iodine (1.12 g, 7.15 mmol), 2-nitroresorcin (0.50 g, 3.25 mmol) and
potassium carbonate (0.89 g, 6.45 mmol) were used as reactant. Product was obtained as 0.57 g white powder, 83% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.26 (dt, $J$ = 7.8 Hz, $J$ = 1.4 Hz, 1 H), 6.58 (d, $J$ = 8.6 Hz, 2 H), 4.10 (q, $J$ = 13.8 Hz, $J$ = 7.0 Hz, 4 H), 1.38 (t, $J$ = 6.8 Hz, 6 H).

**Procedures for Preparing Anilines 4c–4f**

![Chemical Reaction Diagram]

The synthetic routes of compounds 4c–4f followed the general catalyzed hydrogenation method with Pd/C and H$_2$. The substituted nitrobenzene was dissolved in ethanol (40 mL) in a pressure vessel, and 20 mg of Pd/C (10% wt) was added. The vessel was pressurized at 40 psi with hydrogen gas and was stirred for 2 h. The resulting mixture was filtered through celite and the solvent was removed by rotary evaporation to afford the aniline product.

**Preparation of 2-iso-Propoxyaniline (4c)**

Compound 6c (0.16 g, 0.89 mmol) was used as reactant. The product was obtained as brown liquid (0.13 g, 0.86 mmol, 97% yield). The spectra data were in agreement with reported.$^{84}$ $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.90–6.70 (m, 4 H), 4.57 (hp, $J$ = 6.1 Hz, 1 H), 3.80 (brs, 2 H), 1.38 (d, $J$ = 6.1 Hz, 6 H).

**Preparation of 2-n-Butoxyaniline (4d)**

Compound 6d (0.33 g, 1.7 mmol) was used as reactant. Product was obtained as brown oil (0.28 g, 1.7 mmol, 98% yield). The compound is known and has been reported.$^{90}$ $^1$H NMR (300 MHz CDCl$_3$) $\delta$ 6.93–6.86 (m, 5 H), 4.07 (t, $J$ = 6.4 Hz, 2 H), 1.91 (m, 2 H), 1.65 (m, 2 H), 1.12 (t, $J$ = 7.4 Hz, 3 H).
Preparation of 2-Cyclohexyloxyaniline (4e)

Compound 6e (0.27 g, 1.2 mmol) was used as reactant. Product was obtained as brown liquid (0.22 g, 1.1 mmol, 94% yield). The spectra data were in agreement with reported.\(^\text{86}\) \(^1\)H NMR (300 MHz CDCl\(_3\)) \(\delta\) 6.94–6.60 (m, 4 H), 4.27 (m, 1 H), 3.83 (brs, 2 H), 1.30–2.10 (m, 10 H).

Preparation of 2,6-Dimethoxyaniline (4f)

Compound 6f (0.39 g, 2.1 mmol) was used as reactant and was reacted for two days. Product was obtained as yellow solid (0.31 g, 2.0 mmol, 95% yield). The spectra data were in agreement with reported.\(^\text{91}\) \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 6.69 (t, \(J = 8.1\) Hz, 1 H), 6.53 (d, \(J = 8.1\) Hz, 2 H), 3.85 (s, 6 H), 3.82 (s, 2 H).

Preparation of 2,6-Diethoxyaniline (4g)

\[\text{NO}_2\] \[\text{Fe}\] \[\text{CH}_3\text{COOH, H}_2\text{O}\] \[\text{NH}_2\]

Compound 4g is a known molecule.\(^\text{92}\) The synthesis of 4g follows the reduction of a similar nitrobenzene with different substituents.\(^\text{93}\) To the mixture of compound 6g (0.10 g, 0.55 mmol) and acetic acid (0.17 mL, 2.8 mmol) in water (5 mL), iron powder (0.31 g, 5.5 mmol) was added while stirring. The reaction was heated at reflux for 2 h and then neutralized by addition of saturated NaHCO\(_3\) solution. The resulting suspension was extracted 3 times with 30 mL ethyl acetate. The organic layer was combined, and the solvent was removed by rotary evaporation to give compound 4g (0.092 g, 0.51 mmol, 92% yield) as yellow oil. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 6.69 (m, 1 H), 6.54 (d, \(J = 6.5\) Hz, 2 H), 4.06 (q, \(J = 13.9\) Hz, \(J = 6.9\) Hz, 4 H), 3.83 (brs, 2 H), 1.42 (t, \(J = 6.9\) Hz, 6 H).

Preparation of anhydride 5a
Anhydride 5a was synthesized as described in reference. Phencyclone (0.50 g, 1.3 mmol) and maleic anhydride (0.12 g, 1.3 mmol) were mixed in 5 mL of toluene and were heated with a heating gun until the dark green color faded. After cooling, the precipitated product was separated by filtration and washed with cold diethyl ether to give anhydride 5a (0.49 g, 1.0 mmol, 77% yield) as white solid. The crude product was used for next step without further purification. The spectra data were in agreement with reported. $^1$H NMR (300 MHz, CDCl$_3$) δ 8.69 (d, $J = 8.0$ Hz, 2 H), 7.12–7.76 (m, 16 H), 4.75 (s, 2 H).

**Preparation of anhydride 5c**

Anhydride 5c is a known compound, and was synthesized via similar procedure as anhydride 5a. For preparation, 1, 3-diphenylisobenzofuran (0.50 g, 1.9 mmol) and maleic anhydride (0.36 g, 3.7 mmol) were mixed in 5 mL of toluene, and the mixture was heated until the light yellow color faded. After cooling, the precipitated product was separated by filtration and washed with cold diethyl ether to obtain anhydride 5c (0.57 g, 1.0 mmol, 84% yield) as white solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 7.94 (d, $J = 6.8$ Hz, 4 H), 6.94–7.70 (m, 10 H), 4.38 (s, 2 H).

**Procedure for Preparing Molecular Balances 1a–1g, 2a–2c and 3**

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The anhydride and aniline were dissolved in 5 mL of acetic acid, and the reaction mixture was heated at reflux for 24 h. The solvent was then removed by rotary evaporation. The residue was dissolved in 25 mL EtOAc, washed once with 50 mL saturated sodium bicarbonate, and twice with 50 mL water. The solvent of organic layer was then removed under vacuum to give the crude product.

**Preparation of Balance 1a**

Anhydride 5a (0.50 g, 1.0 mmol) and anisidine 4a (0.19 g, 1.5 mmol) were used as reactants, and 10 mL acetic acid was used as solvent. Purified by flash chromatography using silica gel (MeOH/CH$_2$Cl$_2$, v/v = 1/99). White solid, 0.54 g, 0.93 mmol, 93% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.64–8.76 (m, 2 H major, 2 H minor), 8.42 (d, $J = 6.7$ Hz, 2 H minor), 8.38 (d, $J = 6.8$ Hz, 2 H major), 7.09–7.80 (m, 13 H major, 13 H minor), 7.04 (td, $J = 8.1$ Hz, $J = 2.5$ Hz, 2 H major), 6.96 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, 2 H minor), 6.82 (td, $J = 7.5$ Hz, $J = 0.9$ Hz, 2 H minor), 6.74 (d, $J = 8.6$ Hz, 1 H major), 6.44 (dd, $J = 8.5$ Hz, $J = 1.0$ Hz, 1 H minor), 6.28 (td, $J = 7.7$ Hz, $J = 1.4$ Hz, 1 H major), 4.64 (s, 2 H major), 4.62 (s, 2 H minor), 4.54 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, 1 H major), 3.71 (s, 3 H major), 2.16 (s, 3 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 197.19, 173.15, 173.10, 154.22, 133.89, 133.80, 133.68, 133.57, 131.44, 131.11, 131.04, 130.89, 130.51, 130.44, 129.35, 129.28, 129.23, 128.61, 128.46, 128.41, 128.33, 128.30, 127.59, 127.18, 126.84, 126.59, 126.48, 126.32, 126.25, 125.90, 122.96, 122.76, 120.52, 120.07, 119.60, 111.73, 111.54, 63.58, 63.56, 55.72, 53.86, 45.33, 45.00, 29.72. HRMS (EI) calculated for C$_{40}$H$_{27}$NO$_4$: 585.1940; obs: 585.1939.
Figure 3.7: 400 MHz $^1$H NMR spectrum of balance 1a in CDCl$_3$.

Figure 3.8: 100 MHz $^{13}$C NMR spectrum of balance 1a in CDCl$_3$. 
Preparation of Balance 1b

Anhydride 5a (0.37 g, 0.77 mmol) and phenetidine 4b (0.11 g, 0.77 mmol) were used as reactants. Purified by flash chromatography using silica gel (MeOH/CH$_2$Cl$_2$, v/v = 1/99). White solid, 0.36 g, 0.59 mmol, 73% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.3–8.4 (m, 2 H major, 2 H minor), 8.05 (dd, $J$ = 7.9 Hz, $J$ = 1.1 Hz, 2 H minor), 8.02 (dd, $J$ = 6.6 Hz, $J$ = 1.3 Hz, 2 H major), 6.4–7.4 (m, 15 H major, 16 H minor), 6.36 (dd, $J$ = 8.4 Hz, $J$ = 1.0 Hz, 1 H major), 6.06 (dd, $J$ = 8.5 Hz, $J$ = 1.0 Hz, 2 H minor), 5.90 (td, $J$ = 7.7 Hz, $J$ = 1.2 Hz, 1 H major), 4.26 (s, 2 H major), 4.22 (s, 2 H minor), 4.21 (dd, $J$ = 7.8 Hz, $J$ = 1.6 Hz, 1 H major), 3.58 (q, $J$ = 7.0 Hz, 2 H major), 2.13 (q, $J$ = 7.0 Hz, 2 H minor), 0.96 (t, $J$ = 7.0 Hz, 3 H major), −0.21 (t, $J$ = 7.0 Hz, 3 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 197.21, 173.04, 153.59, 133.84, 133.58, 131.13, 130.91, 130.33, 129.52, 129.41, 129.37, 129.33, 129.23, 128.67, 128.62, 128.41, 128.30, 127.58, 127.18, 126.83, 126.60, 126.44, 126.34, 125.91, 122.98, 122.74, 120.37, 119.90, 119.75, 112.58, 64.14, 63.56, 61.80, 45.38, 44.93, 29.73, 14.72, 12.76. HRMS (EI) calculated for C$_{41}$H$_{29}$NO$_4$: 599.2097; obs: 599.2116.
Figure 3.9: 400 MHz $^1$H NMR spectrum of balance 1b in CDCl$_3$.

Figure 3.10: 100 MHz $^{13}$C NMR spectrum of balance 1b in CDCl$_3$. 
Preparation of Balance 1c

Anhydride 5a (0.21 g, 0.43 mmol) and compound 4c (0.13 g, 0.85 mmol) were used as reactants. Purified by flash chromatography using silica gel (EtOAc/Hexane, v/v = 1:5). Yellow crystal, 0.22 g, 0.36 mmol, 85% yield. $^1$H NMR (400 MHz, CDCl$_3$) 8.71 (d, $J = 8.3$ Hz, 2 H), 8.37 (d, $J = 7.8$ Hz, 2 H), 6.81–7.91 (m, 15 H), 6.73 (d, $J = 8.3$ Hz, 1 H), 6.24 (t, $J = 7.7$ Hz, 1 H), 4.61 (s, 2 H), 4.55(dd, $J = 7.7$ Hz, $J = 1.0$ Hz, 1 H), 4.42 (m, 1 H), 1.37 (d, $J = 6.2$ Hz, 6 H). $^{13}$C NMR (100 MHz, CDCl$_3$) 197.43, 173.19, 153.95, 134.09, 133.81, 131.36, 131.14, 130.54, 129.57, 129.44, 128.84, 128.62, 127.81, 127.38, 127.04, 126.59, 126.05, 123.19, 120.56, 120.15, 112.76, 68.48, 63.78, 45.17, 31.27, 19.40, 14.13. HRMS (EI) calculated for C$_{42}$H$_{31}$NO$_4$: 613.2253; observed: 613.2256.

Figure 3.11: 300 MHz $^1$H NMR spectrum of balance 1c in CDCl$_3$. 
Preparation of Balance 1d

Anhydride 5a (0.10 g, 0.21 mmol) and compound 4d (0.068 g, 0.42 mmol) were used as reactants. Purified by flash chromatography using silica gel (MeOH/CH₂Cl₂, v/v = 1:99). Yellow crystal, 0.11 g, 0.17 mmol, 81% yield. ¹H NMR (300 MHz, CDCl₃) δ 8.70 (m, 2 H major, 2 H minor), 8.37 (m, 2 H major, 2 H minor), 7.71 (t, J = 7.6 Hz, 2 H major), 6.90–7.60 (m, 13 H major, 16 H minor), 6.79 (t, J = 8.0 Hz, 1 H minor), 6.73 (d, J = 8.6 Hz, 1 H major), 6.42 (d, J = 8.0 Hz, 1 H minor), 6.27 (t, J = 7.6 Hz, 1 H major), 4.62 (ds, 2 H major, 2 H minor), 4.60 (dd, J = 4.1 Hz, J = 1.3 Hz, 1 H major), 3.87 (t, J = 6.2 Hz, 2 H major, 2 H minor), 1.70 (m, 2 H major), 1.43 (m, 2 H major), 0.98 (t, J = 7.5 Hz, 3 H major), 0.34–0.80 (m, 7 H minor). ¹³C NMR (100 MHz, CDCl₃) δ 197.21, 172.97, 153.71, 133.84, 133.57, 131.12, 130.90, 130.32, 129.35, 129.21, 128.61, 128.40, 127.57, 127.16, 126.81, 126.34, 125.91, 122.97, 122.86, 122.83, 120.13, 119.89, 112.51, 105.00, 68.23, 63.54, 44.93, 31.03, 19.18, 13.91. HRMS (EI) calculated for C₄₃H₃₃NO₄:
627.2410; observed: 627.2416.

**Figure 3.13:** 300 MHz $^1$H NMR spectrum of balance 1d in CDCl$_3$.

**Figure 3.14:** 100 MHz $^{13}$C NMR spectrum of balance 1d in CDCl$_3$. 
Preparation of Balance \textbf{1e}

Anhydride \textbf{5a} (0.27 g, 0.57 mmol) and compound \textbf{4e} (0.22 g, 1.14 mmol) were used as reactants. Purified by flash chromatography using silica gel (MeOH/CH$_2$Cl$_2$, v/v = 1:99). Yellow solid, 0.29 g, 0.44 mmol, 78\% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.71 (d, $J = 8.5$ Hz, 2 H), 8.38 (d, $J = 7.8$ Hz, 2 H), 7.72 (t, $J = 7.8$ Hz, 2 H), 6.94–7.60 (m, 13 H), 6.73 (d, $J = 8.5$ Hz, 1 H), 6.24 (t, $J = 7.8$ Hz, 1 H), 4.61 (s, 2 H), 4.58 (dd, $J = 7.8$ Hz, $J = 1.4$ Hz, 1 H), 4.15 (m, 1 H), 1.20–1.92 (m, 10 H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 197.25, 172.95, 152.64, 133.88, 133.58, 131.12, 130.91, 130.18, 129.36, 129.24, 128.60, 128.38, 127.81, 127.15, 126.81, 126.36, 125.94, 122.96, 120.58, 120.07, 133.62, 63.56, 44.88, 31.58, 25.49, 23.55. HRMS (El) calculated for C$_{45}$H$_{35}$NO$_4$: 653.2566; observed: 653.2553.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{nmr_spectrum.png}
\caption{400 MHz $^1$H NMR spectrum of balance \textbf{1e} in CDCl$_3$.}
\end{figure}
Preparation of Balance 1f

Anhydride 5a (0.47 g, 0.99 mmol) and compound 4f (0.30 g, 2.0 mmol) were used as reactants. The product was recrystallized from MeCN as white crystal, 0.35 g, 0.57 mmol, 58% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.69 (d, $J = 8.5$ Hz, 2 H), 8.45 (d, $J = 7.5$ Hz, 2 H), 7.70 (t, $J = 7.5$ Hz, 2 H), 6.46–7.80 (m, 13 H), 6.42 (d, $J = 8.5$ Hz, 1 H), 6.05 (d, $J = 8.5$ Hz, 1 H), 4.66 (s, 2 H), 3.76 (s, 3 H), 2.17 (s, 3 H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 195.96, 173.06, 155.75, 155.16, 134.03, 133.78, 131.43, 130.56, 129.46, 129.27, 128.40, 128.25, 126.75, 126.66, 126.44, 126.29, 122.67, 103.97, 103.38, 63.60, 56.10, 54.15, 45.29. HRMS (EI) calculated for C$_4$H$_2$NO$_5$: 615.2046; observed: 615.2043.

**Figure 3.16:** 100 MHz $^{13}$C NMR spectrum of balance 1e in CDCl$_3$. 
Figure 3.17: 400 MHz $^1$H NMR spectrum of balance 1f in CDCl$_3$.

Figure 3.18: 100 MHz $^{13}$C NMR spectrum of balance 1f in CDCl$_3$. 
Preparation of Balance 1g

Anhydride 5a (0.11 g, 0.22 mmol) and compound 4g (0.08 g, 0.44 mmol) were used as reactants. Pale yellow solid, 0.12 g, 0.19 mmol, 88% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.69 (d, $J = 8.7$ Hz, 2 H), 8.45 (d, $J = 7.6$ Hz, 2 H), 7.71 (t, $J = 7.6$ Hz, 2 H), 7.08–7.58 (m, 12 H), 7.01 (t, $J = 8.5$ Hz, 1 H), 6.37 (d, $J = 8.3$ Hz, 1 H), 6.04 (d, $J = 8.3$ Hz, 1 H), 4.63 (s, 2 H), 4.00 (q, $J = 13.9$ Hz, $J = 6.95$ Hz, 2 H), 2.56 (q, $J = 13.9$ Hz, $J = 7.0$ Hz, 2 H), 1.38 (t, $J = 7.0$ Hz, 3 H), 0.10 (t, $J = 7.0$ Hz, 3 H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 195.91, 173.08, 154.66, 154.32, 134.06, 133.86, 131.38, 131.03, 130.26, 129.56, 129.41, 129.11, 128.51, 128.37, 128.07, 126.91, 126.66, 126.38, 122.68, 109.36, 104.54, 104.31, 104.08, 103.83, 64.33, 63.59, 62.16, 45.39, 45.11, 14.88, 14.79. HRMS (EI) calculated for C$_{43}$H$_{33}$NO$_5$: 643.2359; observed: 643.2372.

Figure 3.19: 400 MHz $^1$H NMR spectrum of balance 1g in CDCl$_3$. 
**Figure 3.20**: 100 MHz $^{13}$C NMR spectrum of balance 1g in CDCl$_3$.

**Preparation of Balance 2a**

Anhydride 5c (0.22 g, 0.59 mmol) and anisidine 4a (0.11 g, 0.89 mmol) were used as reactants. Pale yellow solid, 0.23 g, 0.48 mmol, 82% yield. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.08 (m, 4 H major, 4 H minor), 6.78–7.60 (m, 13 H major, 14 H minor), 5.68 (dd, $J$ = 7.7 Hz, $J$ = 1.2 Hz, 1 H major), 4.31 (s, 2 H major), 4.28 (s, 2 H minor), 3.76 (s, 3 H major), 3.48 (s, 3 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.17, 154.77, 144.34, 136.46, 128.82, 128.58, 128.37, 128.00, 127.66, 127.39, 127.02, 120.96, 119.80, 90.53, 55.87, 55.65, 54.80, 54.72, 54.68. HRMS (EI) calculated for C$_{31}$H$_{23}$NO$_4$: 473.1627; observed: 473.1613.
**Figure 3.21**: 400 MHz $^1$H NMR spectrum of balance 2a in CDCl$_3$.

**Figure 3.22**: 100 MHz $^{13}$C NMR spectrum of balance 2a in CDCl$_3$. 
Preparation of Balance 2b

Anhydride 5c (0.050 g, 0.13 mmol) and phenetidine 4b (0.023 g, 0.16 mmol) were used as reactants. Pale yellow solid, 0.053 g, 0.11 mmol, 84% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.08 (m, 4 H major, 4 H minor), 6.70–7.58 (m, 13 H major, 14 H minor), 5.69 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, 1 H major), 4.30 (s, 2 H major), 4.28 (s, 2 H minor), 4.00 (q, $J = 14.0$ Hz, $J = 7.0$ Hz, 2 H major), 3.91 (q, $J = 14.0$ Hz, $J = 7.0$ Hz, 2 H minor), 1.31 (t, $J = 7.0$ Hz, 3 H major), 1.12 (t, $J = 7.0$ Hz, 3 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 173.09, 154.12, 144.32, 136.50, 130.66, 128.62, 128.57, 128.46, 128.16, 128.12, 127.26, 127.18, 120.96, 120.44, 120.12, 112.83, 90.51, 64.16, 54.68, 14.65. HRMS (EI) calculated for C$_{32}$H$_{25}$NO$_4$: 487.1784; observed: 487.1778.

Figure 3.23: 400 MHz $^1$H NMR spectrum of balance 2b in CDCl$_3$. 
Figure 3.24: 100 MHz $^{13}$C NMR spectrum of balance 2b in CDCl$_3$.

Preparation of Balance 2c

Anhydride 5c (0.11 g, 0.30 mmol) and aniline 4f (0.07 g, 0.45 mmol) were used as reactants. Yellow solid, 0.11 g, 0.23 mmol, 75% yield. $^1$H NMR (400 MHz, CDCl$_3$) 8.07 (d, $J$ = 7.5 Hz, 4 H), 6.96–7.59 (m, 11 H), 6.52 (d, $J$ = 8.4 Hz, 1 H), 6.42 (d, $J$ = 8.4 Hz, 1 H), 4.33 (s, 2 H), 3.74 (s, 3 H), 3.43 (s, 3 H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.77, 156.18, 155.58, 144.60, 136.91, 128.77, 128.26, 127.78, 127.38, 127.00, 126.53, 124.64, 123.09, 120.88, 104.32, 104.06, 103.86, 103.58, 90.15, 56.20, 55.99, 55.74, 55.49, 54.76, 54.68. HRMS (EI) calculated for C$_{32}$H$_{22}$NO$_5$: 503.1733; observed: 503.1717.
Figure 3.25: 400 MHz $^1$H NMR spectrum of balance 2c in CDCl$_3$.

Figure 3.26: 100 MHz $^{13}$C NMR spectrum of balance 2c in CDCl$_3$. 
Preparation of Balance 3a

It is a known compound that has been reported. Anhydride 5d (0.11 g, 0.68 mmol) and o-anisidine (0.10 g, 0.81 mmol, 0.09 mL) were used as reactants. The crude product was heated in oven (130 °C) for 16 h to give the product as white crystal (0.12 g, 0.45 mmol, 66% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.36 (dt, $J = 7.8$ Hz, $J = 1.4$ Hz, 1 H major, 1 H minor), 6.86–7.06 (m, 3 H major, 3 H minor), 6.28 (s, 2 H major), 6.21 (s, 2 H minor), 3.78 (s, 3 H major), 3.77 (s, 3 H minor), 3.37–3.54 (m, 4 H major, 4 H minor), 1.54–1.82 (m, 2 H major, 2 H minor).

Figure 3.27: 300 MHz $^1$H NMR spectrum of balance 3a in CDCl$_3$. 

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3.5.2 Van’t Hoff Plots

The van't Hoff plots of balances 1–3 were plotted based on the results from variable temperature $^1$H NMR. The full spectra were acquired at 5°C intervals between 25°C–55°C, and the folded/unfolded ratios were obtained via spectral deconvolution of the succinimide alpha singlets (balance 1a, 1b, 2a, 2b in acetone-$d_6$), the methyl singlets or the CH2 quartet on the arm group (balance 1a, 1b, 2a, 2b in CDCl$_3$ because of the overlapped succinimide peaks), or the triplet for ethene protons (of balance 3a). The folded/unfolded ratios were listed as Table 3.3–Table 3.5 and Table 3.7–Table 3.9, and the van’t Hoff plots were as Figure 3.27 and 3.28.

**Table 3.3:** Spectral deconvolution integrations for variable temperature $^1$H NMR of balance 1a and 1b in CDCl$_3$.

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**Table 3.4:** Spectral deconvolution integrations for variable temperature $^1$H NMR of balance 2a and 2b in CDCl$_3$.

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Table 3.5: Spectral deconvolution integrations for variable temperature $^1$H NMR of balance 3a in CDCl$_3$.

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<th>balance 3a</th>
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<tr>
<td></td>
<td></td>
<td>Unfolded</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.553</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>0.559</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.56</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.561</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>0.562</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>0.565</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>0.565</td>
</tr>
</tbody>
</table>

Figure 3.28: Van't Hoff plot of balances 1a, 1b, 2a, 2b, and 3a in CDCl$_3$ based on the information in Table 3.3, Table 3.4, and Table 3.5.

Based on the equation in Chapter 2, the calculation of entropy/enthalpy values errors of balance 1a, 1b, 2a, 2b, and 3a by VT NMR experiments in CDCl$_3$ are listed in Table 3.6. The errors for slopes and intercepts are measured by the regression add-in in excel.
Table 3.6: Calculation of $\Delta G$, $\Delta H$, $\Delta S$, and $-T\Delta S$ and their errors of balance 1a, 1b, 2a, 2b, and 3a by VT NMR experiments in CDCl$_3$.

<table>
<thead>
<tr>
<th>balance</th>
<th>Slope ±</th>
<th>Intercept ±</th>
<th>$\Delta G$ (kcal/mol)</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$\Delta S$ (kcal/mol·K)</th>
<th>$-T\Delta S@25^\circ C$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>45.5 ± 10.3</td>
<td>−0.913 ± 0.033</td>
<td>0.45 ± 0.04</td>
<td>−0.09 ± 0.02</td>
<td>−0.0018 ± 6.6×10$^{-5}$</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>1b</td>
<td>116 ± 14</td>
<td>−1.94 ± 0.05</td>
<td>0.92 ± 0.06</td>
<td>−0.23 ± 0.03</td>
<td>−0.0039 ± 9.3×10$^{-5}$</td>
<td>1.20 ± 0.03</td>
</tr>
<tr>
<td>2a</td>
<td>−353 ± 65</td>
<td>−1.17 ± 0.21</td>
<td>1.4 ± 0.3</td>
<td>0.70 ± 0.13</td>
<td>−0.0023 ± 0.0004</td>
<td>0.69 ± 0.12</td>
</tr>
<tr>
<td>2b</td>
<td>−366 ± 291</td>
<td>−2.18 ± 0.93</td>
<td>2.0 ± 1.1</td>
<td>0.73 ± 0.58</td>
<td>−0.0043 ± 0.0019</td>
<td>1.30 ± 0.55</td>
</tr>
<tr>
<td>3a</td>
<td>−145 ± 26</td>
<td>0.200 ± 0.082</td>
<td>0.17 ± 0.10</td>
<td>0.29 ± 0.05</td>
<td>0.00040 ± 0.00016</td>
<td>−0.12 ± 0.05</td>
</tr>
</tbody>
</table>

Same analysis was done for the data measured in acetone-$d_6$. Although was not discussed in this chapter, the results lead to the same conclusion as data in CDCl$_3$.

Table 3.7: Spectral deconvolution integrations for variable temperature $^1$H NMR of balance 1a and 1b in acetone-$d_6$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>balance 1a</th>
<th>balance 1b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfolded</td>
<td>Folded</td>
<td>ln(F/U)</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>134.14</td>
<td>105.58</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>152.4</td>
<td>116.98</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>108.08</td>
<td>81.19</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>161.14</td>
<td>120.54</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>136.47</td>
<td>104.9</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>155.97</td>
<td>112.2</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>192.98</td>
<td>135.22</td>
</tr>
</tbody>
</table>

Table 3.8: Spectral deconvolution integrations for variable temperature $^1$H NMR of balance 2a and 2b in acetone-$d_6$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>balance 2a</th>
<th>balance 2b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfolded</td>
<td>Folded</td>
<td>ln(F/U)</td>
</tr>
<tr>
<td>25</td>
<td>0.003336</td>
<td>179.05</td>
<td>33.55</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>172.07</td>
<td>32.80</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>169.91</td>
<td>32.44</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>190.93</td>
<td>37.11</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>205.85</td>
<td>40.46</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>183.72</td>
<td>36.22</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>188.30</td>
<td>37.54</td>
</tr>
</tbody>
</table>
Table 3.9: Spectral deconvolution integrations for variable temperature $^1$H NMR of balance 3a in acetone-$d_6$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>Unfolded</th>
<th>Folded</th>
<th>ln(F/U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.54</td>
<td>0.46</td>
<td>−0.160343</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>0.539</td>
<td>0.46</td>
<td>−0.158489</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.545</td>
<td>0.456</td>
<td>−0.178293</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.541</td>
<td>0.459</td>
<td>−0.164369</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>0.543</td>
<td>0.456</td>
<td>−0.174617</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>0.54</td>
<td>0.46</td>
<td>−0.160343</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>0.54</td>
<td>0.46</td>
<td>−0.160343</td>
</tr>
</tbody>
</table>

Figure 3.29: Van't Hoff plot of balances 1a, 1b, 2a, 2b, and 3a in acetone-$d_6$ based on the information in Table 3.7, Table 3.8 and Table 3.9.
<table>
<thead>
<tr>
<th>balance</th>
<th>Slope</th>
<th>Intercept</th>
<th>$\Delta G$ (kcal/mol)</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$\Delta S$ (kcal/mol·K)</th>
<th>$-T\Delta S@25^\circ C$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>316</td>
<td>$-1.30$</td>
<td>0.14</td>
<td>$-0.63$</td>
<td>$-0.0026$</td>
<td>0.77 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>± 82</td>
<td>± 0.26</td>
<td>± 0.32</td>
<td>± 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>307</td>
<td>$-2.04$</td>
<td>0.60</td>
<td>$-0.61$</td>
<td>$-0.0040$</td>
<td>1.20 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>± 143</td>
<td>± 0.46</td>
<td>± 0.56</td>
<td>± 0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>$-198$</td>
<td>$-1.01$</td>
<td>0.99</td>
<td>0.39</td>
<td>$-0.0020$</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>± 14</td>
<td>± 0.05</td>
<td>± 0.06</td>
<td>± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>$-298$</td>
<td>$-1.72$</td>
<td>1.6</td>
<td>0.59</td>
<td>$-0.0034$</td>
<td>1.02 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>± 58</td>
<td>± 0.19</td>
<td>± 0.2</td>
<td>± 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>1.28</td>
<td>$-0.169$</td>
<td>0.10</td>
<td>$-0.003$</td>
<td>$-0.00034$</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>± 32</td>
<td>± 0.103</td>
<td>± 0.12</td>
<td>± 0.064</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.10: Calculation of $\Delta G$, $\Delta H$, $\Delta S$, and $-T\Delta S$ and their errors for balance 1a, 1b, 2a, 2b, and 3a by VT NMR experiments in acetone-$d_6$. 
In previous Chapter 3, our balances system was proved to be sufficiently pre-organized and sensitive to measure aliphatic CH–π interactions. However, because of the existence of lone pair–π interaction caused by the oxygen linker, those balances could only be used to look at single CH–π interaction, even for large alkyl groups with multiple CHs. In fact, in most CH–π interactions, the alkyl groups form multiple interactions with the aromatic surface. The cooperativity of multiple CH–π interactions is commonly observed in solid-state structures, and has been shown to enhance the stability of the polymers complexed inside of nano-channels and stabilizing the interactions between sugars and aromatic side chains in enzyme active sites. Thus, study about this property will be important in the design of supramolecular structures, polymer nano-composites and ligand targeted toward specific receptors. In this chapter, a new series of molecular balances that are able to form more than one intramolecular CH–π interactions were synthesized to study the cooperativity of multiple CH–π interactions.

4.1 DESIGNS OF THE STRUCTURES

The new series of balances (Figure 4.1) shared the same rigid bicyclic \textit{N}–arylimide framework as our previous CH–π balances. To incorporate larger alkyl groups and form multiple CH–π interactions, the oxygen linkers in the former structures (balances \textbf{7e–10e}) was removed, allowing the alkyl groups connected directly to the phenyl rotor. Other than making shortened arms, a side benefit of the new design for
taking out the oxygen linker is that, it will eliminate the repulsive \( \text{O}–\pi \) interaction and can lead to higher folded/unfolded ratios. This result is supported by the conclusion of a recent computational study.\(^{103}\)

![Figure 4.1: Structures of balances 7–10 designed for measuring multiple \( \text{CH}–\pi \) interactions.](image)

Balances 7 and 8 have large phenanthrene or pyrene aromatic shelves were expected to form cooperative \( \text{CH}–\pi \) interaction as shown in Figure 4.2. Balance 9 are control balances with only one benzene ring on the shelf which can only form interaction with the first carbon on the alkyl group, and balances 10 are control balances without aromatic shelf. Balances 7e–10e with methoxy arm were also used for comparison. Balance 7a has been previously reported in literature for the study of \( \text{CH}–\pi \) interaction.\(^{94,104}\) Balances 7e, 9e, and 10e indicate the same structures as balances 1a, 2a and 3a in Chapter 3.

![Figure 4.2: Illustration of (a) single \( \text{CH}–\pi \) interaction in balance 7a, (b, c) multiple \( \text{CH}–\pi \) interactions in balance 7b and 7d, and (d) the long pair–\( \pi \) interaction in previous balance with oxygen linker.](image)
4.2 SOLID-STATE STRUCTURES

The formation of multiple CH–π interactions in the folded conformers of the new balances were first verified and characterized in the solid-state (Figure 4.3). Crystals of the methyl balances 7a and 8a, ethyl balance 7b, and i-Pr balance 7d were obtained in their folded conformations. This was the first indication that the new balances could form more attractive CH–π interactions than previous series of CH–π balances which always crystalized as unfolded conformation. Control balance 9a and 10a crystalized in both folded and unfolded conformations.

![Figure 4.3: X-ray structures of balances (a) 7a, (b) 8a, (c) 7b, (d) 7d, (e) 9a and (f) 10a that obtain the folded conformation. The solvent molecules and the bridge-head phenyl groups for each balance (except 10a with only proton on the bridge-head) are hidden for viewing clarity.]

4.2.1 Geometries of CH₃–π Interactions

All of the solid-state structures obtained for methyl balances 7a, 8a, and 9a
showed expected but slightly different intramolecular CH$_3$–π interactions. Their proton-to-aromatic plane distances ($d$) were all within the typical range of CH–π interactions (2.5–3.0 Å).$^{31}$ Balance 7a forms one clear CH–π interaction with a proton-to-plane distance of 2.571 Å, and balance 8a showed a similar interaction with slightly longer distance ($d = 2.657$ Å). The crystal structure of balance 9a showed three set of folded/unfolded conformers. One of the three folded conformations showed one single CH–π interaction between methyl and the benzene ring ($d = 2.571$ Å), while the other two forms two CH–π interactions at the same time ($d = 2.794$ Å, 2.863 Å and $d = 2.691$, 3.029 Å). The double-interaction geometry was only presented (and preferred) in balance 9a, probably because the different back-side bridge atom (oxygen) on the framework of balance 9 leads to a more restricted environment compared with balances 7 and 8, and the conformation with two protons pointing down to the arene shelf causes less sterics. The two types of CH$_3$–π interactions have similar stability because they showed up together in balance 9a, but in a less restrict environment such as balance 7, the single-interaction geometry is more stable because of a more proper proton-to-arene distance and a moderate sterics.

4.2.2 Geometries of Multiple CH–π Interactions

The solid-state structures of balances 7b and 7d showed expected multiple intramolecular CH–π interactions. In ethyl balance 7b, the interaction between the first carbon and the central ring on arene shelf was shown as the double-interaction geometry ($d = 2.717$ Å, 2.864 Å), probably to adjust the extra steric caused by the additional CH$_3$ compared with 7a. Because of the limitation of the shorter aromatic shelf, this is the only good CH–π interaction that can be formed in balance 7b. The CH$_3$ of the ethyl group is centered over the bay region of the phenanthrene shelf (between the two outer rings),
forming an additional minor and weaker interaction. Although haven’t obtained the crystal, it is possible that the \( n\)-Pr balance 7c have similar situation with balance 7b that with only one good interaction by the first carbon, and the second or third interaction are weak or non-exist. In \( i\)-Pr balance 7d, all three carbons were found to form CH–π interactions in the \textit{folded} structure, and the proton-to-plane distances were 2.785 Å, 2.594 Å (for two CH\(_3\) groups) and 2.643 Å (for CH).

It is important to note that the solid-state structure only provides a snap-shot of one stable conformation of the alkyl arm. Modeling for the balances with longer alkyl groups predicts that the arm would sweep back and forth across the arene shelf in the \textit{folded} conformer. Due to this uncertainty and the similar stability of the two types of interactions formed by the first carbon on alkyl group, the numbers of carbons that possibly form CH–π interaction were used for analysis and comparison, although some make different CH–π interaction than others.

4.2.3 Control Balances

No interaction was observed in control balance 10a with no aromatic shelf, and both \textit{folded} and \textit{unfolded} conformers were found in its crystal structure. The distances between the methyl and double bond on the shelf is too long for any attractive or repulsive interaction. It also helps to make sure that the differences in dipole and solvation of the \textit{folded} and \textit{unfolded} conformers is not biasing the results.

4.3 MEASURING CH–II INTERACTIONS IN SOLUTION

Next, the different intramolecular CH–π interactions were characterized and quantitatively measured in CDCl\(_3\) solution. By analyzing the \(^1\)H NMR spectrums, we were able to compare the strength of CH–π interactions formed in each of the balances (Table 4.1). As expected, due to the absence of the repulsive long pair–π interaction in
the new series, each of these balances was more *folded* than corresponding previous balance with oxygen linkers. This indicates that the replacement of the oxygen with CH\(_2\) successfully replaced the repulsive lone pair–\(\pi\) interaction with an attractive interaction.

**Table 4.1:** The folding energies (\(\Delta G\)) of molecular balances 7–10 in CDCl\(_3\) at 25 °C.

<table>
<thead>
<tr>
<th>Balance</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>shelf</td>
<td>phenanthrene</td>
<td>pyrene</td>
<td>benzene</td>
<td>ethylene</td>
</tr>
<tr>
<td>a (R = Me)</td>
<td>−0.13</td>
<td>−0.23</td>
<td>+0.84</td>
<td>+0.02</td>
</tr>
<tr>
<td>b (R = Et)</td>
<td>−0.27</td>
<td>−0.51</td>
<td>+0.84</td>
<td>+0.07</td>
</tr>
<tr>
<td>c (R = n-Pr)</td>
<td>+0.36</td>
<td>+0.07</td>
<td>+0.86</td>
<td>+0.02</td>
</tr>
<tr>
<td>d (R = i-Pr)</td>
<td>−0.91</td>
<td>−1.10</td>
<td>+0.89</td>
<td>−0.11</td>
</tr>
<tr>
<td>e (R = OMe)</td>
<td>+0.45</td>
<td>+0.25</td>
<td>+1.40</td>
<td>+0.18</td>
</tr>
</tbody>
</table>

4.3.1 Control Balances

The \(\Delta G\) values are close to zero for control balances 10 without arene shelf, which proves that no interaction was formed and balances 10 are reasonable controls for the other balances. Balance 10d showed a slightly lower folding energy because weak interaction might exist between the \(−iPr\) group and the double bond, and balance 10e showed a minor repulsion because of the lone pair on the oxygen linker, but in general, their folding energies were still close to each other.

Balances 9a–9d with benzene shelf showed almost the same folding energy, which matched our expectation that all of these balances form only a single CH–\(\pi\) interaction. However, although being more *folded* than balance 9e with oxygen linker, all these balances preferred the *unfolded* conformer. This suggests that steric still exist between the first carbon on the alkyl group and the aromatic shelf in balances 9. The steric still possibly exist in balances 7 and 8 although they were more *folded* in its solution, but the repulsion should be weaker than that in 9 because with a different bridge group (C=O), their frameworks allow a wider space for the intramolecular interactions.
4.3.2 Strength of Multiple CH–π interactions

In the methyl, ethyl or i-propyl balances 7 and 8, the ΔG values were all negative, demonstrating that the CH–π interactions were attractive. The interactions in balances 8 appeared to be stronger than that in balances 7, because the extended arene shelf strength the dispersion effect. Generally, except for the n-propyl balances, the balances that can form more CH–π interactions showed lower folding energies, although the energies did not change linearly with the number of interactions. The folding energies for both balance series showed similar trends: d (–iPr) < b (–Et) < a (–Me) < c (–nPr).

Compared with balances 7a and 8a that formed single CH–π interaction, balances 7b and 8b showed the expected doubled ΔG values, while balances 7d and 8d showed a much lower folding energies that were more than three times of that of balances 7a and 8a. It is possible that due to the sterics exists between methyl group and arene shelf in balances 7a and 7b, the measured ΔG value turned out to be higher than the actually interaction. Also, in balances 7d and 8d where all three carbons on –iPr group are able to form CH–π interactions with the aromatic surface, the cooperativity of the interactions may lead to a better geometric positioning, and thus strengthened the folded conformation more than three single interactions.

The balances 7c and 8c with linear –nPr group were apparent exceptions among all balances as they favored the unfolded conformer and showed the highest folding energies. It is probably because while forming similar interactions as the ethyl balances due to the limited aromatic area, the alkyl group has less freedom to rotate and thus leads to a larger conformational entropy for the n-propyl group and thus increases the steric and decreases the preference of their folded conformer.
4.4 ENTROPIC AND ENTHALPIC VALUES

To explain the discrepancies above in the folding trends, we hypothesized that they are due to the different entropic penalties imposed by pinning each alkyl group against the arene shelves. In this case, while a larger alkyl group forms more CH–π interactions, it also needs to pay a higher entropic penalty due to the loss of rotational freedom for each C–C single bond in the confined environment of the folded conformer.\(^{105-108}\) To test this hypothesis, the enthalpy (\(\Delta H\)) and entropy (\(–T\Delta S\)) values of CH–π interactions in balances 7–10 were measured using van’t Hoff analysis (Table 4.2) of data from variable temperature \(^1\)H NMR. The \(\Delta G\) values from the analyses match up well with those from the single point r.t. measurement in Table 4.1.

**Table 4.2:** Comparison of \(\Delta G\), \(\Delta H\), and \(–T\Delta S\) for balances 7–10 in CDCl\(_3\) at 25 °C.

<table>
<thead>
<tr>
<th>Balance</th>
<th>Arm</th>
<th>(\Delta G) (kcal/mol)</th>
<th>(\Delta H) (kcal/mol)</th>
<th>(–T\Delta S) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>Me</td>
<td>–0.13 ± 0.06</td>
<td>–0.66 ± 0.03</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>7b</td>
<td>Et</td>
<td>–0.27 ± 0.05</td>
<td>–0.96 ± 0.03</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>7c</td>
<td>(n)Pr</td>
<td>0.36 ± 0.17</td>
<td>–0.34 ± 0.08</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td>7d</td>
<td>(i)Pr</td>
<td>–0.93 ± 0.59</td>
<td>–4.55 ± 0.30</td>
<td>3.62 ± 0.29</td>
</tr>
<tr>
<td>8a</td>
<td>Me</td>
<td>–0.16 ± 0.08</td>
<td>–0.71 ± 0.04</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>8b</td>
<td>Et</td>
<td>–0.42 ± 0.19</td>
<td>–0.98 ± 0.10</td>
<td>0.56 ± 0.09</td>
</tr>
<tr>
<td>8c</td>
<td>(n)Pr</td>
<td>0.06 ± 0.30</td>
<td>–0.57 ± 0.15</td>
<td>0.63 ± 0.14</td>
</tr>
<tr>
<td>8d</td>
<td>(i)Pr</td>
<td>–1.09 ± 0.79</td>
<td>–2.74 ± 0.40</td>
<td>1.65 ± 0.38</td>
</tr>
<tr>
<td>9a</td>
<td>Me</td>
<td>0.84 ± 0.12</td>
<td>0.40 ± 0.06</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>9b</td>
<td>Et</td>
<td>0.84 ± 0.06</td>
<td>0.46 ± 0.03</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>9c</td>
<td>(n)Pr</td>
<td>0.86 ± 0.17</td>
<td>0.80 ± 0.09</td>
<td>0.06 ± 0.08</td>
</tr>
<tr>
<td>9d</td>
<td>(i)Pr</td>
<td>0.89 ± 0.08</td>
<td>0.67 ± 0.04</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>10a</td>
<td>Me</td>
<td>0.02 ± 0.12</td>
<td>–0.62 ± 0.06</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>10b</td>
<td>Et</td>
<td>0.08 ± 0.21</td>
<td>–0.47 ± 0.11</td>
<td>0.55 ± 0.10</td>
</tr>
<tr>
<td>10c</td>
<td>(n)-Pr</td>
<td>0.02 ± 0.07</td>
<td>–0.56 ± 0.04</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>10d</td>
<td>(i)-Pr</td>
<td>–0.11 ± 0.45</td>
<td>–2.60 ± 0.23</td>
<td>2.49 ± 0.22</td>
</tr>
</tbody>
</table>

4.4.1 Comparison between Enthalpy Values

The observed enthalpy components \(\Delta H\) for 7a–7d followed the same trend as the folding energy: 7c (\(n\)Pr) > 7a (–Me) > 7b (–Et) > 7d (–\(i\)Pr). Still, the enthalpy values did not show strict additivity: the \(\Delta H\) of 7b was less than two times of the \(\Delta H\) of 7a,
while balance 7d showed an enthalpy that was more than three times of that of 7a. It is possible that due to the steric exists between methyl group and arenè shelf in balance 7a, the measured ΔH value turned out to be higher than that of the actual interaction. Without the repulsion, the ΔH value for a single CH–π interaction may be $-1.5 \text{kcal/mol}$ or lower, based on the ΔH of balance 7d which contains three CH–π interactions. The n-propyl balance 7c still showed the lowest enthalpy. The balance 7d with branched propyl group was much more stabilized than balance 7c with linear propyl group, probably because the cooperativity of the interactions leads to a better geometric positioning.

4.4.2 Entropy-Enthalpy Compensation

The entropy term ($-T\Delta S$) of balances 7a–7d showed generally similar but opposite sign as the ΔH values. One explanation is that larger alkyl group will lead to larger steric in restricted environment. Similar trend of conformational entropy of Me, Et, nPr and iPr groups, from both calculation and experiments, have been observed in the conformational exchange between axial and equatorial conformers of alkyl-substituted cyclohexanes.\textsuperscript{109-111}

![Figure 4.4: Polts showing the compensation between ΔH and $-T\Delta S$ values of balances 7–10.](image-url)
This can also be attributed to the increasing conformational restriction and entropic penalty of the balance system with higher enthalpic complexation energies, which is also known as the enthalpy/entropy compensation effect (Figure 4.4).\textsuperscript{106,110} For example in balance 7d, all three carbons on \(-iPr\) group are able to form CH–π interactions with the phenanthrene surface, so only one rotamer for the \(-iPr\) is able to be formed in the folded 7d due to the highly restricted rotation of the C\textsubscript{aryl}–C\textsubscript{alkyl} bond. This leads to the highest conformational entropy while showing the lowest \(\Delta H\) value among balance 7.

4.5 SOLVENT EFFECTS

\[ y = 0.0399x - 0.8929 \]
\[ y = 0.0119x - 0.0918 \]
\[ y = 0.0037x + 0.0646 \]
\[ y = 0.0129x - 0.6604 \]

\textbf{Figure 4.5:} Solvent trends for balances 7a–7d in a series of solvents with different E\textsubscript{T}(30) values. The solvents from left to right are: benzene-\(d_6\), bromobenzene-\(d_5\), CDCl\(_3\), acetone-\(d_6\), DMSO-\(d_6\), and acetonitrile-\(d_3\).

The solvent effect on multiple CH–π interaction was also studied. Same as previous balances, these compounds showed excellent solubility in a series of solvents with different polarity. Plots of folding energies vs. E\textsubscript{T}(30) of balances 7a–7d in different solvents were shown in Figure 4.5. Different from result of previous balances
with oxygen linker, there was barely any trend for each of the balances. The strength of CH–π interaction did not change according to the solvent polarity (except for balance 7d with i-Pr group). This suggests that maybe the solvent effect on CH–π interactions are too weak that it only shows up when multiple interactions were formed. It is also possible that the trend observed in previous studies were caused solely by the solvophobic effect of the lone pair–π interactions.

4.6 CONCLUSION

In conclusion, a series of molecular balances were synthesized to study the multiple CH–π interactions. By removing the oxygen linker and eliminate the repulsive O–π interaction, we successfully extended our study to the interactions formed by a larger range of alkyl groups. The geometries of several interactions were characterized in their solid-state, and their folding energies (−ΔG) were compared. It turned out that the CH–π interactions can show certain additivity, but the total strength of the multiple interactions cannot be predicted by simply multiple the strength of one single interaction. The entropic penalty comes from the conformational restriction may be very important on determining the total strength of interactions, leading to the different behaviors of large alkyl groups such as −nPr and −iPr when forming interactions.

4.6 EXPERIMENTAL SECTION

NMR spectra were recorded on Varian 300 MHz and 400 MHz spectrometers. Chemical shifts are reported in ppm (δ) referenced to TMS. All chemicals were purchased from commercial suppliers and used as received unless otherwise specified. Flash chromatography was carried out using silica gel from Sorbent Technologies (60 Å, 200–400 mesh). Thin layer chromatography (TLC) was performed using pre-coated TLC plates (Merck pre-coated 0.25 mm silica gel 60 F254 plates).
4.6.1 Synthesis

![Figure 4.6: Overview of synthesis of balances 7–10 via condensation between aniline 11 and anhydride 5.](image)

The general synthetic route for balances 7–10 (Figure 4.1) was as shown in Figure 4.6. All balances were synthesized via the condensation between anilines 11 and anhydrides 5. Anilines 11 are all commercially available, and the synthetic routes of anhydrides 5a, 5c and 5d and balances 7e, 9e and 10e (balances 1a, 2a and 3a in Chapter 3) have been described in Chapter 3. The detailed synthesis for the rest of these compounds and the characterization data are shown as follows.

**Preparation of pyrene-4, 5-dione**

![Preparation of pyrene-4, 5-dione](image)

This precursor for making anhydride 5b was prepared as described in reference. To a solution of pyrene (2.0 g, 10 mmol) in 40 mL methylene chloride and 40 mL MeCN, NaIO₄ (10.0 g, 46.8 mmol), RuCl₃ (0.20 g, 0.96 mmol), and water (50 mL) were added. The dark brown suspension was stirred at rt. for 14 h. The reaction mixture was then poured into 500 mL water and the organic phase was separated. The
aqueous phase was extracted with methylene chloride (3×50 mL), and the extracts were combined and washed with water (3×200 mL) to give a dark orange solution. The solvent of combined organic phase was removed under pressure to give a dark orange solid (2.11 g) as crude product. Column chromatography was run with methylene chloride, and the pure product was given as an orange solid (1.13 g, 48.7% yield). 1H NMR (300 MHz, CDCl$_3$) δ 8.41 (dd, $J = 0.9$ Hz, $J = 7.2$ Hz, 2 H), 8.11 (dd, $J = 1.2$ Hz, $J = 8.1$ Hz, 2 H), 7.78 (s, 2 H), 7.70 (t, $J = 7.5$ Hz, 2 H).

**Preparation of 9,11-diphenyl-10H-cyclopenta[e]pyren-10-one**

![Chemical Structure](image)

This is also a precursor for making anhydride 5b, and was prepared as described in reference. Pyrene-4,5-dione (0.200 g, 0.86 mmol) and diphenyl acetone (0.199 g, 0.95 mmol) was dissolved in 100 mL methanol, and the mixture was heated to reflux. Potassium hydroxide (0.058 g, 1.03 mmol) in 50 mL of methanol was then added, and the reaction was heated at reflux for 2 h. The reaction mixture was then cooled down with ice water bath, and the precipitate was isolated via suction filtration and collected as the crude diene (dark green solid, 0.100g, 29% yield). The product was used for the next step directly without purification.
Preparation of anhydride 5b

The crude diene (9,11-diphenyl-10H-cyclopenta[e]pyren-10-one) (0.099 g, 0.24 mmol) and maleic anhydride (0.060 g, 0.61 mmol) were mixed in 5 mL of toluene and were heated with a heating gun until the dark green color faded. After cooling with ice-water bath, the precipitated product was separated by suction filtration and washed with cold diethyl ether to give anhydride 5b (0.080 g, 66% yield) as white solid. The crude product was used for next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.26 (d, $J = 7.77$ Hz, 2 H), 8.12 (d, $J = 7.60$ Hz, 2 H), 8.02 (s, 2 H), 7.78 (t, $J = 7.60$ Hz, 2 H), 7.65 (t, $J = 7.98$ Hz, 2 H), 7.60 (t, $J = 7.41$ Hz, 2 H), 7.47 (t, $J = 7.60$ Hz, 2 H), 7.37 (d, $J = 7.98$ Hz, 2 H), 7.27 (d, $J = 7.60$ Hz, 2 H), 4.81 (s, 2 H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.46, 134.09, 132.69, 131.58, 130.97, 129.63, 128.91, 128.90, 128.58, 127.64, 126.56, 125.97, 125.48, 124.81, 123.13, 123.11, 63.34, 46.26. HRMS (EI) calculated for C$_{35}$H$_{20}$O$_4$: 504.1362; obs: 504.1363.
Figure 4.7: $^1$H NMR spectrum of anhydride 5b (CDCl$_3$, 400 MHz).

Figure 4.8: $^{13}$C NMR spectrum of anhydride 6b (CDCl$_3$, 100 MHz).
General procedure for preparing molecular balances 7–10

For the condensation reaction, the corresponding anhydride and aniline were dissolved in acetic acid, and the mixture was heated at reflux for 24 h. The solvent was then removed by rotary evaporation. The residue was dissolved in 25 mL EtOAc, washed once with 50 mL saturated sodium bicarbonate, and twice with 50 mL water. The solvent of organic layer was then removed under vacuum, and the crude product was purified via flash chromatography using silica gel (MeOH/CH$_2$Cl$_2$, v/v = 1/99). Among the balances that were synthesized, balances 7c–7d, 8a–8e, 9b–9d and 10c are new compounds. The other balances are known molecules, and their $^1$H NMR spectra matched the previously reported spectra.

Preparation of balance 7a

Without further purification, anhydride 5a (0.100 g, 0.21 mmol) was reacted with o-toluidine (0.033 g, 0.31 mmol) in 5 mL acetic acid. After work-up and purification, balance 7a was obtained as light yellow solid (0.081 g, 0.14 mmol, 67% yield). It is a known compound and its characterization data matched with the previous publication.$^{113}$ $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.71 (d, $J = 8.6$ Hz, 2 H minor), 8.66 (d, $J = 8.6$ Hz, 2 H major), 8.37 (t, $J = 8.6$ Hz, 2 H), 7.80–6.86 (m, 17 H major, 16 H minor), 6.73 (d, $J = 7.3$ Hz, 1 H major), 6.46 (t, $J = 7.3$ Hz, 1 H minor), 4.64 (s, 2 H), 4.34 (d, $J = 7.3$ Hz, 1 H minor), 2.05 (s, 3 H minor), $-0.03$ (s, 3 H major).

Preparation of balance 7b

Anhydride 5a (0.100 g, 0.21 mmol) was reacted with 2-ethylaniline (0.037 g, 0.31 mmol) in 5 mL acetic acid. After work up and purification, balance 7b was obtained as white solid (0.098 g, 0.17 mmol, 80% yield). It is a known compound, and the
characterization data was matched with the reference.\textsuperscript{94} $^1$H NMR (400 MHz, CDCl\textsubscript{3}) $\delta$ 8.81–8.73 (m, 2 H minor, 2 H major), 8.47–8.39 (m, 2 H major, 2 H minor), 7.80–6.87 (m, 18 H major, 16 H minor), 6.50 (dt, $J = 7.9$ Hz, $J = 1.1$ Hz, 1 H minor), 4.81 (s, 2 H major, 2 H minor), 4.43 (dd, $J = 7.9$ Hz, $J = 1.2$ Hz, 1 H minor), 2.41 (q, $J = 7.6$ Hz, 2 H minor), 1.15 (t, $J = 7.6$ Hz, 3 H minor), 0.20 (q, $J = 7.5$ Hz, 2 H major), $-0.08$ (t, $J = 7.4$ Hz, 3 H major).

Preparation of balance 7c

Anhydride 5a (0.050 g, 0.10 mmol) was reacted with 2-propylaniline (0.028 g, 0.21 mmol) in 5 mL acetic acid. After work up and purification, balance 7c was obtained as yellow solid (0.057 g, 0.095 mmol, 95% yield). $^1$H NMR (400 MHz, CDCl\textsubscript{3}) $\delta$ 8.80 (d, $J = 8.5$ Hz, 2 H major), 8.77 (d, $J = 8.5$ Hz, 2 H minor), 8.49–8.40 (m, 2 H major, 2 H minor), 7.85–6.88 (m, 16 H major, 18 H minor), 6.51 (t, $J = 7.5$ Hz, 1 H major), 4.72 (s, 2 H major), 4.70 (s, 2 H minor), 4.33 (d, $J = 7.9$ Hz, 1 H major), 2.38 (t, $J = 7.5$ Hz, 2 H major), 1.56 (m, 2 H major), 0.96 (t, $J = 7.4$ Hz, 3 H major), 0.52 (m, 2 H minor), 0.26 (t, $J = 6.4$ Hz, 2 H minor), $-0.30$ (t, $J = 7.4$ Hz, 3 H minor). $^{13}$C NMR (100 MHz, CDCl\textsubscript{3}) $\delta$ 196.93, 195.67, 173.81, 173.67, 139.40, 139.01, 133.84, 133.78, 133.72, 133.51, 131.54, 131.23, 131.18, 131.01, 130.92, 129.85, 129.46, 129.38, 129.34, 129.23, 129.18, 128.67, 128.54, 128.49, 128.42, 128.05, 127.83, 127.60, 127.31, 127.18, 126.93, 126.70, 126.61, 126.59, 126.39, 126.37, 126.32, 126.17, 125.96, 123.03, 122.99, 68.17, 63.62, 63.61, 45.51, 44.88, 38.74, 33.11, 30.38, 28.95, 28.44, 23.03, 19.42, 14.03, 11.45. HRMS (EI) calculated for C\textsubscript{42}H\textsubscript{31}NO\textsubscript{3}: 597.2304; obs: 597.2303.
Figure 4.9: $^1$H NMR spectrum of balance 7c (CDCl$_3$, 400 MHz).

Figure 4.10: $^{13}$C NMR spectrum of balance 7c (CDCl$_3$, 100 MHz).
Preparation of balance 7d:

Anhydride 5a (0.050 g, 0.10 mmol) was reacted with 2-isopropylaniline (0.028 g, 0.21 mmol) in 5 mL acetic acid. After work up and purification, balance 7d was obtained as white solid (0.055 g, 0.092 mmol, 92% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.73 (d, $J = 8.42$ Hz, 2 H), 8.40 (d, $J = 7.74$ Hz, 2 H major, 2 H minor), 7.76–6.82 (m, 18 H major, 17 H minor), 4.68 (s, 2 H major), 4.66 (s, 2 H minor), 4.17 (d, $J = 8.04$ Hz, 1 H minor), 2.62 (m, 1 H minor), 1.10 (d, $J = 6.85$ Hz, 6 H minor), –0.02 (m, 1 H major), –0.27 (d, $J = 6.77$ Hz, 6 H major). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 195.57, 173.98, 146.59, 133.82, 133.70, 131.54, 130.99, 129.78, 129.37, 129.27, 128.97, 128.51, 128.38, 127.42, 127.18, 126.60, 126.37, 126.18, 123.05, 63.63, 45.50, 27.62, 22.41. HRMS (EI) calculated for C$_{42}$H$_{31}$NO$_3$: 597.2304; obs: 597.2296.

Figure 4.11: $^1$H NMR spectrum of balance 7d (CDCl$_3$, 400 MHz).
Figure 4.1: $^{13}$C NMR spectrum of balance 7d (CDCl$_3$, 100 MHz).

Preparation of balance 8a:

Anhydride 5b (0.050 g, 0.099 mmol) was reacted with o-toluidine (0.013 g, 0.12 mmol) in 5 mL acetic acid. After work up and purification, balance 8a was obtained as a white solid (0.056 g, 0.094 mmol, 95% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.52–8.40 (m, 2 H major, 2 H minor), 8.15–6.76 (m, 19 H major, 18 H minor), 6.55 (d, $J = 7.8$ Hz, 1 H major), 6.07 (t, $J = 7.8$ Hz, 1 H minor), 4.72 (s, 2 H minor), 4.70 (s, 2 H major), 3.78 (d, $J = 7.9$ Hz, 1 H minor), 2.02 (s, 3 H minor), −0.58 (s, 3 H major). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 197.34, 196.43, 173.56, 173.27, 135.61, 134.80, 134.53, 134.34, 133.73, 133.67, 131.40, 131.34, 131.15, 131.08, 130.59, 130.56, 130.44, 129.95, 129.46, 129.44, 129.33, 128.98, 128.70, 128.62, 128.58, 128.54, 127.59, 127.52, 127.33, 126.46, 126.21, 126.18, 126.12, 126.08, 125.98, 125.95, 125.57, 125.51, 125.34, 125.06, 123.53, 123.44, 63.86, 63.81, 45.46, 45.02, 17.58, 14.47. HRMS (EI) calculated for C$_{42}$H$_{27}$NO$_3$: 593.1991; obs: 593.1981.
Figure 4.13: $^1$H NMR spectrum of balance 8a (CDCl$_3$, 400 MHz).

Figure 4.14: $^{13}$C NMR spectrum of balance 8a (CDCl$_3$, 100 MHz).
Preparation of balance 8b:

Anhydride 5b (0.050 g, 0.099 mmol) was reacted with 2-ethylaniline (0.018 g, 0.15 mmol) in 5 mL acetic acid. After work up and purification, balance 8b was obtained as a white solid (0.050 g, 0.082 mmol, 83% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.52–8.40 (m, 2 H major, 2 H minor), 8.14–6.52 (m, 20 H major, 18 H minor), 6.01 (t, $J = 7.6$ Hz, 1 H minor), 4.72 (s, 2 H minor), 4.70 (s, 2 H major), 3.66 (d, $J = 7.9$ Hz, 1 H minor), 2.31 (q, $J = 15.2$ Hz, $J = 7.6$ Hz, 2 H minor), 1.05 (t, $J = 7.6$ Hz, 3 H minor), –0.32 (q, $J = 14.8$ Hz, $J = 7.6$ Hz, 2 H major), –0.90 (t, $J = 7.5$ Hz, 3 H major). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 197.34, 196.21, 173.85, 173.63, 141.03, 140.65, 134.62, 134.36, 133.71, 133.68, 131.48, 131.40, 131.16, 131.07, 130.01, 129.51, 129.47, 129.42, 129.35, 129.23, 128.67, 128.55, 128.51, 127.95, 127.58, 127.56, 127.25, 126.31, 126.21, 126.19, 126.16, 126.11, 126.04, 125.98, 125.50, 125.38, 125.34, 125.06, 123.61, 123.48, 63.86, 63.84, 45.58, 45.05, 23.94, 20.88, 14.18, 10.08. HRMS (EI) calculated for C$_{43}$H$_{29}$NO$_3$: 607.2147; obs: 607.2149.

Figure 4.15: $^1$H NMR spectrum of balance 8b (CDCl$_3$, 400 MHz).
Figure 4.1: $^{13}$C NMR spectrum of balance 8b (CDCl$_3$, 100 MHz).

Preparation of balance 8c:

Anhydride 5b (0.050 g, 0.099 mmol) was reacted with 2-propylaniline (0.020 g, 0.15 mmol) in 5 mL acetic acid. After work up and purification, balance 8c was obtained as a white solid (0.056 g, 0.094 mmol, 95% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.56–8.43 (m, 2 H major, 2 H minor), 8.17–6.65 (m, 20 H major, 18 H minor), 6.03 (t, $J$ = 7.8 Hz, 1 H major), 4.75 (s, 2 H major), 4.73 (s, 2 H minor), 3.68 (d, $J$ = 7.8 Hz, 1 H major), 2.30 (t, $J$ = 7.8 Hz, 2 H major), 1.55–1.40 (m, 2 H major), 0.88 (t, $J$ = 7.8 Hz, 3 H major), –0.19 (t, $J$ = 6.5 Hz, 2 H minor), –0.31––0.43 (m, 2 H minor), –0.88 (t, $J$ = 7.8 Hz, 3 H minor). HRMS (EI) calculated for C$_{44}$H$_{31}$NO$_3$: 621.2304; obs: 621.2294.
**Figure 4.17:** $^1$H NMR spectrum of balance 8c (CDCl$_3$, 400 MHz).

**Preparation of balance 8d:**

Anhydride 5b (0.050 g, 0.099 mmol) was reacted with 2-propylaniline (0.020 g, 0.15 mmol) in 5 mL acetic acid. After work up and purification, balance 8d was obtained as a white solid (0.053 g, 0.085 mmol, 86% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.55–8.43 (m, 2 H major, 2 H minor), 8.14–6.68 (m, 20 H major, 18 H minor), 5.97 (t, $J = 7.6$ Hz, 1 H minor), 4.76 (s, 2 H major), 4.74 (s, 2 H minor), 3.56 (d, $J = 7.8$ Hz, 1 H minor), 2.59 (m, 1 H minor), 1.07 (d, $J = 6.8$ Hz, 6 H minor), $-0.38$ (m, 1 H major), $-0.95$ (d, $J = 6.7$ Hz, 6 H major). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 196.10, 173.98, 146.46, 134.67, 133.67, 131.60, 131.17, 129.70, 129.46, 129.43, 128.52, 128.48, 128.46, 128.48, 127.57, 127.37, 126.48, 126.32, 126.04, 125.90, 125.49, 123.76, 63.84, 45.63, 27.25, 21.68. HRMS (EI) calculated for C$_{44}$H$_{31}$NO$_3$: 621.2304; obs: 621.2304.
Figure 4.18: \(^1\)H NMR spectrum of balance 8d (CDCl\(_3\), 400 MHz).

Figure 4.19: \(^{13}\)C NMR spectrum of balance 8d (CDCl\(_3\), 100 MHz).
Preparation of balance 8e:

Anhydride 5b (0.050 g, 0.099 mmol) was reacted with o-anisidine (0.013 g, 0.11 mmol) in 5 mL acetic acid. After work up and purification, balance 8e was obtained as a white solid (0.056 g, 0.091 mmol, 93% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.57–8.40 (m, 2 H major, 2 H minor), 8.15–6.60 (m, 18 H major, 19 H minor), 6.23 (d, \(J = 8.2\) Hz, 1 H minor), 5.93 (t, \(J = 7.5\) Hz, 1 H major), 4.72 (s, 2 H major), 4.70 (s, 2 H minor), 3.98 (dd, \(J = 7.8\) Hz, \(J = 1.1\) Hz, 1 H major), 3.68 (s, 3 H major), 1.41 (s, 3 H minor).

Preparation of balance 9a:

Anhydride 5c (0.050 g, 0.14 mmol) was reacted with o-toluidine (0.022 g, 0.20 mmol) in 5 mL acetic acid to produce 9a as light yellow solid (0.051 g, 0.11 mmol, 80% yield) after work-up and purification. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.00–8.12 (m, 2 H major, 2 H minor), 6.92–7.60 (m, 15 H major, 16 H minor), 5.51 (d, \(J = 7.8\) Hz, 1 H major), 4.34 (s, 2 H minor), 4.30 (s, 2 H major), 2.09 (s, 3 H major), 1.08 (3 H minor). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 173.25, 144.29, 136.37, 135.55, 130.79, 130.35, 128.71, 128.67, 128.62, 128.34, 128.25, 121.03, 90.56, 54.63, 54.59, 17.68. HRMS (EI) calculated for C\(_{31}\)H\(_{23}\)NO\(_3\): 457.1678; obs: 457.1680.
Figure 4.20: $^1$H NMR spectrum of balance 14a (CDCl$_3$, 400 MHz).

Figure 4.21: $^{13}$C NMR spectrum of balance 14a (CDCl$_3$, 100 MHz).
Preparation of balance 9b:

Anhydride 5c (0.100 g, 0.27 mmol) was reacted with 2-ethylaniline (0.039 g, 0.33 mmol) in 5 mL acetic acid to produce 9b as light yellow solid (0.127 g, 0.27 mmol, 95% yield) after work-up and purification. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.11–8.01 (m, 2 H major, 2 H minor), 6.89–7.57 (m, 15 H major, 16 H minor), 5.43 (d, $J = 7.9$ Hz, 1 H major), 4.35 (s, 2 H minor), 4.31 (s, 2 H major), 2.38 (q, $J = 7.5$ Hz, $J = 15.2$ Hz, 2 H major), 1.13 (t, $J = 7.6$ Hz, 3 H major), 1.09 (q, $J = 7.5$ Hz, $J = 15.2$ Hz, 2 H minor), 0.85 (t, $J = 7.5$ Hz, 3 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.61, 144.34, 141.33, 136.38, 129.80, 129.72, 128.90, 128.69, 128.66, 128.61, 128.24, 128.17, 127.50, 127.13, 127.11, 126.65, 121.13, 121.05, 90.55, 54.74, 54.65, 24.06, 14.19. HRMS (EI) calculated for C$_{32}$H$_{35}$NO$_3$: 471.1834; obs: 471.1836.

Figure 4.22: $^1$H NMR spectrum of balance 9b (CDCl$_3$, 400 MHz).
Figure 4.23: $^{13}$C NMR spectrum of balance 9b (CDCl$_3$, 100 MHz).

Preparation of balance 9c:

Anhydride 5c (0.100 g, 0.27 mmol) was reacted with 2-propylaniline (0.056 g, 0.41 mmol) in 5 mL acetic acid to produce 3c as light yellow solid (0.134 g, 0.26 mmol, 95% yield) after work-up and purification. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.87–7.09 (m, 17 H major, 17 H minor), 6.90 (d, $J = 7.7$ Hz, 1 H major), 6.77 (d, $J = 7.7$ Hz, 1 H minor), 3.76 (s, 2 H minor), 3.72 (s, 2 H major), 2.28 (t, $J = 7.8$ Hz, 3 H minor), 1.13 (t, $J = 7.8$ Hz, 3 H major), 1.49–1.28 (m, 2 H minor, 2 H major), 0.91 (t, $J = 7.3$ Hz, 3 H major), 0.79 (t, $J = 7.3$ Hz, 3 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 196.61, 172.77, 172.68, 146.44, 146.42, 140.04, 140.02, 137.18, 134.00, 133.77, 133.01, 130.49, 130.38, 129.83, 129.78, 129.68, 129.44, 129.28, 128.34, 128.22, 127.87, 126.52, 126.52, 126.25, 126.04, 119.78, 91.24, 91.20, 53.99, 53.96, 33.28, 33.01, 23.30, 22.97, 14.20, 13.99. HRMS (EI) calculated for C$_{33}$H$_{27}$NO$_3$: 485.1991; obs: 485.1993.
Figure 4.24: $^1$H NMR spectrum of balance 9c (CDCl$_3$, 400 MHz).

Figure 4.25: $^{13}$C NMR spectrum of balance 9c (CDCl$_3$, 100 MHz).
Preparation of balance 9d:

Anhydride 5c (0.100 g, 0.27 mmol) was reacted with 2-propylaniline (0.056 g, 0.41 mmol) in 5 mL acetic acid to produce 9d as light yellow solid (0.114 g, 0.23 mmol, 85% yield) after work-up and purification. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.88–7.03 (m, 17 H major, 17 H minor), 6.90 (d, $J = 7.6$ Hz, 1 H major), 6.76 (d, $J = 7.6$ Hz, 1 H minor), 3.75 (s, 2 H minor), 3.70 (s, 2 H major), 2.71 (m, 1 H major), 2.58 (m, 1 H minor) 1.08 (d, $J = 6.8$ Hz, 6 H minor), 0.79 (t, $J = 6.8$ Hz, 6 H major). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 172.99, 172.72, 146.41, 146.32, 145.92, 140.02, 137.19, 134.01, 133.84, 133.01, 130.38, 129.90, 129.84, 129.68, 129.62, 129.45, 128.39, 128.34, 128.32, 128.24, 127.97, 127.87, 128.87, 126.51, 126.32, 126.25, 126.10, 126.01, 119.80, 104.48, 91.20, 91.15, 56.45, 54.18, 54.05, 28.62, 27.83, 23.59, 23.49. HRMS (EI) calculated for C$_{33}$H$_{27}$NO$_3$: 485.1991; obs: 485.1989.

Figure 4.26: $^1$H NMR spectrum of balance 9d (CDCl$_3$, 400 MHz).
Figure 4.27: $^{13}$C NMR spectrum of balance 9d (CDCl$_3$, 100 MHz).

Preparation of balance 9e:

Anhydride 5c (0.22 g, 0.59 mmol) was reacted with anisidine (0.11 g, 0.89 mmol) in 5 mL acetic acid to produce balance 9e as pale yellow solid (0.23 g, 0.48 mmol, 82% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.08 (m, 4 H major, 4 H minor), 6.78–7.60 (m, 13 H major, 14 H minor), 5.68 (dd, $J$ = 7.7 Hz, $J$ = 1.2 Hz, 1 H major), 4.31 (s, 2 H major), 4.28 (s, 2 H minor), 3.76 (s, 3 H major), 3.48 (s, 3 H minor).

Preparation of balance 10a:

Anhydride 5d (cis-5-Norbornene-endo-2,3-dicarboxylic anhydride) (0.050 g, 0.30 mmol) and o-toluidine (0.039 g, 0.37 mmol) were heated to reflux in 5 mL acetic acid for 24 h. The solvent was removed under vacuum and the reaction mixture was heated in oven (120 °C) for another 12 h. Then balance 10a was obtained as white solid (0.047 g, 0.19 mmol, 62% yield). Its characterization data matches up with the reference. $^1$H
NMR (400 MHz, CDCl$_3$) $\delta$ 7.24–7.38 (m, 3 H), 7.03 (d, $J$ = 7.5 Hz, 1 H minor), 6.91 (d, $J$ = 7.5 Hz, 1 H major), 6.35 (t, $J$ = 1.6 Hz, 2 H), 3.45–3.58 (m, 4 H), 2.18 (s, 3 H major), 2.15 (s, 3 H minor), 1.80–1.88 (m, 1 H), 1.60–1.69 (m, 1 H). HRMS (EI) calculated for C$_{16}$H$_{15}$NO$_2$: 253.1103; obs: 253.1099.

**Preparation of balance 10b:**

Anhydride 5d (0.100 g, 0.61 mmol) was reacted with 2-ethylaniline (0.089 g, 0.73 mmol) in 5 mL acetic acid. After work up and purification, balance 10b was obtained as a white solid (0.142 g, 0.53 mmol, 87% yield). It is a known compound, and the characterization data was matched with the reference.$^1$ $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.41–7.20 (m, 3 H), 6.97 (d, $J$ = 7.8 Hz, 1 H minor), 6.86 (d, $J$ = 7.8 Hz, 1 H major), 6.31 (s, 2 H), 3.54–3.39 (m, 4 H), 2.45 (q, $J$ = 7.7 Hz, $J$ = 15.2 Hz, 2 H major), 2.40 (q, $J$ = 7.7 Hz, $J$ = 15.2 Hz, 2 H minor), 1.79 (t, $J$ = 9.0 Hz, 1 H), 1.60 (t, $J$ = 7.9 Hz, 1 H), 1.22–1.08 (m, 3 H major, 3 H minor).

**Preparation of balance 10c:**

Anhydride 5d (0.050 g, 0.30 mmol) was reacted with 2-propylaniline (0.049 g, 0.37 mmol) in 5 mL acetic acid. After work up and purification, balance 10c was obtained as a white solid (0.073 g, 0.26 mmol, 85% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.34–7.10 (m, 3 H), 6.88 (d, $J$ = 7.6 Hz, 1 H major), 6.78 (d, $J$ = 7.6 Hz, 1 H minor), 6.31–6.18 (m, 2 H), 3.52–3.32 (m, 4 H), 2.40–2.19 (m, 2 H major, 2 H minor), 1.75 (t, $J$ = 10.3 Hz, 1 H), 1.54 (t, $J$ = 8.9 Hz, 1 H), 1.52–1.37 (m, 2 H major, 2 H minor), 0.89 (t, $J$ = 7.3 Hz, 3 H minor). 0.84 (t, $J$ = 7.3 Hz, 3 H major). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 177.23, 176.98, 140.45, 140.11, 135.18, 134.65, 130.86, 130.80, 130.04, 129.90, 129.47.

---

129.44, 128.53, 128.15, 126.96, 126.84, 52.96, 52.32, 46.85, 45.77, 45.44, 45.16, 33.51, 33.24, 23.67, 23.06, 14.12, 14.10. HRMS (EI) calculated for C$_{18}$H$_{19}$NO$_2$: 281.1416; obs: 281.1418.

**Figure 4.28:** $^1$H NMR spectrum of balance 10c (CDCl$_3$, 400 MHz).

**Figure 4.29:** $^{13}$C NMR spectrum of balance 10c (CDCl$_3$, 100 MHz).
Preparation of balance 10d:

Anhydride 5d (0.100 g, 0.61 mmol) was reacted with 2-isopropylaniline (0.089 g, 0.73 mmol) in 5 mL acetic acid. After work up and purification with column, balance 10d was obtained as a white solid (0.097 g, 0.34 mmol, 56% yield). It is a known compound, and the characterization data was matched with the reference.\(^1\) \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.43–7.31 (m, 2 H), 7.28–7.16 (m, 1 H), 6.92 (d, \(J = 7.7\) Hz, 1 H major), 6.81 (d, \(J = 7.7\) Hz, 1 H minor), 6.29 (m, 2 H minor, 2 H major), 3.53–3.39 (m, 4 H), 2.79–2.63 (m, 1 H major, 1 H minor), 1.79 (t, \(J = 9.5\) Hz, 1 H), 1.60 (t, \(J = 8.3\) Hz, 1 H), 1.17 (d, \(J = 6.9\) Hz, 6 H minor), 1.14 (d, \(J = 6.9\) Hz, 6 H major).

Preparation of balance 10e:

Anhydride 5c (0.11 g, 0.68 mmol) and o-anisidine (0.10 g, 0.81 mmol, 0.09 mL) were reacted in 10 mL acetic acid. The crude product was heated in oven (130 °C) for 16 h to give the product as white crystal (0.12 g, 0.45 mmol, 66% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.36 (dt, \(J = 7.8\) Hz, \(J = 1.4\) Hz, 1 H major, 1 H minor), 6.86–7.06 (m, 3 H major, 3 H minor), 6.28 (s, 2 H major), 6.21 (s, 2 H minor), 3.78 (ds, 3 H major, 3 H minor), 3.37–3.54 (m, 4 H major, 4 H minor), 1.54–1.82 (m, 2 H major, 2 H minor).

4.6.2 Variable Temperature \(^1\)H NMR Experiments:

The van’t Hoff plots of balances 7–10 were plotted based on the results from variable temperature \(^1\)H NMR. The full spectra were acquired at 5°C intervals between 25°C–55°C, and the folded/unfolded ratios were obtained via spectral deconvolution of the succinimide alpha singlets, the alkyl peaks (balances 7–9), or the triplet for ethene protons (balances 10). The folded/unfolded ratios (F/UF) were listed as Table 4.3–Table 4.10, and the van’t Hoff plots were as Figure 4.30–4.33.
Table 4.3: Results from variable temperature $^1$H NMR experiments of balance 7a and 7b in CDCl$_3$.

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Table 4.4: Results from variable temperature $^1$H NMR experiments of balance 7c and 7d in CDCl$_3$.

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Figure 4.30: Van't Hoff plot of balances 7a–7d in CDCl$_3$ based on the information in Table 4.3 and Table 4.4.
Table 4.5: Results from variable temperature $^1$H NMR experiments of balance 8a and 8b in CDCl$_3$.

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Table 4.6: Results from variable temperature $^1$H NMR experiments of balance 8c and 8d in CDCl$_3$.

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Figure 4.31: Van't Hoff plot of balances 8a–8d in CDCl$_3$ based on the information in Table 4.5 and Table 4.6.
Table 4.7: Results from variable temperature $^1$H NMR experiments of balance 9a and 9b in CDCl$_3$.

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Table 4.8: Results from variable temperature $^1$H NMR experiments of balance 9c and 9d in CDCl$_3$.

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Figure 4.32: Van't Hoff plot of balances 9a–9d in CDCl$_3$ based on the information in Table 4.7 and Table 4.8.
Table 4.9: Results from variable temperature $^1$H NMR experiments of balance 10a and 10b in CDCl$_3$.

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<td>−0.122</td>
<td>0.832266</td>
<td>−0.1836</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>0.87815</td>
<td>−0.12994</td>
<td>0.804918</td>
<td>−0.21702</td>
</tr>
</tbody>
</table>

Table 4.10: Results from variable temperature $^1$H NMR experiments of balance 10c and 10d in CDCl$_3$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>F/UF</th>
<th>ln(F/UF)</th>
<th>F/UF</th>
<th>ln(F/UF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.964435</td>
<td>−0.03621</td>
<td>1.21065</td>
<td>0.191157</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>0.945449</td>
<td>−0.05609</td>
<td>1.146235</td>
<td>0.136482</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.937693</td>
<td>−0.06433</td>
<td>1.062383</td>
<td>0.060515</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.926644</td>
<td>−0.07619</td>
<td>0.956232</td>
<td>−0.04476</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>0.903053</td>
<td>−0.10197</td>
<td>0.878613</td>
<td>−0.12941</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>0.892207</td>
<td>−0.11406</td>
<td>0.850126</td>
<td>−0.16237</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>0.886356</td>
<td>−0.12064</td>
<td>0.844441</td>
<td>−0.16908</td>
</tr>
</tbody>
</table>

Figure 4.33: Van't Hoff plot of balances 10a–10d in CDCl$_3$ based on the information in Table 4.9 and Table 4.10.
Based on the data above and the equation in Chapter 2, the calculation of entropy and enthalpy values of balance 7–10 with errors are listed in Table 4.11. The errors for slopes and intercepts are measured by the regression add-in in Excel.

**Table 4.11:** Calculation of ΔG, ΔH, ΔS, and −TΔS and their errors of balance 7–10 by VT NMR experiments in CDC13.

<table>
<thead>
<tr>
<th>balance</th>
<th>Slope</th>
<th>Intercept</th>
<th>ΔG  (kcal/mol)</th>
<th>ΔH  (kcal/mol)</th>
<th>ΔS  (kcal/mol·K)</th>
<th>−TΔS@25°C (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>334.28 ± 14.81</td>
<td>−0.9029 ± 0.0474</td>
<td>−0.1296 ± 0.05749</td>
<td>−0.6642 ± 0.02943</td>
<td>−0.001794 ± 9.4×10⁻⁵</td>
<td>0.5346 ± 0.0281</td>
</tr>
<tr>
<td>7b</td>
<td>481.98 ± 13.72</td>
<td>−1.1565 ± 0.0439</td>
<td>−0.2729 ± 0.05326</td>
<td>−0.9577 ± 0.02726</td>
<td>−0.002298 ± 0.000087</td>
<td>0.6848 ± 0.0260</td>
</tr>
<tr>
<td>7c</td>
<td>170.29 ± 40.94</td>
<td>−1.1827 ± 0.1566</td>
<td>0.3619 ± 0.1741</td>
<td>−0.3384 ± 0.0814</td>
<td>−0.00235 ± 0.00031</td>
<td>0.7003 ± 0.1927</td>
</tr>
<tr>
<td>7d</td>
<td>2289 ± 152</td>
<td>−6.106 ± 0.486</td>
<td>−0.9339 ± 0.5898</td>
<td>−4.549 ± 0.302</td>
<td>−0.01213 ± 0.00097</td>
<td>3.6155 ± 0.2878</td>
</tr>
<tr>
<td>8a</td>
<td>354.88 ± 19.78</td>
<td>−0.9171 ± 0.0633</td>
<td>−0.1621 ± 0.0768</td>
<td>−0.7051 ± 0.0393</td>
<td>−0.001822 ± 0.00013</td>
<td>0.5430 ± 0.0375</td>
</tr>
<tr>
<td>8b</td>
<td>493.38 ± 48.74</td>
<td>−0.9515 ± 0.1560</td>
<td>−0.4169 ± 0.1892</td>
<td>−0.9803 ± 0.0969</td>
<td>−0.001891 ± 0.00031</td>
<td>0.5634 ± 0.0924</td>
</tr>
<tr>
<td>8c</td>
<td>285.86 ± 76.13</td>
<td>−1.0576 ± 0.2436</td>
<td>0.05823 ± 0.2955</td>
<td>−0.5680 ± 0.1513</td>
<td>−0.00210 ± 0.00048</td>
<td>0.6262 ± 0.1442</td>
</tr>
<tr>
<td>8d</td>
<td>1377.4 ± 202.2</td>
<td>−2.7861 ± 0.6472</td>
<td>−1.0872 ± 0.7850</td>
<td>−2.7369 ± 0.4018</td>
<td>−0.00554 ± 0.00129</td>
<td>1.6497 ± 0.3832</td>
</tr>
<tr>
<td>9a</td>
<td>−201.9 ± 30.03</td>
<td>−0.7397 ± 0.0956</td>
<td>0.8392 ± 0.1163</td>
<td>0.4012 ± 0.0597</td>
<td>−0.001470 ± 0.00019</td>
<td>0.4380 ± 0.0566</td>
</tr>
<tr>
<td>9b</td>
<td>−230.51 ± 14.58</td>
<td>−0.6472 ± 0.0466</td>
<td>0.8412 ± 0.0565</td>
<td>0.4580 ± 0.0290</td>
<td>−0.001286 ± 0.00093</td>
<td>0.3832 ± 0.0276</td>
</tr>
<tr>
<td>9c</td>
<td>−402.54 ± 44.85</td>
<td>−0.1040 ± 0.1435</td>
<td>0.8614 ± 0.1741</td>
<td>0.7998 ± 0.0891</td>
<td>−0.00021 ± 0.00029</td>
<td>0.0616 ± 0.0850</td>
</tr>
<tr>
<td>9d</td>
<td>−339.56 ± 22.33</td>
<td>−0.3682 ± 0.0714</td>
<td>0.8927 ± 0.0867</td>
<td>0.6747 ± 0.0444</td>
<td>−0.00073 ± 0.00014</td>
<td>0.2180 ± 0.0423</td>
</tr>
<tr>
<td>10a</td>
<td>309.73 ± 31.99</td>
<td>−1.0726 ± 0.1023</td>
<td>0.01968 ± 0.12414</td>
<td>−0.6154 ± 0.06356</td>
<td>−0.002131 ± 0.00002</td>
<td>0.6351 ± 0.0606</td>
</tr>
<tr>
<td>10b</td>
<td>238.3 ± 53.5</td>
<td>−0.9297 ± 0.1711</td>
<td>0.07700 ± 0.20754</td>
<td>−0.4735 ± 0.1062</td>
<td>−0.001847 ± 0.00034</td>
<td>0.5505 ± 0.1013</td>
</tr>
<tr>
<td>10c</td>
<td>284.17 ± 17.69</td>
<td>−0.9902 ± 0.0566</td>
<td>0.02168 ± 0.06866</td>
<td>−0.5646 ± 0.0351</td>
<td>−0.00197 ± 0.00011</td>
<td>0.5863 ± 0.0335</td>
</tr>
<tr>
<td>10d</td>
<td>1309.5 ± 116.3</td>
<td>−0.2047 ± 0.3722</td>
<td>−0.1123 ± 0.4515</td>
<td>−2.6019 ± 0.2311</td>
<td>−0.00835 ± 0.00074</td>
<td>2.4897 ± 0.2204</td>
</tr>
</tbody>
</table>
CHAPTER 5
INVESTIGATION OF DEUTERIUM ISOTOPE EFFECT ON ALIPHATIC CH–π INTERACTIONS

Except for changing the environment and numbers of the interactions as we did in Chapter 3 and Chapter 4, another potentially powerful method for studying the interactions is to use the D/H isotope effects, which has been successfully applied to the study of other non-covalent interactions. The presence of a pronounced D/H isotope effect for the CH–π interactions could be used to verify their formation and to probe their stability trends. The enhanced CH–π interactions of deuterated molecules could also be used to design better pharmaceuticals and asymmetric catalysts.

However, whether hydrogen and deuterium form different strength CH–π interactions remains unclear. Several studies have observed significant deuterium isotope effects: Rebek et al. and Iwata et al. found deuterated species forming stronger interaction within different molecular capsules, and differences on retention times between protic and deuterated species were observed in chromatographic studies. Other studies have observed little or no D/H isotope effects for the CH–π interaction. A possible reason for these discrepancies is that many of these studies were carried out within the confined environments of molecular capsules, which are very sensitive to small differences in molecular volume. Thus, the observed enhancements in the stability of deuterated guests could be due to their reduced steric interactions arising from their shorter C–D bonds, as opposed to stronger attractive CD–π interactions.
Therefore, the goal of this Chapter was to study the D/H isotope effect in CH–π interactions within less constrained environments in which steric interactions were minimized. An experimental approach was carried out using our molecular balance system (Figure 5.1), and only minor differences between CD–π and CH–π interactions was found. The computational approach was also carried out in collaboration with Dr. C. David Sherrill’s group. They applied density functional theory (DFT) to a methane–benzene system, and the results also suggested the same conclusion.

**Figure 5.1:** Schematic representation of the *folded/unfolded* conformational equilibrium of the molecular balances that can be used to measure changes in the strength of the intramolecular CH–π interactions in the folded conformer.

5.1 DESIGNS OF BALANCES

First, the differences in CH–π and CD–π interactions were experimentally studied using molecular balances 12–15 (Figure 5.2). These balances provide a range of different CH–π interaction geometries and environments, affording a comprehensive study of the interaction. For example, balances 12 and 13 have large phenanthrene aromatic shelves, whereas balances 14 and 15 have smaller benzene shelves. The geometry and steric interactions of the *ortho*-methyl group are attenuated by subtle differences in the bicyclic framework. Specifically, the different bridges (Z in Figure 5.1 = –CO−, –O−, –m-C₆H₄−) on the backside of the balances attenuate the distance and steric interactions between the methyl group and aromatic shelf. Finally, balance 16 without aromatic shelf served as
controls which cannot form a CH–π interaction.

![Figure 5.2: Folded conformers of protic and deuterated molecular balances 12–15 that were designed to form intramolecular CH–π interactions and control balance 16.](image)

Noticeable, the balances 12–15 form intramolecular CH–π interactions within relatively open environments with a minimum of steric interactions. Therefore, these model systems are less susceptible to repulsive interactions that could mask and attenuate the CH–π interactions of interest.

Balances 12–16 were all synthesized via similar modular routes, which allowed the preparation of protic (12a–16a) and deuterated (12b–16b) balances.74,75 Protic balances 12a, 13a, 14a and 16a are same structures as balances 1a, 7a, 9a and 10a in Chapter 3 and 4. Balance 15a had been previously described in the literature was used to study CH–π interactions.81,113,123

5.2 SOLID-STATE STRUCTURES

The solid-state structures of balances 13a–16a by X–ray structure analyses was shown in Figure 5.3. The structures of balances 13a, 14a and 16a have been discussed in Chapter 4 (as 7a, 9a and 10a), and the crystal structure of balance 15a was from the literature.122 X-ray structure analysis confirms the existence of well-defined
intramolecular CH–π interactions in balances 13a–15a (Figure 5.3, a–c). It also confirmed the absence of an intramolecular CH–π interaction in the folded conformation in the control balance 16a (Figure 5.3, d).

Figure 5.3: X–ray structures of the folded conformers of (a) 13a, (b) 14a, (c) 15a, and (d) 16a. The bridgehead phenyl groups in 13a and 14a were partially hidden for better viewing clarity. The unfolded conformers were also present in the crystal structures of 14a, 15a, and 16a but are not shown.

Although balances 13a–15a all formed intramolecular CH–π interactions, the number (one hydrogen versus two), geometry, and distances of these interactions varied considerably. The structural parameters (d, θ, and α) used to compare the balances are shown in Figure 5.4, and a comparison of the measurements from the crystal structures of the balances are shown in Table 5.2. The “hinge” angle (θ) defined by the succinimide and arene planes provides a measure of how closely the ortho-methyl group is held against the arene shelf. For example, balance 14a has the smallest θ, fixing the ortho-
methyl tightly against the arene shelf. This strain is evident from the \(N\)-aryl group being pushed upward out of the succinimide plane (\(\alpha = +21^\circ\)). Balances 13a and 15a, in contrast, have larger \(\theta\) values, positioning their ortho-methyl groups at more optimal distances with less strain (\(\alpha = +5^\circ\) and +4\(^\circ\)).

![Diagram](image)

**Figure 5.4:** Definitions of the distance and angular measurements used to characterize balances 13a–16a.

**Table 5.1:** The \(d\), \(\theta\) and \(\alpha\) measured from the crystal structures of balances 13a–16a.

<table>
<thead>
<tr>
<th>balance</th>
<th>(d) (Å)</th>
<th>(\theta)</th>
<th>(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>2.68</td>
<td>58(^\circ)</td>
<td>5(^\circ)</td>
</tr>
<tr>
<td>14a(^a)</td>
<td>2.69</td>
<td>52(^\circ)</td>
<td>21(^\circ)</td>
</tr>
<tr>
<td>15a</td>
<td>2.61</td>
<td>58(^\circ)</td>
<td>4(^\circ)</td>
</tr>
<tr>
<td>16a</td>
<td>–</td>
<td>–</td>
<td>2(^\circ)</td>
</tr>
</tbody>
</table>

\(^a\) The \(d\), \(\theta\), and \(\alpha\) values were averages from the three unique folded conformers present in the unit cell of 14a.

The larger hinge angle \(\theta\) of balances 14 with benzene shelf indicate that balances with the same benzene shelf and O bridge-atom may not be a good control for balances 13. This also explains the observation that balances with the same framework as balances 14 always prefer the unfolded conformation. Thus, some comparisons made in Chapters 3 and 4 involving this series of balances may not be appropriate. In this chapter, they are only being considered as a different environment for the formation of CH–π interactions. The X–ray structure of deuterated balance 13b was also examined and compared with its protic counterpart, 13a. The structures were nearly identical.
5.3 COMPARISON OF FOLDING ENERGIES OF CH$_3$ AND CD$_3$ BALANCES

Next, the strengths of the CH–π interactions in balances 12–15 were measured in solution by $^1$H NMR. In each case, separate peaks for the folded and unfolded conformers were observed at room temperatures, enabling facile measurement of the folded/unfolded ratios. In particular, large upfield shifts were observed for the ortho-methyl groups in the folded conformers, which are consistent with the formation of CH–π interactions. The folded methyl singlets of 12a–15a were shifted upfield by −1.55 ppm, −2.08 ppm, −1.01 ppm and −1.04 ppm, respectively, compared with the peaks for the unfolded methyl groups. By comparison, control balance 16a, which cannot form a CH–π interaction, had almost identical chemical shifts for the folded and unfolded methyl protons ($\Delta\delta = −0.03$ ppm). The $^1$H NMR spectra of the deuterated balances were identical to their protic counterparts except for the absence of the deuterated ortho-methyl peaks.

![Bar chart showing the comparison of the folded/unfolded ratios of balances 12–16 in CDCl$_3$ at 25°C measured by integration of the $^1$H NMR spectra with a ±5% integration error.](image)

**Figure 5.5:** Comparison of the folded/unfolded ratios of balances 12–16 in CDCl$_3$ at 25°C measured by integration of the $^1$H NMR spectra with a ±5% integration error.
Comparison of the \textit{folded/unfolded} ratios for the protic balances showed the differences in their CH–π interactions (Figure 5.5). These ratios were measured from the integration of the singlets for the succinimide protons in the $^1$H NMR spectra. Integration of \textit{ortho}–methyl groups also gave similar \textit{folded/unfolded} ratios, but they were not used for comparisons because of the absence of this peak in the deuterated balances. As expected, control balances 16 had a nearly 1:1 \textit{folded/unfolded} ratio, suggesting that differences in dipole and solvation of the conformers are not biasing the \textit{folded/unfolded} ratios. Despite the presence of intramolecular CH–π interactions in balances 12–15, only balance 13 displayed a preference for the \textit{folded} conformer. We attribute this to the presence of repulsive interaction. The rigid bicyclic framework positions the methyl group slightly too close to the arene shelf, resulting in destabilizing steric interactions. As predicted from the crystal structures, the repulsive interaction is most evident in 14a, which also has the lowest \textit{folded/unfolded} ratio. The repulsive interactions complicate the measurement of the absolute strengths of the CH–π interactions. However, they do not diminish the utility of these balances in measuring the isotope effects of the CH–π interaction.

Differences in the strengths of intramolecular CH–π and CD–π interactions were assessed by comparison of the \textit{folded/unfolded} ratios and the corresponding folding energies (Table 5.2). The folding energies for protic and deuterated balances 12–15 were almost identical. The differences ($\Delta\Delta G_{H-D}$) were very small and were within the error of the analysis ($\pm 0.03$ kcal/mol), which was calculated based on a conservative estimate of $\pm 5\%$ for the $^1$H NMR integration error.$^{18}$ The folding energies of the protic and deuterated balances were also compared in acetone–d$_6$ (see experimental section). Again,
nearly identical folding energies were observed with even smaller errors.

Table 5.2: The folding energies of protic (Δ\(G_H\)) and deuterated (Δ\(G_D\)) balances 12–16 in CDCl\(_3\) at 25 °C.

<table>
<thead>
<tr>
<th>balance</th>
<th>Δ(G_H) (kcal/mol)</th>
<th>Δ(G_D) (kcal/mol)</th>
<th>ΔΔ(G_{H-D}) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.45</td>
<td>0.44</td>
<td>+0.01</td>
</tr>
<tr>
<td>13</td>
<td>−0.10</td>
<td>−0.13</td>
<td>+0.03</td>
</tr>
<tr>
<td>14(^a)</td>
<td>0.84</td>
<td>0.81</td>
<td>+0.03</td>
</tr>
<tr>
<td>15</td>
<td>0.07</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>16</td>
<td>0.02</td>
<td>0.06</td>
<td>−0.04</td>
</tr>
</tbody>
</table>

5.4 THERMODYNAMIC EXPERIMENT

To confirm the above single point measurements, more comprehensive multipoint van’t Hoff analyses were carried out. The folded/unfolded ratios for balances 12–15 were measured over a range of temperatures (25°C to 55°C) in CDCl\(_3\), and the Δ\(G_{fold}\) were calculated from the measured Δ\(H\) and Δ\(S\) values (Table 5.3). This study led to the same conclusion that the small differences in the Δ\(G_{fold}\) values of the protic and deuterated balances were well within the error of the analysis.

Table 5.3: Comparison of calculated Δ\(G_{fold}\) values between protic and deuterated balances 12–15 in CDCl\(_3\) and acetone–\(d_6\) at 25 °C with errors.

<table>
<thead>
<tr>
<th>balance</th>
<th>Δ(G_H) in CDCl(_3) (kcal/mol)</th>
<th>Δ(G_D) in CDCl(_3) (kcal/mol)</th>
<th>Δ(G_H) in acetone–(d_6) (kcal/mol)</th>
<th>Δ(G_D) in acetone–(d_6) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.44 ± 0.15</td>
<td>0.43 ± 0.12</td>
<td>0.14 ± 0.32</td>
<td>0.14 ± 0.09</td>
</tr>
<tr>
<td>13</td>
<td>−0.12 ± 0.05</td>
<td>−0.14 ± 0.11</td>
<td>−0.23 ± 0.16</td>
<td>−0.26 ± 0.37</td>
</tr>
<tr>
<td>14</td>
<td>0.83 ± 0.28</td>
<td>0.80 ± 0.19</td>
<td>0.66 ± 0.07</td>
<td>0.65 ± 0.07</td>
</tr>
<tr>
<td>15</td>
<td>0.07 ± 0.14</td>
<td>0.08 ± 0.38</td>
<td>−0.05 ± 0.26</td>
<td>−0.06 ± 0.10</td>
</tr>
</tbody>
</table>

5.5 CONCLUSION

The above experimental studies found only small differences in the strengths of the CH–\(\pi\) and CD–\(\pi\) interactions that were smaller than the experimental error of the analyses. These results were corroborated by theoretical calculations that compared the interaction energies of methane and benzene.\(^{76}\) Therefore, we concluded that there was either no deuterium isotope effect for the CH–\(\pi\) interaction or that the effect was too
small to be accurately measured by our model system. Another explanation is that the isotope effects for the attractive CH–π and repulsive steric interactions perfectly cancel out in all three balances. However, this possibility was seems unlikely. First, the attractive and repulsive isotope effects would have to balance perfectly for all three models systems, despite their differences in geometries and conformational constraints. Second, the repulsive steric interactions in balances 12–15 are very small (<1.0 kcal/mol) and do not change significantly with small differences in the lengths of the C–D and C–H bonds. A third reason that this explanation is unlikely is because it requires the attractive CD–π interaction to be weaker than the CH–π interaction. However, all reports that observed deuterium isotope effects for the CH–π interaction found the opposite trend.

Thus, previous reports of isotope effects were probably due to other factors such as the size difference between CH₃ and CD₃ groups placed within more confined environments, rather than an attenuation of the CH–π interaction. This steric hypothesis was supported by the theoretical calculations, which showed that differences in energy arose when the interacting groups were brought closer than the optimal CH–π interaction distance. While the lack of an isotope effect eliminates the possibility of using deuteration to enhance the CH–π interaction, it validates the use of deuteration for spectroscopic and labeling purposes, as this introduces a minimal perturbation of the system. Results obtained in this Chapter have been published and were reprinted with permission (Copyright © 2012, American Chemical Society).

5.6 EXPERIMENTAL SECTION

NMR spectra were recorded on Varian 300 MHz and 400 MHz spectrometers. Chemical shifts are reported in ppm (δ) referenced to TMS. All chemicals were
purchased from commercial suppliers and used as received unless otherwise specified.

Flash chromatography was carried out using silica gel from Sorbent Technologies (60 Å, 200–400 mesh). Thin layer chromatography (TLC) was performed using pre-coated TLC
plates (Merck pre-coated 0.25 mm silica gel 60 F254 plates).

5.6.1 Synthesis and Spectrums

![Diagram of synthesis](image)

**Figure 5.6:** Overview of synthesis of balances 13–16 via condensation reactions between deuterated or protic o-toluidine and anhydride 5.

**General procedure for preparing molecular balances 12–16**

For the condensation reaction, the corresponding anhydride and aniline were dissolved in acetic acid, and the mixture was heated at reflux for 24 h. The solvent was then removed by rotary evaporation. The residue was dissolved in 25 mL EtOAc, washed once with 50 mL saturated sodium bicarbonate, and twice with 50 mL water. The solvent of organic layer was then removed under vacuum, and the crude product was purified via flash chromatography using silica gel (MeOH/CH₂Cl₂, v/v = 1/99). The synthesis of balances 12a, 13a, 14a and 16a has been described in previous chapters as compound 1a (Chapter 3), 7a, 9a, and 10a (Chapter 4).
Preparation of \textit{o-Anisidine-}d$_3$

The corresponding deuterated nitrobenzene was synthesized first. To the stirring solution of potassium hydroxide (0.080 g, 1.42 mmol) in THF (3 mL), methanol-d$_4$ (0.100 mL, 2.49 mmol) and 1-fluro-2-nitrobenzene (0.100 g, 0.709 mmol) was added drop wise. The mixture was stirred for 24 h in room temperature. The solvent was then removed under vacuum, and the residue was dissolved with 30 mL ethyl acetate and washed with 50 mL water for 3 times. The solvent was dried to get deuterated \textit{1-methoxy-2-nitrobenzene} as yellow liquid (0.131 g, 0.84 mmol, > 95% yield). \textsuperscript{1}H NMR (300 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J = 7.84$ Hz, $J = 0.94$ Hz, 1 H), 7.54 (dt, $J = 7.52$ Hz, 1.25 Hz, 1 H), 7.08 (d, $J = 8.46$ Hz, 1 H), 7.03 (t, $J = 7.52$ Hz, 1 H).

The nitrobenzene was then reduced via catalyzed hydrogenation with Pd/C and H$_2$. The deuterated \textit{1-methoxy-2-nitrobenzene} (0.131 g, 0.84 mmol) was dissolved in ethanol (20 mL) in a pressure vessel, and 20 mg of Pd/C (10% wt) was added. The vessel was pressurized at 40 psi with hydrogen gas and was stirred for 2 h. The resulting mixture was filtered through celite and the solvent was removed by rotary evaporation to afford the \textit{o-anisidine-}d$_3$ as brown oil (0.130 g, > 95% yield). \textsuperscript{1}H NMR (300 MHz, CDCl$_3$) $\delta$ 6.90–6.58 (m, 4 H), 3.72 (br s, 2 H).

Preparation of balance \textit{12b}:

Anhydride \textit{5a} (0.50 g, 1.0 mmol) and \textit{o-anisidine-}d$_3$ (0.19 g, 1.5 mmol) were used as reactants, and 10 mL acetic acid was used as solvent. Purified by flash chromatography using silica gel (MeOH/CH$_2$Cl$_2$, v/v = 1/99). White solid, 0.49 g, 0.84
mmol, 84% yield. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.64–8.76 (m, 2 H major, 2 H minor), 8.42 (d, $J = 6.7$ Hz, 2 H minor), 8.38 (d, $J = 6.8$ Hz, 2 H major), 7.09–7.80 (m, 13 H major, 13 H minor), 7.04 (td, $J = 8.1$ Hz, $J = 2.5$ Hz, 2 H major), 6.96 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, 2 H minor), 6.82 (td, $J = 7.5$ Hz, $J = 0.9$ Hz, 2 H minor), 6.74 (d, $J = 8.6$ Hz, 1 H major), 6.44 (dd, $J = 8.5$ Hz, $J = 1.0$ Hz, 1 H minor), 6.28 (td, $J = 7.7$ Hz, $J = 1.4$ Hz, 1 H major), 4.64 (s, 2 H major), 4.62 (s, 2 H minor), 4.54 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, 1 H major).

Preparation of balance 13b:

Anhydride 5a (0.10 g, 0.21 mmol) and $o$-toluidine-$d_3$ (0.034 g, 0.31 mmol) were reacted in 5 mL acetic acid. After work up steps and purification, balance 13b was obtained as light yellow solid (0.098 g, 0.17 mmol, 81% yield). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.71 (d, $J = 8.6$ Hz, 2 H minor), 8.65 (d, $J = 8.6$ Hz, 2 H major), 8.37 (t, $J = 8.5$ Hz, 2 H), 7.80–6.86 (m, 17 H major, 16 H minor), 6.72 (d, $J = 7.3$ Hz, 1 H major), 6.46 (t, $J = 7.3$ Hz, 1 H minor), 4.64 (s, 2 H), 4.34 (d, $J = 7.3$ Hz, 1 H minor). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 196.94, 195.94, 173.56, 173.28, 135.79, 133.80, 133.75, 133.71, 133.50, 131.73, 131.24, 131.01, 130.95, 130.74, 130.56, 130.20, 129.41, 129.39, 129.27, 129.17, 129.14, 128.69, 128.60, 128.50, 128.46, 128.08, 127.84, 127.60, 127.41, 127.30, 127.19, 126.92, 126.76, 126.55, 126.51, 126.48, 126.33, 126.07, 125.95, 123.02, 122.99, 122.96, 63.62, 63.60, 45.35, 44.90. HRMS (EI) calculated for C$_{40}$H$_{24}$D$_3$NO$_3$: 572.2179; obs: 572.2181.
Figure 5.7: $^1$H NMR spectrum of balance 13b (CDCl₃, 400 MHz).

Figure 5.8: $^{13}$C NMR spectrum of balance 13b (CDCl₃, 100 MHz).
Preparation of balance 14b:

Anhydride 5c (0.050 g, 0.14 mmol) and o-toluidine-\textit{d}_3 (0.022 g, 0.20 mmol) were reacted to give balance 14b as light yellow solid (0.054 g, 0.12 mmol, 86% yield) after work up and purification. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.00–8.12 (m, 2 H major, 2 H minor), 6.92–7.60 (m, 15 H major, 16 H minor), 5.51 (d, $J$ = 7.8 Hz, 1 H major), 4.33 (s, 2 H minor), 4.30 (s, 2 H major). $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 173.26, 144.29, 136.37, 135.45, 130.78, 130.39, 128.71, 128.67, 128.62, 128.34, 128.25, 121.20, 121.03, 90.57, 54.63, 54.59. HRMS (EI) calculated for C$_{31}$H$_{20}$D$_3$NO$_3$: 460.1866; obs: 460.1871.

Figure 5.9: $^1$H NMR spectrum of balance 14b (CDCl$_3$, 400 MHz).
Figure 5.10: $^{13}$C NMR spectrum of balance 14b (CDCl$_3$, 100 MHz).

Preparation of anhydride 5e:

Anhydride 5e was synthesized via the description in reference.$^{125}$ The mixture of anthracene (0.10 g, 0.56 mmol), maleic anhydride (0.06 g, 0.56 mmol) and 3 mL xylene were heated to reflux for 2 h under stirring. The reaction mixture was then cooled down to room temperature. After crystallized under ice-water bath for 30 min, the product was separated by filtration and washed with several drops of cold ethanol. The pure product was then obtained as white crystal (0.12 g, 0.43 mmol, 75% yield). $^{1}$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.52 (m, 2 H), 7.36 (m, 2 H), 7.17 (m, 4 H), 4.85 (s, 2 H), 3.55 (s, 2 H).
Preparation of balance 15a:

Anhydride 5e (0.050 g, 0.18 mmol) was reacted with o-toluidine (0.023 g, 0.22 mmol) to give balance 15a as white solid (0.048 g, 0.13 mmol, 73% yield) after work up steps and purification. $^1$H NMR (400 MHz, CDCl$_3$) δ 6.79–7.43 (m, 12 H major, 11 H minor), 5.43 (d, $J = 7.6$ Hz, 1 H minor), 4.76–4.88 (m, 2 H), 3.29–3.41 (m, 2 H), 1.98 (s, 3 H major), 0.97 (s, 3 H minor). HRMS (EI) calculated for C$_{25}$H$_{19}$NO$_2$: 365.1416; obs: 365.1411.

Preparation of balance 15b:

Anhydride 5e (0.033 g, 0.12 mmol) and o-toluidine-$d_3$ (0.020 g, 0.18 mmol) were heated to reflux in 3 mL acetic acid to produce balance 3b as white solid (0.037 g, 0.10 mmol, 83% yield) after purification. $^1$H NMR (400 MHz, CDCl$_3$) δ 6.79–7.43 (m, 12 H major, 11 H minor), 5.43 (d, $J = 7.6$ Hz, 1 H minor), 4.77–4.88 (m, 2 H), 3.28–3.40 (m, 2 H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 176.03, 175.92, 141.94, 141.30, 139.27, 138.85, 130.73, 129.44, 127.72, 127.37, 127.28, 127.18, 126.85, 126.75, 126.62, 125.48, 125.24, 124.34, 124.22, 47.18, 47.17, 45.85, 45.34. HRMS (EI) calculated for C$_{25}$H$_{16}$D$_3$NO$_2$: 368.1604; obs: 368.1604.
Figure 5.11: $^1$H NMR spectrum of balance 15b (CDCl$_3$, 400 MHz).

Figure 5.12: $^{13}$C NMR spectrum of balance 15b (CDCl$_3$, 100 MHz).
Preparation of balance 16b:

*cis*-5-Norbornene-endo-2,3-dicarboxylic anhydride 5d (0.031 g, 0.19 mmol) and *o*-toluidine-\(d_3\) (0.025 g, 0.23 mmol) were heated to reflux in 3 mL acetic acid for 24 h. The solvent was removed under vacuum and the reaction mixture was heated in oven (120 °C) for another 12 h. Then balance 4b was obtained as white solid (0.038 g, 0.15 mmol, 78% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.17–7.35 (m, 3 H), 6.82–7.02 (m, 1 H), 6.30 (t, \(J = 1.6\) Hz, 2 H), 3.38–3.56 (m, 4 H), 1.75–1.85 (m, 1 H), 1.56–1.67 (m, 1 H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 176.85, 176.56, 135.28, 134.62, 131.18, 131.03, 129.39, 129.33, 128.23, 127.82, 126.84, 126.78, 52.74, 52.33, 46.78, 45.81, 45.47, 45.17. HRMS (EI) calculated for \(\text{C}_{16}\text{H}_{12}\text{D}_3\text{NO}_2\): 256.1290; obs: 256.1291.

**Figure 5.13:** \(^1\)H NMR spectrum of balance 16b (CDCl\(_3\), 400 MHz).
5.6.2 Folding Energies in Acetone-$d_6$

The folding energies of balances 12–16 in acetone-$d_6$ were also calculated based on the same qualification method, and the results are listed in Table S5.

Table 5.4: Comparison of folding energies of protic and deuterated balances 12–16 in acetone-$d_6$ at 25 °C.

<table>
<thead>
<tr>
<th>balance</th>
<th>$\Delta G_H$ (kcal/mol)</th>
<th>$\Delta G_D$ (kcal/mol)</th>
<th>$\Delta\Delta G$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.13</td>
<td>0.14</td>
<td>-0.01</td>
</tr>
<tr>
<td>13</td>
<td>0.24</td>
<td>0.24</td>
<td>+0.00</td>
</tr>
<tr>
<td>14</td>
<td>-0.64</td>
<td>-0.64</td>
<td>+0.00</td>
</tr>
<tr>
<td>15</td>
<td>-0.05</td>
<td>-0.06</td>
<td>+0.01</td>
</tr>
<tr>
<td>16</td>
<td>0.01</td>
<td>-0.01</td>
<td>+0.02</td>
</tr>
</tbody>
</table>
5.6.3 Van’t Hoff Plots

The van’t Hoff plots of the $\ln(\text{folded/unfolded})$ versus the reciprocal of temperature are linear. Curve-fits of these lines have slopes corresponding to $-\Delta H/R$ and y intercepts of $\Delta S/R$. The full spectra were acquired at 5°C intervals and the folded and unfolded ratio were obtained via spectral deconvolution using VNMRJ software “fitspec” command at corresponding areas on $^1$H NMR spectra.

**Table 5.5**: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectrums of balances 13a and 13b in CDCl$_3$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>balance 13a</th>
<th>balance 13b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F/UF</td>
<td>$\ln$(F/UF)</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>1.23</td>
<td>0.209</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>1.20</td>
<td>0.186</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>1.17</td>
<td>0.161</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>1.15</td>
<td>0.140</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>1.12</td>
<td>0.113</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>1.11</td>
<td>0.104</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>1.09</td>
<td>0.083</td>
</tr>
</tbody>
</table>

**Table 5.6**: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectrums of balances 13a and 13b in acetone-$d_6$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>balance 13a</th>
<th>balance 13b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F/UF</td>
<td>$\ln$(F/UF)</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>1.49</td>
<td>0.401</td>
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<td>30</td>
<td>0.003300</td>
<td>1.41</td>
<td>0.342</td>
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<tr>
<td>35</td>
<td>0.003247</td>
<td>1.41</td>
<td>0.345</td>
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<tr>
<td>40</td>
<td>0.003195</td>
<td>1.36</td>
<td>0.309</td>
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<td>0.003145</td>
<td>1.33</td>
<td>0.286</td>
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<tr>
<td>50</td>
<td>0.003096</td>
<td>1.30</td>
<td>0.260</td>
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<td>55</td>
<td>0.003049</td>
<td>1.27</td>
<td>0.236</td>
</tr>
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</table>
Figure 5.15: The van’t Hoff plots of balances 13a and 13b in CDCl$_3$ and acetone-$d_6$.

Table 5.7: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectrums of balances 14a and 14b in CDCl$_3$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>balance 14a</th>
<th>balance 14b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F/UF</td>
<td>ln(F/UF)</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.242</td>
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<tr>
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<td>0.257</td>
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</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.249</td>
<td>−1.39</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.248</td>
<td>−1.40</td>
</tr>
<tr>
<td>45</td>
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<td>0.255</td>
<td>−1.37</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>0.256</td>
<td>−1.36</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>0.257</td>
<td>−1.36</td>
</tr>
</tbody>
</table>

Table 5.8: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectrums of balances 14a and 14b in acetone-$d_6$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>balance 14a</th>
<th>balance 14b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F/UF</td>
<td>ln(F/UF)</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.333</td>
<td>−1.10</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>0.328</td>
<td>−1.11</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.330</td>
<td>−1.11</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.333</td>
<td>−1.10</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>0.333</td>
<td>−1.10</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>0.335</td>
<td>−1.09</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>0.340</td>
<td>−1.08</td>
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</table>
Figure 5.16: The van’t Hoff plots of balances 14a and 14b in CDCl$_3$ and acetone-$d_6$.

Table 5.9: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectrums of balances 15a and 15b in CDCl$_3$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>F/UF</th>
<th>ln(F/UF)</th>
<th>F/UF</th>
<th>ln(F/UF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.891</td>
<td>-0.115</td>
<td>0.882</td>
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</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>0.872</td>
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</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.868</td>
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<td>-0.177</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.848</td>
<td>-0.165</td>
<td>0.848</td>
<td>-0.165</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>0.840</td>
<td>-0.174</td>
<td>0.804</td>
<td>-0.218</td>
</tr>
<tr>
<td>50</td>
<td>0.003096</td>
<td>0.843</td>
<td>-0.171</td>
<td>0.837</td>
<td>-0.178</td>
</tr>
</tbody>
</table>

Table 5.10: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectrums of balances 15a and 15b in acetone-$d_6$.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>1/Temp (K$^{-1}$)</th>
<th>F/UF</th>
<th>ln(F/UF)</th>
<th>F/UF</th>
<th>ln(F/UF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.915</td>
<td>-0.0884</td>
<td>0.903</td>
<td>-0.102</td>
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<tr>
<td>30</td>
<td>0.003300</td>
<td>0.938</td>
<td>-0.0640</td>
<td>0.932</td>
<td>-0.0704</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.962</td>
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<td>0.948</td>
<td>-0.0535</td>
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<tr>
<td>40</td>
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<td>-0.0501</td>
<td>0.950</td>
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</tr>
<tr>
<td>55</td>
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<td>1.01</td>
<td>0.00599</td>
<td>0.993</td>
<td>-0.00732</td>
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</table>
Figure 5.17: The van’t Hoff plots of balances 15a and 15b in CDCl$_3$ and acetone-$d_6$.

Table 5.11: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectra of balances 16a and 16b in CDCl$_3$.

<table>
<thead>
<tr>
<th></th>
<th>balance 16a</th>
<th>balance 16b</th>
</tr>
</thead>
<tbody>
<tr>
<td>T ($^\circ$C)</td>
<td>1/Temp (K$^{-1}$)</td>
<td>F/UF</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
<td>0.967</td>
</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>0.951</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>0.926</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>0.930</td>
</tr>
<tr>
<td>45</td>
<td>0.003145</td>
<td>0.915</td>
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<tr>
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<td>0.878</td>
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</tbody>
</table>

Table 5.12: Folded/unfolded ratios obtained from the integrations of corresponding peaks in variable temperature $^1$H NMR spectra of balances 16a and 16b in acetone-$d_6$.

<table>
<thead>
<tr>
<th></th>
<th>balance 16a</th>
<th>balance 16b</th>
</tr>
</thead>
<tbody>
<tr>
<td>T ($^\circ$C)</td>
<td>1/Temp (K$^{-1}$)</td>
<td>F/UF</td>
</tr>
<tr>
<td>25</td>
<td>0.003356</td>
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</tr>
<tr>
<td>30</td>
<td>0.003300</td>
<td>1.14</td>
</tr>
<tr>
<td>35</td>
<td>0.003247</td>
<td>1.05</td>
</tr>
<tr>
<td>40</td>
<td>0.003195</td>
<td>1.03</td>
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<tr>
<td>45</td>
<td>0.003145</td>
<td>1.04</td>
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<tr>
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<td>0.917</td>
</tr>
<tr>
<td>55</td>
<td>0.003049</td>
<td>1.01</td>
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</table>
Figure 5.18: The van’t Hoff plots of balances 16a and 16b in CDCl₃ and acetone-d₆.

The errors for slopes and intercepts are measured by the regression add-in in excel, and the calculated \( \Delta H \) and \( T\Delta S \) values were summarized in Table 5.13 and Table 5.14:

Table 5.13: Calculated \( \Delta G \), \( \Delta H \), \( \Delta S \) at 25°C and \( -T\Delta S \) for balance 13–16 in CDCl₃ with errors via VT \(^1\)H NMR experiment.

<table>
<thead>
<tr>
<th>balance</th>
<th>Slope</th>
<th>Intercept</th>
<th>( \Delta G ) (kcal/mol)</th>
<th>( \Delta H ) (kcal/mol)</th>
<th>( \Delta S ) (kcal/mol·K)</th>
<th>( -T\Delta S@25°C ) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>413±14</td>
<td>−1.18±0.04</td>
<td>−0.123±0.054</td>
<td>−0.821±0.028</td>
<td>−0.00234±0.00009</td>
<td>0.698±0.026</td>
</tr>
<tr>
<td>13b</td>
<td>367±28</td>
<td>−1.00±0.09</td>
<td>−0.136±0.108</td>
<td>−0.730±0.056</td>
<td>−0.00199±0.00018</td>
<td>0.593±0.053</td>
</tr>
<tr>
<td>14a</td>
<td>−134±72</td>
<td>−0.950±0.231</td>
<td>0.829±0.280</td>
<td>0.266±0.144</td>
<td>−0.00189±0.00046</td>
<td>0.562±0.137</td>
</tr>
<tr>
<td>14b</td>
<td>−159±48</td>
<td>−0.824±0.154</td>
<td>0.803±0.186</td>
<td>0.316±0.095</td>
<td>−0.00164±0.00031</td>
<td>0.488±0.091</td>
</tr>
<tr>
<td>15a</td>
<td>228±35</td>
<td>−0.885±0.113</td>
<td>0.071±0.137</td>
<td>−0.453±0.070</td>
<td>−0.00176±0.00022</td>
<td>0.524±0.067</td>
</tr>
<tr>
<td>15b</td>
<td>247±98</td>
<td>−0.965±0.317</td>
<td>0.081±0.383</td>
<td>−0.490±0.195</td>
<td>−0.00192±0.00063</td>
<td>0.572±0.188</td>
</tr>
<tr>
<td>16a</td>
<td>310±32</td>
<td>−1.07±0.102</td>
<td>0.020±0.124</td>
<td>−0.615±0.064</td>
<td>−0.00213±0.00020</td>
<td>0.635±0.061</td>
</tr>
<tr>
<td>16b</td>
<td>147±8</td>
<td>−0.597±0.025</td>
<td>0.061±0.030</td>
<td>−0.292±0.016</td>
<td>−0.00119±0.00005</td>
<td>0.353±0.015</td>
</tr>
</tbody>
</table>
Table 5.14: Calculated $\Delta G$, $\Delta H$, $\Delta S$ (25°C) and $T\Delta S$ for balance 13–16 in acetone-$d_6$ with errors via VT $^1$H NMR experiment.

<table>
<thead>
<tr>
<th>balance</th>
<th>Slope</th>
<th>Intercept</th>
<th>$\Delta G$ (kcal/mol)</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$\Delta S$ (kcal/mol·K)</th>
<th>$-T\Delta S$@25°C (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>501 ± 41</td>
<td>-1.29 ± 0.13</td>
<td>-0.231 ± 0.160</td>
<td>-0.995 ± 0.082</td>
<td>-2.56 ± 0.26</td>
<td>0.764 ± 0.078</td>
</tr>
<tr>
<td>13b</td>
<td>715 ± 95</td>
<td>-1.96 ± 0.31</td>
<td>-0.260 ± 0.370</td>
<td>-1.42 ± 0.19</td>
<td>-3.89 ± 0.61</td>
<td>1.16 ± 0.18</td>
</tr>
<tr>
<td>14a</td>
<td>-121 ± 18</td>
<td>-0.714 ± 0.057</td>
<td>0.664 ± 0.070</td>
<td>0.241 ± 0.036</td>
<td>-1.42 ± 0.11</td>
<td>0.423 ± 0.034</td>
</tr>
<tr>
<td>14b</td>
<td>-51.1 ± 17.4</td>
<td>-0.925 ± 0.055</td>
<td>0.649 ± 0.067</td>
<td>0.102 ± 0.034</td>
<td>-1.84 ± 0.11</td>
<td>0.548 ± 0.033</td>
</tr>
<tr>
<td>15a</td>
<td>292 ± 66</td>
<td>-0.897 ± 0.215</td>
<td>-0.049 ± 0.259</td>
<td>-0.580 ± 0.131</td>
<td>-1.78 ± 0.43</td>
<td>0.531 ± 0.128</td>
</tr>
<tr>
<td>15b</td>
<td>274 ± 26</td>
<td>-0.827 ± 0.085</td>
<td>-0.055 ± 0.103</td>
<td>-0.545 ± 0.053</td>
<td>-1.64 ± 0.17</td>
<td>0.490 ± 0.050</td>
</tr>
<tr>
<td>16a</td>
<td>178 ± 43</td>
<td>-0.529 ± 0.138</td>
<td>-0.040 ± 0.167</td>
<td>-0.353 ± 0.085</td>
<td>-1.05 ± 0.27</td>
<td>0.313 ± 0.081</td>
</tr>
<tr>
<td>16b</td>
<td>27.1 ± 43.3</td>
<td>-0.016 ± 0.139</td>
<td>-0.045 ± 0.168</td>
<td>-0.054 ± 0.086</td>
<td>-0.05 ± 0.28</td>
<td>0.0823 ±</td>
</tr>
</tbody>
</table>
CHAPTER 6

MEASURING AROMATIC CH–Π INTERACTIONS USING MOLECULAR BALANCES

In addition to the previously described studies about face-to-face π–π stacking interactions and aliphatic CH–π interactions, the molecular balances can also be applied to the measurement of other non-covalent interactions via simple modification. In this chapter, the application of our phencyclone-based balance system on the study of aromatic CH–π interactions (edge-to-face arene–arene interactions) will be presented. Similar to aliphatic CH–π interactions, the edge-to-face arene–arene interactions are weakly directional and are results of several different forces of similar magnitudes. Therefore, the prediction of the strength, geometries and solvent dependence of edge-to-face arene–arene interactions is quite complex.

Figure 6.1: Equilibrium between the *unfolded* and *folded* conformers of molecular balances used for measuring edge-to-face arene–arene interaction between naphthalene and aromatic rings.

Several molecular models have been developed to study the edge-to-face arene–arene interactions.\(^{62,126}\) By replacing the phenyl rotor with 1-naphthyl or 5-quinolyl rings, the balances can adopt intramolecular edge-to-face arene–arene interactions in their...
folded conformation with well-defined geometry (Figure 6.1). Our balance system compares favorably with the other balance systems as it showed good solubility in a wider range of solvents and better control over the interacting geometry. It also enables the comparison between the stability of edge-to-face arene–arene interactions and the other non-covalent interactions that were studied using the same balance system.

6.1 BALANCE DESIGNS

![Figure 6.2: Structures of balances designed for measuring edge-to-face arene–arene interactions.](image)

The structures of the edge-to-face balances 17–19 (Figure 6.2) were based on the same bicyclic $N$–arylimide framework used in previous chapters. The design of balance 17a was previously shown to adopt the edge-to-face arene–arene geometry in its folded conformation, but this system was primarily used as a host molecule for small aromatic guests. The new balance 17b with a 5–quinolyl rotor was made for comparison containing a different electrostatic distribution and geometry of the edge ring. Substituted balance 17c with $-\text{OH}$ as $Y$ group and balance 17d with $-\text{CH}_3$ at the $N$–position on the quinolyl ring were synthesized to study the substitution effect. Balances 18 and 19 with smaller shelves were made as control balances.

6.2 SOLID-STATE STRUCTURES

In order to confirm the presence and identify the exact geometries of the edge-to-
face arene–arene interactions in these molecular balances, the X-ray structures of balance 17a and 17b were analyzed. The crystal structure of balance 17a was previously reported, and the crystal structure of balance 17b was obtained through single-crystal X-ray diffraction. Both structures crystallized as the *folded* conformation, which clearly displayed the edge-to-face interaction between the edge of the arene-rotor and the shelf (Figure 6.3).

![Figure 6.3: X-ray structures for folded conformers of balances 17a and 17b suggesting edge-to-face interactions between the edge of rotor rings and phenanthrene-shelf. Parts of the phenyl rings at bridge position were hidden for better viewing clarity.](image)

In the solid-state structure of balance 17a, the 1-naphthyl group was fixed perpendicular to the phenanthrene-shelf with the C–8 proton pointing directly into the face of the center phenanthrene-ring. The hydrogen-to-plane distances for the two edge-protons (on C–8 and C–7) were 2.616 Å and 2.797 Å respectively. Both distances were within the sum of van der Waals' radii of H and C atoms (~ 2.9 Å), which suggested the formation of an attractive non-covalent interaction.

Similar edge-to-face geometry was observed in balance 17b with the 5-quinolyl rotor, which showed the hydrogen-to-plain distances of 2.765 Å and 3.300 Å. The 8–proton of the quinoline ring was further away from the phenanthrene shelf than in 17a. The bond-length of the C–N bond is shorter than the C–C bond, making the heterocyclic
quinoline ring slightly smaller than the naphthyl ring. The distance of 3.300 Å exceeded the typical range that can form a non-covalent bond, so it is possible that the second interaction in balance 17b does not exist or only shows weak strength.

6.3 EDGE-TO-FACE ARENE–ARENE INTERACTIONS IN SOLUTION

The interactions were then quantified by the same methods described in Chapter 2 based on the integration of the succinimide peaks in $^1$H NMR spectrum. Same as other molecular balances in our study, separate peaks for the folded and unfolded conformers were observed in the $^1$H NMR spectrums. Assignment of the folded and unfolded peaks was based on the results from previous studies about balance 17a.$^{94}$

The larger aromatic rotors in these balances enhance the possibility of intermolecular aggregation. In order to rule out aggregation effects on this system, the concentration dependence of the folded/unfolded ratios was investigated. Over a concentration range of 1.6 to 15 mM, only a change of 0.02 kcal/mol in the folding energy was observed, which is within the error (0.03 kcal/mol) for this measurement. This indicates that the aggregation effects were either minor or no existent in the edge-to-face arene–arene balances.

6.3.1 Measurement of Rotational Barrier

The two conformers of these balances showed different $R_f$ values on TLC plates, and exchanged overnight at room temperature or after a matter of minutes at elevated temperature. In one example, one of the conformers of 19a was isolated via a quick column at room temperature. By tracking the change in the folded/unfolded ratio via $^1$H NMR spectra over the course of one hour, the rotational barrier was calculated to be 22.8 kcal/mol (Figure 6.4). This equated to a half-life of 56 min at room temperature. This barrier is higher than that of previous balances such as balance 1a with an OMe arm (20.5
kcal/mol), because the larger size and rigidity of the fused naphthalene ring compared with individual ortho-substituents on a phenyl ring. All the balances in this study were, therefore, allowed to equilibrate for at least 10 half-lives in solution before measurement of the folded/unfolded ratios.

![Graph](image)

**Figure 6.4:** The value of \(\ln[(R_{\text{folded/unfolded}} - R_{\text{eq}})/(R_{\text{folded/unfolded}} + 1)]\) plotting versus time (at 21°C) indicating the rate for exchange between folded and unfolded conformers of balance 19a.

6.3.2 Comparison of Balances with Naphthalene and Quinoline Rotors

The folded/unfolded ratios for balances 17–19 with naphthalene and quinoline rotors are listed in Table 6.1. With phenanthrene shelf, both rotors in balances 17a and 17b preferred the folded conformation in solution, which is consistent with an attractive intramolecular edge-to-face interaction between the rotor edge and the phenanthrene shelf. Balances 18a and 18b showed the lowest folded/unfolded ratios among balances, because only one edge proton–π was able to form an interaction. For control balances 19a and 19b without arene shelves, no interaction is possible, so the folded/unfolded ratios were close to 1.
Table 6.1: The *folded/unfolded* ratios of balance 17a–17b, 18a–18b and 19a–19b in CDCl₃ at 25 °C.

<table>
<thead>
<tr>
<th>rotor/shelf</th>
<th>17 (shelf = phenanthrene)</th>
<th>18 (shelf = benzene)</th>
<th>19 (shelf = norborne)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (rotor = naphthyl)</td>
<td>1.81</td>
<td>0.47</td>
<td>0.81</td>
</tr>
<tr>
<td>b (rotor = quinolyl)</td>
<td>2.98</td>
<td>0.61</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Balance 17b with quinolyl rotor showed a higher *folded/unfolded* ratio than balance 17a with naphthyl rotor, which is in conflict with the observation that the quinolyl rotor forms fewer good interactions in crystal structures. One possible explanation is that the interaction is primarily driven by electrostatic force. The greater electronegativity of the nitrogen atom in the quinolyl ring of 17b makes the edge of the rotor to have greater positive charge. Another possibility is that in balance 17a, the proton at 8–position is too close to the arene shelf, so this interaction contains a greater repulsive component which destabilized its *folded* conformer. The lower *folded/unfolded* ratios of balances 18a and 18b with the benzene shelves agreed with the second hypothesis, because the *folded/unfolded* ratios of the two balances in solution were less than one. A third possibility is that the differences are due to a solvent effect. This possibility will be addressed in the following section 6.3.4.

6.3.3 Substituent Effect

To study the electrostatic contributions to the edge-to-face arene–arene interactions, we varied the electrostatic polarization of the aromatic rotors by introducing substituents on the quinolyl rotor of balances 17b. Balance 17c with an 8-hydroxyquinolyl rotor and balance 17d with N–methylquinolyl rotor were prepared as balances with electron-rich and electron-poor substituents respectively.
The folding energies (−ΔG, in kcal/mol) of balances 17b–17d in acetonitrile–d₃ at 25 °C, shown with errors of 0.03 kcal/mol.

Figure 6.5: The folding energies (−ΔG, in kcal/mol) of balances 17b–17d in acetonitrile–d₃ at 25 °C, shown with errors of 0.03 kcal/mol.

The folding energies (−ΔG) in acetonitrile–d₃ of balances 17b–17d with different substituted quinolyl rotors were compared in Figure 6.5. Balance 17d only showed good solubility in acetonitrile, so acetonitrile–d₃ was the only NMR solvent that dissolves all balances to allow this comparison. Balances 17b with quinolyl rotor and 17c with hydroxyquinolyl rotor showed almost identical folding energies, while balance 17d with methylated quinolyl rotor showed slightly lower folding energy. The observation did not match with our expectation that the electron-rich hydroxyl-substituted balance 17c should be less folded and the electron-poor N–methylquinoline balance 17d should be more folded compared than balance 17b. This discrepancy may be related to the solvent effect as discussed in the next section.

6.3.4 Solvent Effects

The solvophobic effect is one of the important factors that drive the folding of molecular balances, and may be able to explain the unexpected observations. To study the solvent dependent of the edge-to-face arene–arene interaction with balances 17a–17c,
the folding energies (−ΔG) of the two balances were calculated from the folded/unfolded ratios in various solvents and plotted versus the Eₜ(30) values of the solvents (Figure 6.6). The folding trend of balance 17d was not measured, because it only showed good solubility in acetonitrile.

![Figure 6.6](image)

**Figure 6.6:** Measured −ΔG values for balances 17a–17c in a variety of solvents at 25°C plotted versus Eₜ(30) values of each solvent. Solvents from left to right are deuterated benzene, chloroform, acetone, DMSO, and acetonitrile.

The folded conformers were favored for 17a–17c in all five solvents tested. For solvents with relatively lower polarity (benzene, CDCl₃, acetone), the folding energies of balance 17b with quinolyl-rotor were stronger than that of balance 17a with naphthyl-rotor. As the polarity of the solvent increased, balance 17a became more folded while balance 17c become less folded, and the folded/unfolded ratio of balance 17b remained the same. Also, in solvents with relatively high polarity (DMSO and acetonitrile), the folding energies of balances 17a–17c were almost identical.

One possibility for the different solvent trends of balances 17a–17c is that the dipoles of folded and unfolded conformers of each balance are different. Based on the calculation, difference in dipole between folded in unfolded increases showed the
following order: hydroxyquinolyl > quinolyl > naphthyl. The theory was tested experimentally using control balances 19a–c without aromatic shelves (Figure 6.7). However, the observation did not match with our expectation. The folding trends of balances 19a–c in different solvents and did not show expected distinction as that of balances 17a–c. Thus, the dipole difference was not the main reason to cause the folding trends. The other theories that can fully explain this observation are still under investigation.

![Figure 6.7: Measured –ΔG values for balances 19a–19c in a variety of solvents at 25°C plotted versus ET(30) values of each solvent. Solvents from left to right are deuterated benzene, chloroform, acetone, and acetonitrile.](image)

The solvent effects on the folding of the balances were also studied in mixed solvents. The folding energies of 17a–17c were measured in the mixtures of methanol–d₄ and CDCl₃ with different ratios (Figure 6.8). Although the polarity of mixed solvents changed when the fraction of methanol increased, the folding energies for each balance stayed relatively consistent and were similar to those in pure CDCl₃. It is possible that because of the poor solubility of balances 17a–17c in methanol, so changes on the concentration of methanol only have limited effect on the folding preference. Another
possibility is that methanol is an H-bond donor that could interact with the basic nitrogen on quinolyl rotor.

\[ -\Delta G (\text{kcal/mol}) \]

\[ \text{ET}(30) \]

\[ 37 \]

\[ 42 \]

\[ 47 \]

\[ 52 \]

![Graph showing measured $-\Delta G$ values for balances 17a–17c in a series of mixtures of CDCl3 and methanol–d4 at 25°C plotted versus ET(30) values.](image)

Figure 6.8: Measured $-\Delta G$ values for balances 17a–17c in a series of mixtures of CDCl3 and methanol–d4 at 25°C plotted versus ET(30) values. The fraction of methanol in each mixture was 0, 20%, 50%, 57% and 66% from left to right, and the ET(30) values were estimated based on literature.\textsuperscript{127,128}

6.3.5 Balances with Quinoline and \textit{iso}-Quinoline as Rotors

![Structures of balances 17b, 20 and 21 with quinoline and \textit{iso}-quinoline arms.](image)

Figure 6.9: Structures of balances 17b, 20 and 21 with quinoline and \textit{iso}-quinoline arms.

Balances 20 and 21 with N atom at different position of the rotor ring were also made for comparison (Figure 6.9). The folding energies for balances 17b, 20, and 21 were compared in different solvents (Figure 6.10). Balance 17b and 20 showed almost identical folding energies in each solvent being tested, and the energy values did not change according to the increasing polarity. This indicates that the electrostatic property of the two protons interacting with the aromatic shelf was the same when the N atom on
the rotor ring is at 5– or 6– position. Their interactions with the solvent molecule did not change either. Balance 21 showed much lower folding ratios because of the existence of lone-pair π interaction in its folded conformer. It can also act as a good control because of its lack of edge-to-face interaction.

Figure 6.10: Folding energies of balances 17b, 20 and 21 in different solvents at 25°C.

6.4 CONCLUSION

In summary, edge-to-face arene–arene interactions were verified by a series of control experiments. The geometries of edge-to-face interaction in the balances were characterized in the solid-state structure. Solvents and substitutions were found to affect the ratio of folded and unfolded conformers, but the relative magnitudes of forces that cause the observed trends are still unclear. Further studies will be conducted to give a better understanding on the questions remains unanswered.

6.5 SYNTHESIS

Edge-to-face balances 17a–b and balances 18–21 were prepared from 1-naphthyl amine, 5-aminoquinoline, 5-aminoisoquinoline or 8-aminoquinoline in one step with corresponding anhydrides 5. The reaction that made balances 17c required the
participation of base. Balance 17d was made from 17b via methylation.

**Figure 6.11:** Overview of synthesis of balances 17a–b, 18, and 19 via condensation reactions.

**Preparation of balance 17a:**

Anhydride 5a (0.17 g, 0.35 mmol) and 1-naphthylamine (0.10 g, 0.70 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 17a was obtained as white solid (0.19 g, 0.31 mmol, 90% yield). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.83 (d, $J = 8.5$ Hz, 2 H major), 8.76 (d, $J = 8.5$ Hz, 2 H minor), 8.41 (d, $J = 7.8$ Hz, 2 H major), 8.38 (d, $J = 8.3$ Hz, 2 H minor), 7.1–7.8 (m, 19 H major, 16 H minor), 7.02 (t, $J = 7.6$ Hz, 2 H minor), 6.74 (t, $J = 7.9$ Hz, 1 H major), 6.04 (t, $J = 7.7$ Hz, 2 H minor), 4.79 (s, 2 H minor), 4.75 (s, 2 H major), 4.65 (dd, $J = 8.6$ Hz, $J = 0.8$ Hz, 1 H major), 4.63 (dd, $J = 7.4$ Hz, $J = 1.1$ Hz, 1 H minor). Characterization data matched with the literature.$^{94}$

**Preparation of balance 17b:**

Anhydride 5a (0.17 g, 0.35 mmol) and 5-aminoquinoline (97%, 0.10 g, 0.69 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 17b was obtained as white solid (0.20 g, 0.33 mmol, 94% yield). $^1$H NMR (300 MHz, CDCl$_3$) δ 8.82 (d, $J = 8.44$ Hz, 2 H major, 1 H minor), 8.76 (d, $J = 8.44$ Hz, 2 H minor, 1 H major).
Hz, 2 H minor), 8.47 (dd, J = 4.01 Hz, J = 1.19 Hz, 1 H major), 8.38 (d, J = 7.71 Hz, 2 H major), 8.35 (d, J = 7.71 Hz, 2 H minor), 7.96 (d, J = 8.62 Hz, 1 H major), 7.90–7.12 (m, 16 H major, 17 H minor), 6.98 (t, J = 8.19 Hz, 1 H minor), 6.05–5.98 (m, 1 H major), 4.94 (d, J = 8.29 Hz, 1 H major), 4.79 (s, 2 H minor), 4.78 (s, 2 H major), 4.68 (d, J = 7.46 Hz, 1 H minor).

Preparation of balance 17c:

Anhydride 5a (0.049 g, 0.102 mmol), potassium carbonate (0.028g, 0.204mmol) and 5-amino-8-hydroxyquinoline dihydrochloride (95%, 0.050 g, 0.204 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 17c was obtained as purple solid (0.078 g, 0.129 mmol, > 90% yield). $^1$H NMR (300 MHz, CDCl$_3$) δ 8.81 (d, J = 8.37 Hz, 2 H major), 8.75 (d, J = 8.75 Hz, 2 H minor), 8.69 (dd, J = 4.06 Hz, J = 1.16 Hz, 1 H minor), 8.46–8.27 (m, 3 H major, 2 H minor), 8.15 (brs, 1 H major), 7.91–6.96 (m, 16 H major, 17 H minor), 6.41 (d, J = 8.14 Hz, 1 H minor), 6.09 (dd, J = 8.39 Hz, J = 3.98 Hz, 1 H major), 4.88 (dd, J = 8.36 Hz, J = 1.02 Hz, 1 H major), 4.75 (s, 2 H minor), 4.74 (s, 2 H major), 4.58 (d, J = 8.19 Hz, 1 H minor).

Preparation of balance 17d:

To a solution of balance 17b (0.096 g, 0.158 mmol) in acetonitrile (10 mL), iodomethane (0.02 mL, 0.316 mmol) was added drop wise while stirring under nitrogen. After heated to reflux for 3 days, the solvent was removed in vacuum, and balance 17d was obtained as yellow solid (0.089 g, 0.150 mmol, 95% yield). $^1$H NMR (300 MHz, CDCl$_3$) δ 9.09 (d, J = 5.72 Hz, 1 H minor), 9.00 (d, J = 8.57 Hz, 2 H major), 8.90 (d, J = 8.57 Hz, 2 H minor), 8.70 (d, J = 5.72, 1 H major), 8.45–7.05 (m, 19 H major, 20 H minor), 6.57 (dd, J = 8.62 Hz, J = 5.80 Hz, 1 H major), 5.47 (d, J = 8.77 Hz, 1 H major),

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5.12 (s, 2 H minor), 5.07 (s, 2 H major), 4.88 (d, J = 7.48 Hz, 1 H minor), 4.44 (s, 3 H minor), 4.35 (s, 3 H major).

**Preparation of balance 18a:**

Anhydride 5c (0.100 g, 0.26 mmol) and 1-naphthylamine (0.056 g, 0.40 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 18a was obtained as white solid (0.113 g, 0.223 mmol, 85% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.18–6.99 (m, 20 H major, 20 H minor), 5.79 (d, J = 7.14 Hz, 1 H major), 5.41 (d, J = 8.51 Hz, 1 H minor), 4.44 (s, 2 H major), 4.43 (s, 2 H minor).

**Preparation of balance 18b:**

Anhydride 5c (0.085 g, 0.23 mmol) and 5-aminoquinoline (0.050 g, 0.35 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 18b was obtained as white solid (0.098 g, 0.20 mmol, 86% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.96–8.78 (m, 1 H major, 1 H minor), 8.17–7.01 (m, 18 H major, 18 H minor), 5.88 (d, J = 7.36 Hz, 1 H major), 5.74 (d, J = 8.34 Hz, 1 H minor), 4.45 (s, 2 H major), 4.44 (s, 2 H minor).

**Preparation of balance 19a:**

Anhydride 5d (0.100 g, 0.61 mmol) and 1-naphthylamine (0.131 g, 0.91 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 19a was obtained as white solid (0.158g, 0.546 mmol, 90% yield). ¹H NMR (300 MHz, CDCl₃) δ 7.96–7.17 (m, 6 H major, 7 H minor), 7.12 (d, J = 7.28 Hz, 1 H major), 6.55 (m, 2 H minor), 6.37 (m, 2 H major), 3.64–3.49 (m, 4 H major, 4 H minor), 1.97–1.60 (m, 2 H major, 2 H minor).
Preparation of balance 19b:

Anhydride 5d (0.095 g, 0.58 mmol) and 5-aminoquinoline (0.100 g, 0.69 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 19b was obtained as white solid (0.145 g, 0.50 mmol, 86% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.97–8.88 (m, 1 H major, 1 H minor), 8.18 (d, $J$ = 8.59 Hz, 1 H major, 1 H minor), 7.96–7.15 (m, 4 H major, 4 H minor), 6.51 (m, 2 H minor), 6.35 (m, 2 H major), 3.63–3.46 (m, 4 H major, 4 H minor), 1.93–1.59 (m, 2 H major, 2 H minor).

Preparation of balance 19c:

Anhydride 5d (0.033 g, 0.20 mmol), potassium carbonate (0.056 g, 0.40 mmol) and 5-amino-8-hydroxyquinoline dihydrochloride (95%, 0.050 g, 0.20 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 19c was obtained as brown solid (0.066 g, 0.20 mmol, >90% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.85–8.74 (m, 1 H major, 1 H minor), 8.44 (brs, 1 H major), 7.85 (t, $J$ = 10.05 Hz, 1 H major, 1 H minor), 7.50–7.40 (m, 1 H major, 2 H minor), 7.29–7.07 (m, 2 H major, 2 H minor), 6.50 (s, 2 H minor), 6.35 (s, 2 H major), 3.62–3.50 (m, 4 H major, 4 H minor), 1.93–1.60 (m, 2 H major, 2 H minor).

Preparation of balance 20:

Anhydride 5a (0.050 g, 0.104 mmol) and 5-aminoisoquinoline (0.018 g, 0.125 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 20 was obtained as white solid (0.059 g, 0.097 mmol, 93% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.13 (s, 1 H minor), 8.95 (s, 1 H major), 8.83 (d, $J$ = 8.59 Hz, 2 H major), 8.75 (d, $J$ = 8.59 Hz, 2 H minor), 8.50 (d, $J$ = 6.18 Hz, 1 H minor), 8.44–7.10 (m, 20 H major, 18 H minor), 6.89 (t, $J$ = 7.83 Hz, 1 H minor), 4.84 (d, 1 H minor),
4.81 (s, 2 H minor), 4.78 (s, 2 H major), 4.42 (d, \( J = 5.98 \) Hz, 1 H major).

**Preparation of balance 21:**

Anhydride 5a (0.050 g, 0.104 mmol) and 8-aminoquinoline (0.018 g, 0.125 mmol) were heated to reflux for 24 h in 5 mL acetic acid. After work up steps and purification, balance 21 was obtained as white solid (0.058 g, 0.095 mmol, 91% yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.89–7.06 (m, 22 H major, 24 H minor), 6.89 (t, \( J = 7.77 \) Hz, 1 H major), 5.05 (dd, \( J = 7.12 \) Hz, \( J = 0.65 \) Hz, 1 H major), 4.84 (s, 2 H major), 4.75 (s, 2 H minor).
CHAPTER 7

OTHER NOTABLE WORKS

By varying the arm group on the structures of our molecular balances and comparing their folding energies, a number of studies about different types of non-covalent interactions were able to be conducted. In this chapter, some additional results that do not fit into any of the previous chapters will be presented.

7.1 DOUBLE-MUTANT CIRCLES FOR MEASURING NON-COVALENT INTERACTIONS

Molecular balances have been proved to be effective tools for measuring weak non-covalent interactions. However, because of the existence of weak secondary effects in the folded structures of balances, it is hard to isolate the actual strengths of each interaction from the total folding energies. In previous chapters, balances were compared with their control balances with smaller arene shelves to eliminate their secondary interactions. However, the single-mutation comparisons do not work perfectly, because some secondary effects still exist after the subtraction, especially when there is a linker between the arm and the phenyl rotor. In addition, the three frameworks used in this study (shelf = phenanthrene, benzene and norborene) have different bridge atom (C=O, O or CH₂) at the backside and may adopt slightly different angles between the rotor ring and the shelf planes.

Double-mutant cycles provide a way to isolate individual weak interactions from the multiple interactions (Figure 7.1). This method was originally proposed by Fersht et al in 1984,¹²⁹ and their application on quantification of non-covalent interactions has been
reviewed. In this section, the double-mutant cycles designed with our balance system for a more precise measurement on the non-covalent interactions will be introduced.

Figure 7.1: General schematic representing a supramolecular double-mutant cycle for measuring the intramolecular interaction between X and Y.

7.1.1 Structures of Molecular Balances

Balances 22 and 23 were synthesized to make double-mutant cycles that can isolate primary and secondary interaction of X and Y with the central and outer rings on the shelves (Figure 7.2). Balances 23b, 23c and 23e have been previously studied as balances 9a, 9b in Chapter 4 and balance 2a in Chapter 3. Balance 22 is similar to the previous balances with phenanthrene shelves, but their backside bridge was changed from a C=O into an O to match the O–bridge in balance 23.

Figure 7.2: Molecular balances 22 and 23 designed for double-mutant cycles analyzing intramolecular primary interactions (blue arrows) and secondary interactions (red dash).
A range of different intramolecular interactions were formed by these molecular balances. In balances 22 with phenanthrene shelf (Y ≠ H), both the linker X and end group Y are able to form primary interactions with the aromatic surfaces below. In addition, the secondary interactions between Y and the central ring and X with the outer rings must be accounted for. In the corresponding balances 23 with the same X and Y combination, the interacting environment remains the same except for the absence of interaction between Y and the outer ring and the secondary interaction of X with the outer ring. This made balances 23 good reference balances in this mutation to isolate Y-to-outer ring interaction and X-to-central ring interaction. In cooperation with balances 22c and 23c that only forms interactions with X group, each of two primary and two secondary interactions were able to be isolated using the double mutant cycles.

7.1.2 Folding Energies of Balances 22 and 23

Characterization of balances 22 and 23 in solution followed the same method as previous balances. The folded/unfolded ratios and folding energies of these balances were measured in CDCl3 at rt., and are listed in Table 7.1.

**Table 7.1:** Folded/unfolded ratios and folding energies of balances 22–23 measured in CDCl3 at 25 °C.

<table>
<thead>
<tr>
<th>balances</th>
<th>shelf</th>
<th>X</th>
<th>Y</th>
<th>F/UF</th>
<th>ΔG (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22a</td>
<td>phenanthrene</td>
<td>CH₂</td>
<td>Ph</td>
<td>0.41</td>
<td>0.53</td>
</tr>
<tr>
<td>22b</td>
<td>phenanthrene</td>
<td>CH₂</td>
<td>CH₃</td>
<td>0.58</td>
<td>0.32</td>
</tr>
<tr>
<td>22c</td>
<td>phenanthrene</td>
<td>CH₂</td>
<td>H</td>
<td>0.53</td>
<td>0.38</td>
</tr>
<tr>
<td>22d</td>
<td>phenanthrene</td>
<td>O</td>
<td>Ph</td>
<td>0.15</td>
<td>1.12</td>
</tr>
<tr>
<td>22e</td>
<td>phenanthrene</td>
<td>O</td>
<td>CH₃</td>
<td>0.18</td>
<td>1.01</td>
</tr>
<tr>
<td>23a</td>
<td>benzene</td>
<td>CH₂</td>
<td>Ph</td>
<td>0.16</td>
<td>1.10</td>
</tr>
<tr>
<td>23b</td>
<td>benzene</td>
<td>CH₂</td>
<td>CH₃</td>
<td>0.24</td>
<td>0.84</td>
</tr>
<tr>
<td>23c</td>
<td>benzene</td>
<td>CH₂</td>
<td>H</td>
<td>0.24</td>
<td>0.84</td>
</tr>
<tr>
<td>23d</td>
<td>benzene</td>
<td>O</td>
<td>Ph</td>
<td>0.10</td>
<td>1.39</td>
</tr>
<tr>
<td>23e</td>
<td>benzene</td>
<td>O</td>
<td>CH₃</td>
<td>0.09</td>
<td>1.40</td>
</tr>
</tbody>
</table>
7.1.3 General Design of Double-Mutant Cycles

The general double-mutant cycles designed for this study are shown in Figure 7.3. Balances A and B makes a single mutation. A parallel mutation between control balances C and D can cancel out the secondary effect between Y group and the central ring within the mutation between A and B. Similarly, the comparison between mutations from A to C and B to D cancels out the secondary interaction between X and the outer ring. The actual interacting energy between Y and the outer ring (\(\Delta\Delta G\)) can be calculated with equation:

\[
\Delta\Delta G = (\Delta G_A - \Delta G_B) - (\Delta G_C - \Delta G_D) = \Delta G_A - \Delta G_B - \Delta G_C + \Delta G_D.
\]

**Figure 7.3:** General design of the double-mutant cycle based on our molecular balances for measuring non-covalent interaction between Y and the outer ring on shelf.

Two double-mutant cycles were formed for the measurement of face-to-face \(\pi-\pi\) interaction (balances 22a, 22c, 23a, 23e) and CH–\(\pi\) interaction (balances 22b, 22c, 23b, 23c), respectively. Balances 22d, 22e, 23d, 23e were not able to form complete double-mutant cycles, because corresponding balances with \(X = O\) and \(Y = H\) showed very low rotational barriers and their folded and unfolded conformers did not show distinct set of peaks in the \(^1\)H NMR spectra. However, balances 22d, 22e, 23d, 23e can still been used
for the comparison between \(\pi-\pi\) stacking and CH–\(\pi\) interactions, because they shared the same \(X (X = O)\) atom, and the difference on folding energies only comes from the difference between the two types of interactions.

### 7.1.4 Measuring \(\pi-\pi\) Stacking Interactions with Double-Mutant Cycle

The double-mutant cycle formed by balances \(22a, 22c, 23a, 23c\) were used to calculate the face-to-face \(\pi-\pi\) interaction and the secondary effects within \(22a\) (Figure 7.4). Based on the equation \((\Delta G_{22a} - \Delta G_{22c} - \Delta G_{23a} + \Delta G_{23c})\), the \(\pi-\pi\) interaction between the phenyl arm and the outer ring was calculated to be \(-0.11\) kcal/mol, which is attractive.

![Figure 7.4: Double-mutant cycle formed by balances 22a, 22c, 23a, 23c for measuring \(\pi-\pi\) stacking interaction.](image)

The secondary interaction between the phenyl arm and the central ring was calculated to be \(0.26\) kcal/mol \((\Delta G_{23a} - \Delta G_{23c})\). This repulsive effect may be caused by the increased steric from the phenyl ring or the conformational entropy change of CH\(_2\) linker because of the extra substitution. Another secondary interaction formed between
CH$_2$ and the outer ring was calculated to be $-0.46$ kcal/mol ($\Delta G_{23a} - \Delta G_{23c}$). This force is stabilizing, and it is possibly because larger arene shelf leads to stronger dispersion and more chance for the CH$_3$ form interaction.

7.1.5 Double-Mutant Cycle for Measuring CH–π Interactions

The double-mutant cycle formed by balances 22b, 22c, 23b, 23c were used to calculate the CH–π interactions and the secondary effects within 22b (Figure 7.5). The interaction between CH$_3$ on ethyl and the side ring was calculated to be $-0.06$ kcal/mol ($\Delta G_{22b} - \Delta G_{22c} - \Delta G_{23b} + \Delta G_{23c}$). The interaction appeared to be very weak. The possibly reasons is that the CH$_3$ is located above the edge of arene surface, and may only form a minor interaction.

![Figure 7.5: The double-mutant cycle formed by balances 22b, 22c, 23b, 23c for measuring π–π stacking interaction.](image)

Balances 23b and 23c showed the same $\Delta G$ values, suggested that the second CH$_3$ on ethyl did not interact with the central ring. Compared with 23a with phenyl arm, the CH$_3$ intend to cause smaller steric and less rotational restriction for the CH$_2$ linker.
7.1.6 Double-Mutant Cycle for Comparing CH–π interactions to O–π and π–π Stacking Interactions

Other than isolating the primary non-covalent interaction from complicate environment, the double-mutant cycles can also be applied for the comparison between different interactions. This provides an indirect way to study some of the interactions that cannot be measured directly (e.g. O–π interaction). The difference between CH–π and O–π interactions at linker position was measured with two double-mutant cycles: (a) balances 22a, 22d, 23a and 23d, and (b) the balances 22b, 22e, 23b and 23e. The difference was calculated to be −0.30 kcal/mol and −0.13 kcal/mol, respectively. The two numbers are close enough considering the errors, and proved that CH–π interaction was more stabilizing than O–π interaction.

The difference between the π–π and CH–π interactions was also experimentally compared with two double-mutant cycles: (a) balances 22a, 22b, 23a and 23b, and (b) balances 22d, 22e, 23d and 23e. The difference was calculated to be −0.05 kcal/mol and 0.12 kcal/mol. This indicates that the two interactions showed very similar strength and the different folding energies of 22a and 22b primarily comes from the secondary interaction in balance, which was shown as the difference between balances 23a and 23b with benzene shelf.

7.1.7 Conclusion

In conclusion, a series of double-mutant cycles based on molecular balances were designed and were proved to be effective on isolating primary and secondary non-covalent interactions formed within the molecular balances. The strengths of weak non-covalent interactions, including face-to-face π–π interaction and CH–π interaction, were measured with high accuracy. This method also provides a more reliable way to measure
certain non-covalent interactions that could not be measured directly.

7.2 SOLVENT EFFECTS ON BALANCES WITH DIFFERENT LINKERS

In Chapter 3 and 4, the solvent dependent of balances 1 and 7 were discussed. Balance 1h is from our previous study for measuring π–π stacking interactions. For each series of balances, the folding energies showed similar trends when measured in different solvents. It was true for balances with different interactions and even for control balances that could not form an interaction. However, the folding energies of the balances 1 and 7 showed different solvent trends. Thus, we hypothesized that the solvent trends might be primarily due to the linker atom between arm and the phenyl rotor.

The importance of linkers on determining the solvent effects was tested by comparing the solvent trends of balances with the same end group but different linkers on the arm. Several balances with same arm group (Me or Ph) and different linkers were then synthesized (Figure 7.6). In addition of balances 1 and 7 with oxygen and carbon linkers, balance 24 with N linker and 25 with S linker were also synthesized. The four linker groups have different abilities to associate with solvent molecules, so we expected the balances to show different solvent trends.

![Figure 7.6](image)

**Figure 7.6:** Structures of balances 1, 7, 24 and 25 with different linkers for the comparison of different solvent effect.

The folding energies (−ΔG) of balances 1, 7, 24 and 25 were measured in different solvents, and the values were plotted according to the ET(30) values of each
solvent (Figure 7.7). The $E_T(30)$ values were used as the parameter indicating the polarities of solvents because it shows great correlation to the folding energies of balances in previous study. The deuterated solvents used were (from left to right on the $x$-axis): benzene-$d_6$, bromobenzene-$d_5$, THF-$d_4$, CDCl$_3$, TCE-$d_2$, acetone-$d_6$, DMSO-$d_6$ and acetonitrile-$d_3$. Not all solvents were applied for study each of the balances because of the lack of data. The solvent trends of balances 1 and 7 should be consistent with less data because they are close to linear. The folding energies of balances 24 and 25 will be tested in more solvent in the future, but the difference on the solvent trends was obvious with the existing data.

![Graph showing comparison of folding energies in different solvents](image)

**Figure 7.7**: Comparison of the folding energies ($-\Delta G$) of balances 1a, 1h, 7b, 7e (left) and balances 1h, 7e, 24 and 25 (right) in different solvents.

Comparison among 1a, 1h with oxygen linkers and 7b, 7e with carbon linkers (Figure 7.7, left) showed that the trends were similar for balances with the same linker even when they formed different intramolecular interactions (CH–π or π–π stacking). Comparison of balances 1h, 7e, 24 and 25 (Figure 7.7, right) with the same π–π interaction but different linkers showed very different trends. It seems that for balance 24
and 25, the folding energies in acetone and acetonitrile were similar to each other, and were different from that in solvents with relatively low polarity. These observations were possibly caused by the different linker-solvent interactions or the solvophobic driving-force for the folding of balances. A complete set of solvent study and a deeper understanding of the polarity scales for both solvent and the linker groups are needed for a clear explanation.

7.3 MOLECULAR BALANCE FOR STUDY NH$_2$–π INTERACTION

Molecular balance 26 with NH$_2$ as the arm group (Figure 7.8, left) was synthesized to measure the interaction between NH$_2$ group and the phenanthrene rings. Due to its restricted rotation of C$_{aryl}$–N$_{imide}$ bond, the balance 26 was able to show separate peaks for the two conformations in $^1$H NMR spectrum. This indicated a rotational barrier that was higher than that of the balance with OH arm (which are not able to show distinct signal on $^1$H NMR spectrum). The reason for the enhanced barrier may be that the extra proton on the NH$_2$ group makes its conformation less flexible than OH group, and thus increases the energy for the transition-state during rotation.

![Figure 7.8](image_url)

**Figure 7.8:** Structure of balance 26 that designed to form the NH–π interaction and its crystal structure obtained from X-ray analysis. The bridge phenyl groups in the crystal structure were hidden for better viewing clarity.
The balance 26 was able to be crystalized from its solution in acetonitrile. Only *unfolded* conformer was observed in the obtained solid-state structure (Figure 7.8, right). However, the *folded* conformation was favored in CDCl₃ in room temperature. The $-\Delta G$ value of balance 26 was measured to be 0.35 kcal/mol at 25°C in CDCl₃, which is stronger than CH₃–π interaction formed in 7a under the same condition ($-\Delta G = 0.10$ kcal/mol).

**Figure 7.9:** Comparison of the folding energies ($-\Delta G$) of balances 24 and 26 in different solvents.

Same as balance 24 mentioned on above section, the folding energy of balance 26 may show different values in solvents with different polarities (Figure 7.9). The trend is also very similar as balance 24, which verified the importance of linker on determining the solvent effect. The factors that make up the solvent effect on the NH–π interaction are still under investigation.
7.4 MOLECULAR BALANCE FOR STUDY IMIDAZOLE–π INTERACTION

Figure 7.10: Structure of balance 27 that designed to form the imidazole–π interaction.

Balance 27 with imidazole arm (Figure 7.10) was synthesized to study the interaction between imidazole ring and the aromatic shelf. Its solid-state structure was characterization in crystal with X-ray analysis (Figure 7.11). A stacking interaction with was observed in the structure. Although the imidazole ring located above the central space between two side rings, the distance from the centroid of the five-member ring to the shelf plane (3.409 Å) was within a typical range of a non-covalent interaction, and one of the protons on the imidazole can still interact with the side ring. In addition, a clear CH–π interaction was also formed between the CH$_2$ linker and the shelf. It could be another stabilization force for the folded conformer of balance 27.

Figure 7.11: Crystal structure of balance 27 with (a) side view and (b) top view of the stacking interaction. Part of the structure was hidden for a better viewing clarity.
The *folded* conformer of balance 27 was favored in balance at room temperature in CDCl₃, and the accordingly −ΔG value was 0.19 kcal/mol. This interaction is slightly weaker compared with the phenyl–π interaction obtained in similar environment: the −ΔG value of balance 7e with CH₂ linker and phenyl arm was measured to be 0.29 kcal/mol.

Containing imidazole structure, the balance 27 showed a potential ability to be soluble in solvents with high polarity. However, its solubility in water and methanol was poor based on the experiments. Further research about this balance could be the study on interactions formed by protonated or alkylated imidazole ring.

7.5 MOLECULAR BALANCE WITH SPLIT PHENYL RINGS ON SHELF

![Figure 7.12: Structure of balance 28 with separate phenyl rings on the shelf.](image)

The structure of balance 28 (Figure 7.12) contains two separate phenyl rings on the shelf (excluding the two bridge phenyl rings) were originally designed in hope of forming interactions between the two rings. However, the crystal structure obtained for this molecule (Figure 7.13) indicates that the space between the rings may not be enough for further interactions. Rather than twisting away and leave more space in between, the two rings intend to be parallel to each other. Still, one possible CH–π interaction was observed between the CH₃ and one of the side rings, but it was not within good geometry and cannot form strong interaction.
Figure 7.13: Crystal structure of balance 28 with (a) side view and (b) front view with both unfolded and folded conformers. Part of the structure was hidden for a better viewing clarity.

The assignment of folded and unfolded conformers was similar to that of previous balances. The interaction of this balance ($-\Delta G = -1.08$ kcal/mol with $\text{folded/unfolded} = 0.16$) is much weaker than the balance 7a ($-\Delta G = 0.13$ kcal/mol with $\text{folded/unfolded} = 1.25$) with connected phenyl rings (phenanthrene shelf) and the methyl arm. Further investigation with similar structures containing larger alkyl arms is still undergoing by undergraduate student Darya Kaborda.

7.6 SYNTHESIS

7.6.1 Balances that forms Double-Mutant Cycles

The synthesis of balances 22 were via the Diels-Alder reaction between corresponding maleic imides 29 and diene 30 (Figure 7.14). The synthesis of balances 23 followed the same procedure as previous balances. Balances 22c, 22d and 22e were synthesized by Ping Li, and balances 23b, 23c and 23e have been discussed in previous sections as balances 9b, 9a (or 14a) and 2a. Balances 23d were synthesized for previous study.74
Figure 7.14: Overview of synthesis of balances 22 via Diels-Alder reaction between maleic imides 29 and the diene 30 with phenanthrene shelf.

To make imides 29, maleic anhydride and corresponding aniline were heated to reflux in acetic acid for 2 d. The crude products were purified by running column with EtOAc/Hexane (v/v = 1/7).

Preparation of imides 29a:

2-Benzylaniline (0.187 g, 1.02 mmol) was reacted with maleic anhydride (0.100 g, 1.02 mmol) in 10 mL acetic acid to produce imide 29a as yellow oil (0.126 g, 0.48 mmol, 47% yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 7.51-6.90 (m, 9 \text{ H}), 6.68 (s, 2 \text{ H}), 3.89 (s, 2 \text{ H})\).

Preparation of imides 29b:

2-Ethylaniline (0.494 g, 4.0 mmol) was reacted with maleic anhydride (0.400 g, 4.0 mmol) in 15 mL acetic acid to produce imide 29b as yellow oil (0.477 g, 2.4 mmol, 59% yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 7.46-7.22 (m, 3 \text{ H}), 7.08 (d, J = 7.83 \text{ Hz}, 1 \text{ H}), 6.87 (s, 2 \text{ H}), 2.46 (q, J = 7.38 \text{ Hz}, J = 14.75 \text{ Hz}, 2 \text{ H}), 1.15 (t, J = 7.38 \text{ Hz}, 3 \text{ H})\).

Preparation of imides 29c:

\(o\)-Toluidine (0.220 g, 2.0 mmol) was reacted with maleic anhydride (0.200 g, 2.0 mmol) in 10 mL acetic acid to produce imide 29c as yellow oil (0.256 g, 1.37 mmol, 68%
yield. \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 7.39–7.23 (m, 3 H), 7.11 (d, \(J = 7.14\) Hz, 1 H), 6.85 (s, 2 H), 2.16 (s, 3 H).

**Preparation of diene 30:**

![Chemical Reaction Diagram]

Phencyclone (2.0 g, 5.2 mmol) in xylene (40 mL) was heated open to air to reflux for 24 h. The oxidation product was purified by running column with EtOAc and hexane (v/v = 1/10), and was obtained as colorless crystal (0.36 g, 0.93 mmol, 18% yield). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 8.81 (d, \(J = 8.7\) Hz, 2 H). 7.66–7.90 (m, 8 H), 7.48–7.60 (m, 4 H), 7.30–7.42 (m, 4 H).

The acetone precursor (0.30 g, 0.78 mmol) was then dissolved in methanol (30 mL) and reacted with NaBH\textsubscript{4} (0.80 g, 21 mmol). After stirring for 3 h under nitrogen, HCl aqueous solution (3 N, 40 mL) was added to quench the reaction. The mixture was extracted with 30 mL CH\textsubscript{2}Cl\textsubscript{2} for twice, and washed with 30 mL water and 30 mL brine. The organic layer was combined and dried under vacuum to get diene 30 as white solid (0.20 g, 0.54 mmol, 69% yield). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) δ 8.37 (dd, \(J = 8.0\) Hz, 2 H), 8.18 (dd, \(J = 8.0\) Hz, \(J = 1.2\) Hz, 2 H), 7.84 (dd, \(J = 7.7\) Hz, \(J = 0.7\) Hz, 4 H), 7.57–7.38 (m, 8 H), 7.30–7.16 (m, 2 H).

**Preparation of balance 22a:**

Imide 29a (0.034 g, 0.135 mmol) and diene 30 (0.050 g, 0.135 mmol) were dissolved in toluene (5 mL) and heated to reflux for 24 h. The crude product was purified by running column with EtOAc/Hex (v/v = 1/5), and balance 22a was obtained as white
solid (0.064 g, 0.128 mmol, 95% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.74 (d, $J = 8.27$ Hz, 2 H minor), 8.61 (d, $J = 8.44$ Hz, 2 H major), 8.41–8.38 (m, 21 H major, 22 H minor), 6.22 (d, $J = 7.36$ Hz, 1 H minor), 5.98 (d, $J = 7.36$ Hz, 1 H major), 4.74 (2 H minor), 4.25–4.25 (m, 3 H major), 3.81 (2 H minor), 3.67 (s, 2 H major).

Preparation of balance 22b:

Imide 29b (0.029 g, 0.143 mmol) and diene 30 (0.053 g, 0.143 mmol) were dissolved in toluene (5 mL) and heated to reflux for 24 h. After work up steps and purification, balance 22b was obtained as white solid (0.058 g, 0.105 mmol, 73% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.81–8.67 (m, 2 H major, 2 H minor), 8.19 (brs, 2 H major, 2 H minor), 7.91–6.70 (m, 14 H major, 16 H minor), 6.49 (t, $J = 7.62$ Hz, 1 H major), 4.81 (s, 2 H minor), 4.75 (s, 2 H major), 4.25 (d, $J = 7.62$ Hz, 1 H major), 2.51 (q, $J = 15.07$ Hz, $J = 7.67$ Hz, 2 H major), 2.35 (q, $J = 15.07$ Hz, $J = 7.67$ Hz, 2 H minor), 1.20 (t, $J = 7.39$ Hz, 3 H major), 1.10 (t, $J = 7.39$ Hz, 3 H minor).

Preparation of balance 23a:

The synthesis of 23a was similar to previous balances with benzene shelves. Anhydride 5c (0.100 g, 0.272 mmol) and 2-benzylaniline (0.054 g, 0.298 mmol) in acetic acid (5 mL) were heated to reflux for 24 h. After work up and purification steps, the balance 23a was obtained as yellow oil (0.155 g, > 90% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.10–7.90 (m, 4 H major, 4 H minor), 7.79–6.66 (m, 13 H major, 14 H minor), 5.56 (d, $J = 7.72$ Hz, 1 H major), 4.39 (s, 2 H major), 4.28 (s, 2 H minor), 3.76 (s, 3 H major), 3.48 (s, 3 H minor).
7.6.2 Balances used in Solvent Studies

The synthesis of balance 1a and 7b was discussed in Chapter 3 and Chapter 4, and balance 1h have been synthesized for previous study.\textsuperscript{74}

Preparation of balance 7e:

The synthesis of 7e was similar to balances in previous chapters with phenanthrene shelves. Anhydride 5a (0.050 g, 0.104 mmol) and 2-benzylaniline (0.023 g, 0.125 mmol) in acetic acid (5 mL) were heated to reflux for 24 h. After work up and purification steps, the balance 7e was obtained as white solid (0.063 g, 0.098 mmol, 94% yield). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 8.70 (d, \(J = 8.44\) Hz, 2 H minor), 8.41 (d, \(J = 7.84\) Hz, 2 H major), 8.21 (d, \(J = 7.84\) Hz, 2 H minor), 8.06 (d, \(J = 8.44\) Hz, 2 H major), 7.84–6.74 (m, 20 H major, 21 H minor), 6.56 (dt, \(J = 8.21\) Hz, \(J = 2.05\) Hz, 1 H minor), 6.31 (d, \(J = 7.52\) Hz, 1 H major), 5.94 (d, \(J = 7.21\), 2 H major), 4.70 (s, 2 H minor), 4.42 (d, \(J = 7.65\) Hz, 1 H minor), 4.14 (s, 2 H major), 3.99 (s, 2 H minor), 3.82 (s, 2 H major).

Preparation of balance 24:

Anhydride 5a (0.050 g, 0.104 mmol) and N-phenyl-o-phenylenediamine (0.023 g, 0.125 mmol) in acetic acid (5 mL) were heated to reflux for 24 h. After work up and purification steps, the balance 24 was obtained as yellow solid (0.056 g, 0.086 mmol, 83% yield). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \(\delta\) 8.69 (d, \(J = 8.30\) Hz, 2 H major), 8.40 (d, \(J = 7.47\) Hz, 2 H minor), 8.29 (d, \(J = 7.47\) Hz, 2 H major), 8.01 (d, \(J = 8.30\) Hz, 2 H minor), 7.73 (t, \(J = 7.58\) Hz, 4 H major), 7.64–6.64 (m, 16 H major, 21 H minor), 6.44–6.26 (m, 2 H major), 5.82–5.67 (m, 2 H minor), 5.41 (s, 1 H major), 4.70 (s, 2 H minor), 4.59 (dd, \(J = 7.94\) Hz, \(J = 0.92\) Hz, 1 H major), 4.38 (s, 2 H major), 3.23 (s, 1 H minor).
Preparation of balance 25:

Anhydride 5a (0.050 g, 0.104 mmol) and 2-aminophenyl-phenylsulfide (0.025 g, 0.125 mmol) in acetic acid (3 mL) were heated to reflux for 24 h. After work up and purification steps, the balance 25 was obtained as white solid (0.076 g, 0.114 mmol, >90% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.79–8.62 (m, 2 H major, 2 H minor), 8.41 (d, $J$ = 7.54 Hz, 2 H minor), 8.32 (d, $J$ = 7.54 Hz, 2 H major), 8.15 (d, $J$ = 7.54 Hz, 1 H minor), 8.08 (d, $J$ = 8.55 Hz, 1 H major), 7.79–6.86 (m, 21 H major, 18 H minor), 6.69–6.54 (m, 2 H minor), 6.26 (d, $J$ = 7.54 Hz, 2 H minor), 4.68 (s, 2 H minor), 4.58–4.48 (m, 3 H major).

7.6.3 Balance for Measuring NH–π Interaction

Anhydride 5a (0.100 g, 0.208 mmol) and o-phenylenediamine (0.023 g, 0.208 mmol) in DMF (2 mL) were heated to reflux for 5 h. The mixture was quenched with 50 mL water and then extracted with 50 mL EtOAc for 3 times. The organic layer was combined and washed with 50 mL water for 3 times. The solvent was dried under vacuum, the balance 26 was obtained as white solid (0.130 g, 0.114 mmol, >90% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.76–8.60 (m, 2 H major, 2 H minor), 8.47–8.27 (m, 2 H major, 2 H minor), 7.83–6.50 (m, 17 H major, 18 H minor), 6.19 (d, $J$ = 8.44 Hz, 1 H major), 6.07 (t, $J$ = 7.91 Hz, 1 H minor), 4.65 (s, 2 H major), 4.63 (s, 2 H minor), 4.36 (d, $J$ = 7.91 Hz, 1 H minor).

7.6.4 Balance for Measuring Imidazole–π Interaction
Balance 27 was obtained in three steps. First, nitrobenzene precursor 31 was made. To the stirring solution of imidazole (0.019g, 0.278 mmol) in DMF (5 mL), potassium carbonate (0.064 g, 462 mmol) and 2-nitrobenzyl bromide (0.050 g, 0.231 mmol) was added. The reaction was stirred under room temperature for 24 h, and was quenched with 30 mL water. The mixture was then extracted with 50 mL EtOAc for 3 times, and the combined organic layer was washed with saturate 50 mL NaHCO$_3$ (aq.) and dried with MgSO$_4$. After the removal of solvent under vacuum, compound 31 was then obtained as yellow liquid (0.048g, 0.24 mmol, 85% yield). $^1$H NMR (300 MHz CDCl$_3$) $\delta$ 8.15 (d, $J = 7.84$ Hz, 1 H), 7.63–6.90 (m, 5 H), 6.80 (d, $J = 7.61$ Hz, 1 H), 5.57 (s, 2 H).

The nitrobenzene 31 was then reduced into aniline 32 via catalyzed hydrogenation. The substituted nitrobenzene 31 (0.048 g, 0.24 mmol) was dissolved with THF (5 mL) in a pressure vessel, then ethanol (20 mL) and of Pd/C (10% wt, 20 mg) was added. The vessel was pressurized at 40 psi with hydrogen gas and was stirred for 4 h. The resulting mixture was filtered through celite and the solvent was removed by rotary evaporation to afford the product 32 as yellow oil (0.044 g, 0.25 mmol, > 90% yield). $^1$H NMR (300 MHz CDCl$_3$) $\delta$ 8.10 (s, 1 H), 7.19 (t, $J = 7.58$ Hz, 1 H), 7.13 (s, 1 H), 7.06 (d, $J = 7.58$ Hz, 1 H), 6.94 (s, 1 H), 6.79 (t, $J = 7.58$ Hz, 1 H), 6.71 (d, $J = 8.08$ Hz 1 H), 5.16 (s, 2 H), 5.04 (brs, 2 H).
Aniline 32 was reacted with anhydride 5a to produce balance 27. Compounds 32 (0.043 g, 0.248 mmol) and 5a (0.079 g, 0.166 mmol) were dissolved in 5 mL acetic acid and was heated to reflux for 24 h. After work up steps and purification, balance 27 was obtained as yellow solid (0.114 g, 0.179 mmol, > 90% yield). \(^1\)H NMR (300 MHz CDCl\(_3\)) \(\delta\) 8.62 (d, \(J = 8.40\) Hz, 2 H major), 8.28 (d, \(J = 7.98\) Hz, 2 H minor), 8.23 (d, \(J = 8.40\) Hz, 2 H minor), 8.10 (d, \(J = 7.56\) Hz, 2 H major), 7.73–6.86 (m, H major, H minor), 6.72 (s, 1 H minor), 6.33 (s, 1 H minor), 6.05 (d, \(J = 7.81\) Hz, 1 H minor), 5.61 (s, 1 H minor), 4.66 (s, 2 H major), 4.65 (s, 2 H minor), 2.25 (s, 2 H minor), 1.97 (s, 2 H major).

7.6.5 Balance with Split Phenyl Shelf

Imide 29c (0.045 g, 0.24 mmol) and tetraphenylcyclopentadienone (0.093 g, 0.24 mmol) were dissolved in benzene (5 mL) and heated to reflux for 24 h. After work up, the crude product was purified by running column with EtOAc/Hexane (v/v = 1/5). Balance 28 was then obtained as purple solid (0.121 g, 0.21 mmol, 88% yield). \(^1\)H NMR (300 MHz CDCl\(_3\)) \(\delta\) 7.91–6.63 (m, 22 H major, 22 H minor), 4.44 (s, 2 H minor), 4.41 (s, 2 H major), 2.25 (s, 3 H major), 2.05 (s, 3 H minor).
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