## Communications

#### Noncovalent Interactions

#### A Weak Attractive Interaction between Organic Fluorine and an Amide Group\*\*

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Questions regarding the nature and strength of noncovalent interactions formed by organic fluorine atoms are increasingly being addressed and debated in the literature.<sup>[1]</sup> We have been exploring noncovalent interactions of fluorine by carrying out a systematic fluorine scan<sup>[2]</sup> at the active site of thrombin.<sup>[3]</sup> While exploring the hydrophobic D pocket of this serine protease, we noticed that the potency of a closely related family of fluorinated inhibitors was strongly influenced by the position of the fluorine atom (Figure 1).<sup>[3a]</sup> The 4-fluorobenzyl derivative  $(\pm)$ -4 exhibits fivefold better inhibition than any other member of the family. The X-ray structure of the (+)-4-enzyme complex showed two close contacts between the fluorine atom and the  $C_{\alpha}$ -H atom as well as the carbonyl C atom of Asn98 (Figure 1b). Subsequent searches in the Cambridge Structural Database and RCSB Protein Data Bank provided numerous examples of similar sub van der Waals contacts between organic fluorine atoms and carbonyl carbon atoms in chemical and biological samples. These interactions have a characteristic geometry: the fluorine atom tends to reside orthogonally above the pseudotrigonal axis of the carbonyl group, and the C-F bond



*Figure 1.* a) A family of inhibitors designed to probe the fluorophilicity of the D pocket at the thrombin active site. b) X-ray structure showing close contacts between the fluorine atom of inhibitor (+)-4 and the protein backbone of thrombin.

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approaches the plane of the carbonyl group from an angle between 100° and, at very short F…C distances, 140°.<sup>[3a]</sup> Herein we report the first model system to evaluate the energetics of the proposed C–F…amide interactions.

The distinct geometry of the orthogonal C–F…amide interaction presented an unusual challenge. We found an answer in the "molecular torsion balance" derived by Wilcox et al. from the Tröger base—a system designed for the accurate measurement of edge-to-face aromatic–aromatic interactions by the observation of a simple conformational equilibrium.<sup>[4]</sup> We found through examination of existing crystal structures<sup>[5]</sup> that appropriate substitution of the Tröger base skeleton would provide the perpendicular arrangement of functional groups required for our study (Scheme 1). The



**Scheme 1.** The torsion balance of Wilcox and co-workers. Modeling studies predict both edge-to-face aromatic–aromatic interactions and close contacts between substituents  $R^1$  and  $R^2$  for the folded state.

use of a trifluoromethyl group was required to approximate the optimal C-F…amide geometry. Based on the similarity of

the results obtained from database searches for fluorine atoms attached to  $sp^2$ - and  $sp^3$ -hybridized carbon atoms, we anticipated that this substitution would have a negligible effect on the energetics of the proposed interaction.

The second challenge in characterizing fluorine–amide interactions is their expected weakness. The relative  $K_i$  values for compounds  $(\pm)$ -**1**– $(\pm)$ -**7** suggest that the fluorine substitution provides approximately 4 kJ mol<sup>-1</sup> of stabilizing energy. Hunter and co-workers have popularized chemical double-mutant cycles for the measurement of very small interaction energies in supramolec-

ular systems, and have used this method to accurately measure various noncovalent interactions as weak as 1 kJ mol<sup>-1.[6]</sup> The application of this strategy to the Wilcox torsion balance is straightforward. The edge-to-face aromatic–aromatic interaction is the primary force behind the folding of the molecule, and the effect of each substitution on this primary force must be accounted for to determine the incremental folding free enthalpy provided by the two appended functional groups. A double-mutant cycle (Scheme 2) can be used to determine the influence of each appended group on the edge-to-face interaction independently from their interaction with each other. When the folding energies of all four molecules in the double-mutant cycle have



**Scheme 2.** Double-mutant cycle for determining the magnitude of the attraction between orthogonally oriented  $CF_3$  and NHCOCH<sub>3</sub> groups.

been determined, the interaction energy of the two appended functional groups ( $\Delta G_{CF_3 \cdots NHCOCH_3}$ ) is given by Equation (1).

$$\Delta G_{\mathrm{CF}_3\cdots\mathrm{NHCOCH}_3} = \Delta G_{(\pm)\text{-8}} - \Delta G_{(\pm)\text{-9}} - \Delta G_{(\pm)\text{-10}} + \Delta G_{(\pm)\text{-11}} \tag{1}$$

The four molecules of interest were synthesized as shown in Scheme 3. The nitrocarboxylic acid intermediate  $(\pm)$ -**12**, prepared in thirteen steps using literature methods,<sup>[7]</sup> was esterified with 4-(trifluoromethyl)phenol and phenol to give  $(\pm)$ -**13** and  $(\pm)$ -**14**, respectively.



**Scheme 3.** Synthesis of  $(\pm)$ -8– $(\pm)$ -11. a) Et<sub>3</sub>N, BOP, 4-(trifluoromethyl)phenol or phenol, CH<sub>2</sub>Cl<sub>2</sub>, RT, 71–97%. b) H<sub>2</sub> (atm), RaNi, EtOAc/ EtOH, RT, 90–100%. c) Ac<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, RT, 20–72%. d) 1. HCl, NaNO<sub>2</sub>, 5°C; 2. H<sub>3</sub>PO<sub>2</sub>, 5°C, 25–63%. BOP = Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate, RaNi = Raney Nickel.

Reduction of the nitro group with H<sub>2</sub>/RaNi gives the corresponding anilines. In divergent steps, each aniline is then acetylated to yield target compounds  $(\pm)$ -8 and  $(\pm)$ -10, or converted into its corresponding diazonium salt, which is in turn reduced with H<sub>3</sub>PO<sub>2</sub> to afford compounds  $(\pm)$ -9 and  $(\pm)$ -11.

The interconversion between the two conformations of each molecule is slow on the NMR timescale ( $E_a = 65-75 \text{ kJ mol}^{-1}$ ).<sup>[4a,8]</sup> Direct integration of the <sup>1</sup>H and/or <sup>19</sup>F resonances for each conformer allows the folding equilibrium to

be determined with well-defined estimations of error. The singlets corresponding to the methyl resonances of each folded state are resolved at 300 MHz, and the identity of each conformer is easily determined by analogy with related compounds (Figure 2).<sup>[4]</sup> A summary of the folding energies



**Figure 2.** <sup>1</sup>H NMR spectra (methyl region,  $C_6D_6$ , c=10 mM) of: a) (±)-**8**, b) (±)-**9**, c) (±)-**10**, d) (±)-**11**. The sensitivity of the acetamide COCH<sub>3</sub> resonance to the proximity of the trifluoromethyl group in the folded state is highlighted.

for compounds  $(\pm)$ -8– $(\pm)$ -11 in three different solvents is given in Table 1. The application of the double-mutant cycle in the form of Equation (1) provides the incremental free enthalpy for the interaction of the two appended functional groups. The interaction is weak, but measurable, in CDCl<sub>3</sub>  $(-1.05\pm0.25 \text{ kJ mol}^{-1})$  and C<sub>6</sub>D<sub>6</sub>  $(-0.85\pm0.25 \text{ kJ mol}^{-1})$ , while the value is essentially zero in CD<sub>3</sub>OD  $(-0.10\pm$  $0.25 \text{ kJ mol}^{-1})$ .

*Table 1:* Folding free enthalpies for compounds  $(\pm)$ -8- $(\pm)$ -11.

Compound	$\Delta G~[\pm 0.12~ ext{kJ} ext{mol}^{-1}]^{[a]}$		
	CDCl <sub>3</sub>	$C_6D_6$	CD₃OD
(±)- <b>8</b>	-2.46	-3.78	-2.88
(±)-9	-1.95	-3.31	-2.37
(±)- <b>10</b>	-0.45	-1.46	-1.72
(±)-11	-1.00	-1.84	-1.31

[a] Determined by integration of signals in the NMR spectra of 10 mm solutions at 298 K. For a complete error analysis, see the Supporting Information.

Two main conditions must be satisfied for a doublemutant cycle to be valid. Firstly, the core must not undergo major structural rearrangements between different members of the cycle. With few degrees of freedom, the scaffold of Wilcox and co-workers derived from the Tröger base is exceptionally well organized. To determine the effect of trifluoromethyl substitution on the folded structure we solved the crystal structure of synthetic precursor  $(\pm)$ -13 (Figure 3).<sup>[9]</sup> Comparison with several existing crystal structures<sup>[5]</sup> of related torsion-balance molecules shows there is little deviation in the folded edge-to-face geometry throughout the series. The crystal structure of precursor  $(\pm)$ -13 also

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**Figure 3.** ORTEP plot with ellipsoids at 50% level showing the folded state of  $(\pm)$ -**13**. Distances between F(32) and the atoms of the nitro group are given in Å.

displays the desired perpendicular approach of the appended functional groups (in this case a trifluoromethyl and a nitro group). The trifluoromethyl group experiences no attraction to the adjacent nitro group (see below), and accordingly the close interatomic contacts involving F(32) are greater than the sum of van der Waals radii (Figure 3). Modeling studies on  $(\pm)$ -8 suggest that the minute (<0.3 Å) vertical adjustment required to bring the fluorine atom close to the plane of the acetamide group can be accommodated, despite the rigidity of the scaffold. However, the modeling study also shows that for  $(\pm)$ -8 the fluorine atom closest to the acetamide group may not reach its ideal position above the pseudotrigonal axis of the carbonyl unit. Instead, it may lie somewhere above the bond connecting the nitrogen atom to the carbonyl carbon atom, which suggests there is a geometric factor that can account for the slightly weaker than expected value measured for the fluorine-amide interaction.

In the absence of an X-ray structure of  $(\pm)$ -8, we turned to NMR studies to provide evidence for the proximity of the trifluoromethyl and acetamide groups in solution. The difference in the chemical shifts observed for the acetamide CH<sub>3</sub> group in the folded and unfolded states is larger for  $(\pm)$ -8  $(\Delta \delta = 0.30 \text{ ppm})$  than for control compound  $(\pm)$ -10  $(\Delta \delta =$ 0.14 ppm), which suggests that the acetamide residue experiences decreased shielding in the folded state of  $(\pm)$ -8 because of the nearby fluorine atoms (Figure 2).

The validity of a double-mutant cycle also rests on the linear additivity of the secondary energy perturbations arising from each functional group mutation. A closer analysis of the data in Table 1 shows that the addition of an acetamide group to the "face" component of the primary edge-to-face interaction (from compound  $(\pm)$ -**11** to  $(\pm)$ -**10**) causes a small unfavorable change in the folding free enthalpy ( $\Delta(\Delta G) = +0.4$  kJ mol<sup>-1</sup> in C<sub>6</sub>D<sub>6</sub>). In comparison, the addition of a trifluoromethyl group to the "edge" component (from com-

pound (±)-**11** to (±)-**9**) causes a much larger favorable secondary effect ( $\Delta(\Delta G) = -1.5 \text{ kJ mol}^{-1}$  in C<sub>6</sub>D<sub>6</sub>). This difference arises from the acidification of the neighboring *ortho* proton, which increases the strength of the CH- $\pi$  interaction at the heart of the folded conformation.

To get a better understanding of the effect of the trifluoromethyl group on the core edge-to-face interaction we synthesized a series of compounds in which the "face" component bears different substituents. The folding equilibrium constant of each was determined by using the same method as for compounds  $(\pm)$ -8– $(\pm)$ -11, and the entire series was examined using a linear free energy relationship between the folding free enthalpy and the Hammett constants  $\sigma_{meta}$  (Figure 4).<sup>[10]</sup> The correlation is excellent ( $R^2 > 0.98$ ), thus



**Figure 4.** A linear free energy relationship showing substituent effects for edge-to-face interactions in a family of trifluoromethyl-substituted molecules.

showing that the primary CH– $\pi$  interaction follows a wellbehaved continuum that depends on the electronic properties of the "face" component. The sole significant departure from this trend arises for the acetamide-substituted compound  $(\pm)$ -8.<sup>[11]</sup> This compound is more folded by 1.5±0.7 kJ mol<sup>-1</sup> than substituent effects would predict—this value is similar to that obtained from the double-mutant cycle analysis.

What is the origin of the attraction between the appended trifluoromethyl and acetamide groups? It is unlikely that the amide NH group can be appropriately oriented to donate a hydrogen bond to the fluorine atoms, and the lack of any attraction for the more acidic phenol compound (Figure 4) confirms that this mode of weak hydrogen bonding does not stabilize the folded state.<sup>[1a]</sup> It is possible that the model suggested by our previous database findings, in which the electronegative end of the carbon–fluorine dipole interacts favorably with the electropositive carbonyl carbon atom, is operative.<sup>[3a]</sup> This dipolar electrostatic model accounts for the absence of any measurable attraction in the polar solvent CD<sub>3</sub>OD. Alternatively, one can consider the approach of the unpolarizable, partially negative surface of the trifluoro-

methyl group<sup>[12]</sup> close to the polarizable delocalized  $\pi$  cloud of the amide group. Attraction arises in this model from chargeinduced dipole and dipole-induced dipole interactions. Arguments against such a model are indicated by a recent study in which a trifluoromethyl group is not attracted by the face of an aryl ring, regardless of the electrostatic potential of the ring.<sup>[13]</sup> In the absence of structural information for compound  $(\pm)$ -**8**, it is impossible to conclude whether an atom-centered model or a functional-group-scale model of this attraction is more appropriate. We hope that further studies using different fluorine-containing functional groups and different carbonyl functional groups will shed light on this issue.

In summary, we have presented the first efforts to quantify the interactions of organic fluorine with the face of an amide functional group. A novel combination of the torsion balance developed by Wilcox and co-workers with two distinct physical methods—a double-mutant cycle and a linear free energy relationship—provided the initial measurement of an attractive interaction with a value of 0.8–1.5 kJ mol<sup>-1</sup> in nonpolar solvents. The effects of fluorination on the hydrophobicity, distribution, metabolism, and pharmacokinetics of potential drug molecules are increasingly well understood.<sup>[14]</sup> Model studies such as those presented here help clarify the role played by fluorine atoms in altering the binding affinity and selectivity<sup>[3,14d]</sup> of enzyme inhibitors.

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