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Modulation of the H-Bond Basicity of Functional Groups by α-Fluorine-Containing Functions and its Implications for Lipophilicity and Bioisosterism

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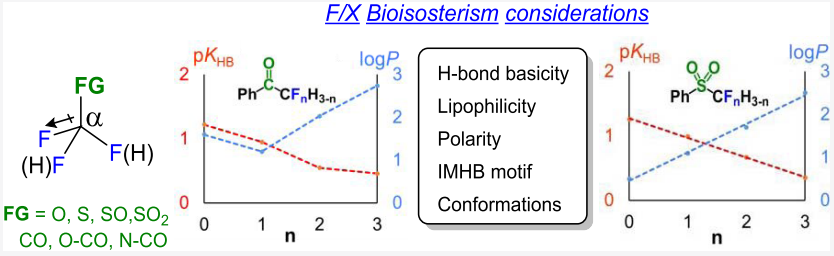

ABSTRACT: Modulation of the H-bond basicity (pK_{HB}) of various functional groups (FGs) by attaching fluorine functions and its impact on lipophilicity and bioisosterism considerations are described. In general, H/F replacement at the α-position to H-bond acceptors leads to a decrease of the pK_{HB} value, resulting, in many cases, in a dramatic increase in the compounds' lipophilicity ($\log P_{o/w}$). In the case of α-CF_2H, we found that these properties may also be affected by intramolecular H-bonds between CF_2H and the FG. A computational study of ketone and sulfone series revealed that α-fluorination can significantly affect overall polarity, charge distribution, and conformational preference. The unique case of α-di- and trifluoromethyl ketones, which exist in octanol/water phases as ketone, hemiketal, and gem-diol forms, in equilibrium, prevents direct $\log P_{o/w}$ determination by conventional methods, and therefore, the specific $\log P_{o/w}$ values of these species were determined directly, for the first time, using Linclau's ^{19}F NMR-based method.

INTRODUCTION

Incorporation of a fluorine atom or fluorine-containing functions in organic compounds is frequently implemented to modify their physicochemical properties, an approach that applies to many chemistry fields[1,2] and, particularly, to medicinal chemistry.[3−10] The origin of the many effects resulting from the introduction of a fluorine atom is its high electronegativity and small size, leading to a highly polar yet poorly polarizable C−F bond. In medicinal chemistry, the use of fluorine atom(s) or fluorine-containing functions in structure−activity relationship (or in bioisosterism) studies is a very practical way to alter physicochemical properties of bioactive compounds, having high relevance to drug development. For instance, the replacement of a hydrogen atom by fluorine, mainly in the popular surrogates Ar-H/Ar-F or Ar-CH_3/Ar-CF_3, is extensively applied in drug development to improve properties such as metabolic stability, lipophilicity, bioavailability, and affinity. The last two decades have witnessed a considerably increased interest in utilizing fluorine-containing groups as bioisosteres, affecting properties such as polarity, hydrogen bonding capacity, lipophilicity, conformational preference, and so on.[11−20] This approach is manifested, for instance, by the replacement of CH_3, OH, or SH by the H-bond-donating group CF_2H, which may affect all of these properties, as a function of structural motifs.[21−25]

H-bonds play a pivotal role in many biological systems and are deeply involved in the activity of numerous drugs and biologically active compounds.[26−30] At the interface between H-bonding and the fluorine effects, one can find two important topics: (1) the intrinsic ability of fluorine functions to act as H-bond donors (e.g., CF_2H group)[14,18,31] or acceptors[32−36] (via the lone pair electrons of fluorine known as very weak acceptors; such interactions exist, mainly, in cases in which the fluorine atom is located in close proximity to a HB donor) and (2) the influence of fluorine functions on the hydrogen bonding capacity of other functional groups (FGs). For instance, the influence of one or more fluorine atoms on the H-bond acidity (pK_{AHY}) of hydroxyl groups in various conforma-

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tionally flexible or rigid substrates was thoroughly investigated by Linclau and Graton and co-workers, who showed that this property can be tuned, up or down, depending on molecular structural motifs such as an intramolecular H-bond (IMHB) and conformational preference or rigidity (Figure 1A).[11,37]

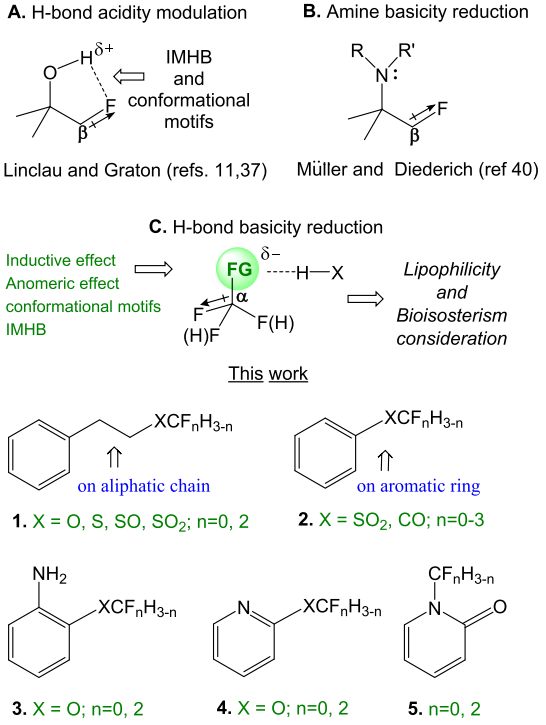


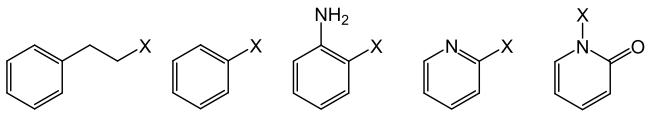
Figure 1. Influence of fluorination on the H-bond acidity (A), amine basicity (B), and H-bond basicity of various functional groups (C, bottom; structures studied in this work).

However, the effect of fluorine atom(s) on the H-bond basicity (pK_{HB}) of the adjacent functional groups, i.e., at the α position, has not yet been systematically studied. In fact, the only systematic study that was reported on this issue is the effect of fluorine on pyridines, which resulted in tuning their H-bond basicity values.[38] The effect fluorine substitutions have on the pK_a values (i.e., of the conjugate acid R_3NH^+) of vicinal amines (from the β position onward) is well documented and implemented in drug design.[39−42] The trend clearly shows that fluorine substituents reduce the basicity, i.e., the pK_a values of amine bases, depending on the position and the amount of fluorine atoms (Figure 1B), an effect that significantly affects adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of biologically active compounds. However, it was found in many cases that there is no correlation between the Brønsted proton basicity (pK_{BH+}) values of various functional groups and their H-bond basicity (pK_{HB}),[26] and therefore, the role fluorine plays on the latter property should be systematically studied.

The inductive effect of fluorine functions on the H-bond basicity of an attached functional group may be of great relevance not only to the lipophilicity and ADMET properties of bioactive compounds but also to their recognition and binding to the biological target as H-bond acceptors.[43] A fluorine effect on the binding affinity of several thrombin inhibitors, both α-fluorinated sulfones and ketones, was recently described by Hangauer and Nasief and co-workers,[43a] who describe a reduction (qualitatively) of their H-bond basicity via fluorination, which resulted in an increase in their binding affinity, an effect that was attributed to the reduction in the dehydration energy. These very interesting counter-intuitive results show that reducing the H-bond basicity of biologically active compounds may pose a benefit in the desolvation penalty during the ligand−protein binding. Additional interesting examples for which α-fluorination to H-bond-accepting groups such as sulfones[43b] and ketones[43c] (vide infra, many other examples) may strongly affect their bioactivity have been recently reported.

One of the findings described in our latest report on the mutual influence between various functional groups and the CF_2H moiety at the α position is the significant increase in

Table 1. pK_{HB} Values of α-Mono-, Di-, and Trifluorinated Substitution of Various Functional Groups



run	C.	x	pK_{HB}	C.	x	pK_{HB}
1	**1a**	OCH_3	0.79 ± 0.06	**1a-F2**	OCF_2H	0.04 ± 0.1
2	**1b**	SCH_3	−0.33 ± 0.08	**1b-F2**	SCF_2H	−0.38 ± 0.1
3	**1c**	$SOCH_3$	2.56 ± 0.02	**1c-F2**	$SOCF_2H$	1.66 ± 0.01
4	**1d**	SO_2CH_3	1.38 ± 0.04	**1d-F2**	SO_2CF_2H	0.84 ± 0.05
5	**2a**	SO_2CH_3	1.26 ± 0.04	**2a-F**	SO_2CFH_2	0.99 ± 0.05
6				**2a-F2**	SO_2CF_2H	0.67 ± 0.02
7				**2a-F3**	SO_2CF_3	0.35 ± 0.03
8	**2b**	$C(O)CH_3$	1.22 ± 0.02[a]	**2b-F**[a]	$C(O)CFH_2$	0.95 ± 0.03
9				**2b-F2**[a]	$C(O)CF_2H$	0.55 ± 0.04
10				**2b-F3**[a]	$C(O)CF_3$	0.46 ± 0.02
11	**2c**	CO_2CH_3	0.89 ± 0.02	**2c-F2**	CO_2CF_2H	0.46 ± 0.07
12	**3**	OCH_3	0.91 ± 0.04	**3-F2**	OCF_2H	0.75 ± 0.04
13	**4**	OCH_3	0.88 ± 0.03	**4-F2**	OCF_2H	0.23 ± 0.09
14	**5**	CH_3	2.47 ± 0.03	**5-F2**	CF_2H	1.47 ± 0.03

[a]These values were determined by ^{19}F NMR according to ref 47.

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lipophilicity when this fluorine function is adjacent to polar groups such as sulfoxide, sulfones, and ethers.[18] We hypothesized that this effect might be attributed to not only a decrease in polarity but also a decrease in the H-bond basicity of these functional groups. Herein, we report the first systematic study, both experimentally and computationally, of the effect fluorine has on the H-bond basicity of various adjacent functional groups and the implication this effect has on the lipophilicity of compounds holding this motif (Figure 1C). The relevant bioisosterism considerations, for instance, CFH_2 and CF_2H as lipophilic bioisosteres of the NH_2 group in amides and sulfonamides and of an α-CH_3 in ketones and sulfones, are also discussed.

RESULTS AND DISCUSSION

Compounds and Synthesis. A broad range of α-fluorinated functional groups were selected for the present study including ethers, sulfides, sulfoxides, sulfones, ketones, anilines, esters, pyridines, and 2-pyridones. All compounds also contain an aryl moiety for practical reasons, namely, having low volatility and UV absorbance (see Figure 1 and Table 1). In this study, we focused mainly on functional groups characterized by medium to strong H-bond basicity, i.e., having relatively high pK_{HB} values, leaving enough room for the expected changes from fluorine influence along with minimizing standard deviations. For the sulfones (**2a-Fn** series) and ketones (**2b-Fn** series), which are well known as highly important functional groups in medicinal chemistry, we compared the effects resulting from all variations in α -fluoromethylation. Namely, the α-mono- (**2a-F**, **2b-F**), di- (**2a-F2**, **2b-F2**), and trifluoromethyl (**2a-F3**, **2b-F3**) groups were compared to those of the non-fluorinated parent compounds (**2a** and **2b**, respectively). Both series are commercially available and used without further manipulation. Since the CF_2H moiety is known to act as a H-bond-donating group, which is also an axially anisotropic group and may participate in IMHB (as in the cases of compounds **2c-F2**, **3-F2**, **4-F2**, and **5-F2**), we gave particular attention to this moiety compared to the parent compounds of all other functional groups. Difluoromethylated compounds **1a-d-F2** and **2c-F2** as well as compounds **4-F2** and **5-F2**, depicted in Table 1, were synthesized according to procedures previously reported by us[14,18,44,45] using *O*-diethyl(bromodifluoromethyl) phosphonate as a difluorocarbene precursor.

H-Bond Basicity (pK_{HB}) Measurements. The pK_{HB} values of the above-mentioned matched pairs and the sulfone and ketone series were determined by Fourier transform infrared (FT-IR) spectrometry[46] or by a ^{19}F NMR-based[33,35,47] method. The latter method was used in cases where a technical limitation such as band overlap was observed for FT-IR (vide infra). In all cases, we used *p*-fluorophenol (*p*-FP) as the reference H-bond donor (acid) and measured the formation constant K_f (K_{HB}) of its H-bonded 1:1 complexes with various H-bond acceptors (base, B), in anhydrous CCl_4 at 298 K. Thus, the pK_{HB} values of the fluorinated and non-fluorinated counterparts were determined according to eqs 1 and 2 (for experimental details, see SI).[46,47] The equilibrium concentration of free *p*-FP was determined from the IR absorbance of the OH band at 3614 cm^{-1}. For most compounds studied in this work, the infrared OH band of the *p*-FP complexes was shifted to a lower wavenumber. For the ketone series **2b-F**, **2b-F2** and **2b-F3**, the free *p*-FP band was found to overlap with other bands, preventing the extraction of the complexation constant by FT-IR and therefore an alternative ^{19}F NMR method was used (Figure S26, SI).[33,35,47]

$$B + 4\text{-}FC_6H_4OH \rightleftharpoons 4\text{-}FC_6H_4OH\cdots B \quad (1)$$

$$K_{HB} = [4\text{-}FC_6H_4OH \bullet B]/[4\text{-}FC_6H_4OH][B];\ pK_{HB} = \log_{10} K_{HB} \quad (2)$$

The structures of the various matched pairs studied, i.e., when X is $FGCH_3$ versus $FGCF_nH_{3-n}$, and their pK_{HB} values are summarized in Table 1. Inspection of the data presented in Table 1 reveals that incorporation of fluorine atom(s) at the α position of many FGs, through a broad range of pK_{HB} values, reduces the H-bond basicity of that FG significantly. For instance, the H-bond basicity of methyl ether **1a** (pK_{HB} 0.79) was reduced to almost zero (0.04) upon CH_3/CF_2H replacement at the α position to the oxygen atom (**1a-F2**, run 1). On the other hand, similar replacement on the methyl sulfide analogue **1b**, which is known as a very poor H-bond-accepting group (pK_{HB} −0.33), causes only a slight reduction in the pK_{HB} value (−0.38, run 2). Comparing the fluorine effect on the organosulfur series **1b−d**, i.e., α-difluoromethyl-sulfide, sulfoxide, and sulfone, respectively, clearly shows that the stronger the H-bond basicity of the parent compound, the bigger the effect α-fluorine has on reducing the pK_{HB} values, that is, the $\Delta pK_{HB(CH3\text{-}CF2H)}$ order is sulfoxide > sulfone > sulfide (runs 2−4, vide infra Figure 4). Furthermore, increasing the number of fluorine atoms on the methyl group at the α position to sulfone **2a** resulted in a proportional decrease in the pK_{HB} values in the order CF_3 > CF_2 > CF as shown in Figure 2A (right) and Table 1 (runs 5−7). This trend is clearly

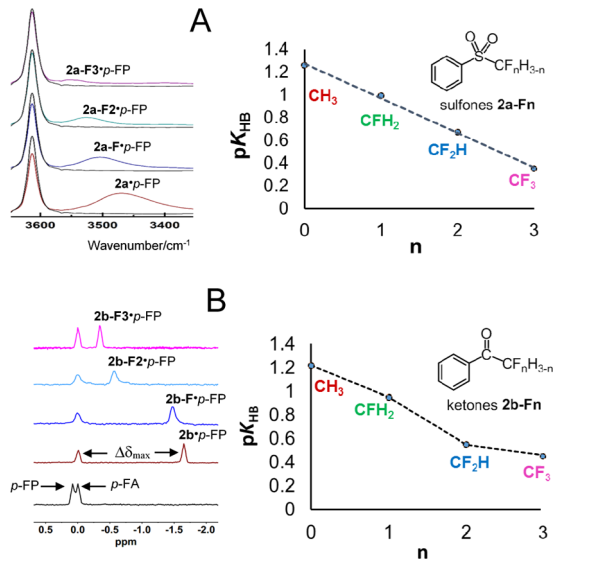

Figure 2. Effect of α-fluorine substitution on the pK_{HB} values of sulfones (**2a-Fn**, top) using FT-IR ($\Delta\nu$(OH), left) and ketones (**2b-Fn**, down) using ^{19}F NMR ($\Delta\delta_{max}$, left) spectroscopies, respectively.

demonstrated in both the magnitude and the shift extent to a lower wavenumber of the IR complex bands; thus, the $\Delta\nu$ values for the complexes **2a-F3**, **2a-F2**, **2a-F**, and **2a** with *p*-FP were found to be 62, 82, 110, and 144 cm^{-1}, respectively (Figure 2A, left). As the H-bond basicity of each oxygen in both S−O bonds is reduced by each dipolar C−F bond added almost linearly, this additive effect may also be expected for other physicochemical properties such as lipophilicity. Interestingly, this trend was also observed for the analogue ketone series **2b-Fn**, however, in no direct proportion to the

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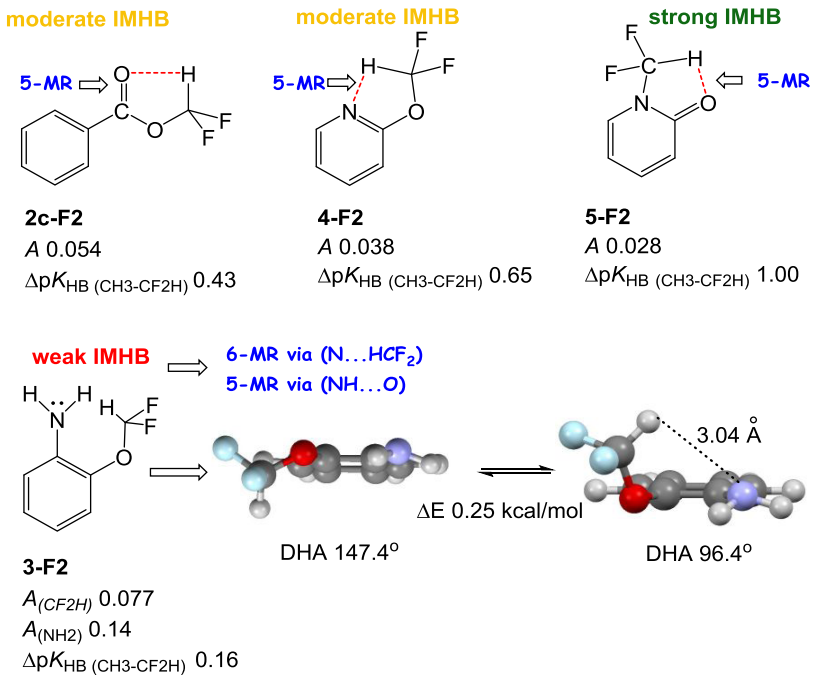

Figure 3. Reducing H-bond basicity of FG and the H-bond acidity of the proximate CF_2H group by IMHB between both groups. Optimized conformational geometries of **3-F2** are calculated at m062x/6-311++g(d,p) levels of theory (gray, carbon; cyan, fluorine; and red, oxygen). DHA is dihedral angle.

consecutive addition of fluorine atoms at α position. For instance, the pK_{HB} value of the difluoromethylated compound (**2b-F2**) was found to be similar to that of its trifluoromethylated counterpart (**2b-F3**). Thus, for this series, the decrease in the pK_{HB} values was found to follow the order $CF_3 \approx CF_2 >$ CF as shown in Figure 2B (right) and Table 1 (runs 8−10). The pK_{HB} values of the ketones were extracted from the ^{19}F NMR chemical shifts of complexed *p*-FP and compared to that of the internal reference *p*-F-anisole (*p*-FA), which are dependent on the $\Delta\delta_{max}$ of each complex, according to previously reported procedures (Figure 2B, left).[33,35,47] Thus, it seems that contrary to the case of the above-mentioned sulfones, in their ketone counterparts, the addition of the third fluorine atom at the α position (C−F bond) only slightly contributes to further reduction of the charge on the oxygen in the carbonyl function.

It is reasonable to assume that the difference between these two families lies deeply in the fundamental differences between the nature of the polar bond of their functions, i.e., S═O versus C═O, and the conformations they adopt relative to the groups at the α position. A further fundamental difference between S═O and C═O bonds that may be of great relevance to the fluorine-induced physicochemical differences is the fact that the former group exists only as a polar double bond, while the latter group exists in an enol−ketone equilibrium, participate in $\pi-\pi$ coupling with an adjacent π system and stabilizes α-carbanion by delocalization. These issues will be widely discussed in the following theoretical analyses, which include a conformational analysis as well as a study of the atomic charges (by both NBO and Hirshfeld analyses) of the oxygen atoms and relative dipole moments of each conformer, using density functional theory (DFT) analysis. As will also be shown, these motifs are important determinants to the differences in the observed physicochemical properties of these two families.

Next, following our previous insights into the H-bond-donating nature of the CF_2H group,[14,18] we hypothesized here that a reduction in the H-bond basicity of various FGs can be achieved not only inductively due to the high electronegativity of the fluorine atom but also via an IMHB between the neighboring basic group and this unique fluorine function. This can occur in compounds such as **2c-F2**, **3-F2**, **4-F2**, and **5-F2** as illustrated in Figure 3. IMHB is a very important motif in general chemistry and particularly when applied to medicinal chemistry.[48] Such intramolecular interactions exist in cases where a donor and an acceptor are in proximity on the same molecule, which may lead to a "cyclic" stable conformer in which the polarity of both functional groups is "hiding" from the environment. This may in turn lead to not only changes in binding affinity but also enhancement of lipophilicity and modulation of membrane permeability.[49] As far as we know, an IMHB between CF_2H and a proximate H-bond-accepting function has rarely been reported,[31,50] and herein, it is the first time that the IMHB effect of the CF_2H function on the H-bond basicity of a nearby FG is described. Thus, for studying this issue, we chose four different matched pairs, in which the parent compounds ester, aniline, pyridine, and 2-pyridone were characterized by medium (**2c**, **3**, and **4**) or strong (**5**) H-bond basicity, respectively. It is known that IMHB can reduce solute H-bond acidity (*A*, the ability of a molecule to act as a H-bond donor), due to a decrease in H-bonding with the solvent.[51] Recently, we have reported on the H-bond acidity of various compounds holding the CF_2H moiety using a ^1H NMR method.[14,18] We found that the CF_2H group acts as a H-bond donor with a range of *A* values (0.035−0.165) that strongly depend on the attached FG (when FG = EWG, the *A* value is higher). Therefore, it is reasonable to assume that IMHB will reduce not only the pK_{HB} of the FG but also the *A* value of the proximate CF_2H group. Indeed, we found that in one of the above-mentioned compounds, i.e., in 2-pyridone **5-F2**, both

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groups mutually, significantly affect each other, so that a prominent reduction of pK_{HB} (FG) and a very low A (CF_2H) value were observed (Figure 3). Therefore, this compound seems to hold a strong IMHB. Specifically, measurement of the H-bond basicity of the difluorinated compounds **2c-F2**, **3-F2**, **4-F2**, and **5-F2** reveals that except for aniline **3-F2** all cases show a significant reduction in pK_{HB} values with **5-F2** having the largest $\Delta pK_{HB(CH3\text{-}CF2H)}$ value, while **2c-F2** and **4-F2** are affected moderately. It is well known that IMHBs via six-membered rings (MRs) are stronger than those formed via five-MRs.[48,51] However, the case of aniline **3-F2**, for which both a 6- and 5-MR may be theoretically considered, showed a very low $\Delta pK_{HB(CH3\text{-}CF2H)}$ value, suggesting that no IMHB occurred.

Conformational analysis of **3-F2** by a DFT study reveals the existence of two conformers with minimum energy, differing by the orientation of the CF_2H group (nearly perpendicular versus nearly planar to the plane of the ring), which are energetically comparable. However, both conformers do not support any IMHB, supporting the experimental observations of the A values for NH_2 (similar to aniline itself) and CF_2H groups, as well as the pK_{HB} value of the NH_2 group. These effects are currently under investigation both experimentally and computationally.

Focusing on the difluoromethylated compounds of the type FG-CF_2H, one may conclude that the noteworthy influence of this fluorine function on the H-bond basicity of various functional groups can be attributed to a variety of effects (Figure 4). Starting with ether, the decrease in the H-bond basicity, i.e., the high $\Delta pK_{HB(CH3\text{-}CF2H)}$, observed can mainly be attributed to the inductive effect of the C−F bond and the anomeric effect that characterizes the α-halo ethers.[52] For sulfoxide and sulfones, it is well known that these groups are affected only inductively, and therefore, it is reasonable to assume that the effect α-fluorine has on these functions is mainly inductive. On the other hand, for their ketone counterparts, which hold the C═O π double bond that may exist in enol-ketone equilibrium and may adopt different conformations (together with its $\pi-\pi$ coupling with an adjacent π system), the effect may not be only inductive as will be discussed in the following theoretical section. Finally, as demonstrated above, we show that the $\Delta pK_{HB(CH3\text{-}CF2H)}$ value may be influenced also by IMHB between the FG and the H-bond-donating group CF_2H, as was clearly observed for the 2-pyridone **5-F2** and to some extent in the ester **2c-F2** and pyridine **4-F2**.

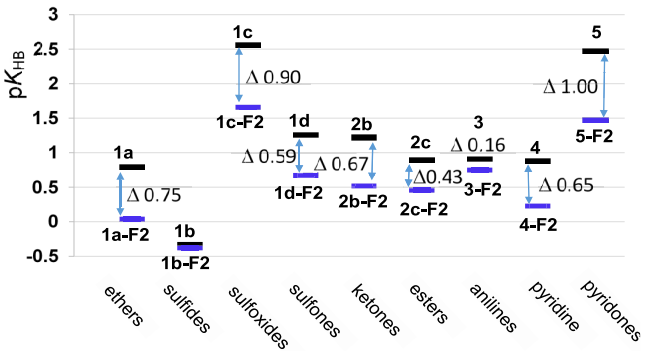

Figure 4. Effect of α-difluoromethyl substitution on the pK_{HB} values of various functional groups.

Lipophilicity (log $P_{o/w}$) Measurements. The influence of fluorine atom(s) on the H-bond basicity can impact the rational design of compounds relevant to many chemistry fields. Here, we are particularly interested in the effects of the H-bond basicity change on the lipophilicity, a physicochemical property with great relevance to drug development. In our previous paper, we studied the influence of the difluoromethyl function on the lipophilicity of various compounds and found that in some cases such as alkyl ethers, sulfoxides, and sulfones, there is a larger increase in lipophilicity relative to other functions such as anisoles, thioanisoles, methylarenes, and thioethers.[18] We assumed that the increase in lipophilicity in these compounds is affected not only by the interplay between volume and polarity but also by H-bond basicity modulation. In this context, in the present study, we decided to investigate the effect of the fluorine function on the lipophilicity of all of the above-mentioned compounds (depicted in Table 1), with respect to the influence of their H-bond basicity on this property. Therefore, we measured and compared the lipophilicity of all series and new matched pairs depicted in Table 1 (Figure 5). As previously reported by us, the replacement of CH_3 by CF_2H at the α position to ether leads to a significant increase in lipophilicity, whereas for thioethers, the change is relatively minor ($\Delta \log P_{o/w}$ 1.0 vs 0.2, respectively). Observing the effect of the H-bond basicity described above, one may conclude that this difference in increase of lipophilicity between ether and thioether may be explained by the finding that there was a significant drop in H-bond basicity for the

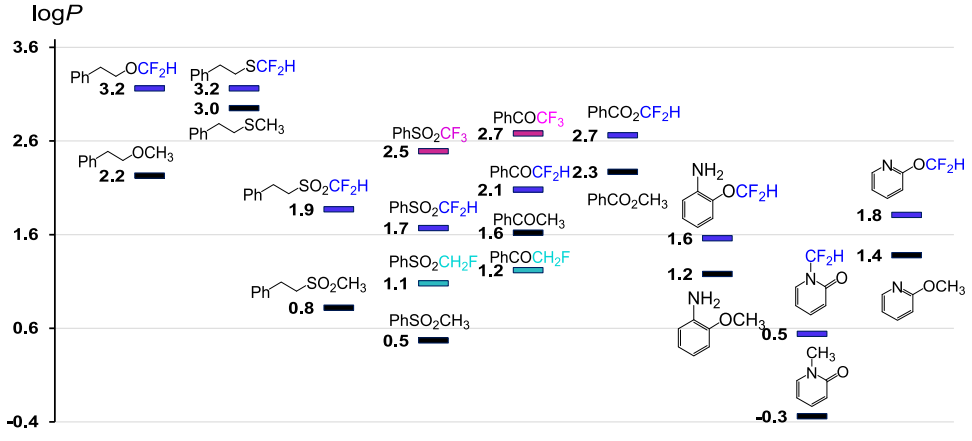

Figure 5. log $P_{o/w}$ values of various compounds having α-mono, di, and trifluorinated substitution of various functional groups.

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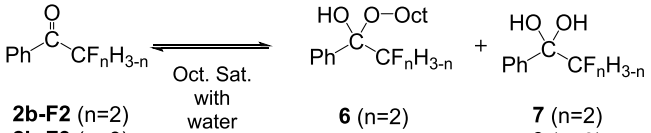
ether, whereas there was no such change for the thioether. Note that, typically, H/F replacement in compounds leads to an addition of ca. 0.2 units of log *P* for each F atom. A similar behavior was recorded for most other compounds described above, namely, a significant fluorine-induced decrease in H-bond basicity led to a large increase in lipophilicity as well. This trend is clearly demonstrated for the sulfone series **2a-Fn**, which display a linear increase in the log $P_{o/w}$ values with the number of F/H replacements at the α position (see Figure 7 vide infra, right). The effect was also found to be relevant for the compounds holding an IMHB between a H-bond-accepting function and the CF_2H moiety as clearly observed for the 2-pyridones **5**, **5-F2** matched pair. The results obtained for 2-pyridone **5-F2** are interesting and highly important since the CF_2H moiety in such *N*-difluoromethylated amides can act as a bioisostere of OH in bioactive hydroxamic acids, which are excellent ligands for metals and are a key pharmacophoric element for matrix metalloprotease and histone deacetylase inhibitors.[53,54] The results described in the present work clearly show that due to the IMHB in 2-pyridone **5-F2**, the H-bond acidity of the CF_2H moiety is negligible (*A* 0.028), the H-bond basicity is significantly reduced ($\Delta pK_{HB(CH3\text{-}CF2H)}$ = 1.00), and as a consequence the lipophilicity is significantly increased (Δlog $P_{o/w}$ 0.8) when CH_3 is replaced by CF_2H (see Figures 3 and 5). Therefore, when the difluoromethyl group is applied as a bioisostere of hydroxyl in bioactive hydroxamic acids (OH/CF_2H), which may also exhibit an IMHB, one may expect that both the H-bond acidity and basicity will be reduced significantly and the lipophilicity will increase considerably.

An unusual behavior was recorded, again, for the ketone series, for which a reliable measurement of the log $P_{o/w}$ values, specifically for compounds **2b-F2** and **2b-F3**, was not possible by the standard method. During our attempts to measure the log $P_{o/w}$ values of these fluorinated ketones using the standard "shake-flask" UV−vis-based method, we found, not entirely surprisingly, that for both difluoro- and trifluoromethyl ketones **2b-F2** and **2b-F3** the absorption rapidly decays during the experimental time span (Figure S27, SI). ^{19}F NMR analysis of the separated solutions revealed that in octanol saturated with water, fluoroketones **2b-F2** and **2b-F3** undergo a facile reaction with octanol and water to form the corresponding hemiketals **6** and **8** and gem-diols **7** and **9**, respectively (Scheme 1 and Figures S28 and S29, SI). In dry octanol or in

Scheme 1. Reactions of Ketones 2b-Fn with Octanol and Water



2b-F2 (n=2) **2b-F3** (n=3) **6** (n=2) **8** (n=3) **7** (n=2) **9** (n=3)

pure water, these ketones form solely the appropriate hemiketals **6** and **8** or gem-diols **7** and **9**, respectively (Figures S30 and S31, SI). In octanol, the hemiketal **6** is represented by an ABq system at −130.0 and −132.7 ppm (dd) owing to the diastereotopic nature of the fluorine atoms. It should be noted that the monofluoromethylated ketone **2b-F** was found to be stable in the octanol saturated with the water phase under the same conditions (Figure S32). The higher reactivity in hydration reactions of trifluoromethyl ketones versus their monofluoromethylated counterparts was previously reported;[55] however, as far as we are aware, the comparison between the two former compounds, i.e., **2b-F2** and **2b-F3**, as emerged from the current study, has not yet been reported. Most importantly, from the partition coefficient determination point of view, the fact is that all of the species involved exist in an equilibrium with their parent ketones. The equilibrium constants, determined by ^{19}F NMR, for the fluorinated ketones **2b-F2** and **2b-F3** are as follows: in pure octanol log *K* 0.52 and 1.26 and in pure water log *K* 0.96 and 2.2, respectively. Therefore, a measurement of the apparent octanol/water partition coefficient of **2b-F2** and **2b-F3** using UV−vis spectroscopy does not represent a "true" log $P_{o/w}$ of the ketone species. The law of mass action defines the concentration quotients of each molecular species in equilibrium as a constant, i.e., for the water/octanol system, molecules containing more than one species in solution at equilibrium require elucidation of the true or, in other words, the specific log *P* for each species (Scheme 2). This has been done extensively for ionizable molecules, using the ionization constants measured by different methods such as UV−vis spectroscopy and potentiometric methods to elucidate the micro-log *P* from the measurement of apparent log *P*.[56,57] The specific log *P* of different conformers was also calculated using the macroscopic log *P* determined experimentally in combination with rotamer populations derived from NMR analysis.[58,59] For the above-mentioned fluorinated ketones, although equilibrium constants of the reactions were found using ^{19}F NMR, the macroscopic or apparent log $P_{o/w}$ could not be measured using UV spectroscopy, and therefore these indirect methods are not applicable in our case. Previously, since the octanol/water partition coefficient could not be measured directly, the log $P_{o/w}$ values of some trifluoromethyl ketones were determined using an indirect method. For example, the log *P* value of 3-octylthio 1,1,1-trifluoronpropane-2-one (OTFP), an inhibitor of juvenile hormone esterase and neuropathy target esterase, was measured in the cyclohexane/water system and then octanol log $P_{o/w}$ was calculated using the solvent regression equation.[60]

Based on these considerations and on the observation that each species can be well defined and characterized separately in each phase by a ^{19}F NMR analysis, we hypothesized that the ^{19}F NMR-based method developed recently by Linclau et al.[61] may be appropriate for the specific log $P_{o/w}$ determination of both **2b-F2** and **2b-F3** and even their hydrated forms **7** and **9**, respectively (see Figure 6). The species population in each phase can be simply determined by integrating their signals against the trifluoroethanol reference (TFE), enabling the direct measurement of the specific log $P_{o/w}$ of each species via the following equation: ($\log Px_{(o/w)} = \log P_{(TFE)} + \log (Ix_{(oct)}/Ix_{(w)})$).[61] First we measured the log $P_{o/w}$ value of the stable mono-fluoroacetophenone **2b-F**, for which we obtained the accurate value of 1.32 ± 0.08 determined by the regular UV−vis-based method, and a comparable value of 1.22 ± 0.03 was obtained by the ^{19}F NMR-based method (Figure S33, SI). Next, we measured the log $P_{o/w}$ values of the difluoro- and trifluoromethyl ketones **2b-F2** and **2b-F3** by Linclau's method and obtained the values 2.08 ± 0.03 and 2.68 ± 0.04, respectively (Figures S34 and S35, SI). We were able to also extract the log $P_{o/w}$ values of the diol products **7** and **9** (0.80 ± 0.02 and 1.56 ± 0.05, respectively). The accuracy of these values has been confirmed by measuring a triplicate of different samples that was taken from each experiment (one of them

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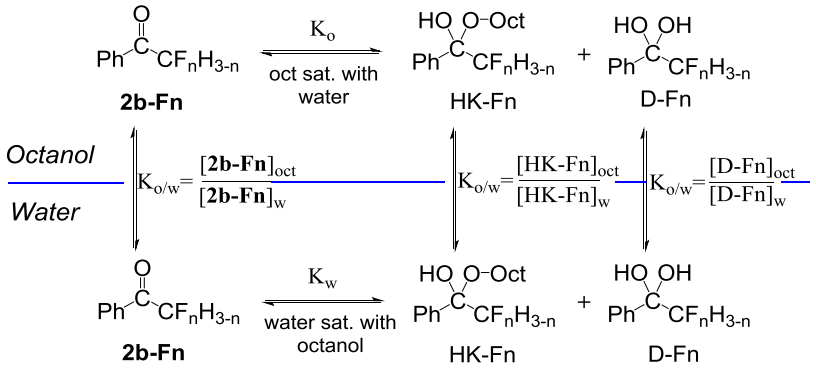
Scheme 2. Equilibrium Constants of 2b-Fn (n = 2, 3) in an Octanol/Water Mixture



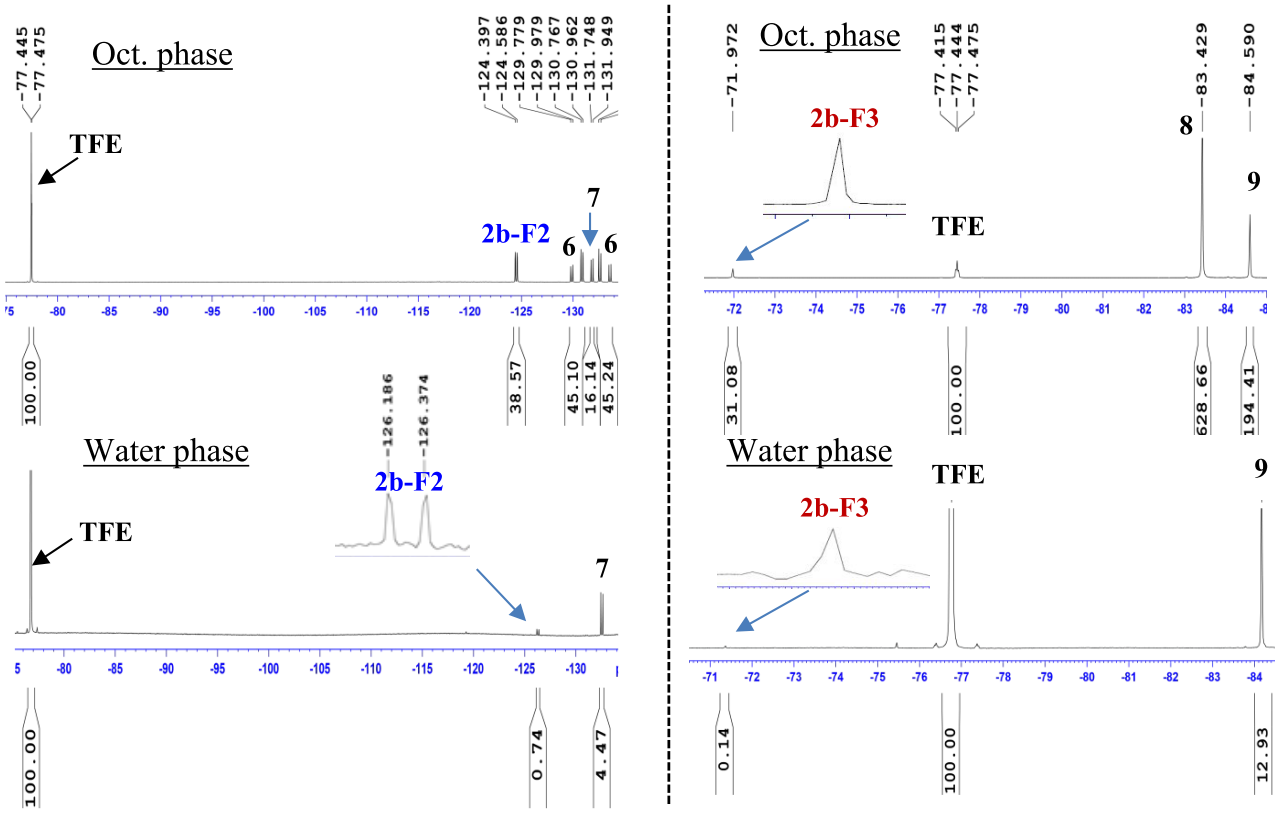


Figure 6. Measurement of the log $P_{o/w}$ values of ketones **2b-F2** and **2b-F3** and diols **7** and **9** by the ^{19}F NMR-based method.

was also run thrice), which gave low standard deviations. It should be noted that despite the high sensitivity of the ^{19}F NMR analysis, both hemiketals **6** and **8** could not be detected in the aqueous phase, indicating their very high log $P_{o/w}$ values, which preclude their partition into the aqueous phase in any detectable amount. As far as we are aware, it is the first time that such a direct analysis of octanol/water partition coefficient measurement is reported for a mixture of various species that exist in equilibrium. Currently, the scope of this approach for other systems in equilibrium is under investigation.

The collective results show, again, a nonlinear behavior of the peculiar fluorinated ketone series, namely, nonadditive outcomes for consecutive H/F replacement, a finding which may be of great relevance for drug design and SAR (bioisosterism) study of carbonyl-containing bioactive compounds (Figure 7, left). This series was found to be significantly different from all of the above-mentioned compounds and particularly from its sulfone series counterpart,

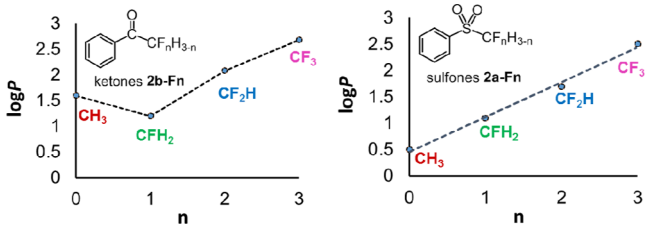


Figure 7. Fluorine effects on the lipophilicity (log $P_{o/w}$) of sulfones (**2a**, right) and ketones (**2b**, left).

namely, a lack of correlation between physicochemical properties (pK_{HB} and log $P_{o/w}$) and the consecutive H/F replacement at the α-methyl moiety (see Figures 2 and 7). This point is demonstrated in the fluoromethyl ketone **2b-F** where the pK_{HB} value was reduced compared to its non-fluorinated analogue **2b** (Δp$K_{HB(CH3\text{-}CFH2)}$ 0.27), yet, at the

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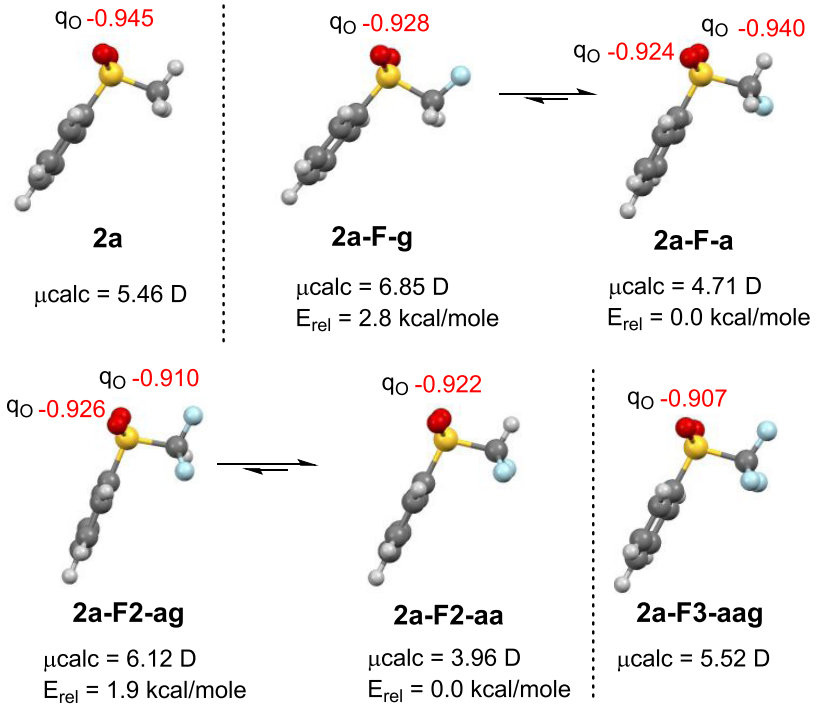

Figure 8. Optimized conformational geometries (anti (a); gauche (g)), relative dipole moments, and charge distribution of the FG's oxygen atom(s) (NBO) of the sulfone series **2a**, **2a-F**, **2a-F2**, and **2a-F3** calculated at the m062x/6-311++g(d,p) level (gray, carbon; cyan, fluorine; red, oxygen; and yellow, sulfur).

same time, its lipophilicity was unexpectedly significantly reduced ($\Delta\log P_{(CH3\text{-}CFH2)}$ −0.4). At the other end, the trifluorinated ketone **2b-F3** reduces the H-bond basicity to nearly the same extent as that of its difluorinated counterpart **2b-F2** ($\Delta pK_{HB(CF3\text{-}CF2H)}$ 0.09), yet possessing a much higher lipophilicity ($\Delta\log P_{(CF3\text{-}CF2H)}$ 0.61).

As mentioned above, we focused our study on both sulfones and ketones due to their importance in drug design and in view of the fact that these functions appear in many bioactive compounds. While in sulfones the fluorine-induced effects described above are mostly inductive and additive, in their ketone analogues, the effects were found to be much more complicated and demonstrate a multitude of influences, which in some cases may form the basis for differing biological activity of the different groups. An excellent example is the trifluoroketones (TFMKs), which, owing to their high activity toward nucleophiles, exhibit high activity as reversible inhibitors for acetylcholinesterase,[62] SARS-Cov 3CL protease,[63] cytosolic phospholipase A2 (cPLA2, studied for neuroprotection and multiple sclerosis and cancer)[64] and so on.[65] For instance, serine proteases interact with the TFMK function to form a stable hemiketal that eventually leads to reversible enzyme inhibition. Similar compounds holding α,α-difluoroketones[66] or arylmethylene ketones,[67] which were found to be effective inhibitors for malarial aspartic protease plasmepsin II and IV or for SARS 3C-like proteinase, respectively, display similar motifs as reversible inhibitor analogous to their TFMK counterparts. Interestingly, the difluoromethyl ketones (DFMKs) are much less abundant in bioactive compounds, and the above-mentioned results clearly position this function as a good potential surrogate for the TFMK function (vide infra). A noticeable example for such replacement was recently reported by Carlier and co-workers who demonstrated the superiority of CF_2H over both CF_3 and CFH_2, when studying the bioactivity of various α-fluoromethyl ketones as potent inhibitors of wild-type and resistant G119S mutant Anopheles gambiae acetylcholinesterase.[68] Docking studies revealed that the lower reactivity of the α-CF_2H carbonyl group relative to its CF_3 analogue is compensated by a H-bond interaction between the hydrogen of the former moiety and the S119 oxygen in the active site, rationalizing the excellent inhibition by the difluoromethylated ketones versus their mono- and trifluoromethylated counterparts. From the equilibrium constants of the ketone series mentioned above, we learn that the fluorination at the α position to ketones modulates the electrophilicity of the carbonyl group toward nucleophiles with the order $CH_2F \ll CF_2H < CF_3$ (for alcohol or water). Therefore, it is obvious that such modulation will significantly affect their interactions with hydroxyl groups in the active site of enzymes. The findings described in the present study clearly show that other effects such as the difference in H-bond basicity or the lipophilicity may be of great relevance to the activity of α-fluoromethylated ketones. The differences in both pK_{HB} and $\log P_{o/w}$ values of the difluoromethyl ketones and their trifluoromethylated analogues, together with the fact that both react with nucleophiles in a similar manner (even if at a different rate), imply the high potential of CF_3/CF_2H replacements in bioisosterism studies.

DFT Study of the Ketone and Sulfone Series. The prominent differences between the series of sulfones **2a-Fn** and ketones **2b-Fn** upon fluorination at the α position, as evidenced by the experimental findings described above, and the importance of these functions in medicinal chemistry encouraged us to further explore the theoretical basis behind the above-mentioned phenomena using a DFT study. Thus, conformational analyses, which included optimized gas phase geometries; relative dipole moments (overall polarity); and charge distribution (by both NBO and Hirshfeld analyses) for

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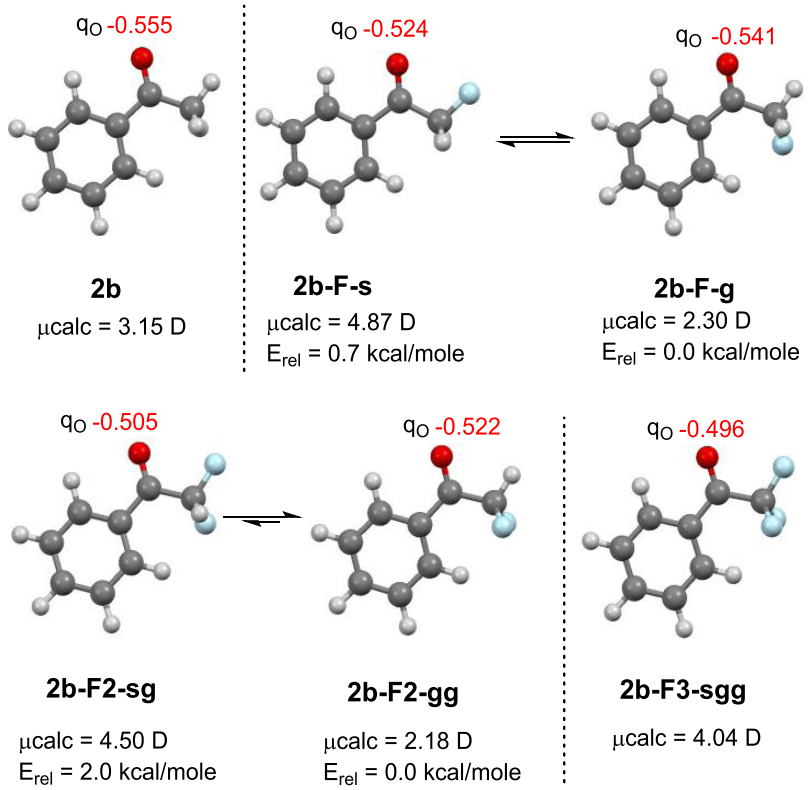

Figure 9. Optimized conformational geometries (syn (s); gauche (g)), relative dipole moments, and charge distribution of the FG's oxygen atom(s) (NBO) of the ketones **2b**, **2b-F2**, **2b-F3**, and **2b-F** calculated at m062x/6-311++g(d,p) levels of theory (gray, carbon; cyan, fluorine; red, oxygen).

all stable conformations of both series were calculated at the m062x/6-311++g(d,p) level (see Figures 8 and 9). For the sulfone series, we found that in all four compounds the staggered conformation (rotation about the SO_2-CF_nH_{3-n} bond) is energetically favored. In addition, for all stable conformations, both the SO bonds lay out of the plane of the benzene ring. Only the mono- and difluoromethyl sulfones **2a-F** and **2a-F2** are axially anisotropic and, therefore, can adopt different conformations having different energies, polarities, and charge distributions. For the monofluoromethyl sulfone **2a-F**, the geometry in which the fluorine atom is in the anti position in relation to the S−O bond (**2a-F-a**) was found to be more stable by 2.8 kcal/mole than that of the gauche rotamer (**2a-F-g**). Expectedly, the overall polarity of the more stable **2a-F-a** rotamer, in which the S−O dipole is offset by the polarized C−F bond, is smaller than that of the less stable **2a-F-g** rotamer and is also smaller than that of the non-fluorinated parent compound **2a**. Similarly, the calculations for the difluoromethylated counterpart **2a-F2** show that the overall polarity of the more stable **2a-F2-aa** rotamer, in which the two S−O dipoles are offset by two C−F bonds, is lower than that of the less stable rotamer **2a-F2-ag** and even lower than that of both non- and mono-fluorinated analogues. Contrary to this trend, the trifluoromethyl analogue **2a-F3** exhibits higher polarity than both mono- and difluoromethylated counterparts (in their stable conformations) and even somewhat exceeds the overall polarity of the non-fluorinated analogue.

These results are rather interesting, as the natural charge average of the oxygens (by both NBO and Hirshfeld analyses) was found to be in linear proportion to the consecutive fluorine addition at the α-methyl moiety (Figure 8 and S36, SI). This correlation is in accordance with the linear proportion that was experimentally found between pK_{HB} and the number of fluorine atoms on the α-methyl sulfone **2a** (see Figure 2). It is well known that the main factors that influence $\log P_{o/w}$ values are the solute H-bond basicity and dipolarity/polarizability, which favor water, and the solute volume, which favors octanol.[69] Hence, the lower the H-bond basicity, the higher the $\log P_{o/w}$ is expected to be. As the experimental lipophilicity of the sulfone series was also found to be dependent linearly on the consecutive addition of fluorine atoms, the above-mentioned calculated results clearly show that it ($\log P_{o/w}$) must be an outcome of the interplay among H-bond basicity, dipolarity/polarizability, and volume, altogether significantly affected by H/F replacement. The relatively high polarity of the trifluoromethyl sulfone, which is an exception to the observed trend for the related mono- and difluoromethyl sulfones, seems to be overcompensated by the comparatively large volume and low H-bond basicity of this group. It should be noted that for all aspects described above, both experimental and theoretical, the effects induced by the fluorine atom(s) in the sulfone series are purely inductive.

In the ketone series, the effect of H/F replacement was found to be, again, more complicated. First, and contrary to their sulfone counterparts, the ketone **2b** adopts an eclipsed conformation, as well as its fluorinated analogues, and even the trifluoromethylated ketone **2b-F3** adopts this geometry as the most stable conformation (Figures 9 and S37, SI). For the latter compound, a conformational diagram, when calculating the energy as the O=C−CF_3 bond is rotated through 10° increments in the dihedral angle F−C−C−O, in the gas phase, shows that the eclipsed conformer is at a minimum energy, while the staggered one is at a maximum energy. Moreover, a similar calculation, in which the carbonyl group has been

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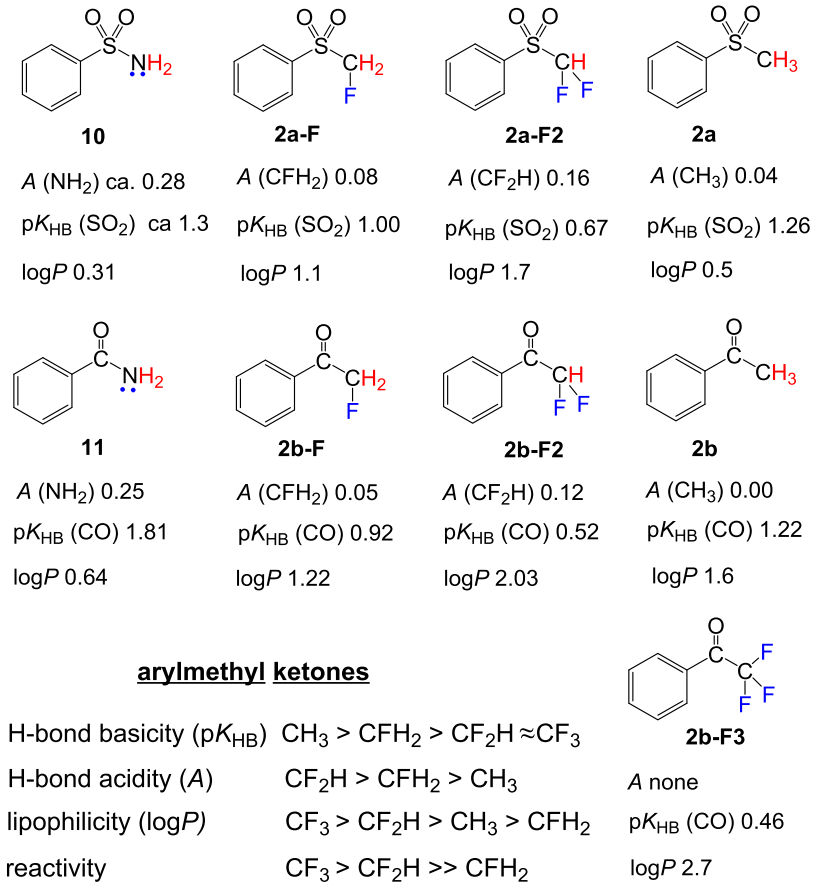

Figure 10. Physicochemical effects expected in bioisosteric replacements of NH_2/CFH_2 or NH_2/CF_2H in amides and sulfone amides or CH_3/CF_nH_{3-n} in sulfones and ketones.

"frozen" perpendicular to the plane of the benzene ring, shows that even in this form, where the loss of the $\pi-\pi$ coupling between the carbonyl and the phenyl ring raises the energy by 5.9 kcal/mole, the preferred conformer is that in which a fluorine atom is in an eclipsed position relative to the carbonyl oxygen (Figure S38, SI). Interestingly, the conformational analysis of the monofluoromethyl ketone **2b-F** shows that this compound exists in two energetically comparable stable conformers (m062x/6-311++g(d,p)), i.e., **2b-F-s** (dihedral angle of 0°, syn) and **2b-F-g** (F-C- C−O dihedral angle of ca 140°, gauche), having different polarities (4.87 and 2.30 D, respectively) as shown in Figure 9. These results are in accordance with those recently reported by Pattison,[70] who calculated the conformational energy profile of monofluoroacetophenone (**2b-F**) and found that in the gas phase gauche (ca 140°) is the optimal geometry, while in ethanol, the more polar conformer, i.e., syn (0°), was found to be the most stable one. In our calculations, in the gas phase, both conformers are energetically comparable, however, as they are very different in terms of polarity, they may be considered to be conformational adaptors to changing environments, as suggested by Müller and co-workers for other partially fluorinated alkoxy groups.[52] The difluoromethyl ketone **2b-F2** exhibits a similar conformational profile, where the eclipsed conformer **2b-F2-gg** is in the lowest energy and more stable than the local minimum conformer **2b-F2-sg**, by 2.0 kcal/mole (Figure 9). Also, here, the more stable rotamer **2b-F2-gg**, where the polar carbonyl group is offset by two C−F bonds, is the less polar conformer (2.18 D) compared to **2b-F2-sg**, which has one syn C−F bond and only one C−F bond in an opposite direction to the carbonyl dipole. The symmetrical trifluoroketone stable conformation **2b-F3-sgg** exhibits higher polarity since one of the three C−F bonds must always be in the syn orientation relative to the carbonyl function in every eclipsed conformation. As in the case of the sulfone series (**2a-Fn**), also for the ketone series (**2b-Fn**), the natural charge calculated for the carbonyl oxygen (average), both via NBO and Hirshfeld analyses, was found to be linearly proportional to the consecutive fluorine addition at the α-methyl moiety (Figure S39, SI). However, this correlation is only partially in accordance with the nonlinear proportion that was experimentally found between the pK_{HB} values and the number of fluorine atoms added to the α-methyl ketone **2b** (see Figure 2). This exception is related to the trifluoroketone **2b-F3** where the pattern drop in the oxygen's charge calculated for **2b-F3-sgg** follows the expected trend and the relatively "higher than expected" pK_{HB} value, observed experimentally, may partially be related to steric effects between the bulky trifluoromethyl group and the *p*-FP in the complex formation. The calculations for both mono- and difluoroketones, **2b-F** and **2b-F2**, respectively, clearly explain the exceptional lipophilicity determined for this ketone series, namely, the negative $\Delta\log P_{(CH3\text{-}CFH2)}$ (−0.4) for the former compound and the positive $\Delta\log P_{(CH3\text{-}CF2H)}$ (+0.4) for the latter one in relation to their non-fluorinated parent ketone **2b**. The computational study shows that both energetically comparable most stable conformers **2b-F-s** and **2b-F-g** hold contrasting properties in terms of factors that determine the octanol/water

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partitioning coefficient. Namely, the former syn conformer is more polar (favoring water) and holds a smaller negative charge on the oxygen atom (favoring octanol), while the latter gauche conformer is less polar (favoring octanol) yet holds a larger negative charge (favoring water). However, the significantly increased polarity of conformer **2b-F-s** (compared to **2b**) is larger than the polarity decrease calculated for conformer **2b-F-g**, and therefore, overall, the interplay among polarity, H-bond basicity, and volume dictates a decrease in lipophilicity after one H/F replacement in **2b**. The difluoromethyl ketone **2b-F2** exhibits a higher log *P* value compared to its non-fluorinated counterpart **2b** as expected in light of the significant decrease in both polarity and the oxygen's negative charge calculated for its most stable conformer (favoring the octanolic phase). As in the case of sulfone **2a-F3**, the relatively high polarity of the trifluoromethyl ketone **2b-F3**, which is an exception to the observed trend for the related mono- and difluoromethyl ketones, seems to be overcompensated by the comparatively large volume and decreased H-bond basicity of this group, leading to a higher log *P* value.

Bioisosterism Considerations. Since numerous drugs contain primary amide or sulfone amide[71] functions, it is important to discuss their potential in fluorine-based bioisosterism, considering the findings described above (Figure 10). Although less abundant in drugs, the methyl sulfonyl and methyl ketone groups are also discussed here in this context. Inspection of the data presented in Figure 10 reveals that the SO_2-NH_2 group in sulfone amides is a moderate H-bond acceptor (SO_2)[26] and donor (NH_2).[72] Replacement of the NH_2 group in sulfone amide **10**, by CFH_2 (**2a-F**) or CF_2H (**2a-F2**), which also act as weak H-bond-donating groups, causes a considerable increase in the compounds' lipophilicity. Therefore, both mono- and difluoromethyl groups can serve as more lipophilic bioisosteres of NH_2 in primary sulfone amides and at the same time can regulate the H-bond basicity of the sulfonyl moiety. Similar changes were observed when analyzing the replacement of the CH_3 group of **2a** by these fluoromethyl groups, i.e., the lipophilicity increased, the H-bond basicity decreased, and to some extent the H-bond acidity increased. Therefore, for methylsulfones ($MeSO_2$), the mono- and the difluoromethyl groups can also act as more lipophilic bioisosteres of the methyl group and as a H-bond basicity modulator. Similar considerations show that replacement of the NH_2 group in the primary amide **11** causes a significant decrease in the H-bond basicity of the carbonyl function along with a significant increase in the compound's lipophilicity. Both fluoromethyl functions in **2b-F** and **2b-F2** can act as weak H-bond donors. Therefore, also here, both CFH_2 and CF_2H can serve as more lipophilic bioisosteres of NH_2 in primary amide compounds. On the other hand, replacement of the methyl group in methylketones such as **2b** by fluoromethyl functions (CFH_2 or CF_2H) can modulate the lipophilicity either up or down along with the expected decrease in the H-bond basicity. The reactivity order of fluoromethyl ketones toward nucleophiles $CF_3 > CF_2H \gg CFH_2$, as well as their metabolic stability (potential hazardous metabolites),[5] should be taken into account when considering replacements between these groups, yet, at the same time, the expected alterations in the lipophilicity and the H-bond abilities should be considered.

CONCLUSIONS

In conclusion, the present work describes the first systematic study on the effect α-fluorine substituents (CF_nH_{3-n}) have on the H-bond basicity of various proximate FGs and its implications for lipophilicity and bioisosterism considerations.

(a) In most compounds investigated including ethers, sulfoxides, sulfones, ketones, esters, anilines, pyridines, and 2-pyridones, the α-fluorine functions cause a significant decrease in the H-bond basicity (pK_{HB}) of the proximate FG. The effects vary and include anomeric effects, inductive dipole offset, and IMHB ($CF_2H\cdots FG$). Thus, the pK_{HB} value of various FGs may be rationally modulated by introducing a CF_nH_{3-n} function at the α position.

(b) In cases in which an IMHB between the H-bond-donating group CF_2H and the FG occurs, both reduction of the H-bond basicity of the FG and H-bond acidity of the CF_2H group were observed.

(c) For most cases (except for that of the α-monofluoro ketone), the reduction in the pK_{HB} value of a compound is accompanied by an increase in the log $P_{o/w}$ value, since the H-bond-accepting ability of a compound, partitioned between octanol and water, significantly influences its interaction with these solvents.

(d) The results show that contrary to the additive effect observed in the sulfone series for both pK_{HB} (decrease) and log $P_{o/w}$ (increase) values for consecutive H/F replacement, a nonlinear behavior of the fluorinated ketone series was observed, a finding that may be of great relevance for drug design and structure activity relationship (SAR; bioisosterism) study of sulfonyl- and carbonyl-containing bioactive compounds.

(e) The unique case of the α-di- and trifluoromethyl ketones (**2b-F2** and **2b-F3**), which exist in octanol/water phases in equilibrium with the corresponding hemiketals **6** and **8** and gem-diols **7** and **9**, prevents direct log $P_{o/w}$ determination by conventional methods. However, we showed that the specific log $P_{o/w}$ values of these species can be determined directly, for the first time for such complicated cases, using Linclau's ^{19}F NMR-based method.

(f) A theoretical study of both sulfone **2a-F$_n$** and ketone **2b-F$_n$** series using DFT calculations shows that the CF_nH_{3-n} function at the α position to these FGs affects the overall polarity of the compounds (n = 1, 2 reduced; n = 3 increased relative to n = 0) and the charge distribution (oxygen's negative charge linearly reduced with consecutive H/F replacement). The conformational analysis, which included optimized gas phase geometries, shows that the sulfones adopt staggered conformations for rotation about the S-CF_nH_{3-n} bond, in which the most stable conformers for CFH_2- and CF_2H-containing sulfones are those where the C−F bond is in the anti position to the S−O bond. The ketones, on the other hand, adopt the eclipsed conformations so that for the monofluoromethyl ketone **2b-F** both conformers, i.e., **2b-F-s** (dihedral angle of 0°, syn) and **2b-F-g** (dihedral angle of ca 140°, gauche), are energetically comparable. However, as they are very different in terms of polarity, they may be considered to be conformational adaptors to changing environments.

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(g) In terms of bioisosterism, when considering CF_2H as a lipophilic hydrogen bond donor bioisostere of NH_2 (or CH_3), placed α to H-bond-accepting functional groups, a pronounced H-bond-donating ability is presented, making this moiety an appealing bioisostere in drug design. Interestingly, even the monofluoromethyl (CFH_2) exhibits in such cases some H-bond-donating ability. Importantly, it should be taken into account that the H-bond basicity of such functional groups as well as the lipophilicity is expected to be significantly altered.

EXPERIMENTAL SECTION

General Methods. All reagents were obtained from commercial suppliers and were dried using standard methods when necessary. 4-Fluorophenol (p-FP) was sublimed under vacuum and dried in an oven-dried Schlenk tube. CCl_4 was dried before use on activated molecular sieves (4 Å). ^1H, ^{13}C, and ^{19}F NMR spectra were obtained using either an 11.7 T spectrometer (500, 125, and 471 MHz, respectively) or a 7.0 T spectrometer (300, 75, and 282 MHz, respectively). Chemical shifts are reported in parts per million (δ, ppm). ^1H NMR chemical shifts were referenced to the residual $CDCl_3$ (δ = 7.26 ppm) and DMSO-d_6 (δ = 2.50 ppm). ^{19}F NMR chemical shifts are reported downfield from external trifluoroacetic acid in D_2O. In ^{13}C NMR measurements, the signal of $CDCl_3$ (δ = 77 ppm) was used as reference. Column chromatography was performed with silica gel 60 (230−400 mesh). UV absorbance for log $P_{o/w}$ calculations was recorded on an UV−vis spectrophotometer from Amersham Biosciences, Ultrospec 2100-pro model. Solutions of 10 mg/mL were prepared in $CDCl_3$ and DMSO-d_6 for the determination of H-bond acidity (A values)[72] with standard deviations in the calculation of A from repeated measurements less than 1%. Fourier transform infrared spectroscopy (FT-IR) analyses were performed using the Bruker Equinox 55 FT-IR instrument. The spectra were recorded at a resolution of 1 cm^{-1}. A 10 mm path length, quartz Infrasil cell was used.

Materials. Difluoromethylated compounds **1a-d-F2** and **2c-F2**, depicted in Table 1, were synthesized according to procedures previously reported by us using *O*-diethyl(bromodifluoromethyl) phosphonate as a difluorocarbene precursor.[14,18,44,45] All compounds used in this study were of high degree of purity (>95%, by ^1H NMR).

*2-(Difluoromethoxy)pyridine (**4-F2**) and 1-(Difluoromethyl)-pyridin-2(1H)-one (**5-F2**).* 2-Hydroxy pyridine (1.9 g, 20 mmol) was dissolved in 100 mL of acetonitrile in a round-bottom flask, which was placed in an ice bath. A solution of 4 M KOH (100 mL, 400 mmol) was then added to the mixture, which was stirred vigorously for a few minutes. The phosphonate (7.1 mL, 40 mmol) was then added dropwise to the reaction mixture, which was kept in the ice bath for an additional ∼30 min. After that time, the ice bath was removed and the reaction mixture was stirred at RT for an additional ∼2.5 h. The organic layer was separated, and the aqueous phase was extracted with ether (∼100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated with no heat to provide a crude mixture that holds products **4-F2** and **5-F2**. Purification of this mixture on silica using a gradient of 10−15% ethyl acetate in hexane provided the desired products in a pure form as observed from their ^1H, ^{13}C, and ^{19}F NMR spectra, which fitted their previously reported data in the literature.[73,74] Product **4-F2** was obtained at 16.7% yield and product **5-F2** at 7.7% yield.

Determination of pK_{HB} Values (IR Measurements). All of the procedures, including the preparation of solutions and filling the IR cell, were conducted in a dry atmosphere of a glove box. The equilibrium constant, for the formation of a 1:1 hydrogen-bonded complex between the acid, *p*-FC_6H_4OH (*p*-FP), and the general base (B), K_f, was calculated using the IR-based method reported by Ouvrard et al.[46] The free OH band absorbance at 3614 cm^{-1} in the IR spectra enabled the calculation of the acid concentration (C_a). The equilibrium concentrations (presented in mM) of the acid (*p*-FP, C_a) were measured, and the concentrations of complex (C_c) and base (C_b) were determined by subtraction; accordingly, K_f was computed. The *p*-FP was used at a concentration of around 2.5 mM, and different excess of base stoichiometry were used (from 3 up to 20) to ensure a 1:1 complexation. Bruker Curve Fit software was used to mathematically resolve overlapping bands into their Gausso−Lorentzian components. Five measurements were performed for each substance, and accordingly the average and standard deviations were calculated. Consequently, after five runs, the ε's were averaged and used. In addition, ε was measured at the same day of analysis to ensure accuracy.

Infrared Wavenumber Shifts. $\Delta\nu$(OH) was calculated as 3614 − ν(OH●●●X) [cm^{-1}] and is equal to the shift of the OH vibration of *p*-FP when the hydrogen bond complex is formed.

Determination of pK_{HB} Values (NMR Measurements). NMR experiments for the determination of pK_{HB} were carried out in CCl_4 at 25 °C. The ^{19}F and ^1H spectra were acquired with proton and fluorine decoupling, respectively. The pK_{HB} values of the ketones were extracted from the ^{19}F NMR chemical shifts of complexed *p*-FP compared to that of the internal reference *p*-F-anisole (*p*-FA), which are dependent on the $\Delta\delta_{max}$ of each complex, according to the procedure reported by Taft and co-workers[47] and Dalvit et al.[33,35] (see SI eq 1). The calculation of K_f where A_0 and B_0 are the initial concentrations of *p*-FC_6H_4OH (*p*-FP) and the base, respectively. In addition, δ is the relative ^{19}F NMR chemical shift (ppm) for the equilibrium mixture to that for 0.01 M (*p*-FP) to 0.01 M *p*-$FC_6H_4OCH_3$ in CCl_4, and Δ_{max} is the maximum ^{19}F NMR chemical shift (ppm) of the formed complex relative to uncomplexed *p*-FP.

Determination of Octanol−Water Partition Coefficients (log *P*): "Shake-Flask" Method (UV−Vis Determination). The partition coefficients were calculated as the logarithm of the ratio of the compound concentration in the octanol phase to its concentration in the aqueous phase. The shake-flask method was used for the determination of log $P_{o/w}$ values.[75] Both octanol and water were presaturated with each other for at least 24 h prior to the experiment. The different compounds were dissolved in water-saturated octanol to obtain a concentration of 10 mM. The maximum wavelength (λ_{max}) for each compound was determined, and the absorbance was recorded using UV−vis spectroscopy. Measurements of the concentrations of compounds were performed on dilute solutions giving absorbance in the range of 0.2−1. Following preliminary experiments, for identifying an optimal water/octanol ratio,[76] to a volume (usually 45 mL) of octanol-saturated water, a volume (usually 0.3 mL) of the water-saturated octanol solution with the dissolved compound was added. In certain cases, other water/octanol ratios were needed. The mixture was gently shaken for 5 min. The solutions were then centrifuged at 3000 rpm for 5 min. An aliquot of the octanolic phase was diluted, and absorbance was measured. The experiment was repeated at least thrice for each sample under identical conditions, and each result is presented as an average with the standard deviation of these measurements. The extraction ratio was obtained by difference, and log $P_{o/w}$ was calculated taking into account the volume ratio between water and octanol.

^{19}F NMR-Based log $P_{o/w}$ Determination. The partition coefficients were calculated using the ^{19}F NMR spectroscopy-based method according to Linclau's protocol.[61] Briefly, octanol (2 mL); **2b-F** (5−10 mg), **2b-F2** (10 mg), or **2b-F3** (16 mg); 2,2,2-trifluoroethanol (1−10 mg); and water (2 mL) were added to a 10 mL flask. The mixture was stirred at room temperature for 2 h and allowed to stand at the same temperature overnight to allow complete phase separation. Samples (0.5−0.7 mL) were gently taken from each layer in such a way so as to obtain a pure single solvent. Each sample was transferred into an NMR tube, and acetone-d_6 was added (0.1 mL) as a locking deuterated solvent for ^{19}F NMR spectroscopy. D1's of 30 and 60 s were used for the octanol and water samples, respectively. The numbers of transients (NSs) were 64 and 768-5900 for the octanol and water samples, respectively, and manual integration was performed. For the separation examination, this process was repeated thrice (for compounds **2b-F2** and **2b-F3**, three different samples were taken from each layer and were analyzed). In

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addition, one of the samples in each layer was analyzed thrice for analysis of repetition examination.

DFT Calculations. All calculations were performed using the Gaussian 09, revision D.01 program. All compounds were calculated at the m062x/6-311++g(d,p) level of theory, and for all species, frequency analysis (at the same base and level) was performed, and all frequencies were verified to be positive. Unless otherwise noted, all four terms of convergence were found to be positive. In cases where this is not the case, the predicted change in energy is provided (in the SI). Where the relative energy between two conformers was reported, it was based on the difference of energies of the sum of electronic and zero point energies (detailed in the SI for the relevant species/conformers). For each species, the following data is provided in the SI: atomic coordinates, charge distribution analysis by NBO, and charge distribution analysis by Hirshfeld.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01868.

Determination of pK_{HB} values; IR spectra of compounds **1**−**5**; NMR spectra for all new compounds and for the ^{19}F NMR-based log *P* determination; and atomic coordinates for the optimized geometries (PDF)

Molecular formula strings, pK_{HB}, *A* and log *P* values (CSV)

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■ ABBREVIATIONS

HB, hydrogen bond; FG, functional group; IMHB, intramolecular hydrogen bond; DFT, density functional theory

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