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FEATURE ARTICLE

Some influences of fluorine in bioorganic chemistry

David O'Hagan[a]† and Henry S. Rzepa[b]‡

[a] *Department of Chemistry, Science Laboratories, South Road, Durham, UK DH1 3LE*
[b] *Department of Chemistry, Imperial College of Science Technology and Medicine, London, UK SW7 2AY*

The stereochemical outcome on processing fluorinated substrate analogues by enzymes can often be controlled by electronic and stereoelectronic factors associated with the fluorine atom.

Substitution of a hydrogen atom or a hydroxy group by fluorine in enzyme substrate analogues has been widely practised in various arenas of bioorganic and medicinal chemistry. It is very difficult however to generalise on the relative abilities of fluorine to act as a hydrogen or hydroxy group mimic and various factors have to be considered in each circumstance. The van der Waals radii of fluorine (1.47 Å) lies between oxygen (1.57 Å) and hydrogen (1.2 Å)[1] and thus fluorine appears to have a particularly close isosteric relationship to oxygen while being larger than hydrogen. However, in solid state X-ray structures, fluorine and hydrogen often interchange, and scanning tunnelling microscopy analyses of monofluorinated stearic acids deposited on graphite show very little distortion in two dimensional packing, suggesting a very close isosteric relationship between hydrogen and fluorine.[2] Consistent with this, the steric impact of replacing hydrogen by fluorine is never too great and binding of analogues to target proteins is not normally impeded. Thus despite the size anomoly fluorine emerges as a reasonable hydrogen mimic.

The ability of fluorine to act as a hydroxy group mimic is less clear and is discussed at some length below. Despite a good size correlation between fluorine and oxygen the electrostatic influence of fluorine is much weaker than that of oxygen, and this compromises its hydrogen bonding ability, a limitation of particular significance in enzyme substrate binding.

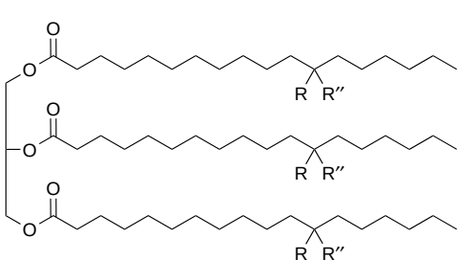
Our work has focused on exploring the behaviour of organofluorine compounds in the bioorganic arena, with a particular emphasis on investigating geometric and stereoelectronic effects which emerge as a consequence of replacing fluorine for hydrogen or oxygen. Stereoelectronic effects, which are generally lower energy phenomenon, are considerably attenuated by indiscriminate solvent interactions (H-bonding, electrostatic, dipolar *etc.*) in solution. The anomeric effect serves as a good example.[3] However the enzyme environment is significantly less polar than aqueous solvent[4] where relative permittivities in the range 15–20 have been estimated and can be compared to 78.5 for water.[5] After binding, the substrate is largely desolvated, with the substrate reaction centre proximate[6,7] and exposed to the cofactor, enzyme or second substrate without intervening solvent. Indeed it has been argued,[8] in the extreme, that enzyme reactions are more closely modelled in the gas phase rather than in the solution state due to these factors. Thus the low polarity of the enzyme active site and the possibilities of conformational control offer an attractive environment in which to assess the stereoelectronic influence of the fluorine atom.

1 Steric and geometric effects in CF, CF_2 and CF_3 systems

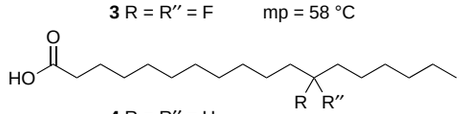
1.1 The CF for CH and CF_2 for CH_2 replacement

Despite the larger van der Waals radii for fluorine over hydrogen, the experimental evidence suggests that, in general terms, the substitution of a single fluorine for a hydrogen atom introduces only small steric and geometric perturbations relative to the hydrocarbon counterpart.[2] Dehydrofluorinated (CH for CF) enzyme substrate analogues usually have a high affinity for their target protein. For example selectively β-fluorinated α-amino acids have been widely explored as suicide substrates[9–11] for decarboxylases, racemases and transaminases, and other fluorinated amino acids are assimilated biochemically into proteins.[12] Fluoroacetyl-CoA is an excellent acetyl-CoA mimic for citrate and malate synthases (see section 3.4) and fluoropyruvate is a pyruvate surrogate for l-lactate dehydrogenase.[13] Thus the fluorine for hydrogen substitution is not generally detrimental to binding, however the high electronegitivity of fluorine can have dramatic mechanistic consequences which can lead to mechanistic deviations and enzyme inhibition.[9–11]

Replacement of a methylene for a difluoromethylene group (CH_2 for CF_2) can be much more dramatic than the single substitution. In a recent study[14] in Durham we have compared the physical properties of tristearins **2** and **3** containing one and two fluorine atoms substituted for the C-12 methylene hydrogens of the hydrocarbon chain. The melting points of these tristearins are shown in Fig. 1. Substitution of one fluorine atom at C-12, in **2**, has very little effect on the melting point, polymorphic phase behaviour and X-ray powder diffraction pattern (data not shown) when compared to tristearin **1** itself, however introduction of the second fluorine atom in **3** leads to a significant lowering of the melting point (72 *vs*. 58 °C) and the polymorphic phase behaviour and X-ray powder diffraction pattern become quite different. The stability of Langmuir films of the corresponding fluorinated stearic acids **4**–**6** was also investigated. 12-Fluorostearic acid **5**, like stearic acid **4**, formed a stable monolayer on the surface of aqueous subphase, however the monolayer of 12,12-difluorostearic acid **6** was unstable and susceptible to collapse/reorganisation at comparable surface pressures, indicative of significant conformational disorder.



1 R = R″ = H mp = 72 °C
2 R = H, R″ = F mp = 73 °C
3 R = R″ = F mp = 58 °C



4 R = R″ = H
5 R = H, R″ = F
6 R = R″ = F

Fig. 1 Melting points of tristearins

A similar lowering in the melting points (by *ca*. 6–10 °C) of CF_2-labelled diacylphosphatidylcholines relative to their hydrocarbon counterparts has also been noted[15] and the phenomenon appears to be general. We attribute this to an increased conformational flexibility of the hydrocarbon as a consequence of CF_2 substitution (Fig. 2). Crystallographic[16] and theoretical evidence[17] support a widening of the C–CX_2–C angle from the sp^3 tetrahedral angle (109.5°) in the hydrocarbon to *ca*. 115–119° in the CF_2-hydrocarbon systems. This angle widening is also apparant from the structures of C–CH_2–P, C–CHF–P and C–CF_2–P phosphonates where the C–C–P angles are 112, 113 and 116° respectively (see section 2.2). The extended zig-zag conformation is favoured in a hydrocarbon chain as disfavoured *gauche* conformations bring 1,4-hydrogens into close contact (*ca*. 1.8 Å) (Fig 3).[18] However, angle widening at the substituted carbon in the CF_2-containing hydrocarbon chains results in increasing this 1,4-H···H distance and in turn lowers the energy of attainment of a *gauche* conformation. A complementary situation occurs when sulfur[19] (but not oxygen) is introduced into a hydrocarbon as the increased C–S bond length (1.8 Å) over that for C–C (1.5 Å) or C–O (1.4 Å) increased the 1,4-hydrogen distance to 2.4 Å and relaxes this interaction.

1.2 The CF_3 for CH_3 replacement

It is generally appreciated that the methyl and trifluoromethyl groups bear very little steric resemblance. A comparison of the molar volumes of a series of related compounds[20] with various substituents, and with a CF_3 group, reveals a size closer to the isopropyl rather than methyl group. Seebach has argued[21] that the CF_3 group is between two and three times larger than CH_3 when the van der Waals hemispheres of these groups are considered (Fig 4). Other assessments[22] suggest a steric influence close to that of an isopropyl group. However there are several cases in asymmetric synthesis[23] where the directing influence of the CF_3 group has been assessed relative to other substituents and a close analogy with the phenyl ring or even a *tert*-butyl group has been found. Of course the origin of this apparantly gross steric effect must be attributed in no small part to polar and electrostatic influences, in a given situation. Such factors are discussed below (section 2.3) in an assessment of lipase resolutions of CF_3 containing compounds. On balance the various lines of evidence place CF_3 significantly larger than methyl and a good upper estimate would equate it with the isopropyl group. Thus the substitution of one, two and three hydrogen atoms on carbon, by fluorine, becomes increasingly distorted in maintaining an isogeometric profile between the fluoro- and hydro-carbon series.

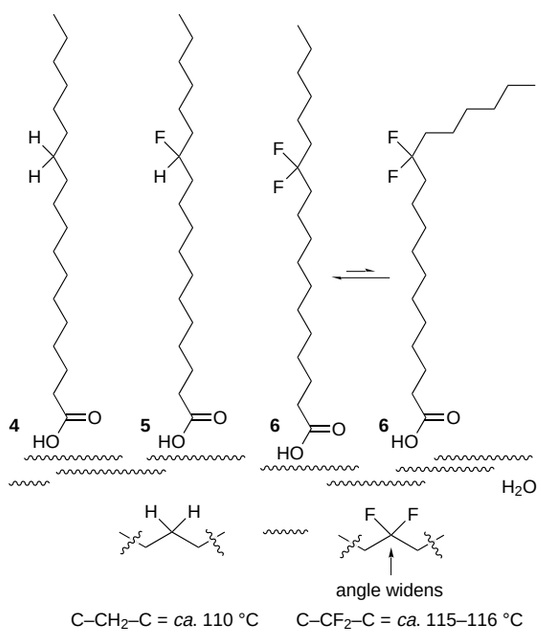


Fig. 2 Schematic representation of C-12 fluorinated stearic acids on the surface of water. The CF_2 stearate **6** posesses significant conformational disorder.

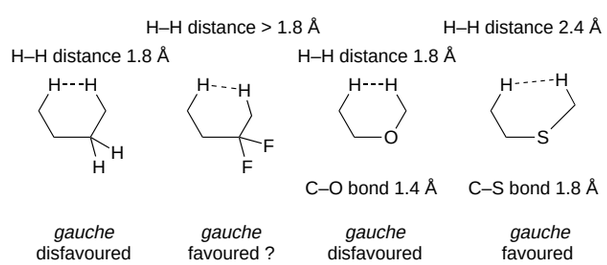


Fig. 3 Schematic representation of 1,4-hydrogen interactions and the preference for *gauche* or zig-zag conformations

2 Electrostatic effects

Despite one extra lone pair, the higher electronegativity and lower polarisibility of fluorine attenuate its electrostatic influence in comparison with oxygen. So despite their apparent similarity in size, electrostatics emerge as a significant factor underlying the limited ability of fluorine to mimic a hydroxy group. In bioorganic chemistry hydrogen bonding is probably the most significant area in which electrostatic interactions are involved, certainly when considering enzyme substrate binding interactions.

2.1 Fluorine as a hydrogen bonding acceptor

The capacity of organically bound fluorine to act as an oxygen surrogate (*e.g.* an OH mimic) and enter into hydrogen bonding as an acceptor has been widely discussed.[24,25] Recent theoretical calculations[26] have measured the strength of an optimum F··H bond (1.9 Å) to be 2.38 kcal mol^{-1} in an adduct between fluoromethane (CH_3F) and water as shown in Fig. 5. Interestingly, the strength of the optimum interaction (1.95 Å) between fluoroethene (CH_2=CHF) and water was only 1.48 kcal mol^{-1}, suggesting that the C(sp^3)–F··H interaction is stronger than the C(sp^2)–F··H interaction. In strength therefore, F··H hydrogen bonds are clearly weaker than O··H hydrogen bonds, which are conservatively estimated[27] to be *ca*. 5 kcal mol^{-1}, but are stronger than O/NH–π interactions.[28,29] The weaker F··HO over O··HO interaction appears to be due to the higher nuclear charge on the F nucleus compacting the surrounding lone pairs.

Calculations[30] measuring the difference in electrostatic potentials around methanol and fluoromethane (Fig. 6) with distance from the nucleus reinforce the view that the C(sp^3)–

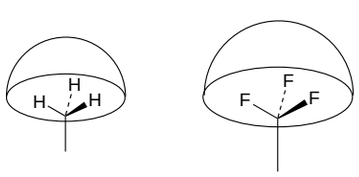


Fig. 4 Van der Waals hemisphere volumes comparing CH_3 and CF_3 (ref. 21)

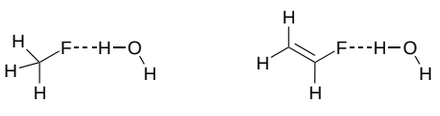


Fig. 5 Calculated strengths of C(sp^3)–FH··H and C(sp^2)–F··H bonds

F···H bond strength is half that of the H···O value. The reduced electrostatic influence of fluorine requires that an incoming hydrogen bond donor must approach the fluorine nucleus more closely than oxygen, and the resulting increase in the nuclear–nuclear repulsions does not allow a strong hydrogen bond to be formed. Thus a best case scenario suggests that fluorine can contribute up to about one half of the binding energy of a hydrogen bond to oxygen. A recent[31] and controversial discussion[32] on low barrier hydrogen bonding (LBHB) suggests that in transition states of enzyme mediated reactions, O···H···O bonds can contribute upto 10–20 kcal mol^{-1} although these arguments appear increasingly tenuous.[33] However if this latter situation is close to reality then the replacement of OH for fluorine would be inadequate if a hydrogen bond was important in stabilising a transition state on the enzyme surface.

Surveys[34] of the organofluorine compounds logged in the Cambridge Crystallographic Data Centre (CCDC) have revealed relatively few situations where fluorine is involved in short contacts to hydrogens. Short contacts between fluorine and less acidic H–C protons are most common whereas those between fluorine and the more acidic O–H or N–H protons are rare, clearly for statistical reasons (*i.e.* more CHs than OH or NHs) but also, acidic hydrogens prefer to find an oxygen or nitrogen acceptor in preference of fluorine. Thus the theoretical and crystallographic evidence force the conclusion that fluorine is a poor hydrogen bond acceptor with only a moderate capacity to replace oxygen (or nitrogen) in this role. In a desolvated and preorganised enzyme cavity, where the fluorine atom is predisposed towards an acidic hydrogen (donor) then these circumstances may amount to an optimal situation for F···H bonding. There are examples in the bioorganic literature which clearly suggest that fluorine is replacing OH as a hydrogen bond acceptor, however they are few and more often than not the substitution proves detrimental.[35]

2.2 CF_2-phosphonates

Phosphate groups are susceptable to hydrolysis by the action of phosphatase enzymes; thus phosphonates, where the bridging oxygen is replaced by carbon, have been explored as phosphatase stable phosphate mimics.[36] A particular refinement of this strategy has been developed by Blackburn[37] and involves the replacement of the bridging oxygen atom by a CF_2 substituent. This is attractive as the fluorine atoms reintroduce the electronegativity lost on removal of the oxygen atom. The degree of electronegativity can be assessed from the pK_a values of the second deprotonation of the phosphonate groups. These values are summarised for the phosphate and various phosphonates in Fig. 7. The CF_2-phosphonate (pK_a = *ca.* 5.64) is approximately one pK_a unit more acidic than the phosphate group it is designed to mimic. The CHF-phosphonate value (pK_a = *ca.* 6.2) is much more closely matched and the CH_2-phosphonate (pK_a = 7.6) is a pK_a unit less acidic. It is generally assumed that the phosphate unit will be fully ionised on protein binding, thus the increased acidity of the CF_2-phosphonate is not judged detrimental in this respect and often CF_2-phosphonates emerge as excellent phosphate surrogates.

Crystal structures[16,38] of the aminophosphonic acids **7**, **9** and **10** reveal that the P–C–C angle of the CF_2-phosphonate (116°) is closer than that of the CH_2- (112°) and CHF- (113°) phosphonates to the C–O–P angle (118°) of the phosphate group of **8**. Thus the CF_2-phosphonate appears to approximate the geometry of the phosphate group most closely in the series, at least in the ground state. Therefore on geometric and ionic grounds the CF_2-phosphonate emerges as a good phosphate mimic. On the other hand the substitution of oxygen for CF_2 will have an increased steric impact, as the fluorine atoms are resident in the space previously occupied by the lone pairs of oxygen.

The electrostatic profile of the CHF and CF_2 groups in comparison to oxygen must also be considered when evaluating these systems. A recent theoretical analysis by Thatcher and Campbell[39] compared the negative electrostatic profile of the phosphate group with the CHF-phosphonate by superimposing the two. Fluorine has a suppressed electrostatic influence relative to oxygen, but placing it one C–F bond length out from the oxygen extends the centre of its electrostatic influence a little beyond that of oxygen when comparing oxygen and C–F. Also the area of maximum negative density for CHF-system is asymmetrically displaced from that of oxygen in the phosphate, by about 1 Å, due to the stereogenic centre. Although the comparison did not extend to the CF_2 system, an extrapolation of Thatcher's results suggests that the CF_2 group will have at least a similar, and possibly greater, electrostatic influence than the phosphate oxygen. Comparative molecular electrostatic potentials (MEPs) of the CHF- and CF_2-phosphonates of the aminophosphonic acids **11** and **12** respectively were evaluated by one of us[40] and reinforces the notion that the CF_2 phosphonate has a significant electrostatic influence (see Fig. 8), in this case on chiral recognition of the substrate *via* the asymmetric binding of the aryl group into a cyclodextrin cavity.

In the case of the CHF-phosphonate **11** the presence of fluorine induces an asymmetric negative potential (red area) on one side of the aryl π-face. This asymmetry is much less evident for the CH_2-phosphonate **13**.[40]

In a recent study[38,41] at Durham, using phosphonates as phosphate mimics, we have drawn the conclusion that an electrostatic interaction between an enzyme and its substrate emerges as an important factor in binding. The CH_2 and CHF-phosphonate analogues **15** and **16** of *sn*-glycerol-3-phosphate **14** have a greater affinity than the CF_2-phosphonate analogue **17** for the enzyme glycerol-3-phosphate dehydrogenase. The enzyme is NAD dependant and oxidised the secondary alcohol of each of the phosphonates to their corresponding dihydrox-

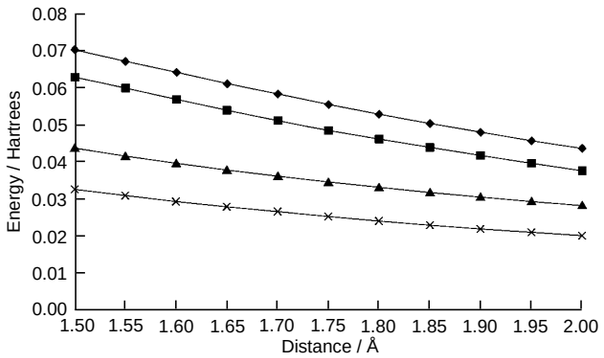


Fig. 6 A plot of calculated electrostatic potential values at the B3LYP/6-31G(d,p) level for methanol (◆), hydroxyethene (■), fluoromethane (▲) and fluoroethene (X) as a function of distance (Å) from the F/O nucleus. The maximum value of the potential at this distance is shown.

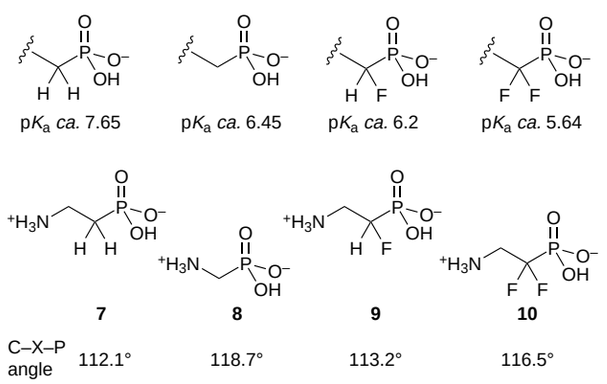


Fig. 7 Phosphate/phosphonate acidity (second deprotonation) and C–X–P angles as obtained from the X-ray structures of compounds **7**–**10**

yacetone phosphate analogues. The relative V_{max} and K_m values are given in Fig. 9. The maximal rates are similar for each substrate, but the K_m value for the CHF-phosphonate analogue is significantly lower than the natural phosphate substrate **14** (0.17 *vs*. 0.29 mm). This low value correlates with previous data[42] on the CH_2-phosphonate **15** and it is perhaps surprising that replacing the bridging oxygen of the phosphate, by carbon, leads to a higher binding affinity. Intriguingly glycerol-3-phosphate dehydrogenase appears to be unoptimised in its binding of the phosphate group of the natural substrate **14**, revealing a sensitivity to the electrostatic influence of oxygen, a situation that relaxes with the CH_2- and CHF-phosphonate analogues. The poorer performance of the CF_2-phosphonate **17** analogue, now with two fluorine atoms, is consistent with an even greater electrostatic influence impacting on the surface of the protein.

Phosphonate analogues of phosphotyrosine have been prepared and incorporated into peptides as phosphatase resistant SH-2 domain inhibitors, in the only other example[43] where CH_2-, CHF- and CF_2-phosphonates have been compared directly in a biological system. The ability of phosphotyrosine residues to bind such domains is important in signal-transducing molecules. A series of pentapeptide analogues (Gly-pX-Tyr-Val-Pro-Met-Leu) were assessed for their ability to bind the C-terminal SH-domain of phosphatidylinositol-3-kinase and were found to bind in the following order; CHOH < CH_2 < CHF < CF_2 = O where the CF_2-phosphonate was the optimal phosphate mimic in this case. Different biological systems respond differently to the CHF- and CF_2-phosphonate analogues and clearly each has to be assessed individually.

2.3 Electrostatic or steric influences in lipase resolutions?

Lipases and other hydrolytic enzymes have been widely used for the kinetic resolution of ester racemates.[44] In general terms the effectiveness of such resolutions is interpreted on the basis of steric interactions between the surface of the protein and the substrate. Indeed steric models for the more widely used lipases have been proposed and refined by identifying hydrophobic and hydrophilic compatible regions.[44] Pig liver esterase is a good example.[45] Such models are used successfully in a predictive way to assess the viability and stereochemical preference of a particular resolution. On the other hand, the kinetic resolution of some CF_3 containing substrates are not so easily rationalised by steric models. For example the *Candida rugosa* lipase (formerly *C. cyclindracea* and also MY-Lipase) esterifies/desterifies the *R*-enantiomer of phenethanol **18** in preference to the *S*-enantiomer.[46]

For trifluorophenethanol however the enzyme prefers alcohol (*R*)-**19** of the opposite configuration (**19** also posesses the *R*-configuration due to a change is assignment priorities).[47] A steric interpretation then suggests that the CF_3 group is larger than the phenyl ring, however as discussed above (section 1.3), various methods of comparison reinforce the trend Ph > CF_3 > CH_3 > H for the steric influence of these substituents. The CF_3 group is larger than the methyl group and will compete more

11 X = CHF
12 X = CF_2
13 X = CH_2

Fig. 8 *Ab initio* electrostatic potential map at the B3LYP/6-31G level for (*a*) the phosphonate **11**, contoured at 0.025 Hartree and (*b*) the F-epimer of **11**. An active three dimensional view of these diagrams encoded in VRML is available *via* the enhanced electronic version of this article. See http://chemcomm.clic.ac.uk/ for further details.

favourably than CH_3 with Ph in steric terms, however it is still smaller than Ph. Thus steric factors alone cannot account for this reversal phenomenon with **19**. If the electrostatic influence of the various substituents is considered then the trend CF_3 > Ph > CH_3 emerges. The electrostatic influence of the CF_3 group is of course greater than CH_3 and it will exert greater repulsion on for example an alkoxide nucleophile (on the enzyme), or on another area of negative density on the protein surface. Also, in an intriguing interplay the CF_3 group in **19** is predicted to reduce the electrostatic influence on one face of the aromatic ring. This was shown in a study by one of us on the chiral resolving agent (*S*)-2,2,2-trifluoro-1-(9-anthranyl)ethanol (Pirkle's reagent).[48,29]

The X-ray structure of **20** shown in Fig. 10 has the CF_3 group lying perpendicular to the plane of the anthracene ring. A molecular electrostatic potential map calculated for **20** also shown in Fig. 10 revealed a distinct difference in the negative potential of the two π-faces of the aromatic. The face *anti* to the CF_3 group is reduced in electron density relative to the face *syn* to the CF_3 group (Fig. 2). The decreased electrostatic potential on the *anti* face is rationalised by negative hyperconjugation due to overlap of the π-orbital system with the σ*-orbital

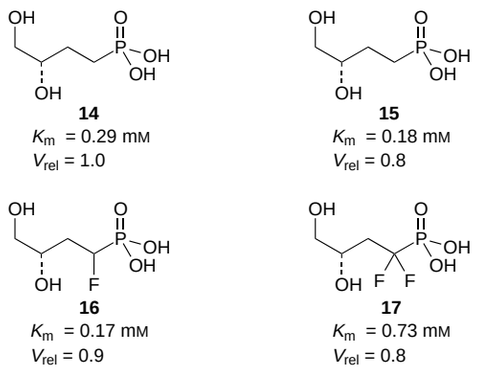
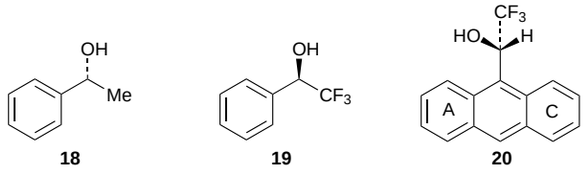


Fig. 9 Kinetic data on **14**–**17** as substrates for glycerol-3-phosphate dehydrogenase[41]



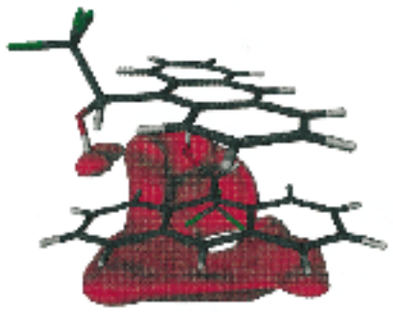


Fig. 10 The X-ray structure of **20** showing the dimeric self-interaction, with a superimposed negative electrostatic potential computed (B3LYP/6-31G, contoured at 0.0175 Hartree) for a single molecule shown in red. The +ve potential is not shown. Note in particular the asymmetry of the π-system, and the coincidence of the residual −ve potential on one face with the position of the πH–O interaction from the second molecule. An active three dimensional view of this diagram encoded in VRML is avaialble *via* the enhanced electronic version of this article. See http://chemcomm.clic.ac.uk/ for further details.

associated with the C–CF_3 bond. Thus the CF_3 group has a synergic effect in possessing a significant electrostatic influence of its own and also depleting that on one face of the aromatic ring. The induced asymmetry extends not only to the two π-faces, but to differentiating between rings A and C on the aromatic system, as a consequence of further interaction between the CF_3 group and the adjacent OH group. The crystal structure shows a OH··π hydrogen bond to the edge of ring A, precisely where the maximum in the negative electrostatic potential on this face is computed to occur. Thus the role of the CF_3 group could be regarded as focusing the role of the two aromatic faces into a π-stacking side and hydrogen bond accepting side. A similar directing influence of a CF_3 group towards an electrophile was observed in the π-facially stereoselective epoxidation of phenyltrifluoromethylpropenol.[49]

In the case of the CH_3- and CF_3-phenethanols **18** and **19** the combined steric, but particularly electrostatic impact of the substituents is now predicted to follow CF_3 > Ph_{CH_3} > Ph_{CF_3} > CH_3. This trend is entirely consistent with the observed stereochemical preferences of the *Candida rugosa* lipase for **18** and **19**.

3 Stereoelectronic effects

3.1 The Fluorine anomeric and Anh–Eisenstein effects

The anomeric effect[50] which in carbohydrates recognises the preference for an axial over an equatorial conformation at the O–C–O anomeric carbon, extends to O–C–F and N–C–F systems.[51] The molecular orbital explanation for the anomeric effect suggests that the lone pair on oxygen lying *anti*-parallel to the C–X bond donates electron density into the σ^*_{C-X} orbital in a stabilising hyperconjugative interaction as illustrated in Fig. 11. Theoretical studies on fluoromethanol ($HOCH_2F$),[51] fluoromethyl methyl ether ($MeOCH_2F$)[52,53] and fluoromethylamine (FCH_2NH_2)[54] suggest a stabilisation of around 6.0 kcal mol^{-1} and X-ray structure data of appropriate compounds[51,52] indicate a consequent shortening of the C–O bond and lengthening of the C–F bond consistent with the hyperconjugation hypothesis.

Anh and Eisenstein,[55] in a theoretical study investigating transition states for hydride attack to 2-chloropropionaldehyde, have suggested that the most stable transition state will have the HOMO of the nucleophile approaching the carbonyl LUMO where the p-orbitals are co-aligned with the σ*-orbital of the vicinal C–Cl bond. An *anti*-parallel approach of the nucleophile to the C–Cl bond is preferred to accommodate mixing of the developing σ-bond electrons with the σ*-orbital of the C–Cl bond and relaxation of any electrostatic repulsion between the halogens, as shown in Fig. 12. This set-up allows negative hyperconjugation where the electron density of the nucleophile is accommodated in the antibonding orbital (σ*-LUMO) associated with the C–Cl bond. The effect was further

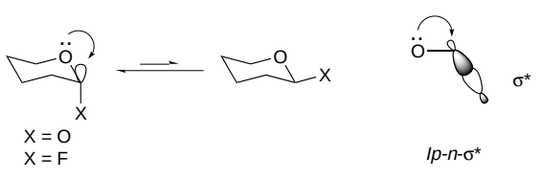


Fig. 11 A molecular orbital representation of the anomeric effect

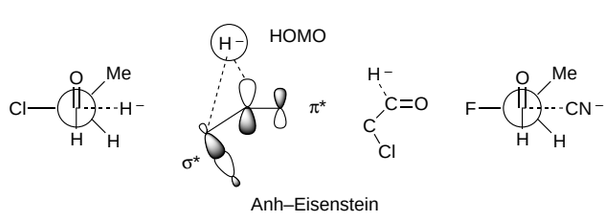


Fig. 12 A molecular orbital description of Anh–Eisenstein type stabilisation of a nucleophile approaching an α-haloaldehyde

evaluated[56] for fluorine in a theoretical study by Wong and Paddon-Row, on transition state geometries of cyanide attack to 2-fluoropropionaldehyde. This effect emerges as an important one in interpreting the stereoselective processing of fluorinated substrates in certain enzyme reactions and is discussed in the next section.

3.2 Anh–Eisenstein stabilisation in enzyme reactions

The ability of lipases to mediate kinetic resolutions by hydrolysis of racemic or *meso* esters has been widely practiced.[45] Active site models have been mapped for the common lipases which identify areas of hydrophobicity and regions that can accommodate small medium and large groups.[44] In general the lipase active site is considered to accommodate a particular substrate enantiomer on the basis of fit and electronic compatibility to the surface of the protein, and this in turn leads to preferential binding and hydrolysis of a single enantiomer. However the lipase resolutions[57,58] of α-fluoro esters shown in Fig. 13 illustrate that in each case the lipase can distinguished completely between the fluorine and hydrogen atoms. This is difficult to reconcile on the basis of a spacial active site model, as the steric influence of fluorine over hydrogen would not be expected to lead to such a high level of selectivity.

On the other hand we have proposed[59] that these resolutions can be rationalised on the basis of Anh–Eisenstein type stabilisation. Each of the enantiomers of the α-fluoro ester give rise to one of two diastereoisomeric transition states where the serine alkoxide nucleophile of the lipase attacks the ester carbonyl. The nucleophile can either approach *anti* to the C–F bond in one case (as shown in Fig. 13) or *gauche* to the C–F bond for the other enantiomer (interchange H for F). *Ab initio* calculations have been used to model the transition states of these hydrolytic reactions. Methoxide was selected as the nucleophile (serine hydroxide surrogate) and methyl 2-fluoropropionate as the substrate ester. It emerged that the transition state with the nucleophile approaching *anti* to the C–F bond, is more stable by up to 2.8 kcal mol^{-1} than the diastereomeric transition state where the hydrogen and fluorine atoms are interchanged. The energy differential between these transition states is sufficient to give 100 : 1 selectivity and can fully account for the observed high enantioselectivities (99% ee).

In a further exploration of Ahn–Eisenstein type stabilisation in an enzyme reaction we have investigated the selectively fluorinated substrate analogue **23** for the enzyme cyclohexenylcarbonyl-CoA reductase.[60] This NADPH dependant enzyme was isolated from *Streptomyces collinus* and operates on the biosynthetic pathway to the antibiotic ansatrienin.[61] The enzyme reduces cyclohexenylcarbonyl-CoA **21** to the corresponding cyclohexane ring product **22**, and offers an ideal framework in which to explore stereoelectronic effects. Hydrogen is added in an *anti* manner across the double bond with hydride from the co-factor delivered to the *si*-face as illustrated in the Fig. 14. In substrate **23** the fluorine at C-3 can occupy two sites. The diastereoisomer where fluorine is sited at C-3 antiperiplanar to the incoming hydride should accommodate the nucleophile, due to Anh–Eisenstein type stabilisation, relative to the diastereisomeric transition state with fluorine in a *gauche* position.

The requisite C-3 fluorinated substrate analogue was synthesised as a 1 : 1 epimeric mixture at C-3.[60] A number of interesting features emerged from the kinetic analysis of this system. The fluorinated substrate had a K_m value of 44 μm indicating a good affinity for the enzyme relative to the natural substrate (K_m = 25 μm). Intriguingly the V_{max} of the fluorinated substrate was five times greater than that of the natural substrate. This observation can be rationalised by the electronegative fluorine atom rendering the site of hydride delivery more electropositive and lowering the transition state energy. This conclusion was reinforced by kinetic isotope data utilising deuterium labelled cofactor (NADP^{2}H). The natural substrate displayed a kinetic isotope effect of k_H/k_D = 3 consistent with hydride delivery as the rate limiting step in the reaction. However this isotope effect was reduced to k_H/k_D = 1.3 with the fluorinated substrate. Thus the transition state energy for hydride delivery, the highest barrier on the reaction course, is lowered as a result of fluorine substitution at C-3, and hydride delivery is now competing with another step (*e.g.* co-factor binding/debinding) as the rate limiting step.

On the issue of Anh–Eisenstein stabilisation, the enzymatic reduction of the fluorinated substrate was monitored by ^{19}F NMR spectroscopy. The stereospecificity of the enzyme reaction, and the preference of the SCoA group to adopt an equatorial conformation, dictate that (3*R*)-**23** will deliver the product **24** with the fluorine atom axial, and (3*S*)-**23** will deliver **24** with the fluorine atom equatorial. Thus the diastereoisomeric products can be distinguished readily by ^{19}F NMR spectroscopy. In the early stages of the reaction there was indeed a small but significant bias in favour of the axial over the equatorial product as predicted *i.e.* at 20% conversion the axial–equatorial ratio was 1.3 (*ax–eq* = 1.3) (see Fig. 5). As the reaction progressed towards > 50% conversion then this ratio reduced to *ax–eq* = 1.0 as expected, as both diastereoisomers of **23** are progressed through the reaction. The stereochemical bias in the early stages of the reaction is consistent with Anh–Eisenstein type hyperconjugation favouring the product **24**, with fluorine in an axial position.

3.3 The gauche *and* cis *effects*

Perhaps the most widely discussed stereoelectronic effects associated with organofluorine compounds are the *gauche*[62,63]

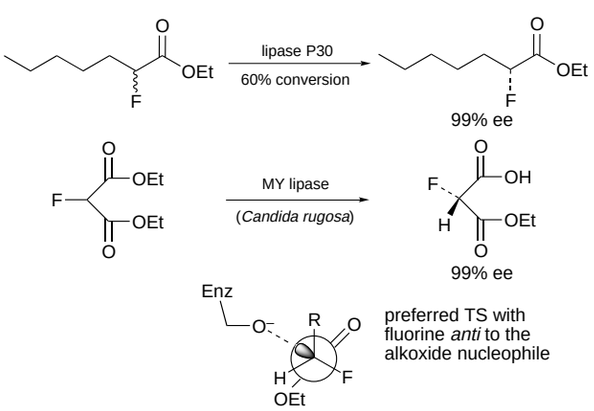


Fig. 13 The high stereospecificity displayed in the resolution of α-fluoro esters is rationalised by Anh–Eisenstein type stabilisation in the transition state

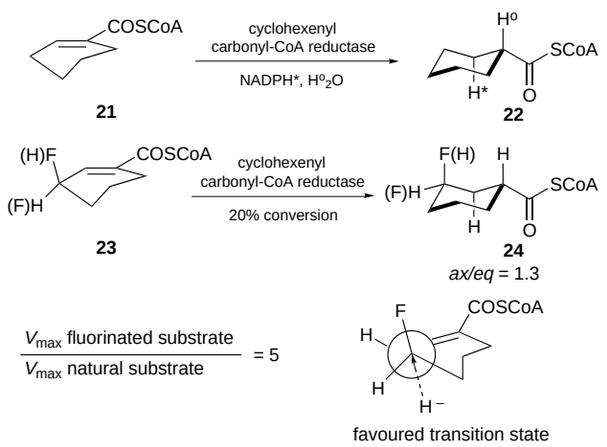


Fig. 14 Cyclohexenylcarbonyl-CoA reductase. Processing of the fluorinated substrate **23** displays a stereochemical bias favouring **24** with fluorine in an axial position.

and *cis* effects.[64] The *gauche* effect recognises that the lowest energy conformation for 1,2-difluoroethane has the fluorine atoms *gauche* rather than *anti* parallel to each other as illustrated in Fig. 15. The *cis* effect is probably related in origin, and recognises that the *cis* geometric isomer is more stable than the *trans* by about 1–2 kcal mol^{-1}. Various hypotheses[65,66] exist to rationalise these observations.[67]

3.4 The cis *effect in enzyme chemistry; citrate synthase*

Citrate synthase, an enzyme of the Kreb's cycle, catalyses the reaction between acetyl-CoA and oxaloacetate to generate citric acid. Fluoroacetyl-CoA, where one hydrogen of the methyl group of acetyl-CoA is replaced by a fluorine atom, is an excellent substrate for the enzyme, generating (2*F*,3*R*)-fluorocitrate **25** as a single stereoisomer[68] as illustrated in Fig. 16. It is this reaction which accounts for the toxicity of fluoroacetate,[69] where the resultant (2*R*,3*R*)-fluorocitrate inhibits aconitase, the next reaction of the Kreb's cycle, and also impedes citrate transport across mitochondrial membranes. The high stereospecificity of this reaction attracted our interest, particularly as a related reaction mediated by malate synthase does not show the same level of stereochemical discrimination. Malate synthase also utilises fluoroacetyl-CoA in place of acetyl-CoA and mediates a condensation with glyoxal to generate two diastereoisomers (2*R*,3*R*) and (2*R*,3*S*) of 3-fluoromalate in an approximately a 3 : 4 ratio.[70,71] Thus in this case the enzyme does not control the stereochemistry of the new chiral centre generated as a consequence of fluorine substitution. These aldol reactions must proceed *via* enzyme bound enol or enolate intermediates of fluoroacetyl-CoA, and clearly these enols can have *Z* or *E* geometries. The high level of stereospecificity, delivering the *R* absolute stereochemistry to C-2 of fluorocitrate **25**, dictates that the *E* enol or enolate of fluoroacetyl-CoA is generated exclusively by citrate synthase, whereas for malate synthase the enzyme appears unable to control this *E*/*Z* geometry to the same extent.

We have calculated[72] the relative energies of such enols and enolates by computational methods. High level *ab initio* calculations demonstrate a significant difference (*ca*. 4 kcal mol^{-1}) in the relative energies of the neutral *E* and *Z* enols generated in a model system from $FCH_2C(O)SH$, whereas this differential was significantly reduced (*ca*. 1.8 kcal mol^{-1}) when the charged enolate intermediates were considered. Thus it would appear that the (2*R*)-stereochemistry in the resultant (2*R*,3*R*)-fluorocitrate arises because the enzyme acts as a general acid and protonates the developing enolate to generate an enzyme bound enol. The intermediacy of a neutral enol in citrate synthase has previously been proposed[73] and is supported by X-ray structure[74] and experimental evidence.[75] X-Ray structural analysis of the enzyme has identified His-274 as the most likely candidate for this general acid as shown in Fig. 17.

The increased stability of the *E* over the *Z* enol in the model system does not have its exclusive origin in an intramolecular bridging hydrogen bond between oxygen and fluorine, as the enol energy is not significantly perturbed when the hydrogen is bridging or turned away from the fluorine atom. Alternatively the stabilisation would appear to be an example of the *cis* effect where the harder fluorine and oxygen atoms pair in preference to the hard/soft interaction between fluorine and sulfur. A corollary of this analysis is that malate synthase, which is less able to control the stereochemistry at the new fluorogenic centre, will have an intermediate with more enolic than enol character. This however remains to be determined.

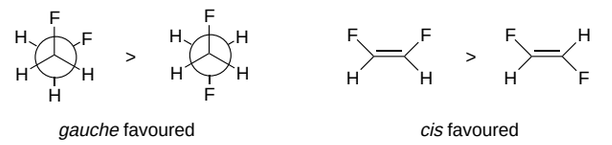


Fig. 15 The *gauche* and *cis* effects favour a *syn* relationship between vicinal fluorine atoms

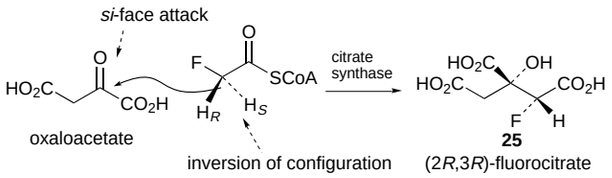


Fig. 16 Citrate synthase generates (2*R*,3*R*)-fluorocitrate **25** as a single stereoisomer after condensation of oxaloacetate and fluoroacetyl-CoA

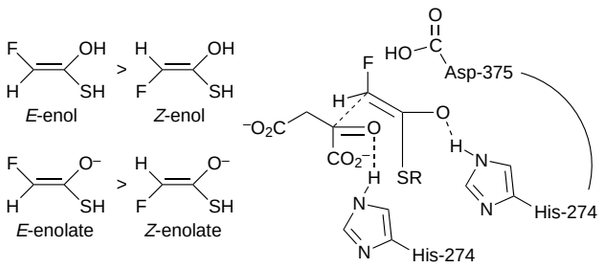


Fig. 17 The *E* enol of $FCH_2C(O)SH$ is more stable than the *Z* enol which is in turn more stable than the *E* enolate followed by the *Z* enolate. This suggests that the high stereochemical control exhibited by citrate synthase in processing fluoroacetyl-CoA involves an enzyme bound *E* enol intermediate.

4 Summary

We have rationalised that the stereoelectronic influence of fluorine can control completely, or influence partially, the stereochemical outcome of given enzyme reactions after an F for H replacement. Interpreting or predicting the behaviour of fluorinated compounds is complex and has led to the term flustrates (fluorine containing substrates) being coined.[21,76] However, a greater appreciation of the electronic and stereoelectronic properties of organic fluorine renders the behaviour of these compounds more interpretable.

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David O'Hagan was born in Glasgow in 1961 and received his BSc from the University of Glasgow in 1982. After graduating with a PhD from the University of Southampton with J. A. Robinson in 1985 he spent a post-doctoral year at the Ohio State University with H. G. Floss. He moved to the University of Durham in 1986 where he is now a Senior Lecturer. His research interests include bioorganic fluorine chemistry, biotransformations and investigations into the biosynthesis of plant, fungal and bacterial secondary metabolites.

Henry S. Rzepa was born in London in 1950, and received both his BSc and PhD degrees from Imperial College, in 1971 and 1974. Following a post-doctoral period with Michael Dewar at the University of Texas, Austin, he returned to Imperial College, where he is now a Reader in Organic Chemistry. His research interests include the theoretical study of stereoelectronic effects, structural studies of unusual forms of hydrogen bonding such as π-facial interactions and those responsible for chiral recognition, and novel applications of the Internet to chemistry.

Footnotes

† E-mail: david.o'hagan@dur.ac.uk
‡ E-mail: rzepa@ic.ac.uk

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