Conformation Analysis

DOI: 10.1002/anie.200700714

An Electrostatic *Gauche* Effect in β-Fluoro- and β-Hydroxy-*N*-ethylpyridinium Cations**

Natalie E. J. Gooseman, David O'Hagan, Michael J. G. Peach, Alexandra M. Z. Slawin, David J. Tozer,* and Robert J. Young*

There has been a recent interest in electrostatic interactions in organic chemistry and in their influence on the conformation and reactivity of organic molecules.[1,2] It emerges that organic cations containing oxygen and fluorine find stabilization through such interactions: polarized C–OH and C–F bonds orient towards charged centers when the molecular conformation allows. This effect has been particularly noted by Snyder, Lankin, and co-workers, who reported the conformational preferences of 3-fluoropiperidinium (**1**) and related ring systems, such as 3-fluoro-*N*,*N*-dimethylpiperidinium (**2**).[3–6] They established that there is a strong preference (calculated energy difference of 4.0–5.4 kcal mol^{-1} between the gas-phase *anti* and *gauche* conformations) for the structures with the fluorine atom in the axial position (**1a**/**2a**) over those with the fluorine atom in the equatorial position (**1b**/**2b**; Scheme 1).[5] In our computational studies, we have explored the conformational preferences of β-fluoroethylamine (**3**) and its protonated counterpart β-fluoroethylammonium (**4**).[7] Our density functional theory (DFT) calculations indicated that there is no intrinsic *gauche* effect for the neutral amine **3**; the neutral molecule only prefers a *gauche* conformation because there is a weakly stabilizing intramolecular N–H···F–C hydrogen bond. When the nitrogen atom is formally charged in **4**, however, the β-fluoroethylammonium cation displays a very strong preference (5.8 kcal mol^{-1}) for the *gauche* conformation (**4a**). This phenomenon extends to 2-fluoroethanol (**5**) and to protonated 2-fluoroethanol (**6**).[7] The slight *gauche* preference in the neutral molecule **5** is almost entirely attributable to a weak bridging hydrogen bond. In contrast, there is a significant intrinsic *gauche* preference in **6** of about 4.4 kcal mol^{-1}, which increases to 7.2 kcal mol^{-1} when one hydrogen atom of the H_2O^+ group is in a bridging *endo* position. These charged systems exhibit a much larger *gauche* preference than do neutral molecules such as 1,2-difluoroethane (**7**), for which the energy difference was calculated to be in the range 0.5–1.0 kcal mol^{-1}.[7]

More recently, we studied the conformation of 3-fluoroazetidinium (**8**).[8] Owing to the ring constraint, it was not possible to establish *gauche* and *anti* conformations, and the energy stabilization of the electrostatic interaction could not be calculated directly. We, therefore, instead investigated the influence of the interaction on the conformation, by comparing structures both with and without a positive charge. For the cation **8**, our DFT calculations were consistent with the single-crystal X-ray structure, indicating a puckered conformation that brings the C–F and N^+–H bonds into proximity. Note that the positive Mulliken charge density on the hydrogen atoms is significant. When an additional electron was added to **8** to give the neutral (but sterically identical) azetidine **9**, however, the ring puckered in the opposite direction, consistent with the removal of a favorable C–F···H–N^+ interaction and the increased steric size of fluorine over hydrogen.

8

9

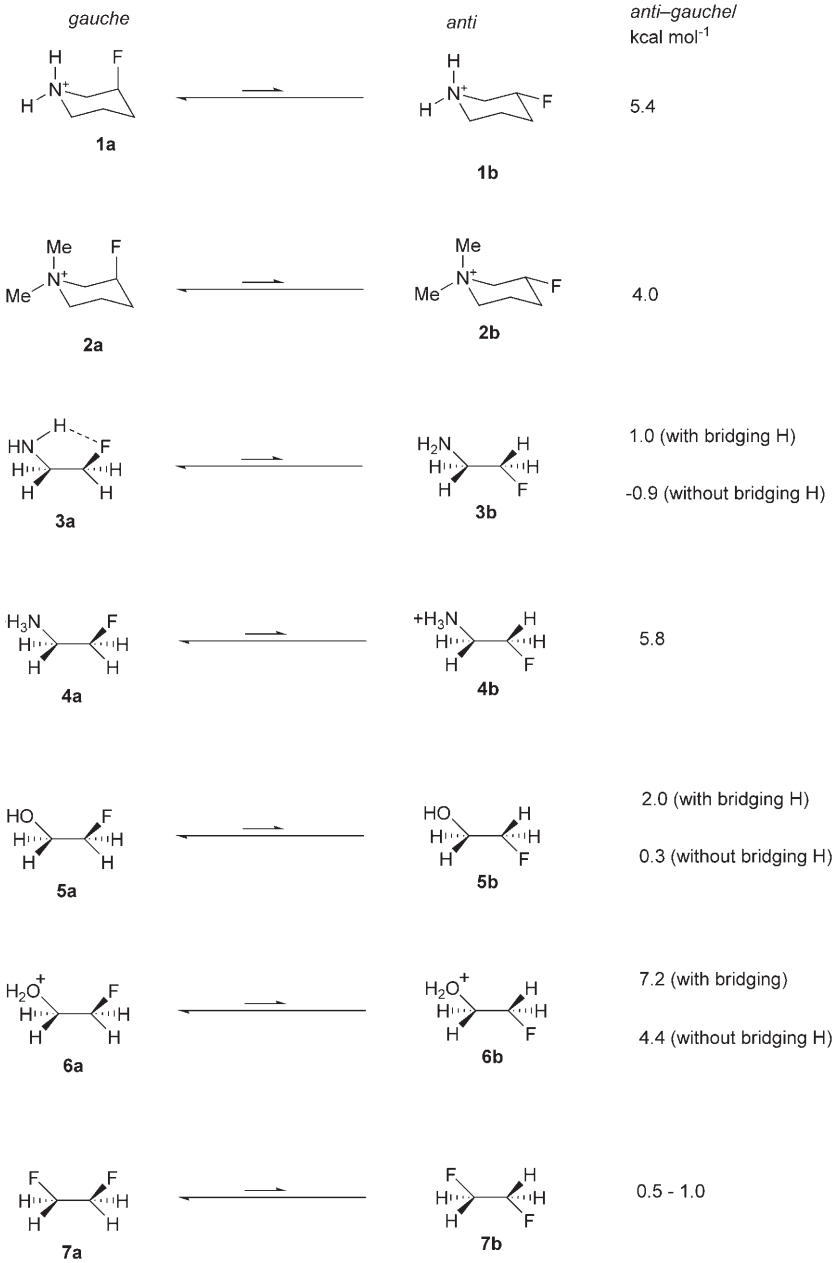
Hydrogen bonding complicates the analysis of these interactions. For example, in cases such as **1** and **4**, it is not possible to deconvolute the intrinsic *gauche* effect from the intramolecular electrostatic hydrogen bonds. In a ring system such as **2**, a significant component of the stabilization involves electrostatic N^+–CH_3···F–C interactions, because the positive charge density is greater on the hydrogen atoms than on the nitrogen atom.[5] Herein, we minimize the influence of such interactions by considering the *N*-(2-fluoroethyl)pyridinium systems **10** and **11** [Eq. (1)]. The salts were prepared by reaction of the corresponding pyridine with the tosyl derivative of 2-fluoroethanol.[9] We also consider the effect of replacing the fluorine atom with a hydroxy group; the salt **12** was prepared by reaction of pyridine with 2-chloroethanol.[10]

The salts **10**–**12** were crystalline, and the structures of the pyridinium cations, determined by single-crystal X-ray dif-

[*] N. E. J. Gooseman, Prof. Dr. D. O'Hagan, Prof. Dr. A. M. Z. Slawin
School of Chemistry and Centre for Biomolecular Sciences
University of St. Andrews
North Haugh, St. Andrews, Fife, KY16 9ST (UK)
Fax: (+44) 1334-463-808
E-mail: do1@st-and.ac.uk
M. J. G. Peach, Dr. D. J. Tozer
Department of Chemistry
University of Durham
Science Laboratories, South Road, Durham, DH1 3LE (UK)
Fax: (+44) 191-3844737
E-mail: d.j.tozer@durham.ac.uk
Dr. R. J. Young
Discovery Medicinal Chemistry
GlaxoSmithKline R&D
Stevenage, SG1 2NY (UK)

[**] D.O'H. thanks the BBSRC and GSK for a CASE Studentship (N.E.J.G.), and D.J.T. thanks the EPSRC for a DTA Studentship (M.J.G.P.). We thank Dr. T. Lebl (St Andrews University) for assistance with NMR stimulation.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

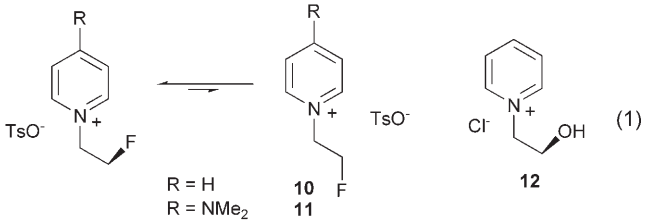


Scheme 1. *Gauche* and *anti* conformations of organic molecules containing fluorine atoms vicinal to different functional groups, and the *anti–gauche* energy difference (from gas-phase DFT calculations). The gauche conformation is strongly preferred when the functional group is cationic.[5,7]

fraction, are shown in Figure 1.[11] In all three cases, there is a very obvious *gauche* relationship between the C–F or C–OH bond and the C–N^+ bond to the pyridinium ring (N-C-C-F 68.1(7)° for **10** and 59.1(4)° for **11**; N-C-C-O 61.2(2)° for **12**). There is no evidence of intramolecular hydrogen bonding to the fluorine atom. For example, in **10** the shortest C–F···H–C contact, which is to the *ortho* hydrogen atom at C(2) of the pyridinium ring, has an F···C distance of 2.71(1) Å, which is beyond a van der Waals contact, and a rather acute F···H–C angle of 98.3(5)°.

Derivative **11** of the parent compound **10** was chosen, as it was anticipated that the dimethylamino group would reduce the positive charge density on the pyridinium ring owing to conjugative effects. In the event, both structures display very clear *gauche* preferences both in the solid and the solution states. For example, the ^{1}H and ^{19}F NMR spectra of **10** and **11** in water reveal average $^3J_{HH}$ coupling constants of 4.3 and 4.7 Hz, respectively, and $^3J_{HF}$ coupling constants of 26.4 and 27.4 Hz, respectively, for the 2-fluoroethyl moieties. The average $^3J_{HF}$ coupling constants are particularly definitive in this respect, as the values are consistent with those of the predicted *gauche* conformer ($^3J_{HH} = 5.0$ Hz and $^3J_{HF} = 26.5$ Hz) and inconsistent with those of the predicted *anti* conformer ($^3J_{HH} = 6.0$ Hz and $^3J_{HF} = 8$ Hz) or of an equally mixed population ($^3J_{HH} = 5.5$ Hz and $^3J_{HF} = 17.3$ Hz).[12]

To quantify the intramolecular *gauche* preference in these three cations, calculations were performed on isolated systems (that is, nonperiodic), using Kohn–Sham DFT with the B97-2 hybrid exchange–correlation-energy functional.[13] We have confirmed that qualitatively similar results are obtained with the widely used B3LYP functional.[14] Following previous methods,[8] all calculations were performed using the TZ2P basis set,[15] augmented with additional s and p diffuse functions on the non-hydrogen atoms. The molecular structures were optimized, and their analytic harmonic vibrational frequencies were calculated to confirm that the located stationary points were minima on the potential-energy surface. The quoted energy differences include zero-point vibrational corrections determined using these harmonic frequencies. All calculations were performed using the Gaussian 03 program.[16]



Consistent with the X-ray structures, the cations of all three compounds **10**–**12** have *gauche* conformers that are 3.1–3.7 kcal mol^{-1} lower in energy compared to their *anti* conformers (Scheme 2). To a first approximation, the optimized *gauche* structures are similar to the X-ray structures, with the plane of the pyridinium ring lying nearly perpendicular to the C–C bond of the 2-fluoroethyl moiety. The calculation of

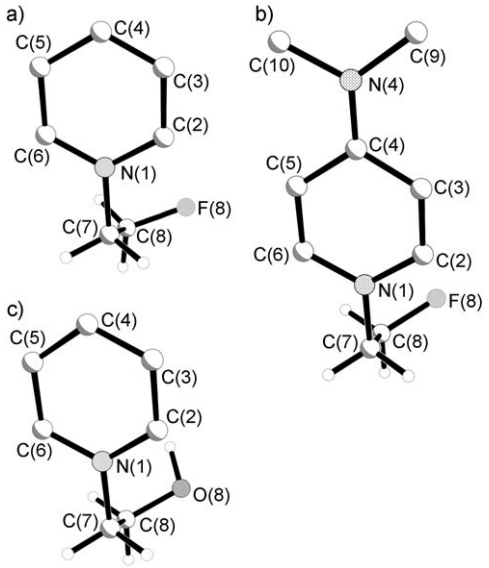
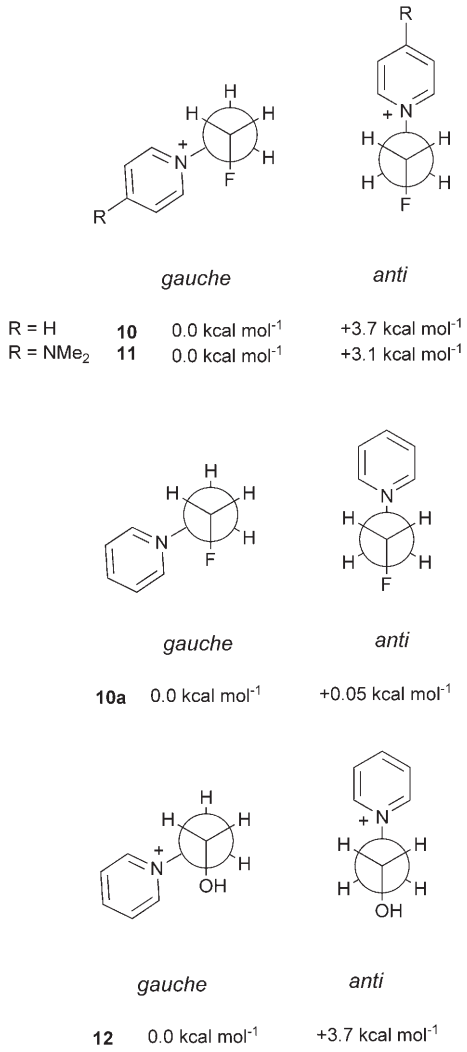


Figure 1. Molecular structures of a) **10** (N-C-C-F 68.1(7)°), b) **11** (N-C-C-F 59.1(4)°), and c) **12** (N-C-C-O 61.2(2)°). Hydrogen atoms and anions are omitted.



Scheme 2. Relative energies of the DFT-optimized *gauche* and *anti* conformers of **10**, **11**, and **12**. The *anti–gauche* energy difference almost disappears in the neutral system **10a**.

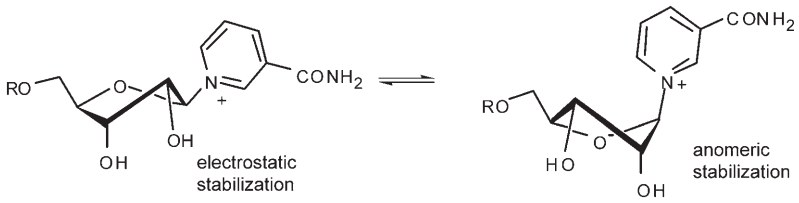
Mulliken charge densities for **10** indicated that the pyridinium nitrogen atom has a +0.29 charge, whereas the adjacent *ortho* carbon (+0.01) and hydrogen (+0.12) atoms carry a significantly less-positive charge. This situation is the reverse of that, for example, in alkylammonium ($[RNH_3]^+$) or alkyltrimethylammonium ($[RN(CH_3)_3]^+$) cations, where the positive charge density resides predominantly on the hydrogen atoms.[5] The electron-donating influence of the *para*-dimethylamino group is apparent in the reduction of the *anti–gauche* energy difference by about 0.6 kcal mol^{-1} for **11** relative to **10**. Following our previous strategy,[8] we also added an electron to cation **10** to generate the chemically counter-intuitive neutral compound **10a**. The *anti–gauche* energy difference for **10a** is reduced to 0.05 kcal mol^{-1}, clearly highlighting the importance of the positive charge in stabilizing the *gauche* structure. In effect, there is no favored conformation for neutral **10a**. Analogous observations were made when an electron was added to **11**.

For the β-hydroxyethyl analogue **12**, the energy difference between the *gauche* and *anti* conformers shown in Scheme 2 is 3.7 kcal mol^{-1}, which reveals a clear *gauche* preference, similar to that found in the β-fluoroethyl system **10**; the replacement of the fluorine atom by a hydroxy group results in a similar phenomenon of similar magnitude. Fluorine, which after helium is the element next in size to hydrogen,[17] is closely isosteric to oxygen in a hydroxy or carbonyl group;[18,19] thus, steric effects are comparable between these systems.

Such a correlation has some precedent in structural studies on collagen where 4-hydroxyproline residues were replaced by 4-fluoroproline residues.[20] This substitution did not weaken the integrity of the triple-helical structures of collagen. The polarization of the C–O bond of the alcohol, rather than its ability to enter into intra- and intermolecular hydrogen bonding, was responsible for the ring conformation and overall helical stability of collagen; fluorine could substitute for oxygen without any detrimental effect.

We assume that a common electrostatic interaction occurs in *N*-ethylpyridinium cations. A review of the structures in the Cambridge Structural Database (CSD) reveals 10 β-hydroxy-*N*-ethylpyridinium cations and related structures.[21] A survey of the N-C-C-O torsion angles reveals a common trend: the *gauche* conformations are observed, without exception. Perhaps of direct relevance to the current study is the conformation of the enzyme cofactor nicotinamide adenine dinucleotide (NAD^+; **13**). Many studies of the conformation of this cofactor have been carried out in solution,[22] in the solid state,[23] and from X-ray structures of the cofactor bound to various enzymes.[24] In different situations, NAD^+ adopts either a C2′-*endo* conformation (**13a**) or a C3′-*endo* conformation [**13b**; Eq. (2)].

It is notable that the C2′-*endo* structure of NAD^+ accommodates the *gauche* electrostatic interaction revealed in this study, whereas the C3′-*endo* structure accommodates the anomeric effect. The two effects are mutually exclusive.



C2′ endo **13a** C3′-endo **13b** (2)

As far as we are aware, the *gauche* electrostatic interaction has not previously been discussed with respect to the origin of the C2′-*endo* conformation of NAD^+, although it should be a significant contributor.

Received: February 15, 2007
Revised: April 4, 2007
Published online: July 3, 2007

Keywords: conformation analysis · density functional calculations · electrostatic interactions · *gauche* effect · pyridinium cations

[1] D. M. Smith, K. A. Woerpel, *Org. Biomol. Chem.* **2006**, *4*, 1195 – 1201.
[2] K. Tenza, J. S. Northen, D. O'Hagan, A. M. Z. Slawin, *Beilstein J. Org. Chem.* **2005**, *1*, 13.
[3] D. C. Lankin, N. S. Chandrakumar, S. N. Rao, D. P. Spangler, J. P. Snyder, *J. Am. Chem. Soc.* **1993**, *115*, 3356 – 3357.
[4] J. P. Snyder, N. S. Chandrakumar, H. Sato, D. C. Lankin, *J. Am. Chem. Soc.* **2000**, *122*, 544 – 545.
[5] A. M. Sun, D. C. Lankin, K. Hardcastle, J. P. Snyder, *Chem. Eur. J.* **2005**, *11*, 1579 – 1591.
[6] D. C. Lankin, G. L. Grunewald, F. A. Romero, I. Y. Oren, J. P. Snyder, *Org. Lett.* **2002**, *4*, 3557 – 3560.
[7] C. R. S. Briggs, M. J Allen, D. O'Hagan, D. J. Tozer, A. M. Z. Slawin, A. E. Goeta, J. A. K. Howard, *Org. Biomol. Chem.* **2004**, *2*, 732 – 740.
[8] N. E. J. Gooseman, D. O'Hagan, A. M. Z. Slawin, A. M. Teale, D. J. Tozer, R. J. Young, *Chem. Commun.* **2006**, 3190 – 3192.
[9] Preparation of **10**: Pyridine (59 mg, 0.74 mmol) and 2-fluoroethyltosylate (100 mg, 0.46 mmol) were heated at 110 °C for 20 h. The solvent was removed, and HCl in ether was added to give a solid, which was recrystallized from ether/ethanol (37 % yield). M.p. 207–210 °C; ^{1}H NMR (D_2O, 300.06 MHz): $\delta = 2.24$ (3 H, s, CH_3), 4.82 (4 H, dm, NCH_2, CH_2F), 7.20 (2 H, d, CH), 7.53 (2 H, d, CH), 7.94 (2 H, t, CH), 8.44 (1 H, t, CH), 8.71 ppm (2 H, d, CH); ^{13}C NMR (D_2O, 75.5 MHz): $\delta = 20.4$ (CH_3), 61.4, (d, $J =$ 18.1 Hz, NCH_2), 81.8 (d, $J = 169.8$ Hz, CH_2F), 125.3, 128.3, 129.4, 139.4, 142.4, 144.7, 146.3 ppm; ^{19}F NMR (D_2O, 282.3 MHz): $\delta =$ −224.2 (m); for NCH_2CH_2F, $^2J_{HF} = 47.1$ Hz, $^3J_{HF} = 26.4$ Hz, $^3J_{HH} = 4.3$ Hz by simulation, Bruker TopSpin/Daisy; HRMS: *m*/*z*: calcd for C_7H_9NF: 126.0719; found: 126.0714 $[M]^+$. Preparation of **11**: A solution of 4-dimethylaminopyridine (44 mg, 0.36 mmol) and 2-fluoroethyltosylate (76 mg, 0.35 mmol) in acetonitrile (1 mL) was heated at 90 °C for 16 h. The solvent was removed, and the salt was recrystallized from ether/ethanol (76 % yield). M.p. 118–120 °C; ^{1}H NMR (D_2O, 300.06 MHz): $\delta = 2.26$ (3 H, s, $ArCH_3$), 3.07 (6 H, s, $N(CH_3)_2$), 4.32 (2 H, dt, $^3J_{HF} = 27.4$ Hz, $^3J_{HH} = 4.7$ Hz, NCH_2), 4.7 (2 H, dt, $^2J_{HF} = 46.4$ Hz, $^3J_{HH} = 4.7$ Hz, CH_2F), 6.76 (2 H, d, CH), 7.23 (2 H, d, CH), 7.55 (2 H, d, CH), 7.88 ppm (2 H, d, CH); ^{13}C NMR (D_2O, 75.47 MHz): $\delta = 20.4$ (CH_3), 39.3 ($N(CH_3)_2$) 57.2 (d, $^2J_{CF} =$ 19.1 Hz, NCH_2), 81.4, (d, $^1J_{CF} =$ 167.6 Hz, CH_2F), 107.5, 125.3, 129.4, 139.3, 142.5, 141.5, 156.4 ppm; ^{19}F NMR (D_2O, 282.3 MHz): $\delta = -224.46$ ppm (tt, $^2J_{HF} = 46.8$ Hz, $^3J_{HF} = 27.4$ Hz); HRMS: *m*/*z*: calcd for $C_9H_{14}N_2F$: 169.1141; found: 169.1135 $[M]^+$.
[10] Preparation of **12**: Pyridine (1.3 mL, 15.9 mmol) and 2-chloroethanol (0.95 mL, 14.2 mmol) were heated at 110 °C for 16 h. The residual pyridine was removed under vacuum, giving a light brown solid. Colorless crystalline needles were obtained after recrystallization in ether/ethanol at 4 °C (98 % yield). M.p. 66–68 °C; ^{1}H NMR (CD_3OD, 300.06 MHz): $\delta = 4.05$ (2 H, t, $^3J_{HH} =$ 4.9 Hz, CH_2OH), 4.79 (2 H, t, $^3J_{HH} = 4.9$ Hz, NCH_2), 8.18 (2 H, d, CH), 8.67 (1 H, t, CH), 9.04 ppm (2 H, d, CH); ^{13}C NMR (CD_3OD, 75.47 MHz): $\delta = 62.1$ (OCH_2), 65.5 (NCH_2), 129.6, 146.9, 147.5 ppm (CH); HRMS: *m*/*z*: calcd for $C_7H_{10}NO$: 124.0762; found: 124.0763 $[M]^+$.
[11] Crystal data for **10**: $C_{21}H_{23}FNNaO_6S_2$, $M_r = 491.51$, triclinic, space group $P\bar{1}$, $a = 9.7646(17)$, $b = 9.8148(16)$, $c = 13.290(2)$ Å, $\alpha = 78.499(8)$, $\beta = 80.851(8)$, $\gamma = 66.472(7)^o$, $V = 1139.9(3)$ Å^3, $F(000) = 512$, $Z = 2$, $\rho_{calcd} = 1.432$ Mg m^{-3}, $\mu = 2.716$ mm^{-1}, $Cu_{K\alpha}$ radiation ($\lambda = 1.54178$ Å), $T = 173$ K, 14680 reflections, $3.41 < \theta < 67.61°$, Rigaku Saturn 92 detector with 007 generator, 3712 unique data, $R_{merg} = 0.1643$, final $R = 0.0948$ (for 2642 reflections with $I \geq 2\sigma(I)$), GOF = 1.090, 292 refined parameters, max. residual electron density: 0.525 e Å^{-3}. Crystal data for **11:** $C_{16}H_{21}FN_2O_3S$, $M_r = 340.41$, triclinic, space group $P\bar{1}$, $a =$ 9.3305(12), $b = 9.4854(13)$, $c = 9.6604(14)$ Å, $\alpha = 78.152(13)$, $\beta = 79.100(15)$, $\gamma = 74.664(14)^o$, $V = 798.70(19)$ Å^3, $F(000) =$ 360, $Z = 2$, $\rho_{calcd} = 1.415$ Mg m^{-3}, $\mu = 0.230$ mm^{-1}, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 93(2)$ K, 5130 reflections, $2.37 < \theta < 25.37°$, Rigaku Mercury CCD diffractometer, 2727 unique data, $R_{merg} =$ 0.0429, final $R = 0.0617$ (for 1688 reflections with $I > 2\sigma(I)$), GOF = 1.048, 213 refined parameters, max. residual electron density: 0.320 e Å^{-3}. Crystal data for **12:** $C_7H_{10}ClNO$, $M_r =$ 159.61, orthorhombic, space group *Pbca*, $a = 12.023(3)$, $b =$ 7.2111(15), $c = 17.605(4)$ Å, $V = 1526.3(6)$ Å^3, $F(000) = 672$, $Z = 8$, $\rho_{calcd} = 1.389$ Mg m^{-3}, $\mu = 0.428$ mm^{-1}, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 93(2)$ K, 8307 reflections, $2.87 < \theta < 25.35°$, Rigaku Mercury CCD diffractometer, 1361 unique data, $R_{merg} =$ 0.0327, final $R = 0.0297$ (for 1265 reflections with $I > 2\sigma$(I)), GOF = 1.108, 96 refined parameters, max. residual electron density: 0.197 e Å^{-3}. CCDC 652106 (**10**), CCDC 652101 (**11**), and CCDC 652108 (**12**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[12] A. M. Ihrig, S. L. Smith, *J. Am. Chem. Soc.* **1970**, *92*, 759 – 763; D. O'Hagan, H. S. Rzepa, M. Schüler, A. M. Z. Slawin, *Beilstein J. Org. Chem.* **2006**, *2*, 19. The $^3J_{HF}$ coupling constants obey Karplus-type relationships: their magnitude varies with the F-C-C-H angle, but also with the electronegativity of the substituent vicinal to the fluorine atom. The high electronegativity of the pyridinium group is consistent with a *antiperiplanar* $^3J_{HF}$ value (that is, for F-C-C-H 180°) of about 45 Hz and a *synclinal* $^3J_{HF}$ value (that is, for F-C-C-H 60 or 300°) of about 8 Hz. The average $^3J_{HF}$ value calculated for the *gauche* isomers ((45 Hz + 8 Hz)/2 = 26.5 Hz) is close to the experimental $^3J_{HF}$ values for both **10** and **11**. However, we appreciate that the mixing of a small population of the *trans* isomer would be difficult to discern, although this population is unlikely to be greater than 1 %, given the energy differences. In the case of **10**, the chemical shifts of both sets of methylene protons in the 2-fluoroethyl moiety are

almost identical. This situation gives rise to a second-order ^{1}H and ^{19}F NMR spectrum, which could be accurately simulated (Bruker Topspin/Daisy) with average $^{3}J_{HF}$ and $^{3}J_{HH}$ values of 26 and 4.3 Hz, respectively.

[13] P. J. Wilson, T. J. Bradley, D. J. Tozer, *J. Chem. Phys.* **2001**, *115*, 9233 – 9242.

[14] A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648 – 5652; C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785 – 789.

[15] T. H. Dunning, *J. Chem. Phys.* **1971**, *55*, 716 – 723.

[16] Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian, Inc., Wallingford, CT, **2004**.

[17] K. Uneyama, *Organofluorine Chemistry*, Blackwell, Oxford, **2006**; A. Bondi, *J. Phys. Chem.* **1964**, *68*, 441 – 451.

[18] J. C. Biffinger, H. W. Kim, S. G. DiMagno, *ChemBioChem* **2004**, *5*, 622 – 627.

[19] R. Paulini, K. Mueller, F. Dederich, *Angew. Chem.* **2005**, *117*, 1820 – 1839; *Angew. Chem. Int. Ed.* **2005**, *44*, 1788 – 1805.

[20] M. D. Shoulders, J. A. Hodges, R. T. Raines, *J. Am. Chem. Soc.* **2006**, *128*, 8112 – 8113; S. K. Holmgren, K. M. Taylor, L. E. Bretscher, R. T. Raines, *Nature* **1998**, *392*, 666 – 667.

[21] See the Supporting Information for a full list. CSD reference codes: **13a**: NOFFAP; **13b**: NOWDUY.

[22] W. Egan, S. Forsen, J. Jacobus, *Biochemistry* **1975**, *14*, 735 – 742; E. Ragg, L. Scaglioni, R. Mondelli, I. Carelli, A. Casini, S. Tortorella, *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **1991**, *1076*, 49 – 60.

[23] R. Parthasarathy, S. M. Fridey, *Science* **1984**, *226*, 969 – 971; B. Guillot, N. Muzet, E. Artacho, C. Lecomte, C. Jelsch, *J. Phys. Chem. B* **2003**, *107*, 9109 – 9121.

[24] S. J. F. Vincent, C. Zwahlen, C. B. Post, J. W. Burgner, G. Bodenhausen, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4383 – 4388; C. Schalk-Hihi, Y.-Z. Zhang, G. D. Markham, *Biochemistry* **1998**, *37*, 7608 – 7616; J. J. Tanner, S-C Tu, L. J. Barbour, C. L. Barnes, K. L. Krause, *Protein Sci.* **1999**, *8*, 1725 – 1732.

