Fluorinated Analogues of Biological Molecules: Accessing New Chemical, Physical and Biological Properties

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Introduction

The introduction of fluorine into biological molecules often results in significant changes in their chemical, physical, and biological properties. As such, fluorinated analogues of biological molecules provide useful tools for probing and modifying the functions of biological systems. Where such modifications are beneficial to humans the fluorinated analogue becomes a potential therapeutic agent.

The potent effect of introducing fluorine is demonstrated here by describing the unique properties of a number of fluorinated biological compounds (amino acids, peptides and sugars). In particular, a focus on compounds that possess a single fluorine atom at a chiral centre serves to demonstrate how even a single F atom can have profound effects on the properties of a biologically active molecule. However, before discussing specific examples let us first consider some of the common effects that the introduction of fluorine can have on an organic molecule.

• As the most electronegative element, fluorine introduction has a potent electronic effect, particularly on nearby functional groups, *e.g.* the acidity of an adjacent carboxylic moiety is increased.

• Fluorine is similar in size to hydrogen and is often treated as a steric isostere of hydrogen. This assumption has been disputed due to the difference in C-H and C-F bond lengths (106 and 134 pm, respectively) which does affect the size of fluorinated molecules, especially those containing more than one fluorine atom, *i.e.* -CF_3 is now considered to be a steric isostere of the –$CH(CH_3)_2$ group.

• The replacement of H with F generally increases lipophilicity, a useful property in the design of medicinal agents.

• The C-F bond strength is relatively high and, in combination with its small size, allows it to be a suitable replacement for labile or oxidizable C-H bonds. Such substitutions can circumvent metabolism issues in the pharmaceutical industry.

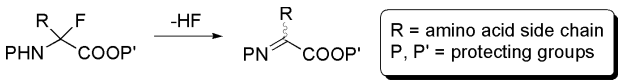
• The presence of fluorine in organic molecules can have a significant effect on the preferred conformation, and when strategically placed they may also influence the preferred conformations of amides and peptides.

• The use of ^{19}F NMR spectroscopy provides an analytical tool that can aid complex organic structure determination.

Fluorinated Amino Acids, Peptides, and Proteins

α-Amino acids

α-Amino acids are the basic building blocks of proteins and peptides. As such, fluorinated analogues of α-amino acids would provide valuable tools in the study of peptide and protein structure and function. Early research, however, revealed that amino acids fluorinated at the α position are unstable, undergoing immediate dehydrofluorination (Scheme 1).[1] Consequently, little use has been found for α fluorinated α-amino acids. Fluorination of the side chain is still possible and can result in derivatives with unique properties, particularly when the fluorinated amino acid is incorporated into a peptide or protein. A notable example of this can be seen in the use of 4-fluoroproline to produce fluorinated analogues of collagen.[2]



Scheme 1

Fluorinated Collagen

Collagen is an extremely well studied protein, no doubt due to its important biological properties and its unusual structure. The stability of its triple helix is due to the high percentage of proline and hydroxyproline residues, which have long been regarded as providing stability by hydrogen bond formation between the hydroxyl groups of the latter and amide carbonyls in the collagen backbone. However, recent work has shown that replacement of the (4*R*)-hydroxyproline residues with their (4*R*)-fluoroproline analogue actually *increases* triple helix stability.[2] Furthermore, incorporation of the diastereomeric (4*S*) isomer greatly destabilises the triple helical structure of the fluorinated collagen analogue. As fluorine rarely participates in H-bond formation (and only then as acceptor), it is now proposed that at least part of the stabilisation in collagen must be attributed to a stereoelectronic effect whereby a (4*R*)-fluorine (or hydroxyl) force the proline residues to adopt a conformation conducive to helix structure.

As well as the insight that this work provides into the formation of the secondary/tertiary structure of collagen, it is also proposed that these new fluorinated (hyperstable) forms of collagen may have potential applications as biomaterials for use in wound healing and tissue repair.

β-Amino acids

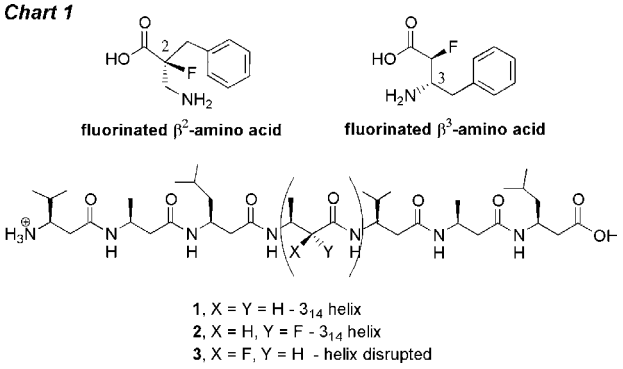
β-amino acids demonstrate many similarities to their α-analogues. For instance β-peptides, oligomers of β-amino

acids, have an ability to form secondary structures such as helices and turns. Hence they have been used as tools for studying peptide structures and functions. β-Peptides also have the advantage of being metabolically much more stable than peptides derived from α-amino acids, making them of great interest to medicinal chemists.

The presence of the CH_2 spacer group in the backbone also allows fluorine to be incorporated into the β-amino acid backbone without the problem of dehydrofluorination, *i.e.* so that it is not adjacent to the amine functionality.

There are two types of β-amino acids, β^2- and β^3-amino acids as shown by the fluorinated phenylalanine analogues in Chart 1. Fluorinated β^3-amino acids have been used to examine the effect of fluorine on secondary structure formation in β-peptides. In 2005, Seebach *et al.*[3] prepared the β-heptamers **1-3** (Chart 1) and examined their secondary structure using NMR spectroscopy. While the non-fluorinated **1** and fluorinated **2** adopt stable 3_{14} helixes, it was found that the presence of the fluorine atom in the non-axial position of **3** is enough to disrupt the 3_{14} helix. While fluorinated β^2 amino acids are not easy to obtain, recent work in our laboratory has provided a new methodology for their preparation. We are now working to see if these compounds can be incorporated into β-peptides and produce similar effects to those observed for fluorinated β^3 peptides.

Chart 1



Conformational effects – amide bonds

Fluorine is known to produce two conformational effects in organic amides. When positioned α to the carbonyl group of an amide it preferentially adopts a conformation *anti* to the carbonyl and *syn* to the C-N bond.[4] On the other hand, when β to the N atom of an amide the fluorine favours a conformation in which the C-F and C-N bonds are *gauche* with a dihedral angle of approx. 63º (Fig. 1).[5]

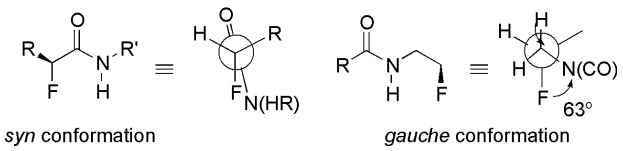


Fig. 1. Conformations of α- and β-fluoroamides.

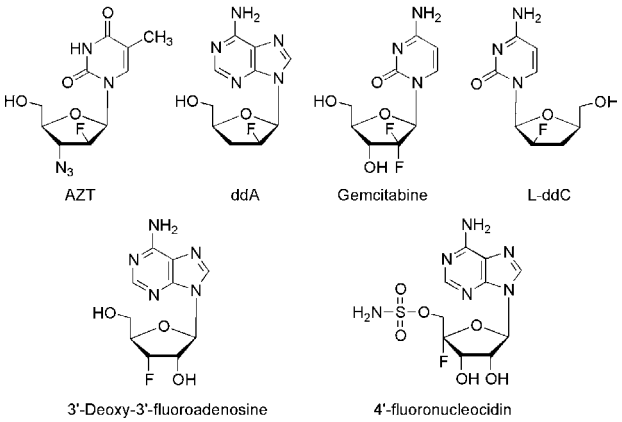
The ability of fluorine to influence the conformation of amide bonds has potential applications in medicinal chemistry where the design and control of the conformation of bioactive compounds is highly desirable. The *gauche* effect of fluorine has also been used to explain the unusual properties of fluorinated analogues of collagen.[2]

Fluorinated Sugars

The many roles of sugars in biological systems make them a key target for modification in the development of new tools for studying biological systems and new medicinal agents.

Nucleosides

Chart 2



The incorporation of fluorinated sugar residues into nucleosides has provided a number of potent therapeutic agents (mainly anticancer and antiviral),[6] as well as additional information on nucleoside function. The location of fluorine in the sugar is a major determinant with regards to its biological and medicinal effect. Fluorination at C2 to give a β-oriented fluorine has provided a number of potent antivirals such as AZT and ddA (Chart 2), both powerful inhibitors of HIV. The glycosylic bond is stabilized against hydrolysis by this adjacent fluorine and the tendency to undergo enzymatic deamination is reduced. The stereoelectronic effect of the F atom also locks the molecule into a preferred conformation. While α-fluorination at C'2 does not usually produce sugars of therapeutic value, the difluorinated C2' compound *Gemcitabine* (Chart 2) is a potent anticancer agent approved by the FDA for the treatment of pancreatic cancer. A further interesting approach to therapeutic C2' fluorosugars has been through synthesis of the unnatural L-configuration such as in L-ddC. These fluorinated L-nucleosides are reported to have strongly antiviral and anticancer properties, but possess lower toxicities than their D-counterparts.

Fluorination of nucleosides at positions other than C2' also introduce some beneficial stabilizing and conformational effects, and has been used to prepare compounds of medicinal and biological interest. 3'-Deoxy-3'-fluoroadenosine, for example, is active as an antiviral and anticancer compound, while fluorination at C4' has been used to produce a fluorinated analogue of the antitrypanosomal antibiotic, nucleocidin.

Enzyme probes and inhibitors

Sugars play important roles in many biological systems. Their role in immunological recognition and as components of genetic material also identifies them as suitable targets for developing therapeutic agents. Consequently, there are many enzymes that use sugars as substrates.

In order to better understand how enzymes process sug-

ars the substrate is often modified and the subsequent changes in enzyme function recorded. Incorporation of fluorine into the sugar analogues provides a strong electron-withdrawing effect expected to alter the mechanism of enzyme action. This has recently been demonstrated by Coward *et al*.[7] who, in initial studies, demonstrated that the 5-fluoro analogue of *N*-acetylglucosamine completely blocks epimerisation by a 4-epimerase enzyme, and also significantly alters the kcat/Km value in catalysis by a glycosyltransferase enzyme.

Conclusions

The incorporation of fluorine into biological molecules is a powerful way of modifying the properties of these molecules. At present the number of methods for incorporating fluorine into organic molecules is quite small, but a recent resurgence in organofluorine chemistry now promises new methods for synthesis. With the development of such methods one can expect an increase in the number of novel fluorinated analogues of biological molecules used to elucidate enzyme mechanisms and develop novel therapeutic agents and biopolymers.

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