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Review

Use of fluorinated functionality in enzyme inhibitor development: Mechanistic and analytical advantages

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ABSTRACT

On the one hand, owing to its electronegativity, relatively small size, and notable leaving group ability from anionic intermediates, fluorine offers unique opportunities for mechanism-based enzyme inhibitor design. On the other, the "bio-orthogonal" and NMR-active 19-fluorine nucleus allows the bioorganic chemist to follow the mechanistic fate of fluorinated substrate analogues or inhibitors as they are enzymatically processed. This article takes an overview of the field, highlighting key developments along these lines. It begins by highlighting new screening methodologies for drug discovery that involve appropriate tagging of either the substrate or an array of potential substrates (i.e. in proteomics screens) with ^{19}F-markers that then report back on turnover and function, respectively, via the NMR screen. Taking this one step further, substrate-tagging with fluorine can be done in such a manner as to provide stereochemical information on enzyme mechanism. For example, substitution of one of the terminal hydrogens in phosphoenolpyruvate, provides insight into the, otherwise latent, facial selectivity of C–C bond formation in KDO synthase. Perhaps, most importantly, from the point of view of this discussion, appropriately tailored fluorinated functionality can be used to form stabilized "transition state analogue" complexes with target enzymes. Thus, 5-fluorinated pyrimidines, α-fluorinated ketones, and 2-fluoro-2-deoxysugars each lead to covalent adduction of catalytic active site residues in thymidylate synthase (TS), serine protease and glycosidase enzymes, respectively. In all such cases, ^{19}F NMR allows the bioorganic chemist to spectrally follow "transition state analogue" formation. Finally, the use of specific fluorinated functionality to engineer "suicide substrates" is highlighted in a discussion of the development of the α-(2′Z-fluoro)vinyl trigger for amino acid decarboxylase inactivation. Here ^{19}F NMR allows the bioorganic chemist to glean useful partition ratio data directly from the NMR tube.

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1. Introduction

The C–F bond is one of the strongest covalent bonds available, with an average bond energy of approximately 105–116 kcal/mol. This contributes significantly to the relative metabolic inertness of carbon–fluorine bonds, particularly those at unactivated sp^2-carbon-centers. Moreover, introduction of a C–F bond imposes only modest steric constraints, as the C–F bond (1.41–1.47 Å) is slightly shorter than a C–OH bond (1.52 Å) [1]. And while fluorine is the most electronegative element in the periodic table, it also has a very a small atomic radius, resulting in an exceptionally low polarizability. DiMagno has pointed out that this feature of organically bound fluorine means that fluoroalkyl groups are less able to engage in dispersion-based interactions with aqueous solvent than simple alkyl groups. He has proposed the term, "polar hydrophobicity" [2] to describe this phenomenon, and points out that this may provide unique opportunities for enhancing ligand binding to a protein target [3]. In terms of specific interactions with functionalities in proteins, while C–F bonds appear to have rather limited H-bond acceptor ability [4–6], in optimally aligned cases F–H–N–amide interactions may make contributions to binding [7–9]. Additionally, more recent observations by Diederich and Müller [10–12] suggest that the hard C–F bond is able to engage amide carbonyls in specific attractive interactions reminiscent of the sort of trajectory-dependent n–π^* (amine-carbonyl) interactions suggested by Bürgi and Dunitz years before [13,14]. Finally, in the context of ionizable groups, such as fluorinated phosphonates as phosphate surrogates, one can use position and degree of organic fluorination to finely tune the pK_a of the surrogate [15]. Thus, the α-monofluorophosphonates are generally "isoacidic" with the phosphate monoesters that they mimic [16,17]. For all of these reasons, incorporation of fluorinated functionality into ligands directed at protein targets is often advantageous, and will likely remain an important stratagem in medicinal chemistry for years to come [9,18–21]. An interesting new development along these lines involves the incorporation of the SF_5-group, in place of CF_3 groups, for example, as has been put forth by Welch and Lim [22].

It is the purpose of this article to focus on the advantage offered by specific fluorinated functional groups, in both inhibitor design, and in mechanistic analysis. In this regard, emphasis will be placed on the possibility of observing protein–ligand interactions through the use of ^{19}F NMR, and on the development of organofluorine functional groups to target active sites of interest, based upon an understanding of mechanism. We will begin with examples in which fluoroorganics are strategically introduced to serve as NMR-based reporting elements to provide (i) the medicinal chemist with a rapid screen for enzyme inhibition; (ii) the functional proteomics investigator with an assay for function and (iii) the mechanistic enzymologist with information on the stereochemical course of a biocatalytic reaction. From there, our discussion will move into organofluorine functionalities that have been specifically tailored to produce either transition state analogue inhibition or irreversible, enzyme-activated inhibition (i.e. suicide substrates).

2. Emergence of ^{19}F-based NMR screens for inhibitor development and functional proteomics

The past decade or so has seen the coming of age of NMR spectroscopy as a screening tool to facilitate the drug discovery process. This is particularly due to the influential work of Fesik and co-workers in developing so-called SAR by NMR techniques [23,24]. The last few years have seen the emergence of a number of creative ^{19}F-based NMR techniques that highlight the utility of fluorinated functionality in such systems. Notable advantages of the fluorine nucleus include its virtual "bio-orthogonality" [25], and its responsiveness to environmental factors. This is particularly true if one considers fluorination of an enzymatic substrate. The ^{19}F isotropic chemical shift is very sensitive to small structural perturbations, resulting in chemical shift changes with substrate turnover, even in cases where the label is distal to the site of the chemistry. Moreover, if one employs CF_3 groups as tags, one increases sensitivity, generating sharp singlets in the ^{19}F spectrum and obviating the need for proton-decoupling, so long as the CF_3 groups are not scalar-coupled to ^1H nuclei. Thus, trifluoromethylated aromatics are ideal platforms for such applications.

This area has really blossomed in past several years, due in no small part to the work of Dalvit [26]. As is shown in Figs. 1 and 2, for screens of enzyme activity on peptide substrates this technique is particularly well-suited. If one employs trifluoromethylated aromatic amino acids, a single CF_3 group suffices to yield clean assays for both peptide phosphorylation, by AKT kinase in this case, or peptide cleavage, by trypsin here (Fig. 1). Note that the fluorinated reporting amino acid does not itself undergo chemical transformation for either reaction being screened. Because CF_3 groups are employed for the reasons elaborated above, Dalvit labels this method 3-FABS (3 Fluorine Atoms for Biochemical Screening) [27]. A similar approach has been taken by Giralt and co-workers to screen for HIV protease inhibitors [28]. Quite recently, the Dalvit group has pointed out that the installation of reporting amino acids with two symmetrically disposed CF_3 groups (Fig. 2) increases sensitivity [29]. Indeed, his group has shown that sensitivity can be improved still further through the application of cryoprobe technology. These studies have established the ability of such CF_3-tagged substrate methods to yield accurate IC_{50} values. Thus, the 3-FABS approach is expected to see wider application for inhibitor screening, in automated manifolds, particularly in combinatorial chemistry applications.

In addition to this, one of the most promising observations to arise from this work is the notion that one might be able to generate a library of tagged substrates to probe for protein function. Thus, the tetradecapeptide illustrated in Fig. 1 gives unambiguous and distinct signature signals for its specific phosphorylation by AKT kinase, and for its cleavage by trypsin (note that though up to four potential tryptic cleavage sites are present, one appears to be preferred). When used as a test substrate for the PAK-4 protein, one see the signature of serine-kinase activity. It remains to be seen how many such test peptide/signature reactions can be screened in parallel, perhaps in a single NMR tube, but the possibilities are intriguing, to be sure. One can imagine, for example, given optimal chemical shift dispersion, rapidly getting a fingerprint for the substrate specificities of newly isolated kinases, phosphatases, proteases or perhaps even histone acetyltransferases and/or histone deacetylases, by such methods. A principal advantage of such ^{19}F-based functional proteomics techniques is the near "bio-orthogonality" of carbon-bound fluorine [25].

3. Use of fluorinated functionality to reveal latent enzyme stereochemistry

Furdui, Anderson and co-workers have provided an elegant current example of how fluorine can be employed as an analytical tool to study enzyme mechanism, even illuminating otherwise latent issues of pi-facial selectivity. Thus, both *E*- and *Z*-isomers of 3-fluoro-PEP serve as substrates for the enzyme KDO (3-deoxy-D-manno-2-octulosonate) 8-phosphate synthase [30]. And while the product stereochemistry requires that the key C–C bond forming step involve attack of the nucleophilic-PEP 3-carbon upon the *re*-face of D-arabinose 5-phosphate, any facial selectivity with respect to PEP would be invisible here. As can be seen in the ^{19}F NMR