Editorial

For reprint orders, please contact: reprints@future-science.com

Future Medicinal Chemistry

Fluorinated nucleosides, nucleotides and sugar nucleotides

Jonathan P Dolan[‡,1], Caecilie MM Benckendorff[‡,1], Robert A Field[2] & Gavin J Miller*[,1]

[1]School of Chemical and Physical Sciences and Centre for Glycoscience, Keele University, Keele, Staffordshire, ST5 5BG, UK

[2]Department of Chemistry and Manchester Institute of Biotechnology, The University of Manchester, Manchester, M1 7DN, UK

*Author for correspondence: g.j.miller@keele.ac.uk

[‡]Authors contributed equally

> "The successful inclusion of fluorine within nucleoside and sugar nucleotide components presents significant opportunity and ensures an exciting future for such capabilities."

First draft submitted: 30 May 2023; Accepted for publication: 26 June 2023; Published online: 19 July 2023

In this editorial we highlight the inclusion of fluorine within the building blocks for biomolecule assembly, namely, within nucleoside analogs, as precursors to nucleotides and nucleic acids, and phosphorylated sugars as the building blocks of glycans. We shape this context across a commonality in structure for these building blocks, specifically that they contain fluorination within either a nucleoside core or sugar component of a nucleotide. We believe this delivers a useful viewpoint regarding the opportunities afforded by including fluorine within these molecular architectures, and that these motifs have a proven capability to traverse the traditional boundaries of chemical biology and medicinal chemistry.

Fluorinated nucleoside analogs

Despite organofluorine compounds being extremely rare among natural products, approximately 20–25% of drugs in current pharmaceutical pipelines contain at least one fluorine atom [1]. The inclusion of fluorine modulates lipophilic, electronic and steric parameters, influencing pharmacodynamic and pharmacokinetic properties and blocking oxidative metabolism [1,2]. Unsurprisingly therefore, the inclusion of fluorine in analogs of the canonical nucleosides has a longstanding and proven capability within medicinal chemistry research for drug discovery [3], with an arsenal of fluorinated analogs now available for the treatment of many cancers and viral infections. This includes fludarabine (lymphocytic leukemia), an analog of adenosine consisting of an arabino-configured pentose and a C2-fluorinated adenine; clofarabine (lymphoblastic leukemia), an analog of adenosine consisting of a C2′-deoxy-2′-fluoro arabino system and a C2-chlorinated adenine; and sofosbuvir (Figure 1) (chronic hepatitis C), a phosphoramidate prodrug analog of uridine, containing a C2′-deoxy-2′-fluoro-2′-methyl modification. Furthermore, gemcitabine, an analog of cytidine bearing a C2′-deoxy-2′-*gem*-difluoro modification, is currently the second most clinically used nucleoside analog toward metastatic pancreatic, bladder, epithelial ovarian and breast cancers. For the majority of nucleoside analogs, their function as antiviral and anticancer agents requires conversion to an active di- and/or triphosphate metabolite, through a series of consecutive phosphorylations by cellular kinases. For many of these pharmaceutical agents, it is the first phosphorylation to the respective nucleotide monophosphate that is efficacy limiting. To circumvent this, a ProTide approach was successfully developed [4], where a masked, permeable monophosphoramidate is delivered into cells, where it is then metabolized to its nucleotide monophosphate counterpart. This type of modification is showcased by the fluorinated nucleoside analog drug sofosbuvir (Figure 1) [5]. Despite a longstanding clinical success of fluorinated nucleoside analogs for therapeutic intervention, they are not without their limitations; frequently they exhibit low oral bioavailability and cell permeability alongside growing resistance profiles. Thus, the development of new generations of nucleoside analog scaffolds to combat these limitations is an ever-present requirement for medicinal chemistry.

A relatively underexplored structural modification is the replacement of the nucleoside furanose ring oxygen with fluorinated carbon fragments, that is, CHF and CF_2. This presents an intriguing opportunity for development, as such scaffolds are perceived to benefit from a combination of metabolic stability (due to the absence of the

newlands press

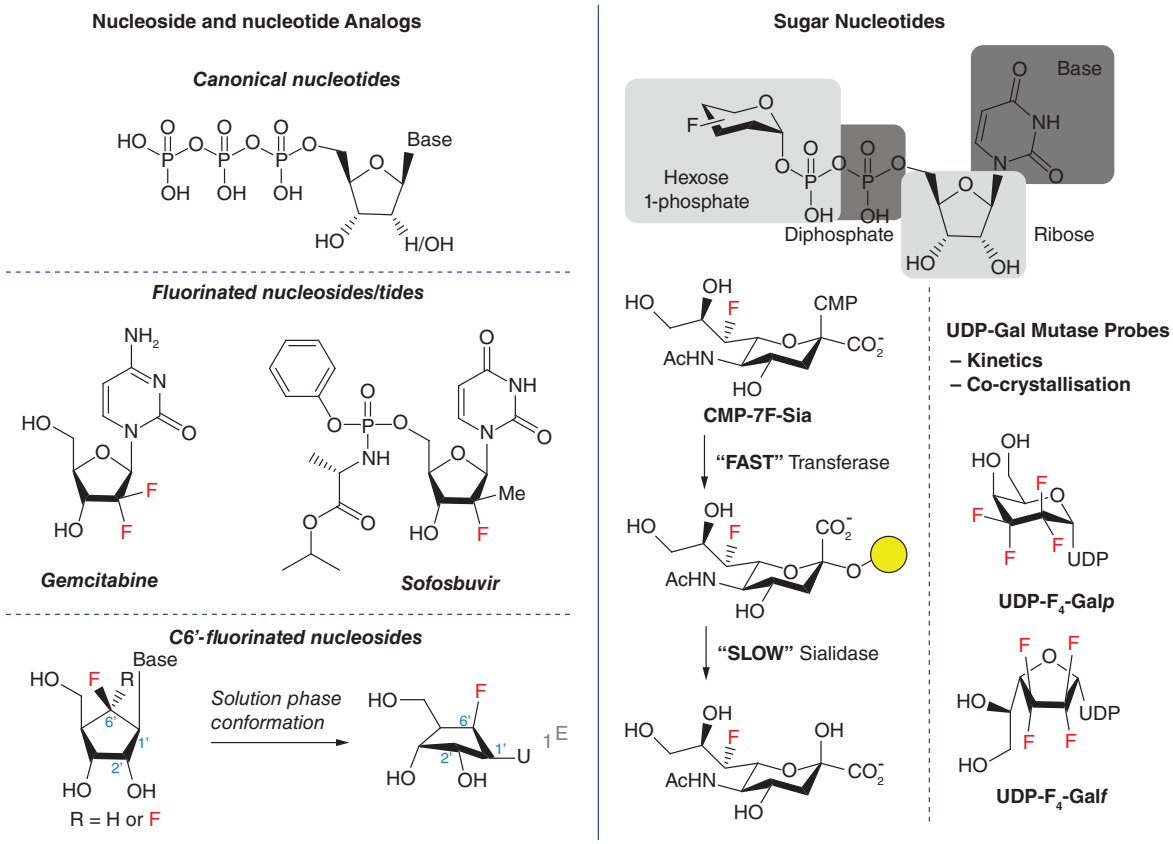

Figure 1. Example fluorinated motifs within nucleoside/-tide components (left side) and sugar nucleotide glycan building blocks (right side).

labile hemiaminal glycosidic linkage) and pharmacokinetic improvements that the inclusion of fluorine can offer. Recent work by Jeong demonstrated the pharmaceutical potential for this modification, with C6′-mono- and *gem*-difluorinated carbocyclic analogs of adenosine displaying potent biological activity as S-adenosylhomocysteine hydrolase inhibitors in +RNA viral assays [6]. Relatedly, arabinose, ribose and deoxyribose pyrimidine analogs containing CHF and CF_2 fragments have been synthesized in our group, and solution-phase NMR conformational analyses for these molecules indicate deviation from that of the parent nucleosides (Figure 1, bottom left) [7,8]. This conformational departure requires further investigation and also presents new opportunities for nucleic acid research. Fluorinated monomer components are already established in oligonucleotide therapeutics (e.g., C2′-deoxy-2′-fluoro) and with the syntheses of the abovementioned building blocks now available, exploring inclusion of C6′-fluorinated monomers within nucleic acid components is realizable.

Fluorinated sugar nucleotides

Moving across from fluorinated nucleoside analogs to fluorinated carbohydrates or glycans, several exciting reviews have recently highlighted the growing effect of fluorine inclusion upon their structure and the use of such motifs as chemical probes for molecular recognition studies [9,10]. Accordingly, the enzymatic production of activated fluorinated sugar nucleotide donor substrates and enzymatic glycosylation with these building blocks and with fluorinated sugar acceptors is an area of intensifying importance. Scope for chemoenzymatic synthesis of (or using) fluorinated sugar nucleotides is rapidly expanding, as aforementioned probes [11], but also as substrates for the enzymatic preparation of fluorinated oligo- and poly-saccharides [12].

Sugar nucleotides are the archetypal activated sugar building block required for the biological synthesis of glycans. Despite fluorination being shown to have a minimal effect on sugar conformation, the success of enzymatic approaches to prepare fluorinated carbohydrates, as a result, relies upon the ability of the enzymes to recognize and efficiently utilize fluorinated substrates [13]. The compatibility of sugar substitution with fluorine is now

demonstrable across a wide range of carbohydrate-active enzymes, including multistep enzymatic reactions and the generation of products containing multiple fluorinated sugar units. Selected recent examples are outlined below.

Mutases

The tolerance of UDP-galactose mutase (UGM) to accept fluorinated sugar nucleotides has been evaluated to probe UGM substrate specificity and the equilibrium position of the enzyme-catalyzed reaction [14]. Substrate fluorination led to only a modest impact on K_m, while k_{cat} was reduced tenfold, 386-fold and 53100-fold on substitution of UDP-galactose with fluorine at the pyranose 6-, 3- or 2-positions, respectively. X-ray crystal analysis of *Mycobacterium tuberculosis* UGM with UDP-F_4-Gal*p* and UDP-F_4-Gal*f* (Figure 1, bottom right) revealed the CF_2-CF_2 motif is involved in multiple interactions with the enzyme, suggesting this dideoxy-tetrafluorination modification results in both stabilizing enthalpic and entropic contributions to binding [15].

Sialidases

Substitution with fluorine adjacent to the anomeric center has been long used to trap intermediates in glycosidase reactions. Subsequent studies have demonstrated the utility of this approach in the development of suicide substrates for sialidases and *trans*-sialidases, with potent antiviral activity evident in this class of compounds. Recently, 7-deoxy-7-fluorosialyl glycosides (Figure 1, right) have been shown to be resistant to hydrolysis, but are readily assembled by sialyltransferases. 7′-fluorination of CMP-sialic acid resulted in a modest impact to K_m and k_{cat} of several sialyltransferases, while the resulting 7-deoxy-7-fluorosialosides were three- to fivefold more resistant to spontaneous hydrolysis and 40- to 250-fold more resistant toward cleavage by GH33 sialidases [16].

Transglycosidases

Using glycogen as a donor substrate, *Escherichia coli* MalQ and cassava DPE1 have been demonstrated to *trans*-glycosylate 2F-Glc, 3F-Glc and 6F-Glc with varying levels of efficiency, forming α-(1→4)-linked glycosides with 12–28% conversion [17]. In contrast, *trans*-glycosylation with *Arabidopsis* DPE2 was shown to readily form discrete α-(1→4)-linked disaccharide products with 2-FGlc, 3F-Glc and 6F-Glc in 15–28% conversions [17].

Taken together, these examples indicate an untapped potential for the enzymatic synthesis of fluorinated oligosaccharides and glycoconjugates, which will no doubt be realized as the prevalence of enzymatic carbohydrate synthesis increases.

Future perspective

The successful inclusion of fluorine within nucleoside and sugar nucleotide components presents significant opportunity and ensures an exciting future for such capabilities. For example, beyond fluorination of the hexose moiety within sugar nucleotides, fluorination of the ribose and nucleobase moieties has been investigated, but not present substrates for glycoprocessing enzymes [18]. In addition, directed evolution and *in silico* design of 'designer' enzymes is rapidly becoming more popular as a method to avoid the challenges of poor substrate acceptance, enzyme kinetics and negative feedback loops [19]; methods such as these will become key to rapidly screening large libraries of fluorinated substrates and evolved enzyme panels [20].

Financial & competing interests disclosure

This work was supported by the UK Research and Innovation Medical Research Council (grant no. MR/T019522/1). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1. Purser S, Moore PR, Swallow S, Gouverneur V. Fluorine in medicinal chemistry. *Chem. Soc. Rev.* 37(2), 320–330 (2008).
2. Gillis EP, Eastman KJ, Hill MD, Donnelly DJ, Meanwell NA. Applications of fluorine in medicinal chemistry. *J. Med. Chem.* 58(21), 8315–8359 (2015).
3. Cavaliere A, Probst KC, Westwell AD, Slusarczyk M. Fluorinated nucleosides as an important class of anticancer and antiviral agents. *Future Med. Chem.* 9(15), 1809–1833 (2017).
4. Mehellou Y, Balzarini J, McGuigan C. Aryloxy phosphoramidate triesters: a technology for delivering monophosphorylated nucleosides and sugars into cells. *ChemMedChem* 4(11), 1779–1791 (2009).

5. Ross BS, Ganapati Reddy P, Zhang H-R, Rachakonda S, Sofia MJ. Synthesis of diastereomerically pure nucleotide phosphoramidates. *J. Org. Chem.* 76(20), 8311–8319 (2011).
6. Yoon J, Kim G, Jarhad DB *et al.* Design, synthesis, and anti-RNA virus activity of 6′-fluorinated-aristeromycin analogues. *J. Med. Chem.* 62(13), 6346–6362 (2019).
7. Benckendorff C, Hawes C, Smith M, Miller G. Chemical diversification of carbocyclic fluorinated pyrimidine nucleosides: introducing 2′-arabino analogues and ring unsaturation. *Synlett* doi: 10.1055/a-2079-9310 2023) (Epub ahead of print).
8. Benckendorff CMM, Slyusarchuk VD, Huang N, Lima MA, Smith M, Miller GJ. Synthesis of fluorinated carbocyclic pyrimidine nucleoside analogues. *Org. Biomol. Chem.* 20(47), 9469–9489 (2022).
9. Linclau B, Ardá A, Reichardt N-C *et al.* Fluorinated carbohydrates as chemical probes for molecular recognition studies. Current status and perspectives. *Chem. Soc. Rev.* 49(12), 3863–3888 (2020).
10. Huonnic K, Linclau B. The synthesis and glycoside formation of polyfluorinated carbohydrates. *Chem. Rev.* 122(20), 15503–15602 (2022).
11. Beswick L, Dimitriou E, Ahmadipour S *et al.* Inhibition of the GDP-D-mannose dehydrogenase from *Pseudomonas aeruginosa* using targeted sugar nucleotide probes. *ACS Chem. Biol.* 15(12), 3086–3092 (2020).
12. Andrade P, Muñoz-García JC, Pergolizzi G *et al.* Chemoenzymatic synthesis of fluorinated cellodextrins identifies a new allomorph for cellulose-like materials. *Chem. Eur. J.* 27(4), 1374–1382 (2021).
13. Council CE, Kilpin KJ, Gusthart JS, Allman SA, Linclau B, Lee SS. Enzymatic glycosylation involving fluorinated carbohydrates. *Org. Biomol. Chem.* 18(18), 3423–3451 (2020).
14. Errey JC, Mann MC, Fairhurst SA *et al.* Sugar nucleotide recognition by *Klebsiella pneumoniae* UDP-D-galactopyranose mutase: fluorinated substrates, kinetics and equilibria. *Org. Biomol. Chem.* 7(5), 1009 (2009).
15. van Straaten KE, Kuttiyatveetil JRA, Sevrain CM *et al.* Structural basis of ligand binding to UDP-galactopyranose mutase from *Mycobacterium tuberculosis* using substrate and tetrafluorinated substrate analogues. *J. Am. Chem. Soc.* 137(3), 1230–1244 (2015).
16. Geissner A, Baumann L, Morley TJ *et al.* 7-Fluorosialyl glycosides are hydrolysis resistant but readily assembled by sialyltransferases providing easy access to more metabolically stable glycoproteins. *ACS Cent. Sci.* 7(2), 345–354 (2021).
17. Tantanarat K, Rejzek M, O'Neill E *et al.* An expedient enzymatic route to isomeric 2-, 3- and 6-monodeoxy-monofluoro-maltose derivatives. *Carbohydr. Res.* 358, 12–18 (2012).
18. Ko H, Fricks I, Ivanov AA, Harden TK, Jacobson KA. Structure–activity relationship of uridine 5′-diphosphoglucose analogues as agonists of the human P_2Y_{14} receptor. *J. Med. Chem.* 50(9), 2030–2039 (2007).
19. Dolan JP, Cosgrove SC, Miller GJ. Biocatalytic approaches to building blocks for enzymatic and chemical glycan synthesis. *JACS Au.* 3(1), 47–61 (2023).
20. Kempa EE, Galman JL, Parmeggiani F *et al.* Rapid screening of diverse biotransformations for enzyme evolution. *JACS Au.* 1(4), 508–516 (2021).