CF PLUS CHEMICALS
Fluoroalkylation and bioconjugation

Fluorinated azides: clickable ^{19}F NMR probes for sensing of protein, nucleic acid and functional material interactions

Chiara Volpe [1], Tomáš Vranka [1], Daniel Pokorný [1], Martin Adamec [1], Lukáš Trantírek [2] and Václav Matoušek [1]

The clickable ^{19}F NMR probes were developed and synthesized by CF Plus Chemicals and their functionality verified by the group of Dr. L. Trantírek.

[1] CF Plus Chemicals s.r.o., Karásek 1767/1, 621 00 Brno-Řečkovice, Czechia, www.cfplus.cz, **sales@cfplus.cz**
[2] Central European Institute of Technology, Kamenice 753/5, 625 00 Brno, Czechia

Introduction

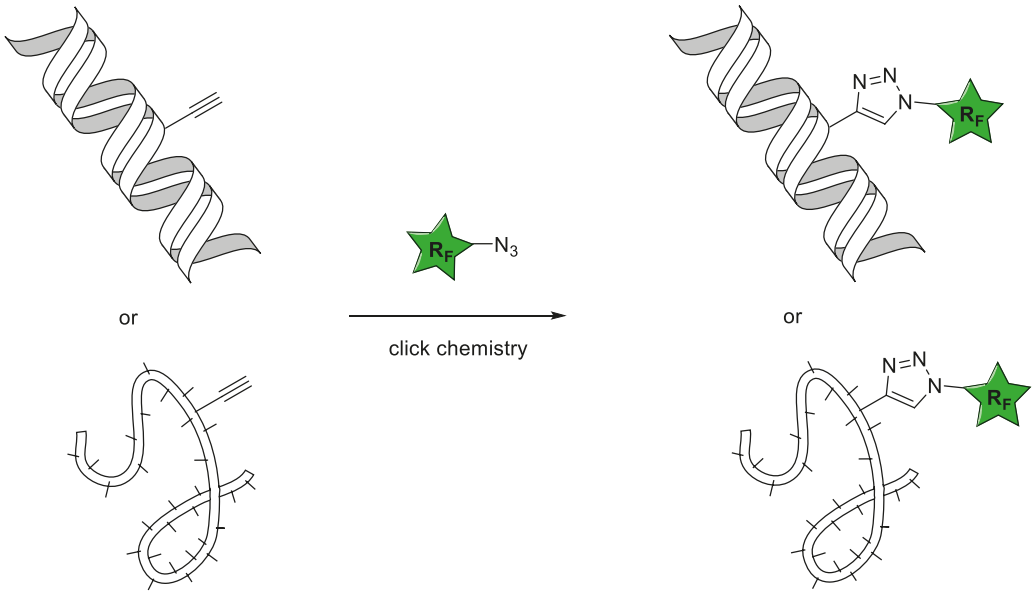
Due to its negligible abundance in living systems, favourable NMR properties (100% natural abundance, high gyromagnetic ratio (83% sensitivity compared to ^{1}H) and large span of chemical shifts that is extremely sensitive to the surroundings of the molecule[1], ^{19}F NMR spectroscopy has recently gained attention as a tool to monitor interactions of small-molecule drugs[2,3], peptides, proteins[4], glycans[5] and nucleic acids[6] in *in-vitro* and also in *in-cell* experiments.[7]

Problem

However, *de-novo* synthesis of complex biomolecules using fluorinated building blocks can be a rather costly and synthetically difficult task. The availability of fluorinated amino acids, glycans or oligonucleotides is limited and the incorporation of the fluorinated building block into the structure of the biomolecule might pose additional synthetic difficulties that can arise from the different chemical behaviour of the fluorinated building block.

Solution

While the availability and synthetic compatibility of biomolecular building blocks featuring different fluorinated moieties is limited, various building blocks containing terminal acetylenes or cyclooctynes are commercially available as they are typically employed for late-stage attachment of various functional groups, such as fluorescent dyes, affinity labels or special ligands. Azide-alkyne cycloaddition, also coined as click reaction, has gained large popularity as a connective reaction in chemical biology due to its robustness, chemocompatibility and a broad general applicability.[8]



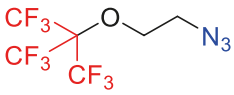
Click chemistry for attachment of ^{19}F NMR probes to biomolecules of interest

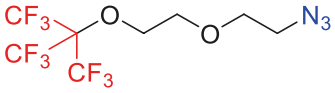
Here we present a collection (kit) of 9 fluoroalkyl azides which can be attached to the alkyne-containing biomolecule of choice. Depending on the desired sensing performance and physicochemical requirements, various probes can be attached, spanning from high-intensity aliphatic probes, azido(aryl/alkyl) trifluoromethylketones, derivatives of azidodifluoroacetic acid, bis(trifluoromethyl)-4-azidophenyl carbinol and a highly lipophilic fluorinated aromatic probe.

Kit containing clickable fluoroalkyl azide probes for ^{19}F NMR

High-intensity probes

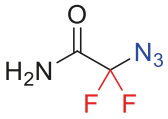
Nonafluoro-*t*-butyl ethers are typical representatives of the high-intensity probes. Depending on whether the reporting group needs to reflect changes in chemical environment close the the attachment point or farther from it, short or medium length fluoroalkyl azide can be selected.

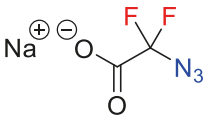


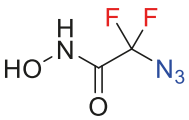


Azidodifluoroacetic acid derived probes

Despite being low in ^{19}F integration intensity, derivatives of azidodifluoroacetic acid are probably one of the least sterically demanding fluoroalkyl azides that also display excellent aqueous solubility without any cosolvents. While sodium azidodifluoroacetate introduces a charged residue, the corresponding primary amide is neutral and the hydroxamic acid is expected to act as a potent metal binder[9] and metalloenzyme inhibitor[10]. All these three azides are expected to have significantly lower pK_a values than their non-fluorinated counterparts.

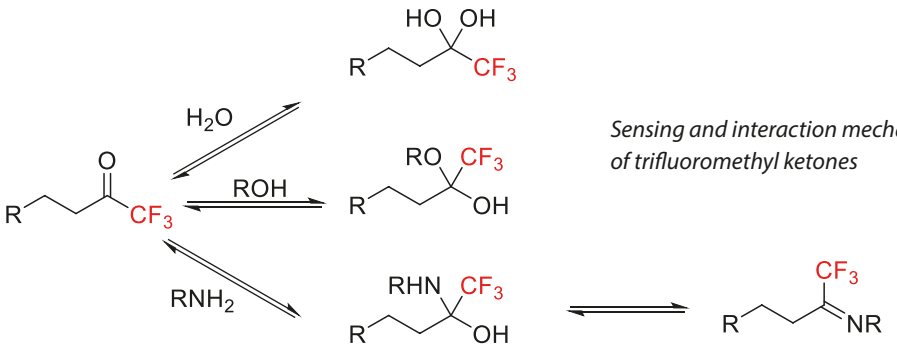






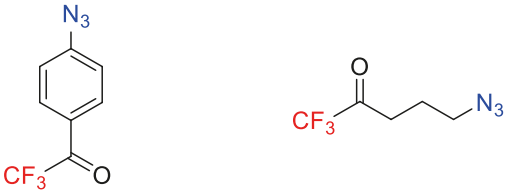
Trifluoromethyl ketone probes

Due to the powerful electron-withdrawing effect of the CF_3-group, trifluoromethyl ketones are excellent carbonyl electrophiles which reversibly form adducts with various nucleophiles, such as water, oxygen- and nitrogen-centered nucleophiles derived from amino acid side chains.[11] Therefore it can be used to sense the local degree of hydration[12], proximity of certain amino acids, such as serine, act as a reversible molecular glue or represent the warhead part of a reactive inhibitor.[13]



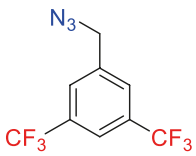
Sensing and interaction mechanisms of trifluoromethyl ketones

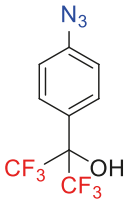
Generally speaking, due to the propensity to form hydrates, trifluoromethyl ketones show appreciable aqueous solubility. The more electron poor aromatic 4-azidophenyl trifluoromethyl ketone shows a higher propensity to form hydrates than its aliphatic counterpart, while also capable of π-π stacking.



Other aromatic probes

Bis(trifluoromethyl) azidomethylbenzene and 4-azidophenyl-bis(trifluoromethyl)carbinol are medium intensity probes. While the sensing performance of the highly lipophilic bis(trifluoromethyl) azidomethylbenzene is based on π-π interactions, the mildly acidic fluorinated tertiary alcohol might additionally act as a hydrogen bond donor.

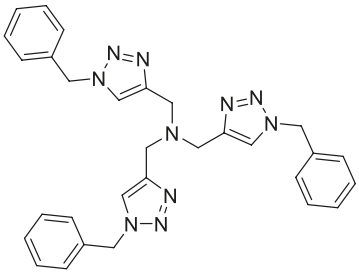




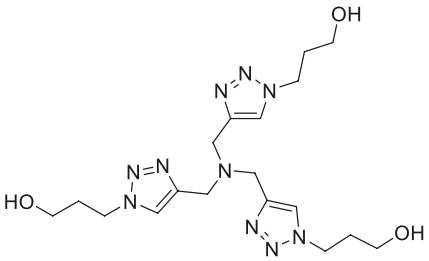
Recommended conditions for click reaction

Standard protocols for click chemistry are recommended[14]:

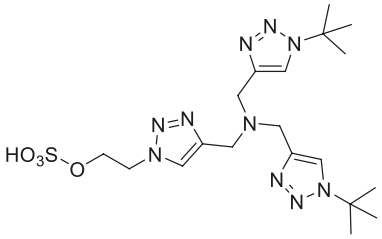
- copper sulfate or acetate as copper source
- sodium ascorbate as reductant
- freshly degassed buffers and cosolvents
- auxiliary ligands that stabilize the Cu^{I} oxidation state, such as TBTA or water soluble THPTA or BTTES ligands
- using only minimal amounts of Cu-coordinating cosolvents, such as DMSO or MeCN
- avoiding the use of highly coordinating anions in the buffer such as Tris, phosphate, MOPS, acetate or HEPES



TBTA



THPTA



BTTES

Recommended auxiliary ligands for CuAAC

References

1) Prog. Nucl. Magn. Reson. Spectrosc., **2005**, *46*, 1–21
2) Prog. Nucl. Magn. Reson. Spectrosc., **2007**, *51*, 243–271
3) Chem. Biol. Drug Des., **2010**, *75*, 237–256
4) ACS Chem. Biol. **2014**, *9*, 6, 1242–1250
5) Angew. Chem. Int. Ed. **2021**, *60*, 13302
6) J. Am. Chem. Soc. **2008**, *130*, 51, 17230–17231
7) J. Am. Chem. Soc. **2010**, *132*, 321–327
8) Angew. Chem., Int. Ed. **2002**, *41*, 2596–2599
9) J. Chrom. A, **1997**, *770*, 85–91
10) ACS Omega **2021**, *6*, 34, 21843–21849;
    Neurotox. Terat., **2008**, *30*(5), 390–394
11) Chem. Commun., **2013**, *49*, 11133–11148
12) J. Org. Chem. **2017**, *82*, 21, 11431–11439
13) Bioorg. Med. Chem. **2021**, *50*, 116457
14) Chem. Rev. **2021**, *121*, 12, 7122–7154