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# Development of potential fluorine-18 radiotracers for imaging of mutant huntingtin fibrils in Huntington's Disease

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## Background

- Huntington's disease is an autosomal dominant inherited neurodegenerative disease whose pathology is linked to the deposition of aggregated mutant huntingtin (mHTT) in the brains of affected individuals.[1]
- Currently, measures of disease progression lack sensitivity. PET imaging of mHTT offers an improved platform for accurate disease staging and progression.
- Using scaffolds from two previously reported mHTT ligands (CHDI-180R and CHDI-650) two new ligands containing a difluoromethyl group have been proposed as potential mHTT PET ligands.[2]

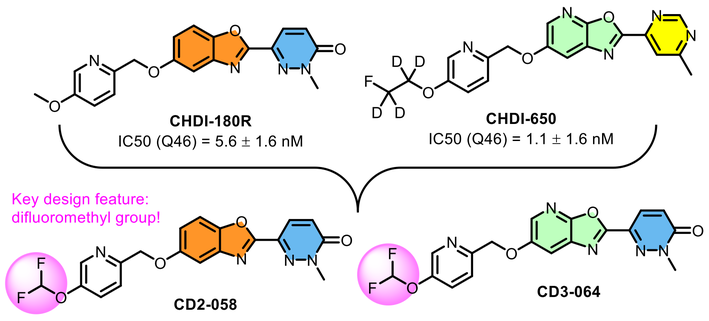
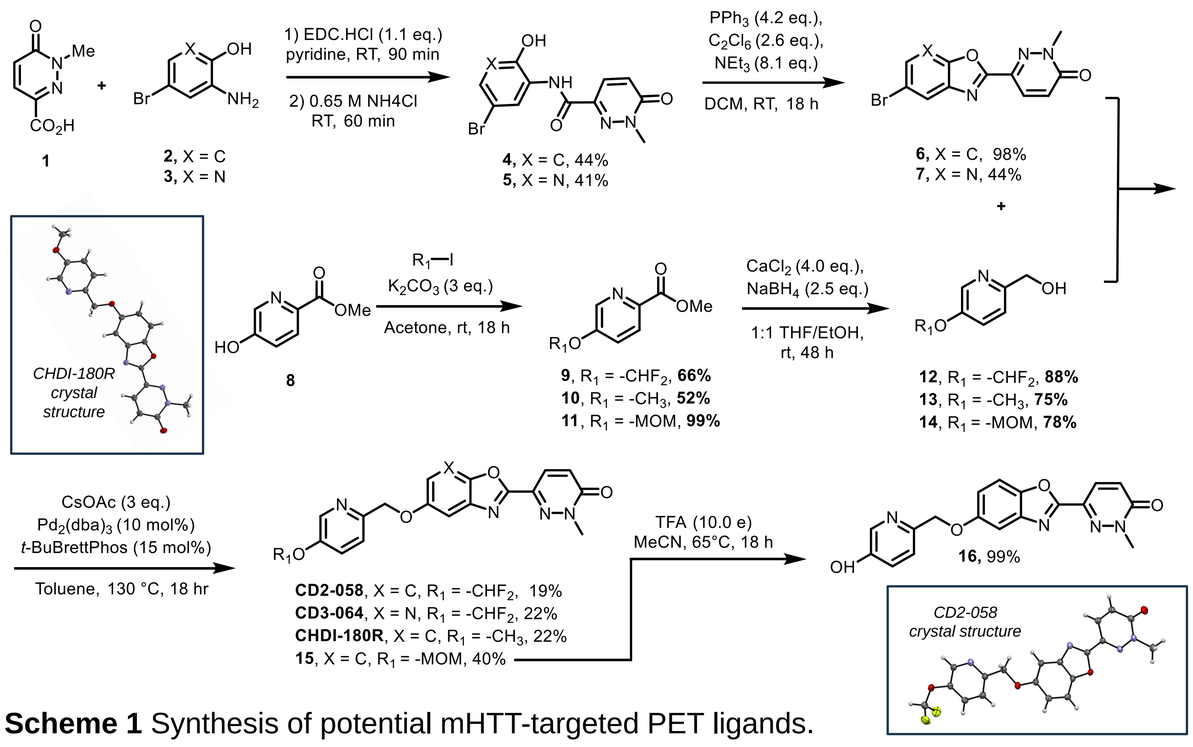



**Figure 1** Design of potential difluoromethyl-based mHTT PET ligands.

## Synthesis of mHTT-targeted radiotracers




**Scheme 1** Synthesis of potential mHTT-targeted PET ligands.

## Binding to mHTT fibrils *in vitro*

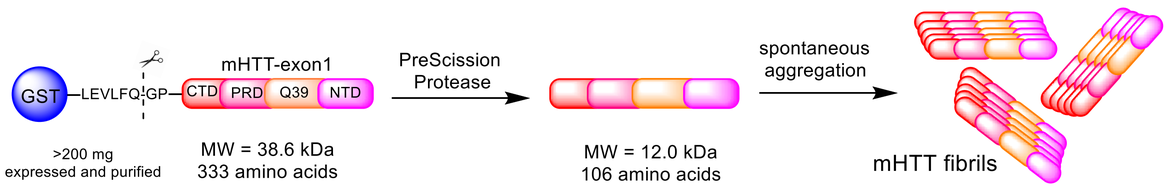



**Figure 2** Generation of mHTT fibrils from recombinant GST-exon 1 Q39 mHTT construct.

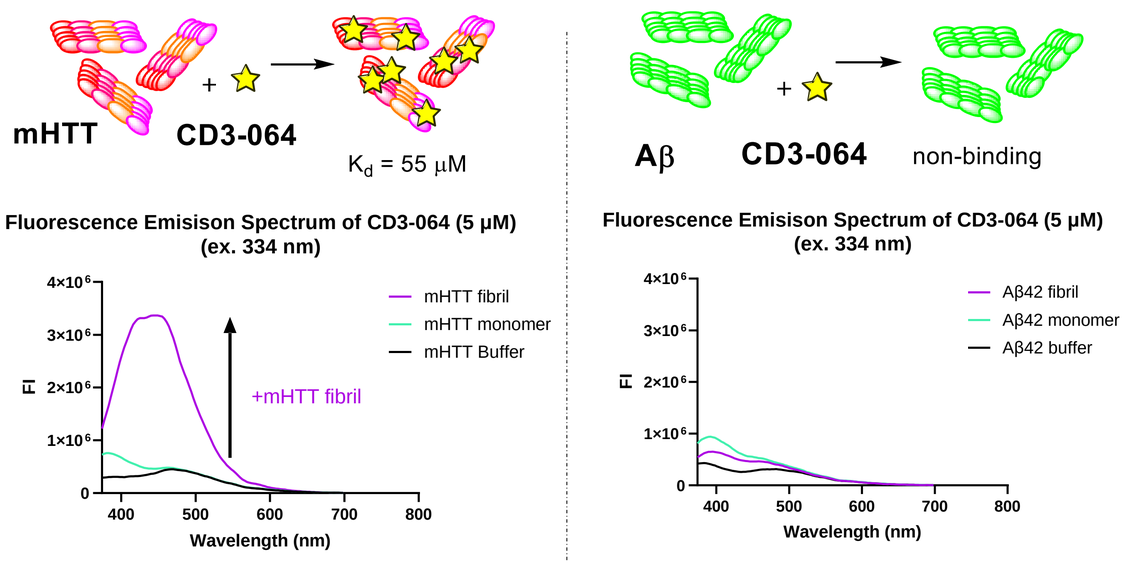



**Figure 3** Principle of fluorescence-based binding assay for determination of binding of CD3-064 to mHTT and amyloid-β fibrils.

## [18F]Difluorocarbene radiosynthesis

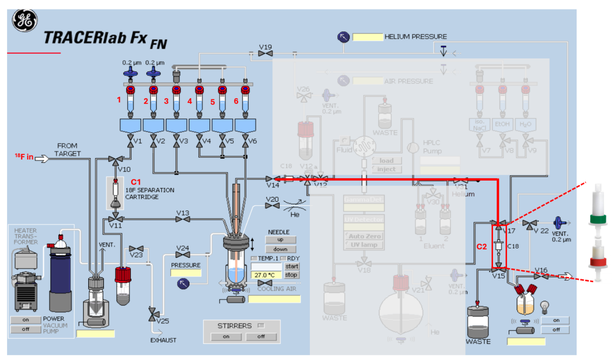



**Figure 4** Schematic of GE $FX_{FN}$ configuration for chromatography-free fluorine-18 difluorocarbene synthesis.

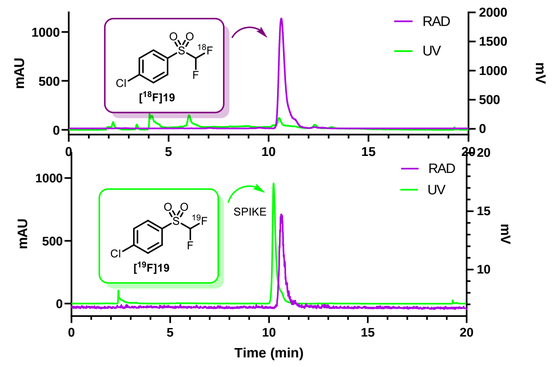
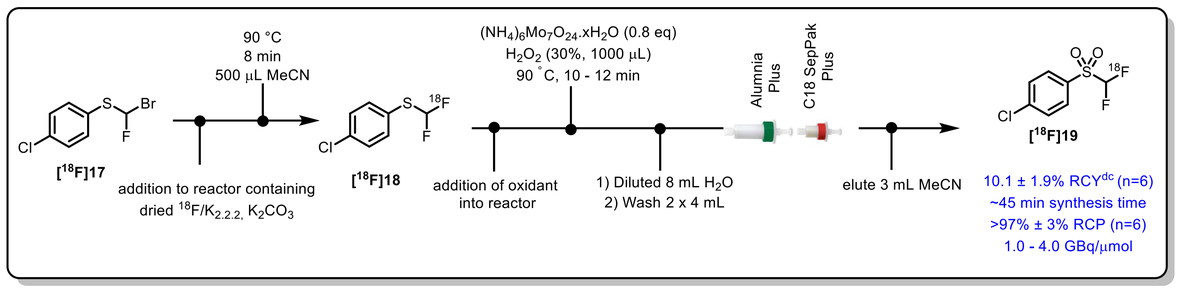
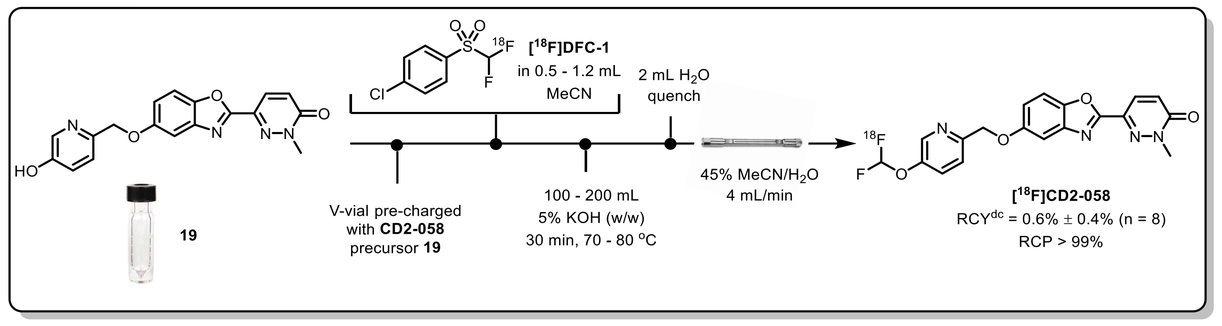



**Figure 5** RAD and UV HPLC traces of [18F]difluorocarbene reagent, **[18F]19**.[3]




**Scheme 2** Automated synthesis of **[18F]19** on the GE $FX_{FN}$ module.[4]

## Radiosynthesis of [18F]CD2-058




**Scheme 3** Manual radiosynthesis of **[18F]CD2-058** *via* [18F]difluorocarbene insertion.

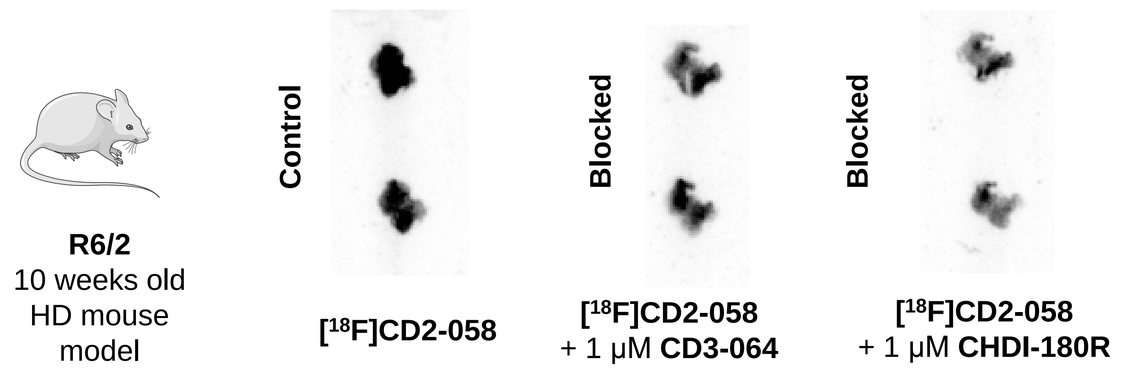



**Figure 6** Autoradiography of **[18F]CD2-058** on R6/2 mouse brain tissue.

## Conclusions

- Two potential PET ligands, **CD2-058** and **CD3-064** targeting mHTT fibrils have been synthesised and characterised.
- Using a fluorescence enhancement-based binding assay, **CD3-064** was found to bind to mHTT fibrils ($K_d$ = 55 μM) but not Aβ fibrils.
- A chromatography-free automated synthesis of difluorocarbene reagent **[18F]19** was developed for the GE FXFN module
- **[18F]CD2-058** was radiosynthesised *via* fluorine-18 difluorocarbene insertion with **[18F]19**.
- **[18F]CD2-058** binds to R6/2 mouse tissue and is blockable with **CD3-064** and **CHDI-180R**

## References

1) Bates *et al*, Huntington disease, *Nature Disease Primers,* **2015**, 1, 1-21; 2) Dickmann *et al*, PET ligands for imaging mutant huntingtin aggregates: A case study in non-for-profit scientific management, ChemBioChem, 2024, e202400152 3) Sap, J. B. I. *et al* [18F]Difluorocarbene for positron emission tomography. *Nature* **606**, 102–108 (2022). 4) Dickmann *et al*, Development of a halofluorocarbon, chromatography-free radiosynthesis of fluorine-18 difluorocarbene, 2025, (under review EJNMMI Radiopharmacy and Chemistry).

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