

Bivalent Dicovalent Ligands for A₁AR Homodimers

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Introduction

- G protein-coupled receptors (GPCRs) are the largest family of transmembrane signaling proteins in the human genome and are the target of 30-40% of current drugs
- GPCRs were traditionally considered to be monomeric protein units, but are now thought to often form complexes with other GPCRs, e.g., homodimers, heterodimers, and higher-order oligomers
- One such receptor is the Adenosine A₁ receptor (A₁AR) which has been shown to form both homodimers as well as heterodimers with other GPCRs^{1,2} These dimers have been implicated in CNS diseases such as Alzheimer's Disease

Aims of the Project

- To develop the first bivalent dicovalent ligands, which contain two covalent ligands, each capable of covalently binding to a receptor monomer of the dimer
- It is hypothesised that these types of novel ligands could be used as tools to evaluate the types, formation, and structure of A₁AR hetero- and homo-dimers. This will allow examination of the biological response (both pathological and therapeutic) of A₁AR dimers.

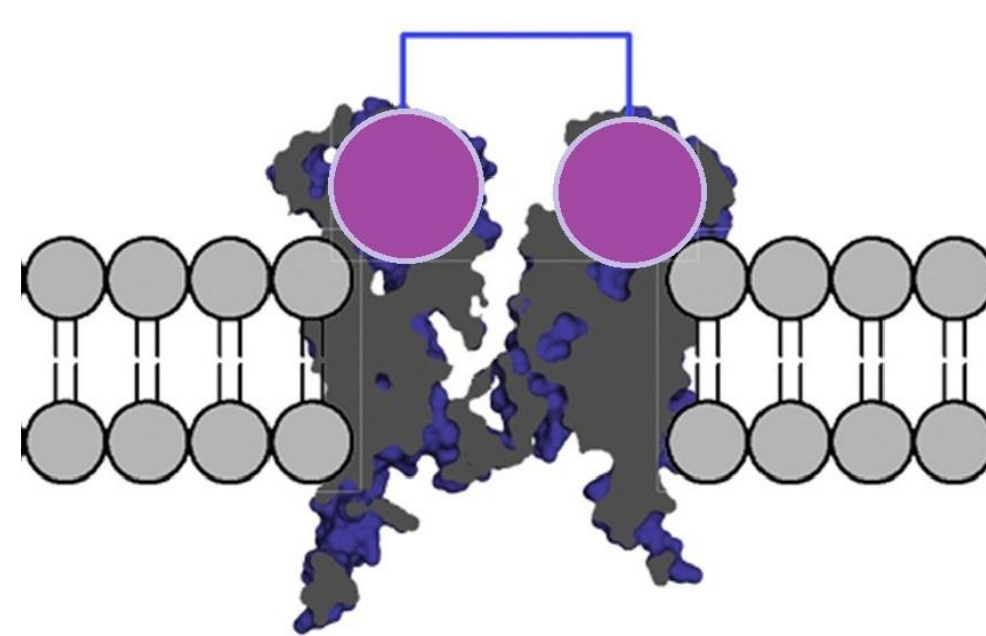


Figure 1. Bivalent ligand cartoon. One ligand with two ligands, each bound to a receptor monomer

Bivalent Dicovalent Ligand Design

- The A₁AR crystal structure (PDB ID: 5UEN)³ complexed with covalent ligand DU172 bound to residue TYR270 (**Figure 2**) served as a starting point for covalent ligand design
- Published work by the Vernall group developed bifunctional (chemoreactive and clickable) ligands for A₁AR⁴. The ligand **UODC_14** (**Scheme 1**) showed high affinity for A₁AR and moderate selectivity for A₁AR over other AR subtypes
- UODC_14 could be adapted to a 'bivalent' approach by joining two ligands together linked via varying length linkers to bridge A₁AR homodimer
- Each ligand 'monomer' contains a fluorosulfonyl (SO₂F) 'arm', which can react with an equivalent tyrosine residue in each monomer of the homodimer (**Figure 3**)

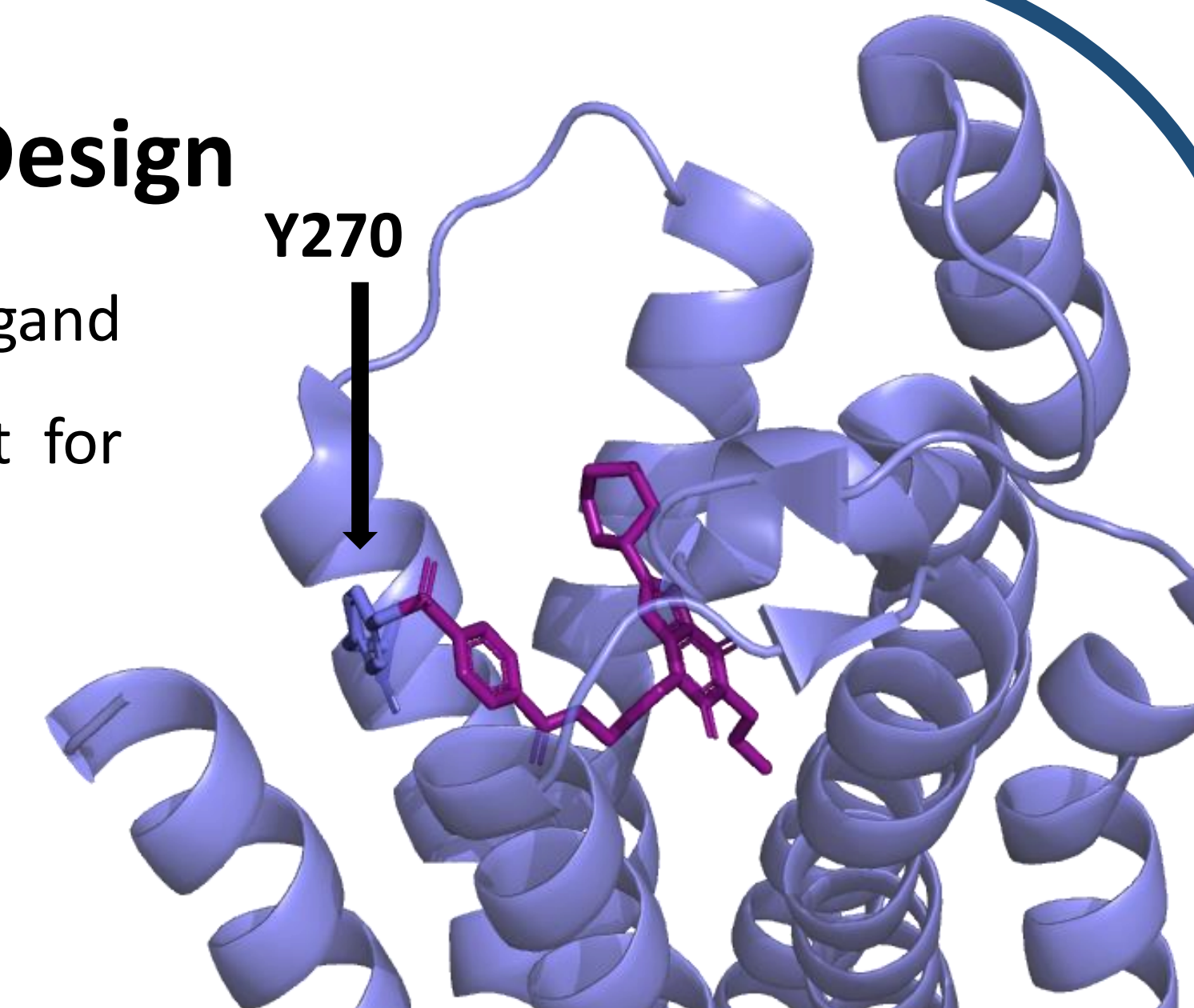


Figure 2. A₁AR crystal structure complexed with high affinity covalent A₁AR ligand, DU172 (PDB ID: 5UEN)

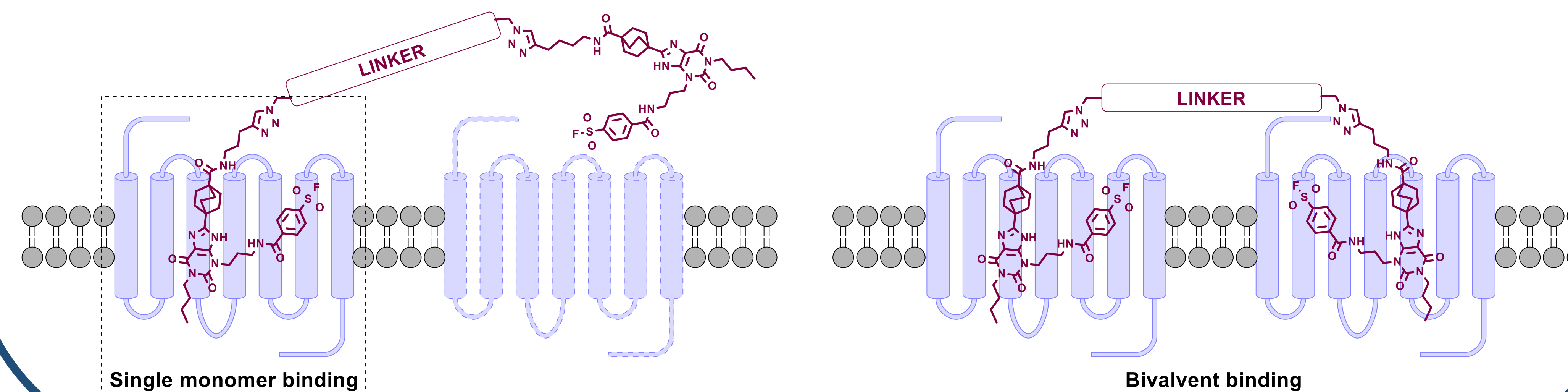


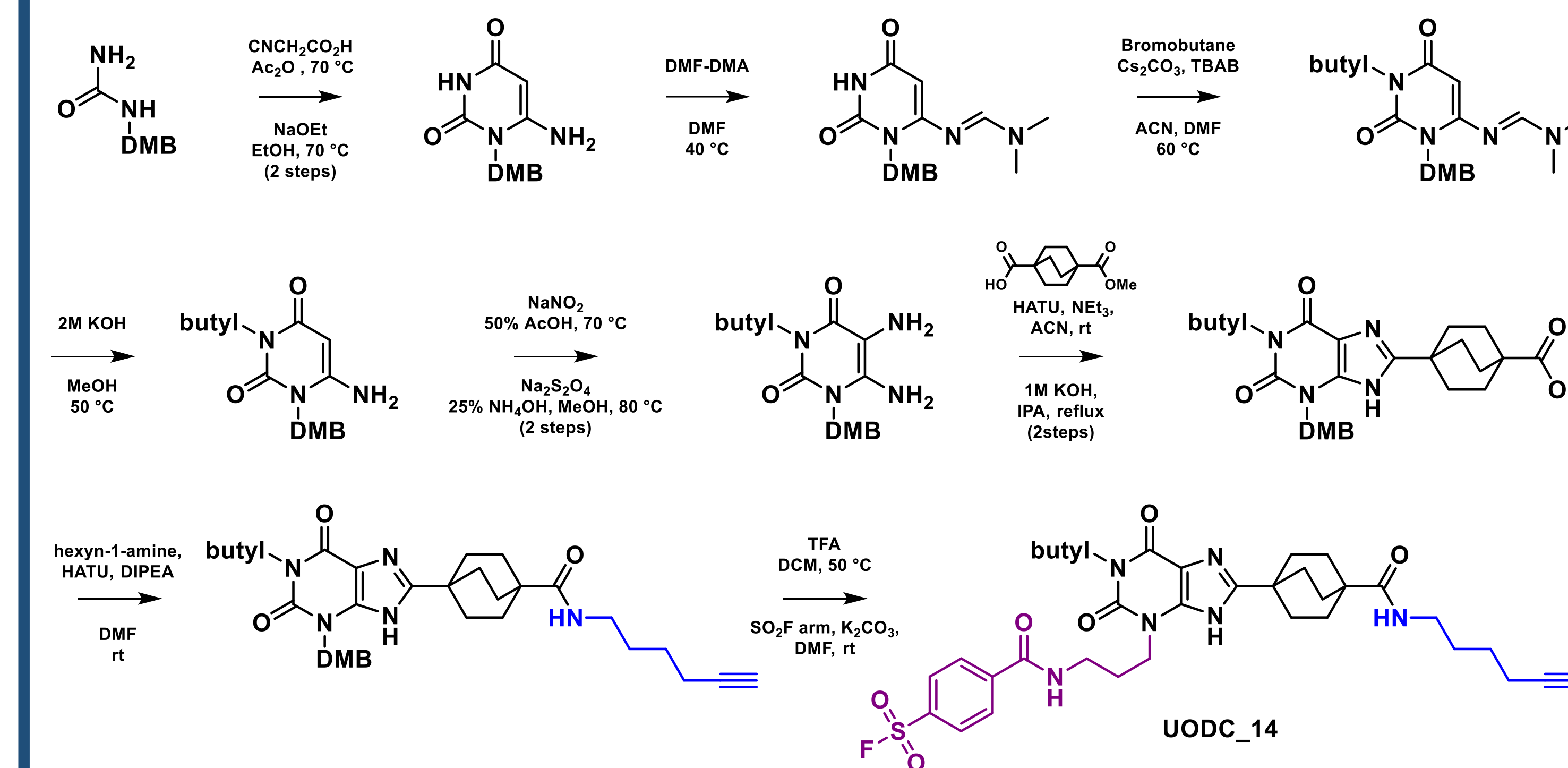
Figure 3. Bivalent dicovalent ligand binding modes. The ligand is represented engaging in binding with an A₁AR monomer and homodimer.

Further Work

- Linkers of different lengths (10–30 atoms) and compositions (e.g. peg-, alkyl- or peptide-based) are being investigated
- Following this, bivalent dicovalent ligands targeted to A₁AR-mGlu₅ heterodimers will be also synthesized, this will be informed by work done to synthesise bivalent dicovalent ligands for the A₁AR homodimer.
- Non-covalent control compounds are also being synthesised with a methyl substituted for the fluorine of the SO₂F arm

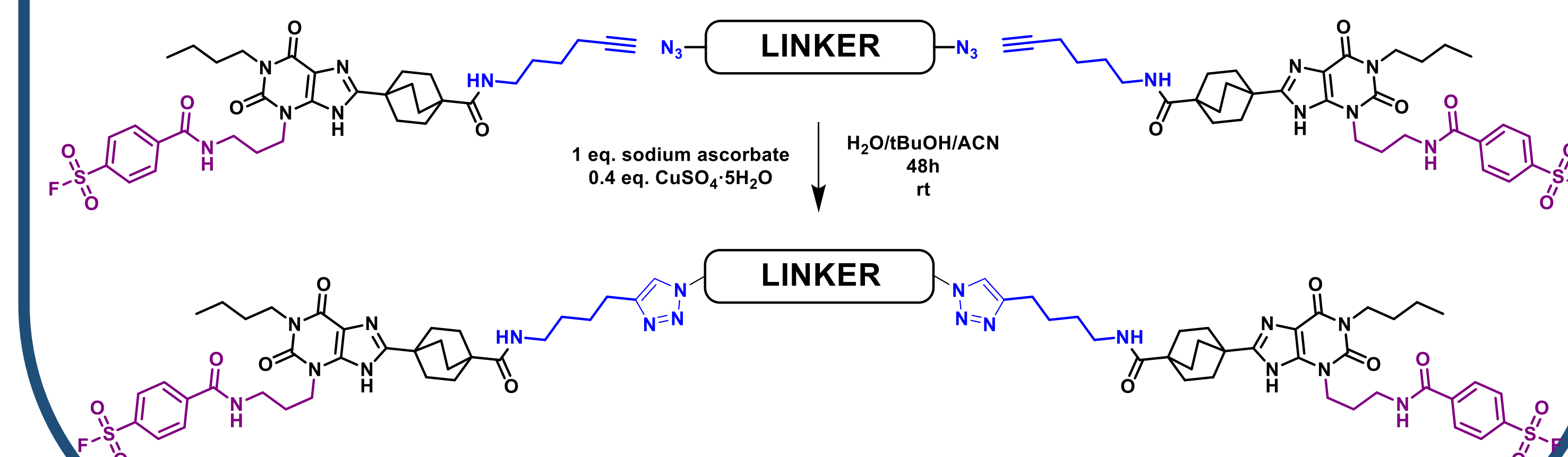
Synthesis

- Synthesis begins with assembly of the xanthine core, following modified procedures from Holschbach *et al.*⁵ followed by addition of the clickable alkyne arm, then installation of the SO₂F 'warhead'



Scheme 1. Synthesis of covalent ligand UODC_14

- Following assembly of the 'monomer', a double-click reaction is used to attach one linker to two monomers



Scheme 2. Generalised final step for synthesis of bivalent dicovalent ligands

References

- Vecchio, E. A., *et al.*, *Br J Pharmacol* **2018**, 175 (21), 4036–4046.
- May, L. T.; *et al.*, *The FASEB Journal* **2011**, 25 (10), 3465–3476.
- Glukhova, A., *et al.* *Cell* **2017**, 168 (5), 867-877.e13.
- Trinh, P. N. H., *et al.*, *J. Med. Chem.* **2021**, 64 (12), 8161-8178.
- Holschbach, M. H., *et al.*, *Org. Lett.* **2009**, 11 (19), 4266-4269.