The Development of Novel Diagnostics Tools for The Neurodegenerative Diseases

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1) Introduction

A key process in the development of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases is the aggregation of proteins to produce fibrillary aggregates with a cross β-sheet structure, amyloid[1,2]. The development of reagents that can bind these aggregates with high affinity and selectivity has potential for early disease diagnosis.

We present a new approach to the capture and detection of protein aggregates using synthetic chemical antibodies. The concept is to pulldown all the aggregates present based on their structure, in this case β -sheet structure (amyloid), rather than their protein composition in order to identify which protein aggregates are present in human biofluids[3].

3) Background and preliminary study

- We recently described a new approach to the capture and detection of protein aggregates using synthetic chemical antibodies. The approach has some real advantages in being able to enhance the selectivity and sensitivity of detection by tuning the chemical structure, as well as increased stability and resistance to degradation compared to conventional antibodies. We have demonstrated the viability by synthesising CAP-1 for capture of amyloid aggregates from solution (Figure 2). The concept is to pulldown all the aggregates present based on their structure, in this case β-sheet structure (amyloid), rather than their protein composition in order to identify which protein aggregates are present in human biofluids (Figure 3,4).

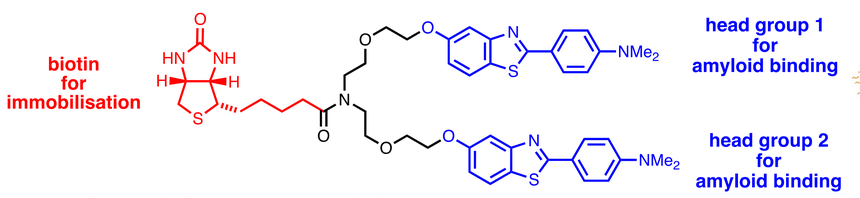


Figure 2. The structure of CAP-1

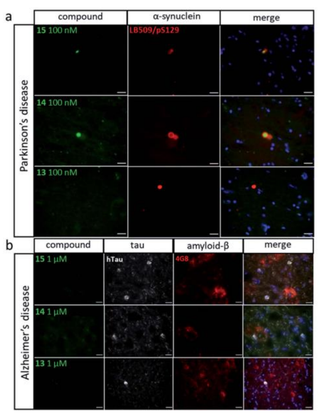


Figure 3. Amyloid-precipitation of CSF spiked with recombinant α-synuclein oligomers.

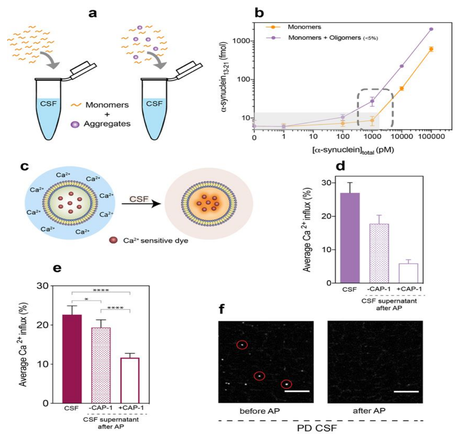


Figure 4. Fluorescence microscope images of human brain sections. (a) Sections from patients with Parkinson's disease labelled with antibodies against a-synuclein (b) Sections from patients with Alzheimer's disease labelled with antibodies against tau (dako tau)

2) Project goal and approach

- To develop new molecules with high affinity and high selectivity for α-synuclein aggregates and to use the new reagents to pulldown α-synuclein aggregates from CSF.

The key coupling chemistries are highlighted: 1. amide coupling using HBTU ; 2. copper-catalysed azide-alkyne cycloaddition using copper(I)-TBTA; 3. Mitsunobu coupling using DIAD/PPh_3 (Note: different permutations of the order in which the coupling reactions are carried out have been investigated) (Figure 1).

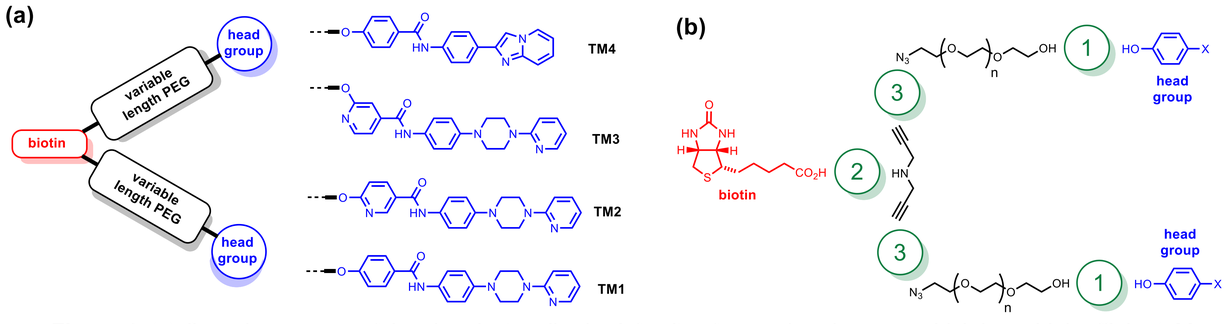


Figure 1: (a) Generic structure of the chemical antibody. A family of target head groups which have high affinity (nM K_d) are illustrated in blue.

(b) Synthetic strategy for assembly of a library of chemical antibodies with different linkers (different values of n) and different head groups.

4) Target molecules

- The target molecules were synthesized with/without biotin by click chemistry (Figure 5).

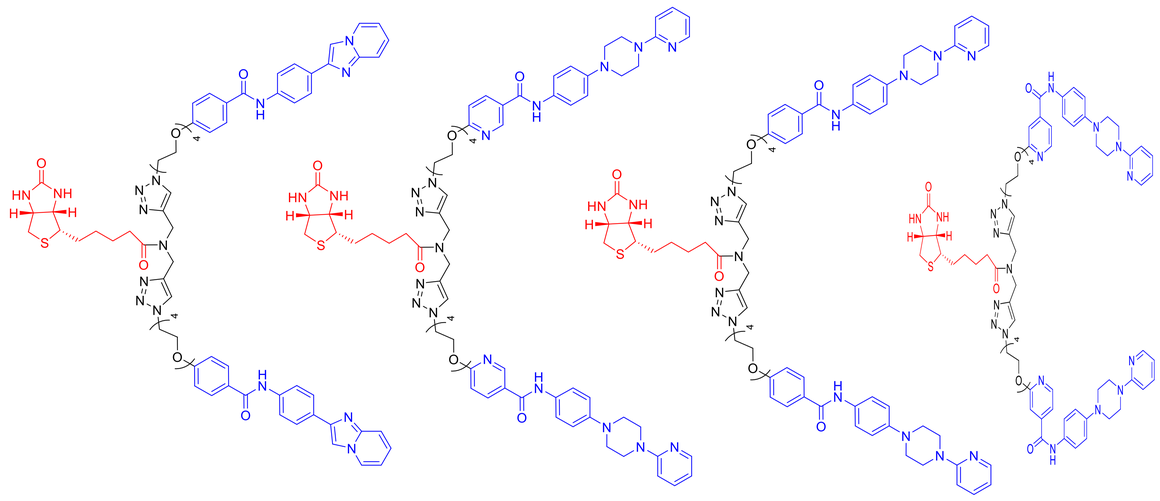
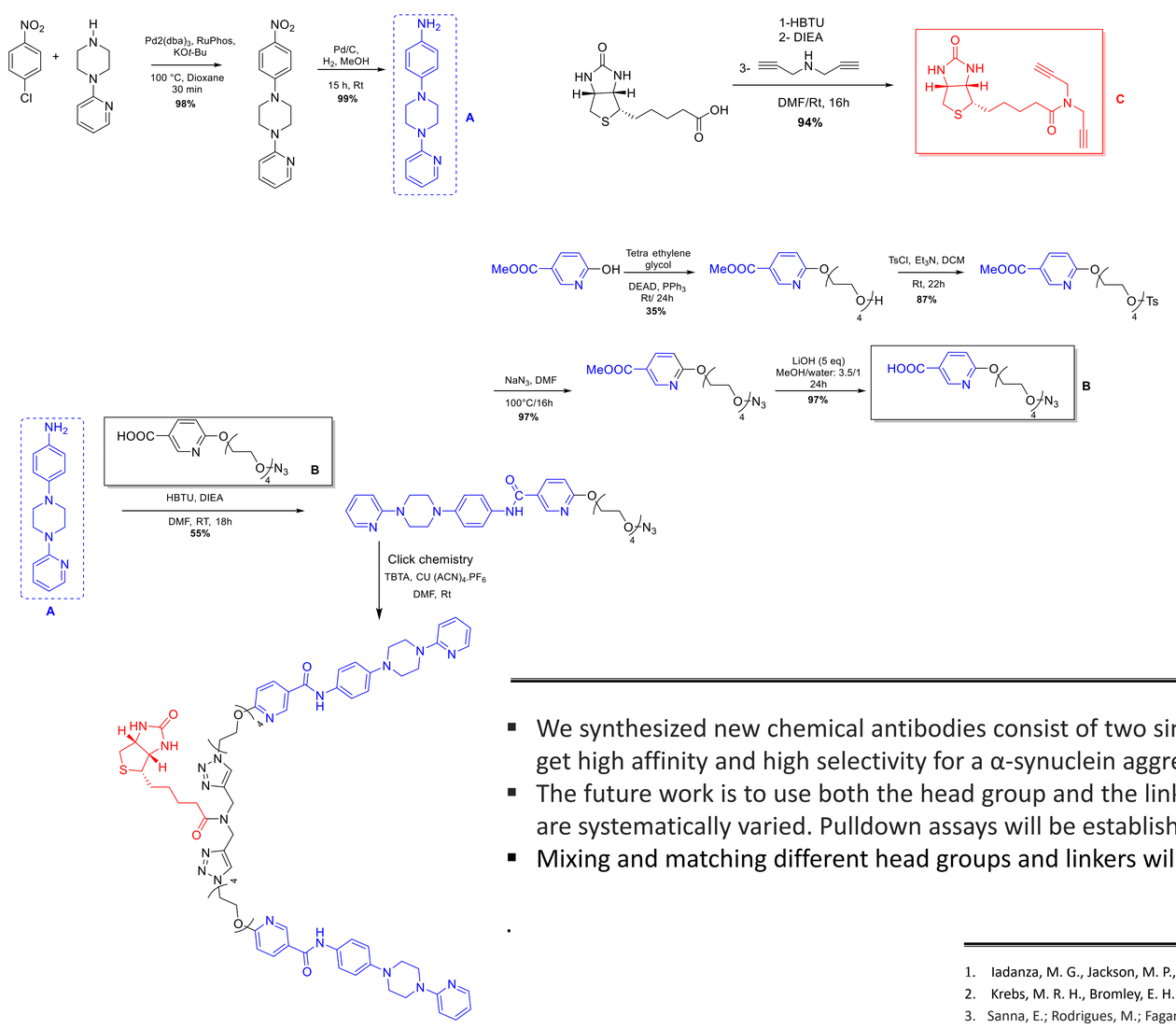


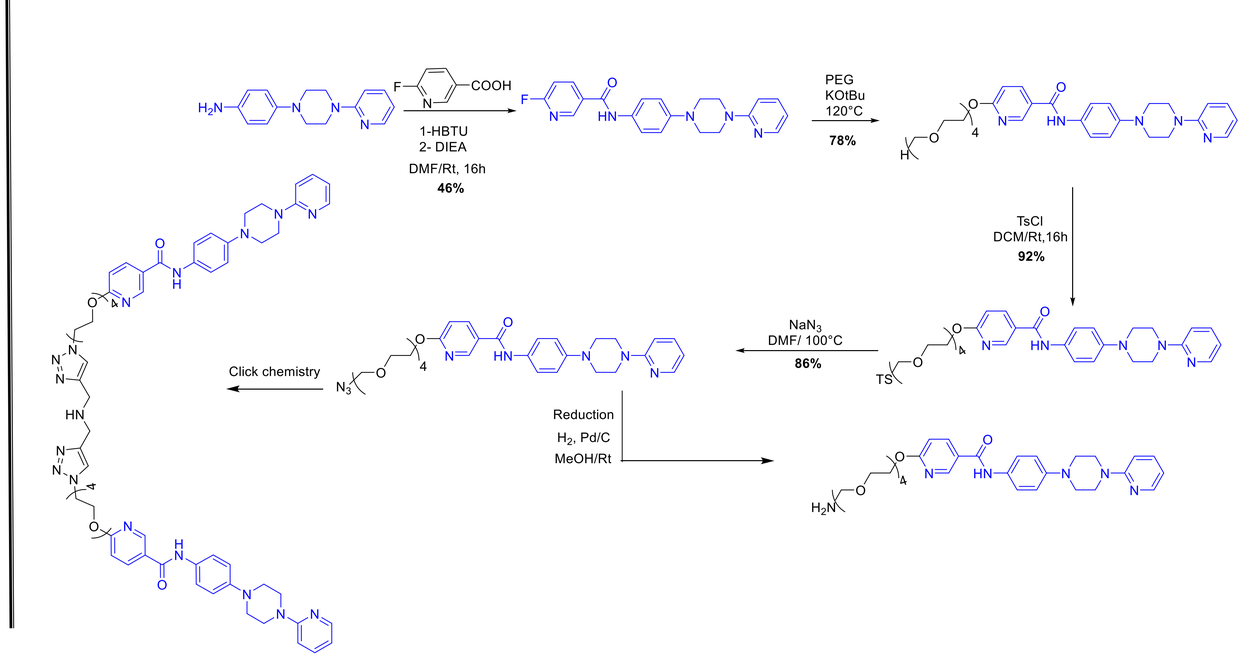
Figure 5. The structure of the target molecules.

5) Results

a) Synthetic strategy 1



b) Synthetic strategy 2



6) Conclusion & perspective

- We synthesized new chemical antibodies consist of two similar head groups linked by variable linker (PEG) length using different types of coupling chemistry aiming to get high affinity and high selectivity for a α-synuclein aggregates and to use the new reagents to pulldown α-synuclein aggregates from CSF.
- The future work is to use both the head group and the linker to tune affinity and selectivity, so libraries of reagents will be synthesized in which these two parameters are systematically varied. Pulldown assays will be established to screen the library and identify the most promising candidates for further development.
- Mixing and matching different head groups and linkers will allow us to rapidly assemble a library of different chemical antibodies for testing.

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7) References

1. Iadanza, M. G., Jackson, M. P., Hewitt, E. W., Ranson, N. A. and Radford, S. E., *Nat. Rev. Mol. Cell Biol.*, **2018**, *19*, 755–773. 3
2. Krebs, M. R. H., Bromley, E. H. C and Donald, A. M., *J. Struct. Biol.*, **2005**, *149*, 30–37.
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