Multivalent 2-alkyl trihydroxypiperidines as potential enhancers of the β-glucocerebrosidase enzyme activity

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The enzyme β-glucocerebrosidase (GCase) is a lysosomal glycosidase whose misfolding and dysfunction is involved in many pathological disorders, such as Gaucher Disease (a Lysosomal Storage Disorder, LSD). A therapeutic strategy for restoring the GCase activity is based on the use of Pharmacological Chaperones (PCs), molecules able to bind and stabilize misfolded GCase resulting in enzyme activity's enhancement [1]. The role of iminosugars as PCs for the treatment of LSDs has been disclosed. In fact, it has been proven that *N*-alkylated 3,4,5-trihydroxypiperidines (such as compound **1**) are promising PCs for GCase (see Figure 1) [2]. Moreover, the multimerization of **1** into multivalent dendrons has shown a significant enhancement in the PCs activity [2].

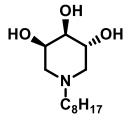
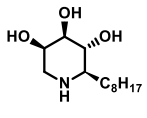
Comp.	 1	 2
Mutations	Mutant GCase activity rescue	
N370S	1.25 at 100 μM	1.90 at 50 μM
L444P	1.56 at 100 μM	1.80 at 100 μM

Figure 1 Chemical structures and biological data of piperidines **1** and **2**.

Recently, it has been discovered that shifting the alkyl chain from the nitrogen to the C-2 atom of the piperidine ring (compound **2**) led to a higher rescue of mutant GCase activity (see Figure 1) [3]. Given the already proven efficacy of multivalency in modulating GCase activity, this project focuses on the synthesis of multivalent systems based on compound **2**. The synthesis of new multivalent derivatives is accomplished through copper catalysed alkyne-azide cycloaddition (CuAAC) reaction of the azido ending piperidine **6** and several propargylated scaffolds **7a-c** (see Figure 2). The synthetic strategy (see Figure 2) makes use of D-mannose as starting material, achieving the key intermediate **3** in five steps with a 50% overall yield. After an addition of a Grignard reagent, presenting a terminal alkene functionality, and a hydroboration-oxidation of the double bond, hydroxylamine **4** is obtained. The piperidine intermediate **5** is generated through debenzylation and reductive amination of **4**. After suitable steps to protect the amine group and to convert the primary hydroxyl group in azide, the key piperidine **6** is obtained with a "clickable" azide tag. Scaffolds displaying terminal alkyne functionalities are commercially available or synthesized through propargylation of the corresponding alcohols. *Ex vitro* biological assays are ongoing to test the ability of these new multivalent systems to act as PCs for GCase.

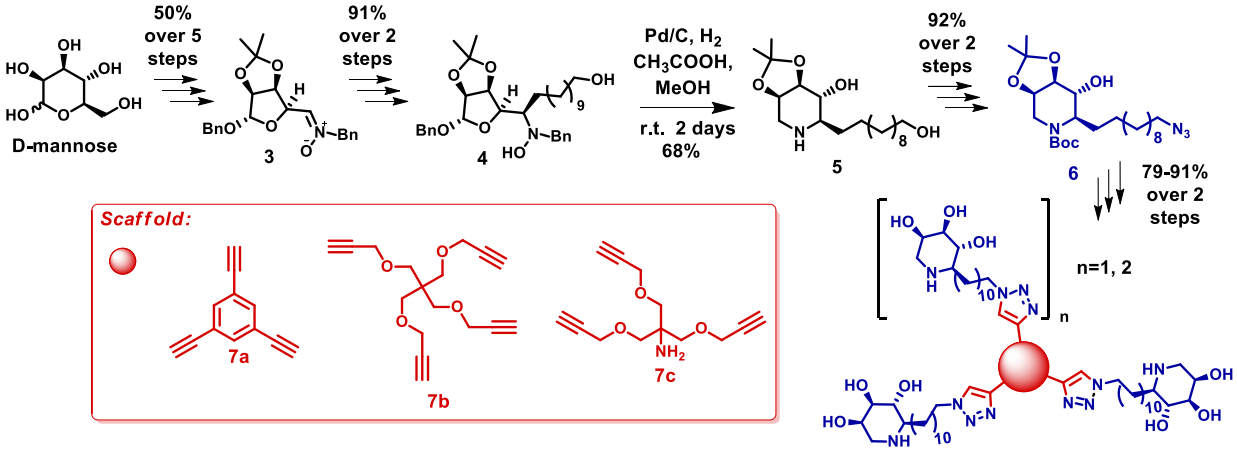


Figure 2 Synthetic strategy of the new multivalent dendrimers, topics of this project.

1. Pereira, D. M., et al., *Chem. Sci.* **2018**, *9*, 1740
2. Martìnez-Bailén, M., et al., *Pharmaceuticals* **2022**, *15*, 823, and references cited therein.
3. Clemente, F., et al., *Bioorg. Chem.* **2020**, *98*, 103740