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Multivalent enzyme inhibition

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Pillar[5]arene glyco(mimetic)rotaxanes for the functional interrogation of multivalency responsive glycosidases

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***Abstract**: Multiconjugation of a pillar[5]ene scaffold and rotaxanation of a glycomimetic-exposing axel has been exploited as a tactic to alter molecular volume and rigidity in hetero(multivalent) constructs purposely conceived to investigate the mechanisms at play in multivalent enzyme inhibition (MEI). With the help of a newly developed glycosidase binding mode screening tool, using reporter lectins and computational docking, the influence of the glycosidase tertiary and quaternary structure in MEI has been evidenced. The results provide a rational for the activation of biomimetic lectin-like associations in the intriguing case of multivalency-responsive glycosidases with deeply located catalytic pockets.*

The observation that the inhibitory potency of the iminosugar-type glycomimetic 1-deoxynojirimycin (DNJ) towards the Jack bean α-mannosidase (JbM) is enhanced by 2000-fold when displayed in twelve copies coating a C_{60}-fullerene ball (**1**)[1] shook the foundations of the generally accepted key-and-lock model for enzyme inhibition by substrate analogues (Figure 1). The phenomenon, generically termed multivalent enzyme inhibition (MEI), has been further confirmed for a variety of glycomimetic architectures,[2,3] depicting a scenario that is reminiscent of that portraying sugars binding to their cognate protein receptors (lectins). The fact that the active site of JbM, a member of the glycosyl hydrolase (GH) family GH38 in the Carbohydrate Active Enzyme (CAZy) classification,[4] is relatively open and that the enzyme is multimeric in solution reinforced this notion, which is consistent with recent X-ray evidence.[5] However, this thesis cannot apply for multivalency responsive enzymes that are either monomeric and/or have deeply buried catalytic pockets, which includes human enzymes of biological and therapeutic relevance such as lysosomal β-glucosidase,[6] hexosaminidases and O-glucosaminidase[7] or heparanase.[8] Most disturbing, sugar glycosides, the putative substrates of glycosidases, were found to turn into inhibitors when multivalently presented at the surface of nanomaterials, resulting in lectin/glycosidase binding promiscuity.[9] The impact of these discoveries in the glycoscience is palpable, promoting a reformulation of multivalency from a "safe" tactic to achieve biologically useful lectin affinities to a "multi-switch" tool with the potential to elicit lectin/glycosidase cross-talk behaviors.[10]

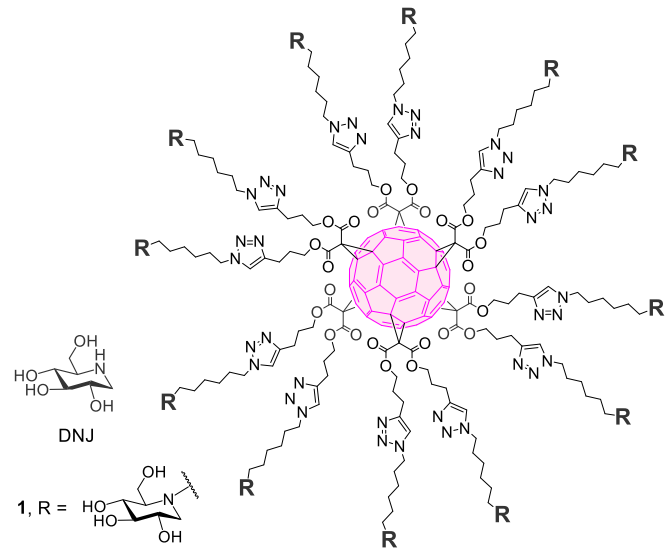

Figure 1. Structures of the iminosugar 1-deoxynojirimycin (DNJ) and the C60 fullerene/DNJ dodecavalent conjugate **1**.

Advancing our understanding on the mechanisms underpinning MEI events beyond the α-mannosidase case is critical to substantiate this paradigm shift and uncover the biological and technological repercussions. The inhibition of enzymes featuring narrow catalytic sites by bulky multivalent conjugates is a counterintuitive phenomenon whose interpretation requires the design of precise (macro)molecular probes and purposely-conceived binding site-informing screening tools. Herein we report a new class

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