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## Hot Paper

## Phosphorus Dendrimers for Metal-Free Ligation: Design of Multivalent Pharmacological Chaperones against Gaucher Disease

My Lan Tran,<sup>[a]</sup> Marc Borie-Guichot,<sup>[a]</sup> Virginie Garcia,<sup>[e]</sup> Abdelouahd Oukhrib,<sup>[d]</sup> Yves Génisson,<sup>[a]</sup> Thierry Levade,<sup>[e]</sup> Stéphanie Ballereau,<sup>[a]</sup> Cédric-Olivier Turrin,<sup>[b, c, d]</sup> and Cécile Dehoux\*<sup>[a]</sup>

The first phosphorus dendrimers built on a cyclotriphosphazene core and decorated with six or twelve monofluorocyclooctyne units were prepared. A simple stirring allowed the grafting of *N*-hexyl deoxynojirimycin inhitopes onto their surface by copper-free strain promoted alkyne-azide cycloaddition click reaction. The synthesized iminosugars clusters were tested as multivalent inhibitors of the biologically relevant enzymes  $\beta$ -glucocerebrosidase and acid  $\alpha$ -glucosidase, involved in Gaucher and Pompe lysosomal storage diseases, respectively. For both enzymes, all the multivalent compounds were more potent than the reference *N*-hexyl deoxynojirimycin. Remarkably, the final dodecavalent compound proved to be one of the best

$\beta$ -glucocerebrosidase inhibitors described to date. These cyclotriphosphazene-based deoxynojirimycin dendrimers were then evaluated as pharmacological chaperones against Gaucher disease. Not only did these multivalent constructs cross the cell membranes but they were also able to increase  $\beta$ -glucocerebrosidase activity in Gaucher cells. Notably, dodecavalent compound allowed a 1.4-fold enzyme activity enhancement at a concentration as low as 100 nM. These new monofluorocyclooctyne-presenting dendrimers may further find numerous applications in the synthesis of multivalent objects for biological and pharmacological purposes.

## Introduction

Multivalency is a bio-inspired concept widely studied in the field of carbohydrate-lectin interactions.<sup>[1]</sup> In order to enhance the affinity for a target protein, a large variety of compounds have been designed to present multiple copies of a sugar onto a multivalent platform. The expected multivalent effect is characterized by a higher relative affinity of the multivalent ligand compared to that of the ligand alone. In 2009, the biological evaluation of di- and tri-valent iminosugars by Gouin, Kovensky et al. led to the first observation of a quantifiable inhibitory multivalent effect against a glycosidase, the Jack bean  $\alpha$ -mannosidase (JB $\alpha$ -man).<sup>[2]</sup> Since this seminal work, the search for multivalent effect in glycosidase inhibition has grown exponentially.<sup>[3–7]</sup> Although various glycosidases such as amyloglucosidases,<sup>[8]</sup>  $\alpha$ -fucosidases,<sup>[9,10]</sup>  $\alpha$ -galactosidases<sup>[11]</sup> or sialidases<sup>[12]</sup> have been evaluated for their sensitivity to multivalency, JB $\alpha$ -man remains both the most studied and the most sensitive glycosidase to multivalent effect known to date. Very recently, an important step in the understanding of multivalent inhibitory effects was taken with the disclosure of the first high-resolution crystal structure of JB $\alpha$ -man complexed with a cyclopeptoid multimeric inhibitor.<sup>[13,14]</sup>

The seminal 1-deoxynojirimycin (DNJ) is archetypical of iminosugar-based glycosidase inhibitors. Two *N*-alkylated derivatives thereof, Zavesca® and Miglitol®, are approved drugs respectively against Gaucher disease<sup>[15]</sup> (GD), the most common lysosomal storage disease<sup>[16,17]</sup> (LSD), and against type II diabetes mellitus.<sup>[18]</sup> Since 2009, the DNJ inhitope has been grafted onto a large variety of multivalent scaffolds such as fullerenes,

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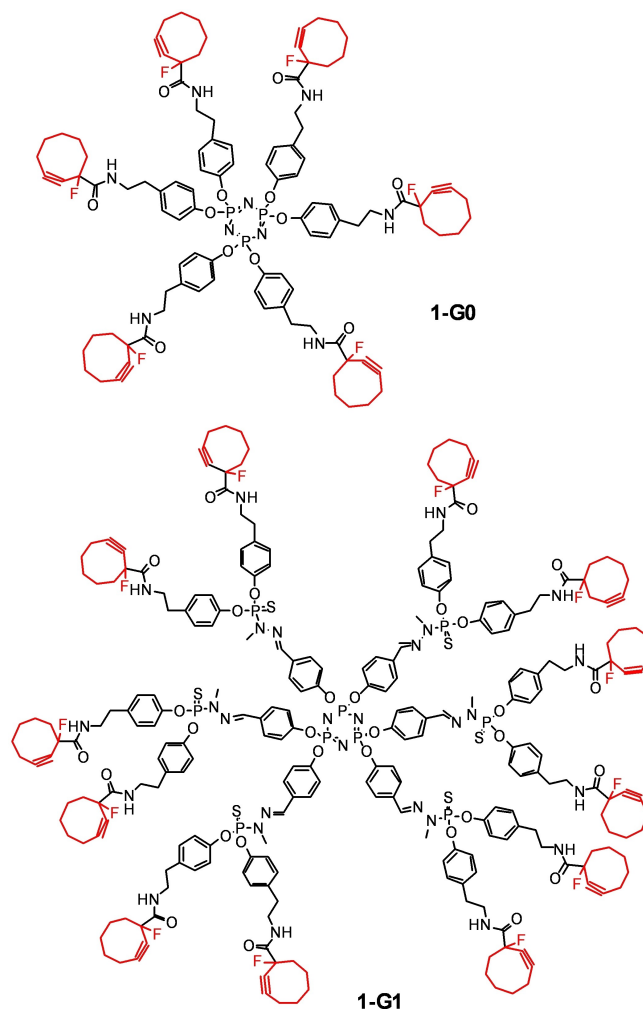
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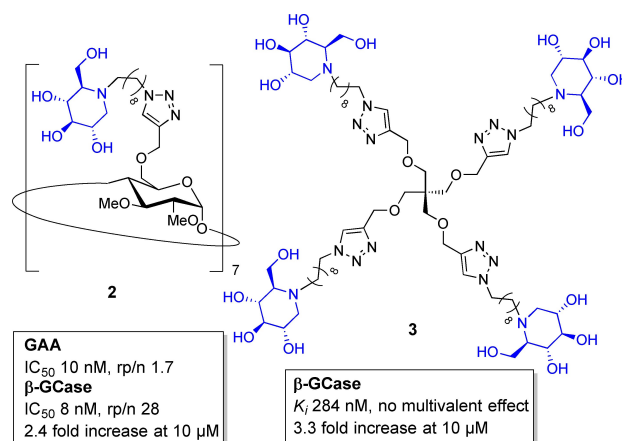
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dendrimers, calixarenes, cyclodextrins, cyclopeptides *inter alia* to generate multivalent glycosidases inhibitors.<sup>[7,19–26]</sup> These studies have highlighted the importance of both platform topology and valency on the resulting biological activity. To graft the inhitope onto the surface of the multivalent scaffold, the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) remains the preferred reaction. Although CuAAC is an efficient and robust transformation that tolerates a variety of conditions, it requires the use of cytotoxic copper.<sup>[27]</sup> So as not to interfere with the biological evaluation, the copper has to be carefully removed after the ligation. In this regard, it should be kept in mind that the copper chelating ability of iminosugars can reduce the metal catalytic efficacy and impair its complete removal, even upon tedious workup procedures. Surprisingly, there are only very rare examples of prepared multivalent iminosugars using alternative click reactions.<sup>[19,28,29]</sup> With the aim of developing a multivalent support on which inhitopes could be grafted by azide-alkyne cycloaddition not-catalyzed by copper, we report here the first dendrimers based on a cyclotriphosphazene core and presenting 6 or 12-monofluorocyclooctyne units grafted onto their surface, i.e., generation 0 (1-G0) and generation 1 (1-G1) dendrimers, respectively (Figure 1). These original dendrimers have been decorated with six or twelve DNJ inhitopes on their surface thanks to strain promoted alkyne-azide cycloaddition (SPAAC) reaction.<sup>[30,31]</sup>

Then, these new DNJ-grafted dendrimers have been assessed against two therapeutically relevant lysosomal glycosidases: the  $\beta$ -glucocerebrosidase ( $\beta$ -GCCase, E.C.3.2.1.45) involved in GD and the acid  $\alpha$ -glucosidase (GAA, EC 3.2.1.20) involved in Pompe disease (PD),<sup>[32]</sup> another LSD. The design of glycosidase inhibitors has recently gained a renewed interest due to the possibility to use these compounds as pharmacological chaperones<sup>[33–35]</sup> (PCs) to contain LSD progression and alleviate their symptoms. PCs are specific low molecular weight ligands able to promote the stabilization or induce the proper folding of a misfolded, yet catalytically active, lysosomal enzyme. Thus, PCs avoid the premature degradation of the protein by the endoplasmic reticulum (ER) associated degradation systems and improve its transport to the lysosome to mitigate the substrate burden.<sup>[19,20]</sup> Paradoxically, in most cases, PCs are competitive glycosidase inhibitors used at sub-inhibitory concentration. The first example of a multivalent PC was reported in 2012 by Compain and co-workers.<sup>[36]</sup> They found modest multivalent effects on the inhibition of  $\beta$ -GCCase and GAA with the multivalent compound **2** bearing seven DNJ-pharmacophores on its surface (Figure 2). Their studies also proved the ability of multivalent iminosugars to cross cell membranes, to reach the ER and to increase the lysosomal  $\beta$ -GCCase activity. However, only a few examples of multivalent PCs of this enzyme have since been described in the literature.<sup>[36–39]</sup> Among them, the best enhancement of  $\beta$ -GCCase activity ( $\times 3.3$  at  $10\ \mu\text{M}$ ) in N370S Gaucher fibroblasts was obtained with the DNJ tetravalent derivative **3** (Figure 2) built from a pentaerythritol core.<sup>[37]</sup> With regard to GAA, compound **2** is the only multivalent inhibitor described to date and no multivalent PC for this enzyme has yet been reported.<sup>[36]</sup>



**Figure 1.** Generation 0 (1-G0) and generation 1 (1-G1) polyphosphoryhydrazone dendrimers terminated with monofluorocyclooctynes moieties for SPAAC grafting strategies.



**Figure 2.** Examples of multivalent inhibitors and PCs of  $\beta$ -GCCase and GAA. rp/n is the valency-corrected relative inhibition potency ( $\text{rp} = K_i(\text{mono})/K_i(\text{multi})$  or  $\text{IC}_{50}(\text{mono})/\text{IC}_{50}(\text{multi})$  and  $n$  = valency of the cluster). A multivalent effect is observed for  $\text{rp}/n > 1$ .

The herein reported dendrimers grafted with six or twelve DNJ inhitopes were evaluated as  $\beta$ -GCase and GAA inhibitors. Moreover, their ability to increase  $\beta$ -GCase activity in GD patient fibroblasts was studied.

## Results and Discussion

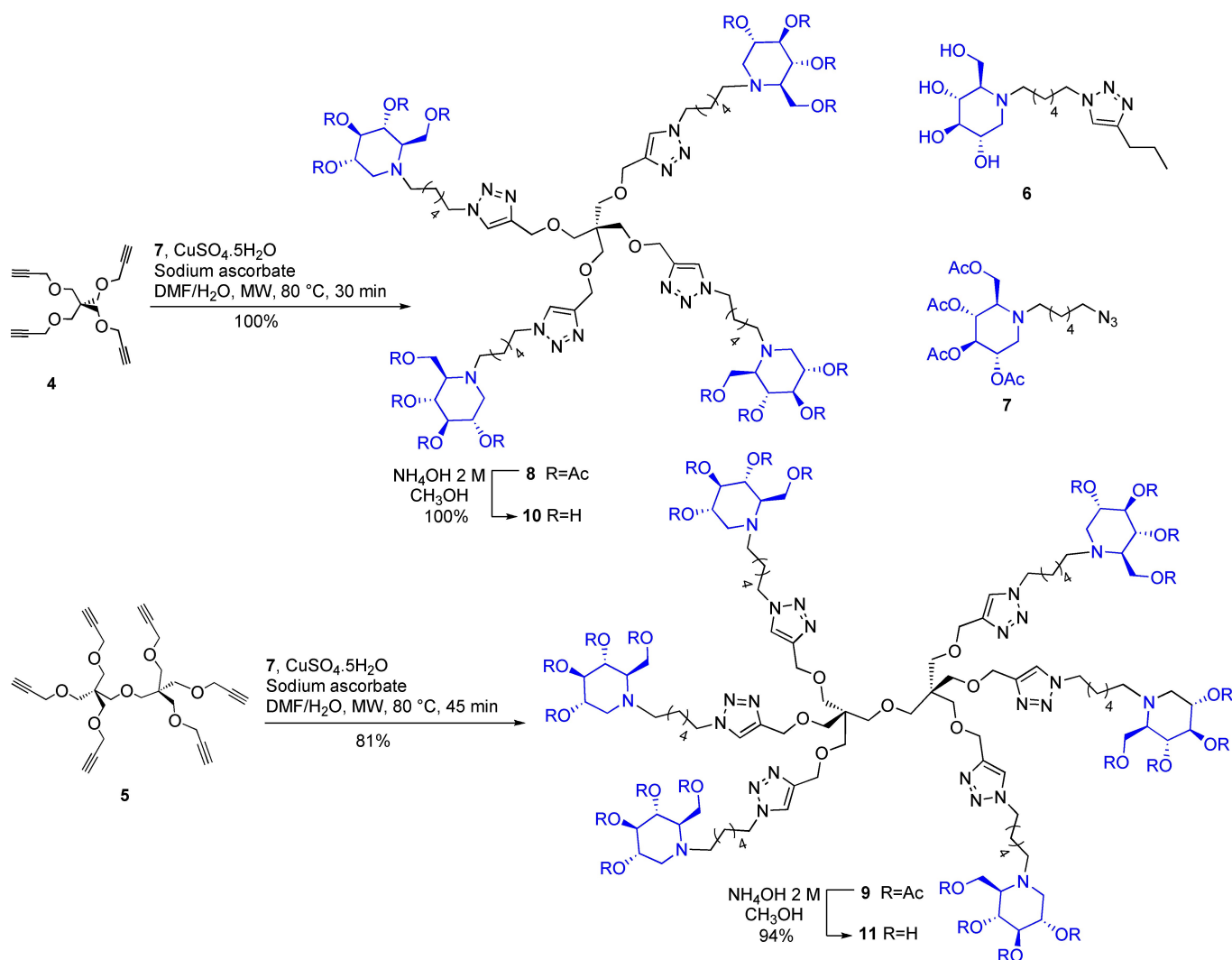
### Synthesis

#### Preparation of pentaerythritol-based multivalent iminosugars by CuAAC reaction

For comparison, scaffolds **4** and **5** with a pentaerythritol core and presenting 4 and 6 alkyne functions, respectively, were prepared following reported procedures (Scheme 1).<sup>[40,41]</sup> Indeed, the tetravalent scaffold **4** has already been used to generate multivalent inhibitors of various glycosidases.<sup>[37,39,42–44]</sup> Noteworthy, Compain et al. have also used this scaffold to prepare the  $\beta$ -GCase PC **3**, as mentioned above. The hexavalent

compound **5** is interesting to obtain the same valency as the multivalent iminosugar synthesized from **1-G0**.

Under standard CuAAC reaction conditions, coupling between platforms **4** or **5** and azido functionalized peracetylated *N*-hexyl DNJ (NH-DNJ) **7**<sup>[45]</sup> did not reach completion. In contrast, microwave activation delivered the pure tetravalent derivative **8**<sup>[46]</sup> as well as the hexavalent derivative **9** with respective yields of 100% and 81% (Scheme 1). Before deacetylation, traces of copper salts needed to be removed by filtration over a short pad of silica gel or by washing with a saturated EDTA solution. Note that this step was simplified by the use of peracetylated NH-DNJ **7** rather than unprotected NH-DNJ **15** (Scheme 3). The straightforward deacetylation step was carried out in an ammonia/methanol mixture to give the final products **10**<sup>[46]</sup> and **11** with respective yields of 100% and 94%. To determine the *rp/n* ratio, a monovalent reference **6** was also synthesized as previously reported.<sup>[45,47]</sup>

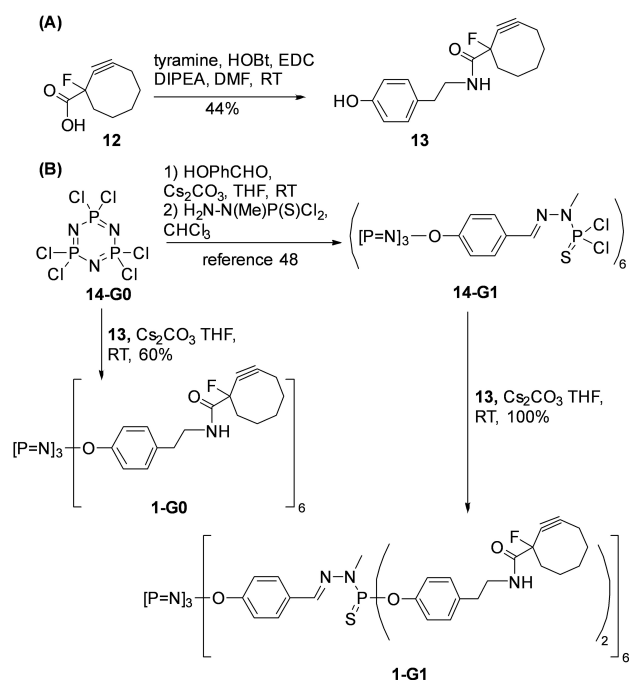


**Scheme 1.** Preparation of multivalent iminosugars **10** and **11** by CuAAC reaction.

### Preparation of multivalent iminosugars with cyclotriphosphazene core by SPAAC reaction

Dendrimers **1-G0** and **1-G1** were prepared respectively from commercially available hexachlorocyclotriphosphazene **14-G0** and the P(S)Cl<sub>2</sub>-terminated generation 1 polyphosphorhydrazone (PPH) **14-G1** dendrimers (Scheme 2). Large batches (5–50 g) of **14-G1** were readily obtained according to routine procedure published in the seminal work of A.-M. Caminade and coworkers.<sup>[48]</sup> The tyramine-based building block equipped with the cyclooctyne moiety **13** (Scheme 2) was easily obtained with a 44% yield by a coupling of tyramine with 1-fluorocyclooct-2-yne-1-carboxylic acid **12** prepared according to a well-established four-step procedure from cyclooctanone.<sup>[49]</sup> The dendrimers were reacted with stoichiometric amounts of 1-fluoro-*N*-(4-hydroxyphenethyl)-cyclooct-2-yne-1-carboxamide **13** in the presence of cesium carbonate at room temperature to afford the corresponding **1-G0** and **1-G1** polycyclooctyne scaffolds in 60% and quantitative yields respectively.

These dendrimers **1-G0** and **1-G1** were then engaged in SPAAC reaction with azido functionalized NH-DNJ **15**. The monovalent reference **16** (Scheme 3) was first prepared by stirring **13** with **15** during 2 h at 40 °C in a THF/methanol mixture. Since the SPAAC reaction is not regioselective, the monovalent reference **16** was obtained as a non-separable mixture of regioisomers with a yield of 87% after purification by chromatography on silica gel. The hexavalent and dodecavalent derivatives **16-G0** and **16-G1** were synthesized in the same way by simple stirring of **15** with **1-G0** or **1-G1** (Scheme 3). Purification on Sephadex LH-20 led to multivalent DNJ **16-G0** and **16-G1** with respectively 71% and 92% yield as



**Scheme 2.** Synthesis of A) the cyclooctyne-functionalized tyramine-based phenol **13**, and B) the dendrimers **1-G0** and **1-G1** resulting from the grafting of **13** on PPH dendrimers.

complex mixtures of non-separable regioisomers. <sup>13</sup>C NMR reaction monitoring was identified as a relevant tool to ensure the complete coupling of all the monofluorocyclooctyne units of the dendrimer. As an example, Figure 3 shows an enlargement of the <sup>13</sup>C NMR spectra of the hexavalent precursor **1-G0** (before SPAAC) and products **16-G0** (after SPAAC). The disappearance of the signals from the alkyne Csp of **1-G0** ( $\delta$  109.4 ppm (d,  $J$  = 10.5 Hz, C<sub>1</sub>) and 87.2 ppm (d,  $J$  = 31.8 Hz, C<sub>2</sub>)) and the splitting of the signal of the **1-G0** Csp<sup>3</sup> carrying the fluorine atom ( $\delta$  94.5 ppm (d,  $J$  = 186.3 Hz, C<sub>3</sub>) into two doublets corresponding to the Csp<sup>3</sup>-F atom of the 1,3- and 1,5-regioisomers ( $\delta$  94.8 ppm (d,  $J$  = 186.9 Hz, C<sub>3</sub>) and 93.9 ppm (d,  $J$  = 187.2 Hz, C<sub>3</sub>')) indicate the total conversion of the cyclooctyne-functionalized dendrimer into the DNJ-grafted dendrimer **16-G0** (Figure 3). Moreover, the purity of the molecules was assessed <sup>13</sup>C, <sup>31</sup>P, and <sup>19</sup>F NMR and by Maldi and ESI mass spectrometries.

### Inhibition of human recombinant $\beta$ -GCase and GAA

The synthesized monomeric and multivalent compounds were evaluated for their inhibition of recombinant human  $\beta$ -GCase and GAA. (4-Methylumbelliferyl)- $\alpha$ -D-glucopyranoside and (4-methylumbelliferyl)- $\beta$ -D-glucopyranoside were used as respective fluorogenic GAA and  $\beta$ -GCase enzyme substrates to release fluorescent 4-methylumbelliferone ( $\lambda_{\text{ex}}$  = 360 nm and  $\lambda_{\text{em}}$  = 455 nm) after enzymatic hydrolysis. For both enzymes, we observed that all the multivalent compounds are more potent than NH-DNJ, a known competitive inhibitor<sup>[36]</sup> of  $\beta$ -GCase and GAA (except for monomeric reference **6** regarding  $\beta$ -GCase inhibition) (Table 1). In general, the multivalent derivatives were better inhibitors versus GAA than  $\beta$ -GCase, except compound **16-G1** which was a highly potent  $\beta$ -GCase inhibitor with an IC<sub>50</sub> of 5.9 nM.

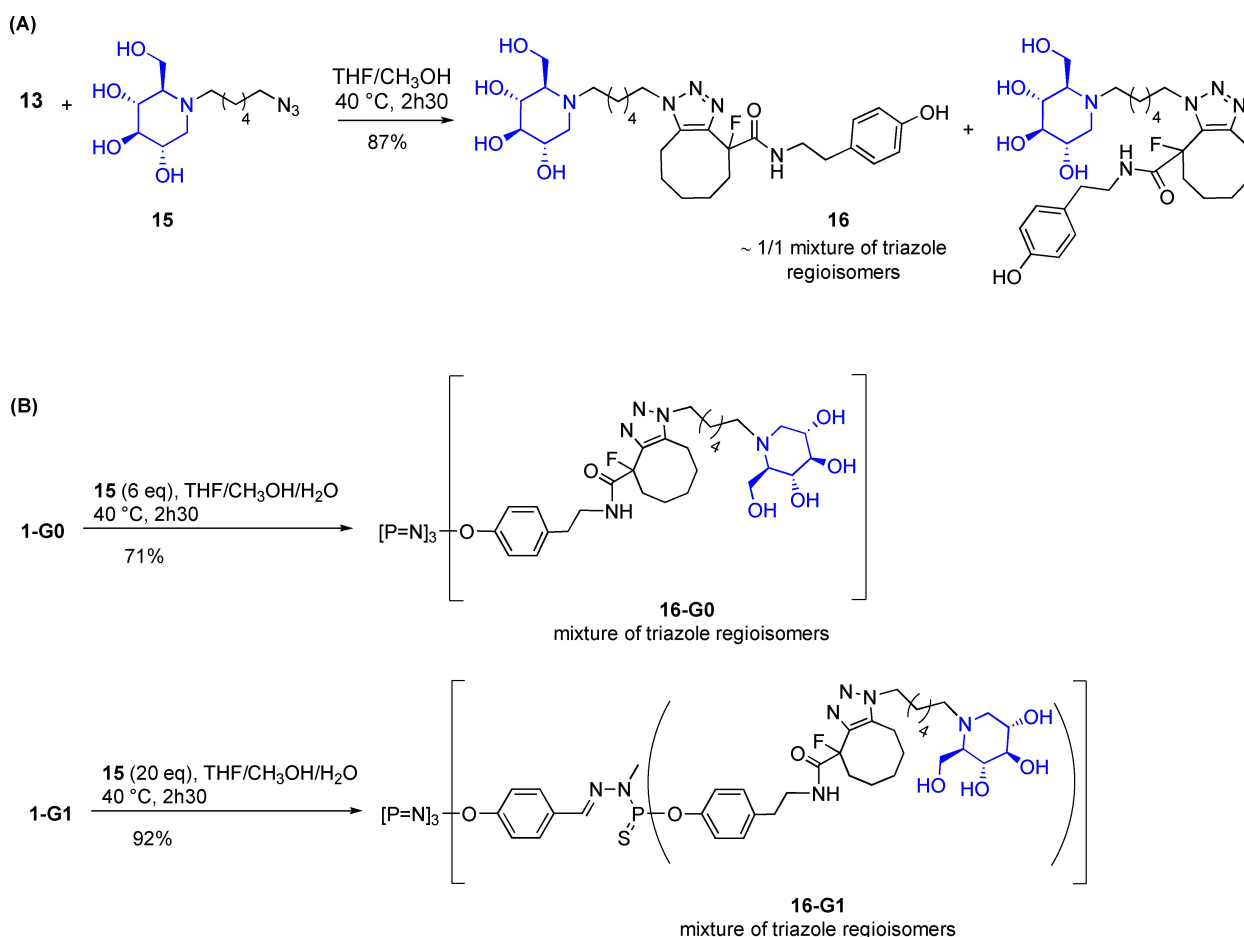
Interestingly, the inhibition potency of compounds obtained by CuAAC (compounds **6**, **10** and **11**) and by SPAAC (compounds **16**, **16-G0** and **16-G1**) was similar for GAA protein. In contrast, compounds **16**, **16-G0**, **16-G1** derived from cyclotriphosphazene core were better  $\beta$ -GCase inhibitors than those with a pentaerythritol core. This trend suggests that monofluorocyclooctatriazole units have a favorable effect on the binding to  $\beta$ -GCase. Moreover, inhibition of  $\beta$ -GCase increased with the valency for the series of compounds obtained by CuAAC as well as by SPAAC. However, this trend was not observed with GAA.

**Table 1.** Inhibition of human recombinant GAA and  $\beta$ -GCase.

Inhibitor	Valency	GAA IC <sub>50</sub> <sup>[a]</sup> [μM]	$\frac{IC_{50}^{\beta-GC}}{IC_{50}^{\text{GAA}}}$	$\beta$ -GCase IC <sub>50</sub> <sup>[a]</sup> [μM]	$\frac{IC_{50}^{\beta-GC}}{IC_{50}^{\text{GAA}}}$
NH-DNJ	1	4 <sup>[b]</sup>	–	13 <sup>[b]</sup>	–
<b>6</b>	1	0.175 ± 0.005	–	34.8 ± 1.8	–
<b>10</b>	4	0.031 ± 0.002	1.4	11.6 ± 2.0	< 1
<b>11</b>	6	0.042 ± 0.004	< 1	2.7 ± 0.2	2.1
<b>16</b>	1	0.163 ± 0.010	–	0.984 ± 0.080	–
<b>16-G0</b>	6	0.034 ± 0.006	< 1	0.345 ± 0.002	4.7
<b>16-G1</b>	12	0.607 ± 0.086	< 1	0.0059 ± 0.0002	14

[a] Results are means from triplicate experiments; [b] data from Ref. [36].





**Scheme 3.** A) Preparation by SPAAC reaction of monovalent iminosugar **16**, and B) preparation of multivalent iminosugars **16-G0** and **16-G1** (only one regioisomer is represented on dendrimers for clarity purposes).

Indeed, for the series of compounds prepared with CuAAC, tetrameric molecule **10** was slightly more potent than its hexamer counterpart **11**. Likewise, in the series derived from SPAAC, hexamer **16-G0** was a better GAA inhibitor than dodecaivalent derivative **16-G1**, suggesting that GAA is sensitive to steric hindrance.

With regard to the multivalent effect, GAA is less sensitive to multivalency than the  $\beta$ -GCase and no multivalent effect ( $rp/n < 1$ ) was observed on GAA for multivalent compounds, except with tetramer **10** ( $rp/n = 1.4$ ). On  $\beta$ -GCase, no multivalent effect was observed for the tetramer **6** in accordance with previous reported results.<sup>[37]</sup> For the hexavalent derivative **11**, a multivalent effect was seen with a  $rp/n$  ratio of 2. The highest multivalent effects were obtained with **16-G0** and **16-G1** dendrimers with  $rp/n$  ratio of 4.7 and 14 respectively. Compared to the literature,<sup>[36–39]</sup> these multivalent effects on  $\beta$ -GCase are moderate. On the other hand, compound **16-G1** is one of the best  $\beta$ -GCase inhibitors described to date.

### Increase of $\beta$ -GCase intracellular activity in Gaucher fibroblasts

The PC activity of compound **16** and dendrimers **16-G0** and **16-G1** obtained by SPAAC reaction was evaluated on N370S homozygous Gaucher fibroblasts. Of note, evaluation of the cytotoxicity of the three compounds showed no significant effect on cell viability at 10  $\mu\text{M}$  (see Supporting Information). In addition, N370S homozygous Gaucher fibroblasts in comparison with normal fibroblasts displayed 10% residual  $\beta$ -GCase activity. Fibroblasts from GD patients were incubated without (control) or with the compounds **16**, **16-G0** and **16-G1** for 3 days.

First, the induced intracellular  $\beta$ -GCase activity enhancement was assessed at 10  $\mu\text{M}$  and compared to that measured with *N*-nonyl-DNJ (NN-DNJ) as a reference. We were pleased to observe that the three compounds crossed the cell membrane since they induce an effect on  $\beta$ -GCase activity. At that concentration, NN-DNJ, **16** and **16-G0** displayed a comparable enzyme activity enhancement (about 2-fold increase at 10  $\mu\text{M}$ ) (Figure 4). In accordance with the strong inhibition observed with the recombinant enzyme, a sharp decrease in  $\beta$ -GCase activity was observed with the dodecaivalent derivative **16-G1** at 10  $\mu\text{M}$ . Assays were further run at lower concen-

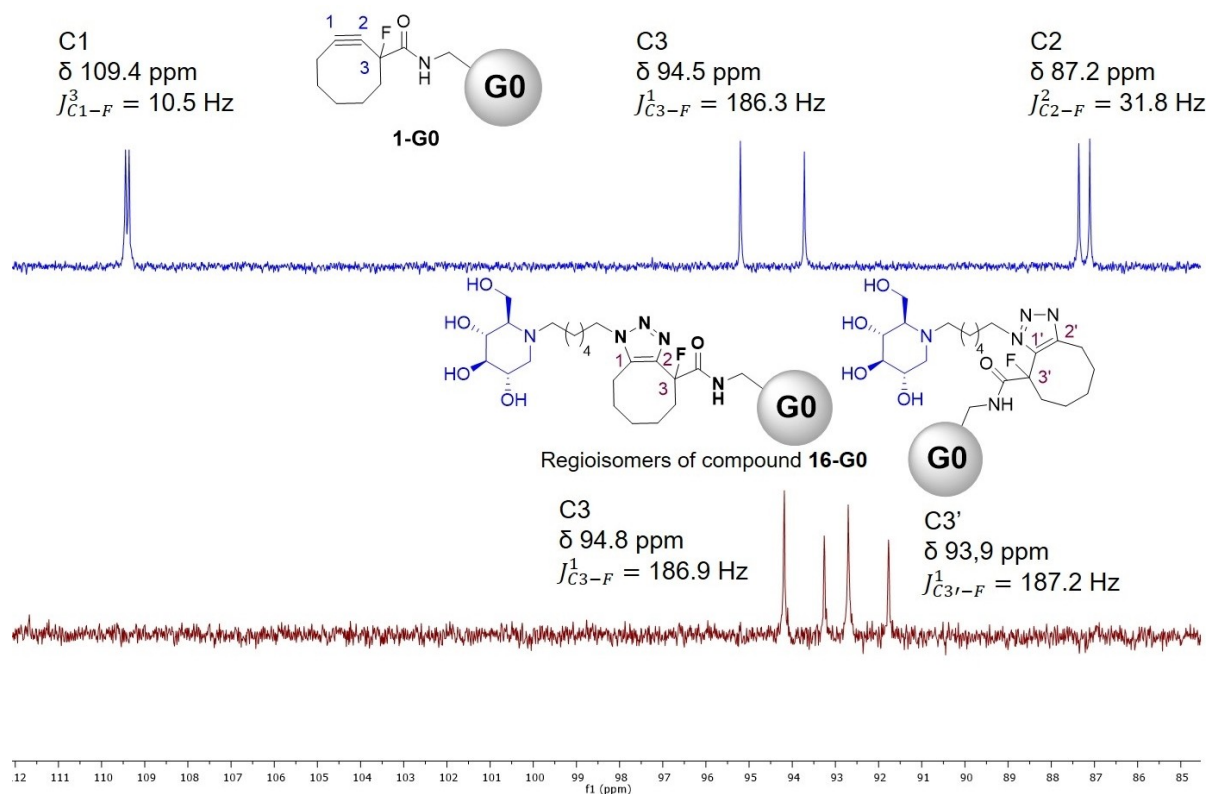


Figure 3. Enlargement of the  $^{13}\text{C}$  NMR spectra of the hexavalent compounds 1-GO (before SPAAC) and 16-GO (after SPAAC).

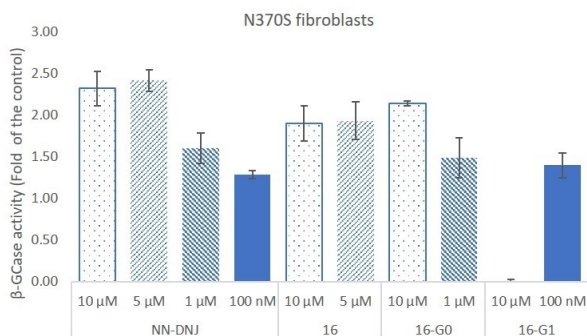


Figure 4. Evaluation of NN-DNJ, 16, 16-GO, 16-G1 as  $\beta$ -GCase enhancers in fibroblasts from GD patients bearing the N370S mutation. The fibroblasts were incubated in the presence of compounds at the chosen concentration for three days and compared to the untreated control assay. Mean values are shown for triplicate experiments.

trations. The strongest enhancement was obtained at 5  $\mu\text{M}$  for NN-DNJ (2.4-fold increase). The monovalent compound 16 led to an enhancement similar than to that recorded at 10  $\mu\text{M}$  (1.93-fold increase at 5  $\mu\text{M}$  vs. 1.90-fold at 10  $\mu\text{M}$ ). At 1  $\mu\text{M}$ , the hexavalent DNJ 16-GO allowed a 1.5-fold enhancement of the  $\beta$ -GCase activity. Remarkably, reducing the concentration of 16-G1 as low as 100 nM allowed a 1.4-fold increase in enzyme activity.

## Conclusions

We described the synthesis of the first dendrimers built on a cyclotriphosphazene core and displaying monofluorocyclooctyne units on their surface. These nano-objects allowed the grafting of 6 and 12 DNJ inhitopes thanks to metal-free SPAAC click reaction by just a stirring of the generation zero and generation 1 dendrimers (1-GO and 1-G1, respectively) and an azide-functionalized *N*-alkyl DNJ.

Then, the prepared compounds 16-GO and 16-G1 were evaluated with respect to two therapeutically relevant enzymes,  $\beta$ -GCase and GAA. For comparison, compounds with a pentaerythritol core and 4 or 6 grafted DNJ inhitopes were also prepared by standard CuAAC reaction. All synthesized compounds proved to be inhibitors of both enzymes. Multivalent effects were mainly observed with  $\beta$ -GCase. Moreover, our results suggest that monofluorocyclooctyne units have a favourable effect on the binding to the  $\beta$ -GCase. The compound 16-G1 was found to be a nanomolar range  $\beta$ -GCase inhibitor and is among the best inhibitors described to date. In order to assess their capacity to behave as PC, cellular evaluations were carried out with the compounds obtained by SPAAC on fibroblasts of Gaucher patients. This study shows that dendrimers 16-GO and 16-G1 can cross cell membrane and increase the  $\beta$ -GCase activity down to nM concentrations in the case of 16-G1.

To conclude, we demonstrate that dendrimers 1-GO and 1-G1 are relevant platforms for the straightforward preparation

of complex iminosugar clusters thanks to a SPAAC-based ligation. Furthermore, the obtained multivalent nanoobjects are fully appropriate to biological studies. These results thus pave the way for further syntheses of multivalent systems based on dendrimeric scaffolds with a cyclotriphosphazene core and/or iminosugar moieties.

## Experimental Section

**Synthetic chemistry:** General procedure for CuAAC under MW conditions: A solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.3 equiv. per alkyne) and L-ascorbic acid sodium salt (3 equiv. per  $\text{Cu}^{\text{II}}$ ) in water ( $[\text{Cu}^{\text{II}}] = 0.05 \text{ M}$ ) were added to a solution of alkyne (1 equiv.) and azide derivative (1.1 equiv. per alkyne) in DMF ( $[\text{Alkyne}] = 0.01 \text{ M}$ ). The mixture was stirred and heated under microwave irradiation at  $80^\circ\text{C}$  and 23 W. Then, the mixture was diluted in EtOAc, washed with saturated EDTA until the aqueous layer is colorless. The organic layer is dried over  $\text{Na}_2\text{SO}_4$  and concentrated to give the desired product.

General procedure for deacetylation: A solution of  $\text{NH}_4\text{OH}$  (2 M in  $\text{CH}_3\text{OH}$ , 130 eq) was added to a solution of peracetylated multivalent cluster (1 equiv.) in  $\text{CH}_3\text{OH}$  (55.5 mL/mmol). The resulting mixture was stirred under nitrogen at r.t. overnight. The resulting deacetylated cluster was obtained by co-evaporation with toluene and  $\text{CH}_3\text{OH}$ .

General procedure for SPAAC reaction: THF,  $\text{CH}_3\text{OH}$  and water were first degassed under argon. Then the monovalent cyclooctyne (1.3 equiv.) or dendrimer derivative (0.7 equiv.) and azide (1 equiv.) were dissolved in a THF/ $\text{CH}_3\text{OH}$  (1:1,  $[\text{Alkyne}] = 0.01 \text{ M}$ ) before adding water (1/3 of the final volume). The mixture was stirred at  $40^\circ\text{C}$  at 1200 rpm.

**Inhibition assays on recombinant  $\beta$ -Gcase:** Recombinant Human  $\beta$ -GCase/GBA (7410-GH), purchased from R&D was used in the inhibition studies. The used substrate 4-methylumbelliferyl- $\beta$ -D-glucopyranoside was purchased by Sigma-Aldrich.  $\beta$ -GCase activity was determined with 4-methylumbelliferyl- $\beta$ -D-glucopyranoside as reported previously. Briefly, enzyme solutions (25  $\mu\text{L}$  from a stock solution containing  $0.6 \mu\text{g mL}^{-1}$ ) in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2) were incubated at  $37^\circ\text{C}$  without (control) or with inhibitor at a final volume of 50  $\mu\text{L}$  for 30 min. After addition of 25  $\mu\text{L}$  4-methylumbelliferyl- $\beta$ -D-glucopyranoside (7.2 mM, McIlvaine buffer pH 5.2), the samples were incubated at  $37^\circ\text{C}$  for 10 min. Enzymatic reactions were stopped by the addition of aliquots (100  $\mu\text{L}$ ) of glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methylumbelliferone formed was determined with a FLUOstar microplate reader (BMG Labtech) at 355 nm (excitation) and 460 nm (emission).

**Inhibition assays on recombinant GAA:** Recombinant Human  $\alpha$ -Glucosidase acid/GAA (8329-GH), purchased from R&D was used in the inhibition studies. The used substrate 4-methylumbelliferyl- $\alpha$ -D-glucopyranoside was purchased by Sigma-Aldrich. Briefly, enzyme solutions (10  $\mu\text{L}$  from a stock solution containing  $2.2 \mu\text{g mL}^{-1}$ ) in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2) were incubated at  $37^\circ\text{C}$  without (control) or with inhibitor at a final volume of 50  $\mu\text{L}$  for 30 min. After addition of 25  $\mu\text{L}$  4-methylumbelliferyl- $\alpha$ -D-glucopyranoside (4.7 mM, McIlvaine buffer pH 4.6), the samples were incubated at  $37^\circ\text{C}$  for 15 min. Enzymatic reactions were stopped by the addition of aliquots (100  $\mu\text{L}$ ) of glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methylumbelliferone formed was determined with a

FLUOstar microplate reader (BMG Labtech) at 355 nm (excitation) and 460 nm (emission).

**Cell viability evaluation:** Cultured primary skin fibroblasts from control individuals were obtained from the Laboratoire de Biochimie Métabolique, CRB, IFB, CHU Toulouse, France and CBC Biotech biobank BB-0033-00046 (3809 F01 and 1541 F02 cell lines), Hospices Civils de Lyon, France. Cells were immortalized after transfection with a plasmid encoding the SV40 large T antigen. Cells were routinely cultured in DMEM medium supplemented with 10% inactivated fetal calf serum. After incubation for 72 h in the presence of the chemical compounds or vehicle (ethanol) only, cell viability was evaluated using the MTT test as reported earlier.<sup>[50]</sup> Briefly, cells were plated in 24-well plates (30,000 cells/well) and at 50% confluency cells were incubated with fresh medium supplemented with 30  $\mu\text{M}$  of compounds. After 72 h, 500  $\mu\text{g}$  of MTT was added to each well. After 2 h of incubation at  $37^\circ\text{C}$ , supernatants were discarded, and the remaining material was dissolved by 1 mL of DMSO. After 30 min, absorbance was measured at 560 nm by using a microplate reader. Background values were subtracted from all other values and viability was expressed as percentage compared with untreated controls.

**Assay of  $\beta$ -GCase activity on human fibroblasts:** Cultured primary skin fibroblasts from patients affected with Gaucher disease (carrying the N370S mutation) were obtained from CBC Biotech biobank BB-0033-00046 (3809 F01 and 1541 F02 cell lines), Hospices Civils de Lyon, France. Cultured primary skin fibroblasts from control individuals were from the Laboratoire de Biochimie Métabolique, CRB, IFB, CHU Toulouse, France. Informed, signed consent was obtained from all patients for molecular genetic studies and cell cultures. All experiments were performed in compliance with the French rules and institute policies on ethics and human material usage. The activity of preparation and conservation of biological material for research purposes is the subject of a declaration to the "Ministère de l'Enseignement Supérieur, de la Recherche et de l'innovation (MESRI)", to the "Comité de protection des Personnes Sud-Est IV" and at "the Agence Régionale d'Hospitalisation" registered under number DC-2008-72 and amendments DC-2011-1437, DC-2015-2566, DC-2019-3464 and DC-2020-3919. The activity of biological material transfer is subject to MESRI authorizations registered under numbers AC-2008-73, AC-2013-1867, AC-2015-2576, AC-2019-3465 and AC-2020-3918. The biobank has also been certified according to the NF S-96 900 standard since 2011. Cells were immortalized after transfection with a plasmid encoding the SV40 large T antigen. Cells were routinely cultured in DMEM medium supplemented with 10% inactivated fetal calf serum. Cells were harvested and pelleted. Cell lysates were prepared in 0.2% Triton X100 by brief sonication.  $\beta$ -GCase enzyme activity was determined on cell lysates in sodium acetate buffer pH 5.6 using 4-methylumbelliferyl- $\beta$ -D-glucopyranoside (Sigma-Aldrich, St Louis, MO, USA) as substrate.

## Supporting Information

Additional references cited within the Supporting Information.<sup>[46,48]</sup>

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## Conflict of Interests

Cédric-Olivier Turrin is cofounder and CEO of IMD-Pharma SAS.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** copper-free click reaction · dendrimers · inhibitors · lysosomal storage diseases · pharmacological chaperones

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