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# Synthesis of N-alkylated noeurostegines and evaluation of their potential as treatment for Gaucher's disease

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# ABSTRACT

The potent and selective inhibitor of  $\beta$ -glucosidases, noeurostegine, was evaluated as an inhibitor of glucocerebrosidase (GCase) to give an IC<sub>50</sub> value of 0.4  $\mu$ M, being 250- and 150-fold better than *N*-butyl and *N*-nonyl noeurostegine, respectively. The parent noeurostegine and its *N*-butyl and *N*-nonyl alkylated congeners were also tested as pharmacological chaperones against a N370S GCase mutant. Of these, only noeurostegine, was found to increase enzyme activity, which in potency was comparable to that previously reported for isofagomine.

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Gaucher's disease is a lysosomal storage disorder caused by inherited genetic mutations in the GBA gene, which results in deficient activity of glucocerebrosidase (GCase). Deficient GCase activity leads to progressive accumulation of glucosylceramide (GlcCer), primarily in the lysosomes of Gaucher's patients, causing hepatosplenomegaly, anaemia, bone lesions, and, in more severe cases, central nervous system impairment.<sup>1</sup> Gaucher's disease is the most common of all lysosomal storage disorders with a prevalence of 1 in 40,000–60,000 in the general population and 1 in 800 among the Ashkenazi Jewish population.<sup>2</sup>

The disease is clinically classified into three types based on the age of onset and the degree of neurological involvement, the most common and mild form being type 1.<sup>3</sup> Although more than 200 different mutations in GCase have been reported, N370S is the most prevalent disease-causing mutation and is associated with type 1 disease.<sup>4</sup> There is no cure for Gaucher's disease, but there are three types of therapies available to patients.

Enzyme replacement therapy (ERT)<sup>5</sup> is available to type 1 Gaucher's patients and involves intravenous infusion of recombinant form of GCase (Cerezyme<sup>®</sup>).<sup>6</sup> This therapy is very expensive, with an estimated annual cost upwards of \$200,000 per patient.<sup>7</sup> Another therapy is called substrate reduction therapy. This involves inhibiting the enzyme responsible for GlcCer biosynthesis, for example, by administrating *N*-butyl deoxynojirimicin (NB-DNJ, Miglustat, Zavesca<sup>®</sup>).<sup>8</sup> With a reduction in the amount of GlcCer being synthesized, less is stored in the lysosome and the clinical course of the disease can be improved. However, several drawbacks concerning this therapy have also been identified.<sup>9</sup>

A very promising therapeutic strategy uses small molecule pharmacological chaperones, often competitive enzyme inhibitors, to facilitate proper folding and trafficking of the lysosomal enzymes. The pharmacological chaperones act as a folding template for mutant enzymes and stabilize the conformation of the enzyme detected by the quality control system, thus avoiding the endoplasmic reticulum-associated degradation (ERAD) pathway.<sup>10</sup>

It is known that calystegines  $A_3(1)$ ,  $B_1(2)$ ,  $B_2(3)$  and  $C_1(4)$ , naturally occurring nor-tropane glycoside hydrolase inhibitors also act as pharmacological chaperones increasing the GCase activity in Gaucher fibroblasts up to two-fold over untreated cells.<sup>11</sup> However, the effective concentrations needed of the calystegines (1–4, Fig. 1) are 2–20-fold larger than observed for isofagomine (5), this indicates that isofagomine (5) is a more effective pharmacological chaperone for GCase. It has also been indicated that the hydroxymethyl present in isofagomine (5) is important for interaction with the protein.<sup>11</sup> On the other hand, isofagomine (5) is lacking the 2-OH which in studies have been shown to contribute significantly to enzyme binding.<sup>12,13</sup>

Recent results from our group have shown that noeurostegine (**7**, Fig. 1) is a potent inhibitor of  $\beta$ -glucosidase from sweet almonds and *T. maritima*.<sup>14</sup> This (**7**) was designed as a hybrid of inherently unstable and unselective noeuromycin (**6**) and the stable and selective calystegine B<sub>2</sub> (**3**), and therefore contains both the hydroxymethyl substituent and the important 2-OH.

Based on our initial promising results with noeurostegine we set out to evaluate whether this nor-tropane and its *N*-butyl and *N*-nonyl analogues too were inhibitors of GCase. Furthermore,

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**Figure 1.** Structure of iminosugars calystegines (1–4), isofagomine (5), noeuromycin (6) and noeurostegine (7).

these compounds effect as pharmacological chaperones for N370S GCase mutants and hence their potential as therapeutics for treating Gaucher's disease was also investigated.

Noeurostegine (**7**) was synthesised from levoglucosan (**8**) in 22-steps as previously described (Scheme 1).<sup>14</sup>

We envisaged that N-alkylated analogues of noeurostegine (7) could be potential inhibitors of GCase since it is reasonable to postulate that GCase, besides a binding site that recognises the glucosyl moiety, also contains a hydrophobic binding site that normally accommodates the ceramide moiety of the natural substrate.<sup>15</sup> Furthermore, addition of an alkyl chain would increase the hydrophobicity of the compound and thereby enhance ER membrane translocation which has a significant influence on cellular activity.<sup>16</sup> The *N*-butyl (**12**) and *N*-nonyl (**13**) noeurostegine were synthesised from tri-O-benzyl ether protected noeurostegine (**9**). Reductive amination using butanal or nonanal, respectively, in combination with sodium cyanoborohydride in acidic methanol gave the desired amines **10** and **11** in good yield. Catalytic hydrogenolysis over Pearlman's catalyst next yielded the desired deprotected N-alkylated noeurostegines **12** and **13** (Scheme 1).<sup>17</sup>

The inhibitory activities of the three sugar-mimicking compounds against human GCase was determined using a partially purified preparation of placental enzyme and fluorescently labelled substrate and are summarised in Table 1. Among the compounds tested only the parent compound was a potent inhibitor of GCase with an IC<sub>50</sub> value of 0.4  $\mu$ M. It is clear that neither the *N*-butyl nor the *N*-nonyl chain improved inhibitory activity, which is in

#### Table 1

Inhibitory activity of  ${\bf 7}$  and derivatives  ${\bf 12}$  and  ${\bf 13}$  against GCase

Inhibitor	IC <sub>50</sub> [μM]
Calystegine $B_2(3)$ Noeurostegine ( <b>7</b> )	1.0* 0.4
<i>N</i> -Butyl noeurostegine ( <b>12</b> )	100
N-Nonyl noeurostegine (13)	60

\* See Ref. 11.

accordance with results previously reported by Zhu et al.<sup>15</sup> and Chang et al.<sup>11</sup> for N-alkylated isofagomine.

To evaluate the pharmacological chaperone activity a cell-based glucocerebrosidase enhancement assay was conducted in the presence of compound 7, 12 or 13. Each compound was added at various concentrations to the cultured medium of Gaucher N370S lymphoblasts cultured for three days in the presence of inhibitor before enzyme activity was determined.<sup>17</sup> Since all three compounds showed some inhibitory activity against GCase a decrease in residual enzyme activity would be expected in cells cultured with the inhibitors at high concentration, since inhibition would start to overwhelm enzyme activity enhancement. The obtained results for noeurostegine (7) are illustrated in Figure 2; no increase in residual enzyme activity was observed for compounds 12 and 13. The maximal increase in activity in N370S Gaucher lymphoblasts for noeurostegine (7) was 1.82-fold that of untreated cells. The positive control N-nonyl 1-deoxynojirimycin (NNDNJ) reached a level of 1.16-fold at 0.1 µM. The effective concentration responsible for this increase was in the range of  $10-100 \mu$ M, comparable to that previously reported for isofagomine.<sup>11</sup> Interestingly, no significant decrease in residual enzyme activity was seen within the investigated concentration range, an observation that has also been made with other imino sugar chaperones for enhancing  $\alpha$ -galactosidase and CGase activity.<sup>18</sup> N-Butyl noeurostegine (12) and N-nonyl noeurostegine (13) did not give any increase in residual enzyme activity in N370S Gaucher lymphoblasts within the same concentration range as (7) (results not shown). The orientation of the *N*-alkyl chain in localising to a hydrophobic pocket adjacent to the active site might restrict binding, reducing potency and increasing the concentration required for a chaperone mediated enhancement of mutant enzyme activity beyond experimental evaluation.



Scheme 1. Synthesis of N-alkyl noeurostegines.



**Figure 2.** Residual glucocerebrosidase activity after treatment with noeurostegine (**7**). N370S lymphoblasts were cultured for three days in the presence of concentrations of compound shown before estimating CGase activity in an in vitro assay as described in the text.

Replacement of the anomeric carbon atom with an endocyclic nitrogen atom in noeurostegine provides a similar inhibition profile for glucosidases to the isofagomines.<sup>19</sup> No inhibition of endoplasmic reticulum (ER)  $\alpha$ -glucosidases I and II was found for compound **7** when incubated with HL60 cells for 24 h at 500  $\mu$ M, as determined by a free oligosaccharide assay that detects the glucosylated products of inhibition (Fig. 3).<sup>20</sup> The free oligosaccharides in untreated cells (Fig. 3a) contained further glucosylated glycans following treatment with NB-DNJ (50  $\mu$ M), with monoglucosylated structures Glc<sub>1</sub>Man<sub>4</sub>GlcNAc<sub>1</sub> (G1M4) and G<sub>1</sub>M<sub>5</sub>GlcNAc<sub>1</sub> (G1M5) evidence of glucosidase II inhibition (Fig. 3b). Treatment with compound **7** revealed that no additional glucosylated oligo-saccharides were present, consistent with a lack of  $\alpha$ -glucosidase I and II inhibition (Fig. 3c).

Noeurostegine (**7**) clearly has access to the ER as evidenced by its ability to enhance CGase activity in Gaucher lymphoblasts so the lack of inhibition must be related to active site affinity. An in vitro analysis using purified  $\alpha$ -glucosidase II (Fig. 4) confirmed that NB-DNJ at 100  $\mu$ M was sufficient to inhibit the enzyme-catalysed conversion of Glc<sub>2</sub>Man<sub>7</sub>GlcNAc<sub>2</sub> to Glc<sub>1</sub>Man<sub>7</sub>GlcNAc<sub>2</sub> and Man<sub>7</sub>GlcNAc<sub>2</sub> (Fig. 4b) whereas compound **7** at 500  $\mu$ M had no  $\alpha$ -glucosidase II inhibitory effect (Fig. 4c).



**Figure 3.** Free oligosaccharide (FOS) analysis of the inhibition of ER- $\alpha$ -glucosidases in cells. HL60 cells were incubated for 24 h in the presence of (a) no inhibitor; (b) NB-DNJ (50  $\mu$ M) and (c) compound **7** (500  $\mu$ M). Oligosaccharides were extracted, labelled with 2-AA and analysed by NP-HPLC as described in the text. G1M4, Glc<sub>1</sub>Man<sub>4</sub>Glc-NAc<sub>1</sub>; M5, Man<sub>5</sub>GlcNAc<sub>1</sub>; G1M5, Glc<sub>1</sub>Man<sub>5</sub>GlcNAc<sub>1</sub>; G3M5, Glc<sub>3</sub>Man<sub>5</sub>GlcNAc<sub>1</sub>.



**Figure 4.** In vitro ER- $\alpha$ -glucosidase II assay. Purified glucosidase II was incubated with 2-AA labelled G2M7, Glc<sub>2</sub>Man<sub>7</sub>GlcNAc<sub>2</sub> for 1.25 h at 37 °C in the presence of (a) no inhibitor; (b) NB-DNJ (100  $\mu$ M) and (c) compound 7 (500  $\mu$ M). The reaction was stopped, deproteinated and oligosaccharides analysed for starting material and glucosidase-mediated reaction products, G1M7, Glc<sub>1</sub>Man<sub>7</sub>GlcNAc<sub>2</sub> and M7, Man<sub>7</sub>GlcNAc<sub>2</sub> by NP-HPLC.

The selectivity of noeurostegine for CGase and the significant improvement of mutant enzyme activity provides an important property for therapeutic evaluation of this and similar compounds.

In conclusion, we have identified noeurostegine (**7**) as a hydrophilic, potent and selective inhibitor for human GCase and a pharmacological chaperone capable of increasing the residual enzyme activity in N370S Gaucher lymphoblasts 1.82-fold that of untreated cells, an increase comparable to that reported for similar compounds, for example, isofagomine and calystegine. The effective concentration to increase N370S enzyme activity in lymphoblasts was in the range 10–100  $\mu$ M, similar to the reported value for isofagomine and lower than the observed value for calystegine B<sub>2</sub>. This indicates that noeurostegine is as effective as a pharmacological chaperone as isofagomine. In addition, we report the efficient synthesis of *N*-alkyl noeurostegine **12** and **13** from a known intermediate in the synthesis of noeurostegine. These novel compounds did not act as potent inhibitors for human GCase nor did they act as pharmacological chaperones.

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# Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.106.

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