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## **Supporting Information**

## Targeted Delivery of Pharmacological Chaperones for Gaucher Disease to Macrophages by a Mannosylated Cyclodextrin Carrier<sup>†</sup>

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## **General methods**

**NMR titration experiments**. Association constants ( $K_{as}$ ) were determined in D<sub>2</sub>O at 313 K by measuring the proton chemical shift variations in the <sup>1</sup>H NMR spectra of a solution of the  $\beta$ CD derivative in the presence of increasing amounts of the corresponding chaperone. In a typical titration experiment, a solution of the host (ManS)<sub>3</sub>- $\beta$ CD (1.63 mM) in D<sub>2</sub>O was prepared, a 500 µL aliquot was transferred to a 5-mm NMR tube, and the initial NMR spectrum was recorded. A concentrated solution (ca. 25 mM) of the chaperone 6S-NOI-NJ and 6S-NAdB-NJ in the previous solution was prepared in order to maintain the host concentration constant all throughout the titration experiment. 10 µL aliquots of this solution were sequentially added to the  $\beta$ CD solution and the corresponding NMR spectra recorded until 90-100% complexation of the guest had been achieved. The chemical shifts of the diagnostic signals obtained at 10-15 different host-guest concentration ratios were used in an iterative least-squares fitting procedure (Figures S4-S5).<sup>1</sup>

**Isothermal titration calorimetry (ITC)**. In addition to information in the Experimental Section of the manuscript, the lectin solns to be used for ITC were prepared in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1 mM Ca<sup>2+</sup> and 0.1 mM Mn<sup>2+</sup>, dialysed against a large volume of the same buffer and centrifuged to remove any insoluble material. The protein concentrations were determined colorimetrically following the Bradford method,<sup>2</sup> measuring the absorbance at 595 nm of a solution (1 mL) containing 200 µL of the commercial reagent (BIO-RAD protein assay), distilled water (780 µL) and 20 µL of protein solution containing 2-10 µg of protein. Calibration of the commercial reagent was carried out using a 10% (w/v) solution of BSA. The extinction coefficient determined for the colorimetric complex was  $\varepsilon_{595} = 45 \text{ mL}\cdot\text{mg}^{-1}\cdot\text{cm}^{-1}$ .



**Preparation** of N-dansyl-2,2,2-tris[5-(α-D-mannopyranosylthio)-2oxapentyl]ethyl amine (8). A solution of 2,2,2-tris[5-(2,3,4,6-tetra-O-acetyl- $\alpha$ -Dmannopyranosylthio)-2-oxapentyl]ethyl azide 9<sup>3</sup> (106 mg, 77 µmol) in MeOH (5 mL) was treated with a NaOMe (10% eq, 1 M methanolic solution, 92 µL) for 3 h. The solution was stirred at room temperature for 30 min, and then neutralised using Amberlite IR-120 (H<sup>+</sup>) ion exchange resin, filtered and concentrated to dryness. The resulting deacetylated azide was then hydrogenated by treatment with 10% Pd/C (20 mg) under a H<sub>2</sub> atmosphere (1 atm) for 16 h. The reaction mixture was filtered and the solvents evaporated. The crude amine 10 thus obtained was dissolved in anhydrous DMF (2.3 mL) and the solution was cooled to 0 °C. Triethylamine (17 µL, 0.12 mmol) and 5-dimethylaminonaphtalene-1sulfonyl chloride (32 mg, 0.12 mmol) were added and the reaction mixture was stirred for 24 hours at room temperature. The solvent was subsequently removed under reduce pressure and the residue was purified by column chromatography using  $50:10:1 \rightarrow 50:10:1$ DCM-MeOH-H<sub>2</sub>O as eluent. Yield: 66 mg (80% over three steps).  $[\alpha]_D$  +97.4 (c 0.7, MeOH); R<sub>f</sub> 0.33 (30:10:1 DCM-MeOH-H<sub>2</sub>O); IR (ATR) v<sub>max</sub> 3351, 1648, 1456, 1067 cm<sup>-1</sup>;

<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  8.61 (bd,  $J_{3,4}$  = 8.5 Hz, 1 H, H-4<sub>Naph</sub>), 8.34 (d,  $J_{8,9}$  = 8.7 Hz, 1 H, H-9<sub>Naph</sub>), 8.22 (dd,  $J_{2,3}$  = 7.3 Hz,  $J_{2,4}$  = 2.0 Hz, 1 H, H-2<sub>Naph</sub>), 7.65 (m, 2 H, H-3<sub>Naph</sub>, H-8<sub>Naph</sub>), 7.32 (d,  $J_{7,8}$  = 7.6 Hz, 1 H, H-7<sub>Naph</sub>), 5.24 (bs, 3 H, H-1<sub>Man</sub>), 3.95 (bd, 3 H,  $J_{1,2}$  = 0.9 Hz, H-2<sub>Man</sub>), 3.91 (m, 3 H, H-5<sub>Man</sub>), 3.84 (dd, 3 H,  $J_{6a,6b}$  = 11.9 Hz,  $J_{5,6a}$  = 2.2 Hz, H-6a<sub>Man</sub>), 3.78 (dd, 3 H,  $J_{5,6b}$  = 5.5 Hz, H-6b<sub>Man</sub>), 3.70 (m, 6 H, H-3<sub>Man</sub>, H-4<sub>Man</sub>), 3.30 (t, 6 H, <sup>3</sup> $J_{H,H}$  = 5.9 Hz, H-3<sub>Pent</sub>), 3.15 (bs, 6 H, H-1<sub>Pent</sub>), 2.94 (m, 2 H, CH<sub>2</sub>NH), 2.92 (s, 6 H, CH<sub>3</sub>), 2.65 (m, 6 H, H-1<sub>Pent</sub>), 1.78 (t, 6 H, H-4<sub>Pent</sub>); <sup>13</sup>C NMR (125.7 MHz, CD<sub>3</sub>OD)  $\delta$  153.3 (C-6<sub>Naph</sub>), 136.1 (C-1<sub>Naph</sub>), 131.4-130.7 (C-2<sub>Naph</sub>, C-4<sub>Naph</sub>, C-5<sub>Naph</sub>, C-10<sub>Naph</sub>), 129.4 (C-8<sub>Naph</sub>), 124.4 (C-3<sub>Naph</sub>), 120.2 (C-9<sub>Naph</sub>), 116.5 (C-7<sub>Naph</sub>), 86.5 (C-1<sub>Man</sub>), 74.9 (C-5<sub>Man</sub>), 73.7 (C-2<sub>Man</sub>), 73.1 (C-3<sub>Man</sub>), 71.2 (C-3<sub>Pent</sub>), 70.6 (C-1<sub>Pent</sub>), 68.7 (C-4<sub>Man</sub>), 62.7 (C-6<sub>Man</sub>), 47.8 (CH<sub>3</sub>), 45.9 (CH<sub>2</sub>NH), 44.9 (C<sub>q</sub>) 30.6 (C-4<sub>Pent</sub>), 28.8 (C-5<sub>Pent</sub>); ESIMS: *m*/z 1099 [M + Na]<sup>+</sup>; Anal. Calcd for C<sub>44</sub>H<sub>72</sub>N<sub>2</sub>O<sub>20</sub>S<sub>4</sub>: C, 49.05; H, 6.74; N, 2.60; S, 11.91. Found: C, 48.67; H, 6.83; N, 2.34; S, 11.56.



N-(13-tert-butoxycarbonylamino-4,7,10-trioxa)tridecanyl-1-

adamantanecarboxamide (13). To a stirred solution of adamantane-1-carbonyl chloride 11 (0.47 g, 2.38 mmol) in DCM (27 mL), a solution of *N-tert*-butoxycarbonyl-4,7,10-trioxa-1,13-tridecanediamine<sup>4</sup> 12 (0.71 g, 2.23 mmol) and Et<sub>3</sub>N (1.02 mL, 5.50 mmol) in DCM (27 mL) was added. The reaction mixture was stirred for 3 h at room temperature,

and then diluted with DCM (30 mL), washed with water (30 mL), an aqueous solution of 1 N HCl (30 mL) and saturated aqueous NaHCO<sub>3</sub> (35 mL), dried (MgSO<sub>4</sub>), and concentrated. The resulting residue was purified by column chromatography using 1:2 acetone-cyclohexane as eluent. Yield: 1.043 g (97%);  $R_f$  0.24 (1:2 acetone-cyclohexane) ; IR (ATR)  $v_{max}$  3344, 2904, 1697, 1638, 1521, 1105 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  6.28 (bs, 1 H, NHCO), 4.96 (bs, 1 H, NHBoc), 3.66-3.49 (m, 12 H, OCH<sub>2</sub>), 3.33 (q, 2 H, <sup>3</sup> $J_{H,H} = J_{NH,H} = 6.1$  Hz, CH<sub>2</sub>NHCO), 3.20 (q, 2 H, <sup>3</sup> $J_{H,H} = J_{NH,H} = 6.1$  Hz, CH<sub>2</sub>NHBoc), 2.00 (bs, 3 H, CH), 1.81 (m, 6 H, CCH<sub>2</sub>), 1.75 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>NH), 1.69 (m, 6 H, CH<sub>2</sub>), 1.41 (s, 9 H, CMe<sub>3</sub>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>)  $\delta$  177.8 (CO amide), 155.9 (CO carbamate), 78.8 (CMe<sub>3</sub>), 70.6, 70.5, 70.4, 70.3, 70.1, 69.5 (OCH<sub>2</sub>), 40.4 (CH<sub>2</sub>NHBoc), 39.2 (CH), 38.5 (CCO), 37.9 (CH<sub>2</sub>NHCO), 36.5 (CCH<sub>2</sub>), 29.6 (CH<sub>2</sub>CH<sub>2</sub>NHBoc), 28.9 (CH<sub>2</sub>CH<sub>2</sub>NHCO), 28.4 (CMe<sub>3</sub>), 28.1 (CH<sub>2</sub>); ESIMS: m/z 505.3 [M + Na]<sup>+</sup>; Anal. Calcd for C<sub>26</sub>H<sub>46</sub>N<sub>2</sub>O<sub>6</sub>: C, 64.70; H, 9.61; N, 5.80. C, 60.84; H, 9.70; N, 5.72.

*N*-(13-amino-4,7,10-trioxa)tridecanyl-1-adamantanecarboxamide (14). To a stirred solution of 13 (130 mg, 0.27 mmol) in DCM (5 mL), TFA (1 mL) was dropwise added. The reaction mixture was stirred at room temperature for 1 h, concentrated under reduced pressure, coevaporated several times with DCM and toluene, suspended in 10:1 water-HCl 0.1 N, freeze-dried, and neutralized with basic resin IRA67. Yield: 103 mg (quantitative);  $R_f$  0.40 (30:1:1 CH<sub>3</sub>CN-H<sub>2</sub>O-NH<sub>4</sub>OH); IR (ATR)  $v_{max}$  3350, 2902, 1634, 1525, 1098 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  6.37 (bs, 1 H, NH), 3.55-3.46 (m, 12 H, OCH<sub>2</sub>), 3.25 (q, 2 H,  ${}^{3}J_{H,H} = J_{NH,H} = 6.3$  Hz, *CH*<sub>2</sub>NHCO), 2.84 (bs, 2 H, NH<sub>2</sub>), 2.77 (bt, 2 H,  ${}^{3}J_{H,H} = 6.3$  Hz, *CH*<sub>2</sub>NH<sub>2</sub>), 1.94 (bs, 3 H, CH), 1.75 (bs, 6 H, CCH<sub>2</sub>), 1.69 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>N), 1.62 (m, 6 H, CH<sub>2</sub>);  ${}^{13}$ C NMR (75.5 MHz, CDCl<sub>3</sub>)  $\delta$  177.8 (CO), 70.3, 70.2, 70.1, 69.9, 69.3 (OCH<sub>2</sub>), 40.3 (CH<sub>2</sub>NH<sub>2</sub>), 39.3 (CCO), 39.0 (CH), 37.7 (*C*H<sub>2</sub>NHCO), 36.4 (CCH<sub>2</sub>), 32.0(*C*H<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 28.9 (*C*H<sub>2</sub>CH<sub>2</sub>NHCO), 28.0 (CH<sub>2</sub>); ESIMS: *m*/z 383.3 [M + H]<sup>+</sup>; Anal. Calcd for C<sub>21</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>: C, 65.93; H, 10.01; N, 7.32. Found: C, 65.79; H, 9.73; N, 6.98.

*N*-(13-dansylamino-4,7,10-trioxa)tridecanyl-1-adamantanecarboxamide (7). A solution of 14 (263 mg, 0.687 mmol) in anhydrous DMF (15 mL) under Ar was cooled to 0  $^{\circ}$ C and triethylamine (92 µL, 0.742 mmol) and 5-dimethylaminonaphtalene-1-sulfonyl

chloride (186 mg, 0.742 mmol) were added. The reaction was stirred for 4 h. Subsequently the solvent was removed under reduced pressure and the residue was purified by column chromatography using 1:2 acetone-cyclohexane as eluent. Yield: 224 mg (53%);  $R_f 0.32$ (1:2 acetone-cyclohexane). IR (ATR)  $v_{max}$  3282, 3056, 2906, 1635, 1525, 1425, 1095 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.47 (d,  $J_{3,4}$  = 8.6 Hz, 1 H, H-4<sub>Naph</sub>), 8.28 (d,  $J_{8,9}$  = 8.6 Hz, 1 H, H-9<sub>Naph</sub>), 8.18 (dd, J<sub>2.3</sub> = 7.3 Hz, J<sub>2.4</sub> = 0.8 Hz, 1 H, H-2<sub>Naph</sub>), 7.52-7.43 (m, 2 H, H-3<sub>Naph</sub>, H-8<sub>Naph</sub>), 7.12 (d,  $J_{7.8} = 7.4$  Hz, 1 H, H-7<sub>Naph</sub>), 6.35 (bt, 1 H, NHCO), 5.99 (t, 1 H,  ${}^{3}J_{H,H} =$ 5.9 Hz, NHSO<sub>2</sub>), 3.59-3.46 (m, 12 H, OCH<sub>2</sub>), 3.27 (q, 2 H,  ${}^{3}J_{H,H} = J_{NH,H} = 5.9$  Hz, CH<sub>2</sub>NHCO), 2.97 (q, 2 H, J<sub>NH,H</sub> = 5.9 Hz, CH<sub>2</sub>NHSO<sub>2</sub>), 2.82 (s, 6 H, CH<sub>3</sub>), 1.91 (bs, 3 H, CH), 1.74, 1.73 (s, 6 H, CCH<sub>2</sub>), 1.71-156 (m, 4 H, CH<sub>2</sub>CH<sub>2</sub>NH), 1.60 (m, 6 H, CH<sub>2</sub>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>) δ 177.0 (CO), 151.6 (C-6<sub>Naph</sub>), 134.9 (C-1<sub>Naph</sub>), 129.9-129.2 (C-2<sub>Naph</sub>, C-4<sub>Naph</sub>, C-5<sub>Naph</sub>, C-10<sub>Naph</sub>), 127.9 (C-8<sub>Naph</sub>), 123.0 (C-3<sub>Naph</sub>), 119.0 (C-9<sub>Naph</sub>), 114.9 (C-7<sub>Naph</sub>), 70.4, 70.2 (2 C), 70.0, 69.7, 69.5 (OCH<sub>2</sub>), 45.2 (CH<sub>3</sub>), 41.7 (CH<sub>2</sub>NHSO<sub>2</sub>), 40.2 (CCO), 38.9 (CH), 37.6 (CH<sub>2</sub>NHCO), 36.3 (CCH<sub>2</sub>), 28.8 (CH<sub>2</sub>CH<sub>2</sub>NHCO), 28.5  $(CH_2CH_2NHSO_2)$ , 27.9  $(CH_2)$ ; ESIMS: m/z 638.6  $[M + Na]^+$ ; Anal. Calcd for C<sub>33</sub>H<sub>49</sub>N<sub>3</sub>O<sub>6</sub>S: C, 64.36; H, 8.02; N, 6.82; S, 5.21. Found: C, 64.02; H, 7.90; N, 6.50; S, 4.88.



Figure S1. <sup>1</sup>H and <sup>13</sup>C NMR (500 MHz, 125.7 MHz, CD<sub>3</sub>OD, 323 K) spectra of 6.



Figure S2. <sup>1</sup>H and <sup>13</sup>C NMR (500 MHz, 125.7 MHz, D<sub>2</sub>O, 323 K) spectra of (ManS)<sub>3</sub>- $\beta$ CD.



Figure S3. <sup>1</sup>H and 1D TOCSY spectra (500 MHz, D<sub>2</sub>O, 323 K) of compound (ManS)<sub>3</sub>- $\beta$ CD.



Figure S4. <sup>1</sup>H and <sup>13</sup>C NMR (500 MHz, 125.7 MHz, CD<sub>3</sub>OD) spectra of compound 8.



Figure S5. <sup>1</sup>H and <sup>13</sup>C NMR (300 MHz, 75.5 MHz, CDCl<sub>3</sub>) spectra of compound 16.



Figure S6.  $^{1}$ H and  $^{13}$ C NMR (300 MHz, 75.5 MHz, CDCl<sub>3</sub>) spectra of compound 14.



Figure S7. <sup>1</sup>H and <sup>13</sup>C NMR (300 MHz, 75.5 MHz, CDCl<sub>3</sub>) spectra of compound 7.



Figure S8. ESI-MS spectra of compounds 6 (A),  $(ManS)_3$ - $\beta$ CD (B), 7 (C), and 8 (D).



**Figure S9**. (a) Stacked <sup>1</sup>H RMN spectra (anomeric region) of  $(ManS)_3$ - $\beta$ CD upon increasing concentration of 6S-NOI-NJ and (b) plot of the  $\delta_{H^{-1}(I)}$  variation vs. [6S-NOI-NJ].



**Figure S10**. (a) Stacked <sup>1</sup>H RMN spectra (anomeric region) of  $(ManS)_3-\beta CD$  upon increasing concentration of 6S-NAdB-NJ and (b) plot of the  $\delta_{H-1}$  variation vs. [6S-NAdB-NJ], with indication of the averaged  $K_{as}$ .



**Figure S11**. Lineweaver-Burk plot for  $K_i$  determination (9.9  $\mu$ M) of complex (ManS)<sub>3</sub>- $\beta$ CD:6S-NOI-NJ against bovine liver  $\beta$ -glucosidase (pH 7.3).



**Figure S12**. Lineweaver-Burk Plot for  $K_i$  determination (0.29 μM) of complex (ManS)<sub>3</sub>βCD:6S-NOI-NJ against almonds β-glucosidase (pH 7.3).



**Figure S13**. Lineweaver-Burk plot for  $K_i$  determination (5.8  $\mu$ M) of complex (ManS)<sub>3</sub>- $\beta$ CD:6S-NAdB-NJ against almonds  $\beta$ -glucosidase (pH 7.3).



**Figure S14**. Inhibition of the adhesion of rhMMR to an immobilized polymannoside (yeast mannan) in the presence of increasing concentration of  $(ManS)_3$ - $\beta$ CD ( $\blacklozenge$ ) in comparison with representative members of the high mannose oligosaccharide family (**Man5** and **Man6**, **and A**; structures are also depicted).

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