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Lysosomal targeting of β -cyclodextrin

Andrea Mascherpa,^a Nozomii Ishii,^a Ayelen Tayagui,^a Jiang Liu,^b Matthieu Sollogoub^{b,*} and Antony J. Fairbanks^{a,*}

a) School of Physical and Chemical Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand.

b) Sorbonne University, CNRS, Institut Parisien de Chimie Moléculaire (IPCM), UMR 8232, 4, place Jussieu, F-75005 Paris, France

E-mails: antony.fairbanks@canterbury.ac.nz, matthieu.sollogoub@sorbonne-universite.fr

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Abstract: β -Cyclodextrin (β -CD) and derivatives are approved therapeutics in >30 clinical settings. β -CDs have also shown promise as therapeutics for treatment of some lysosomal storage disorders, such as Niemann-Pick disease type C, and other disease states which involve metabolite accumulation in the lysosome. In these cases, β -CD activity relies on transport to the lysosome, wherein it can bind hydrophobic substrate and effect extraction. The post-translational attachment of N-glycans terminated in mannose-6-phosphate (M6P) residues is the predominant method by which lysosomal enzymes are targeted to the lysosome. In this work we covalently attach a synthetic biantennary bis-M6P-terminated N-glycan to β -CD and study the effect of the added glycans in a mammalian cell line. The formation of a host guest complex with a Cy5 fluorophore allows study of both cellular internalisation and transport to the lysosome by fluorescence microscopy. Results indicate that the rates of both internalisation and lysosomal transport are increased by the attachment of M6P-glycans to β -CD, indicating that M6P-glycan conjugation may improve the therapeutic effectiveness of β -CD for the treatment of disorders involving hydrophobic metabolite accumulation in the lysosome.

The cyclodextrins¹ (CDs) are a family of cyclic oligosaccharides, comprising 6 (α -CD), 7 (β -CD), 8 (γ -CD), or more, α -(1 \rightarrow 4)-linked D-glucopyranose residues. Besides numerous other uses, they have found applications in >30 clinical settings² as drug delivery systems,³ taking advantage of their lipophilic central cavity to bind to hydrophobic drugs,⁴ increasing their solubility, stability, and bioavailability. Chemical modification of CDs, may improve their efficacy. For example, 2-hydroxypropyl- β -CD and sulfobutylether- β -CD⁵ are both clinically used as pharmaceutical excipients for poorly water-soluble drugs.

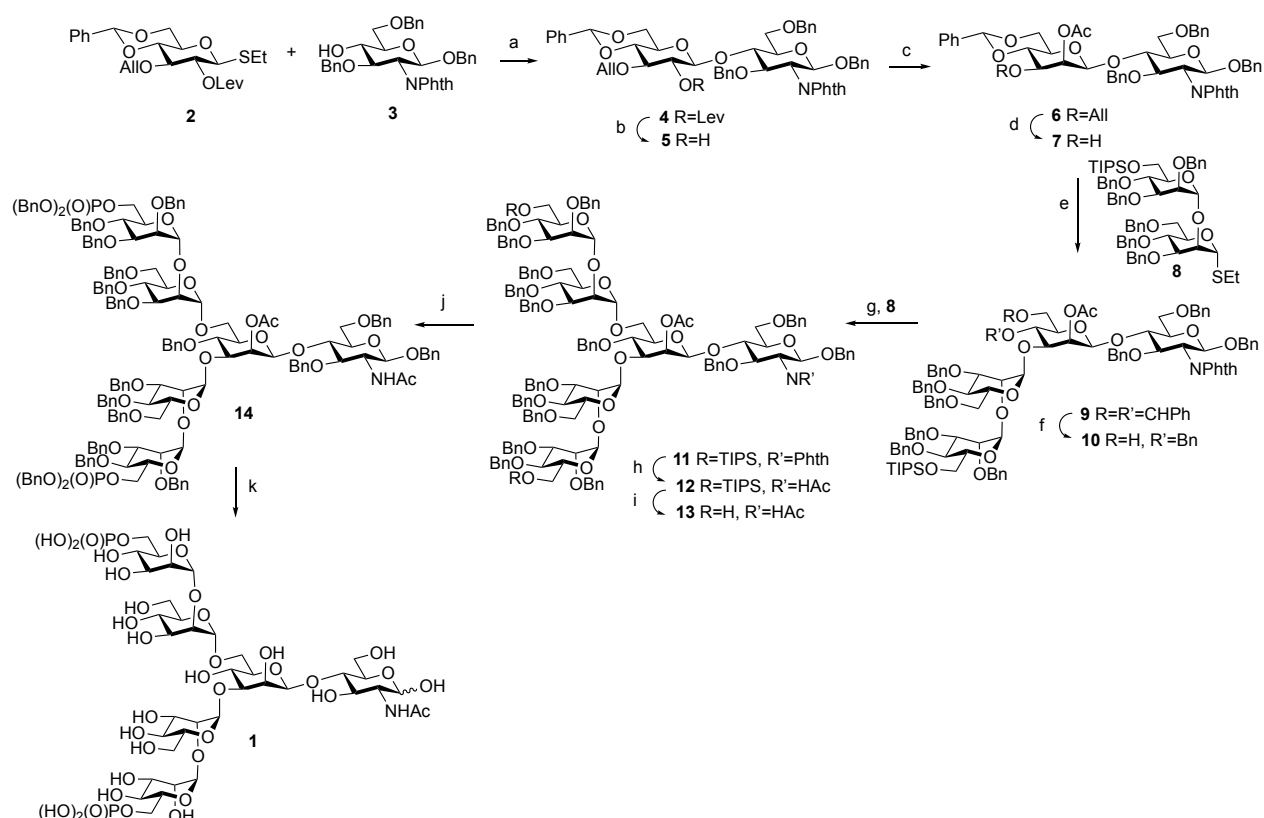
The lysosomal storage disorders (LSDs)⁶ are a series of >70 rare, but incurable and often fatal, genetically inherited diseases, the clinical manifestations of which arise from the build-up of undegraded lysosomal metabolites. In general, each disease is caused by the lack of a functional enzyme in the lysosome to catalyse a required degradation pathway, resulting in the gradual occupation of undegraded metabolites (also termed accumulating substances).

Amongst several treatment strategies⁷ that have been investigated against the LSDs, such as enzyme replacement therapy (ERT),⁸ gene therapy, chaperone therapy and substrate reduction therapy, a novel emerging and complimentary strategy is reduction of the accumulated substance. In the case of Niemann-Pick disease type C,⁹ the major accumulated metabolites are glycosphingolipids and unesterified cholesterol; hydrophobic guests than can be accommodated inside the cavity of β -CD, as demonstrated by the use of β -CDs to modulate the cholesterol content of plasma membranes.¹⁰ Testing of 2-hydroxypropyl- β -CD in both murine¹¹ and feline disease models for Niemann-Pick type C revealed beneficial effects,¹² so that a compassionate FDA-approved human trial of 2-hydroxypropyl- β -CD therapy was undertaken.¹³ Although objective improvement in clinical outcomes was observed in the majority of patients, the doses required for beneficial effect in the murine model caused significant adverse effects in humans.¹⁴

β -CDs and related compounds also have potential for therapeutic intervention in other disease states which result in the build-up of hydrophobic metabolites. For example, the accumulation of lipofuscin bisretinoids (LBs) in the lysosomal compartment of cells in the retinal pigment epithelium is thought to be the cause of retinal degradation in Stargardt's disease,¹⁵ and is also a putative etiological agent for age-related macular degeneration. Administration of β -CDs resulted in a significant reduction in the build-up of LB's in the retinal pigment epithelium in an animal model,¹⁶ indicating the potential for β -CD-based therapy development.

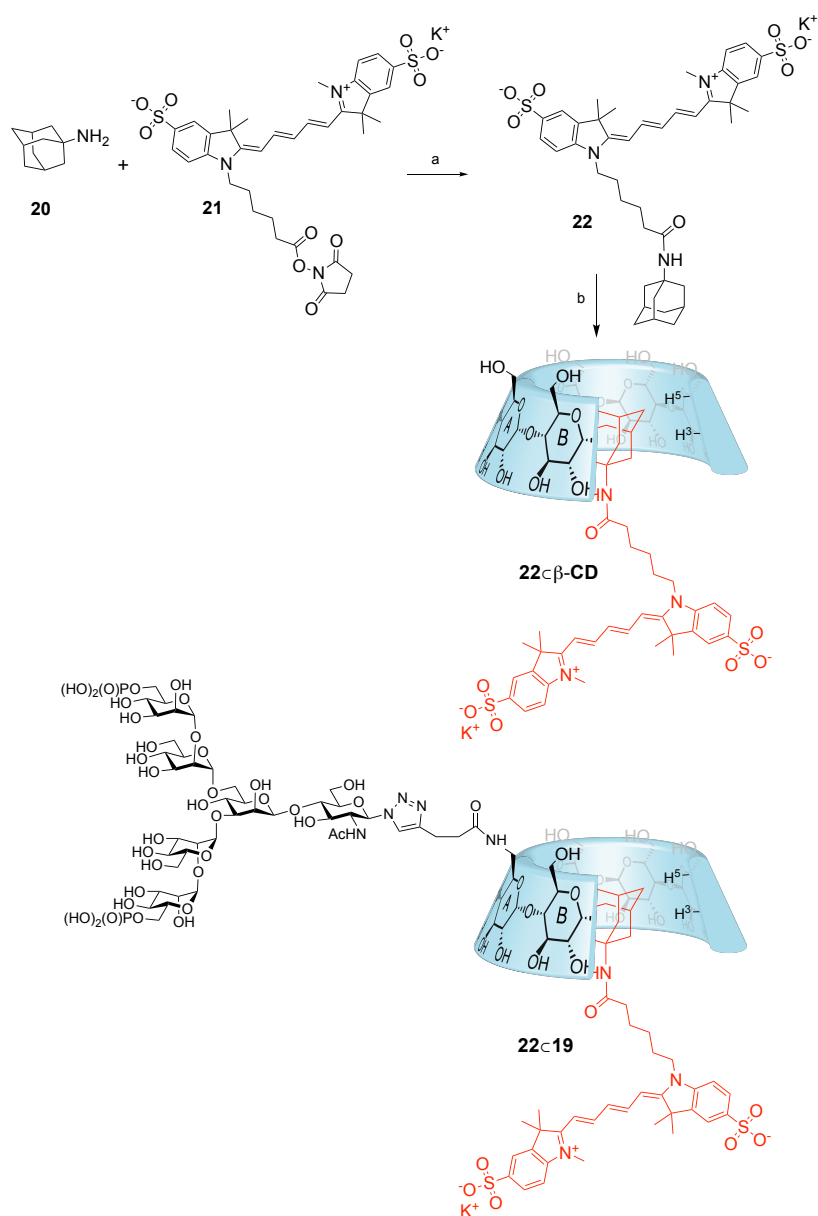
Improvement of the efficacy of administered functionalised β -CD, so that beneficial effects may be achieved at much lower dose levels so obviating adverse effects, may plausibly be achieved by specifically targeting the administered β -CD to the lysosome. Opportunely a natural 'lysosomal targeting system' exists, as, following their assembly in the endoplasmic reticulum (ER), the majority of lysosomal enzymes are trafficked to the lysosome by the phosphorylation of some the terminal mannose residues of their attached N-glycans. Installation of these mannose-6-phosphate (M6P) residues at the non-reducing termini of high mannose oligosaccharides during glycan processing in the Golgi then effects trafficking of proteins carrying such M6P-glycans to the lysosome *via* interactions with the two mannose-6-phosphate receptors (M6PRs);¹⁷ namely the ~46-kDa cation dependent mannose-6-phosphate receptor (CD-M6PR), and the ~300-kDa cation independent mannose-6-phosphate receptor (CI-M6PR).¹⁸ The M6PR system can therefore be regarded as an endogenous targeting mechanism, which may be hijacked by externally administered

molecules in order to deliver them to the lysosomes. In particular the CI-M6PR migrates to the cell surface and, amongst its other functions, mediates endocytosis of externally circulating species bearing M6P-glycans¹⁹ before then delivering them to the lysosome *via* intermediate endosomes. Thus, the attachment, either enzymatic²⁰ or chemical,²¹ of M6P-terminated glycans to recombinant human lysosomal enzymes has been an area of significant interest in attempts to increase the efficacy of ERT's for the LSDs. Furthermore, CDs have been conjugated to carbohydrates, often in a multivalent fashion, to interact with proteins, often lectins.²² Logical extension of these ideas engenders the hypothesis that chemical modification of β -CD by the attachment of M6P glycans may significantly increase the efficacy of lysosomal targeting, by CI-M6P mediated interactions. In this study we therefore undertook the chemical synthesis of a modified β -CD to which a biantennary glycan containing two M6P-residues was then covalently attached. *In vitro* fluorescence microscopy studies using a mammalian cell line, as a precursor to more detailed clinical investigations, then demonstrated the effect of M6P-glycan attachment, both on the rate of endocytosis and lysosomal transport. Based on a report in which the binding affinity of a series of phosphorylated oligomannosides to the CI-M6PR was probed,²³ a biantennary hexasaccharide structure **1**, in which the two M6P-residues were $\alpha(1\rightarrow2)$ -linked to the adjacent mannose units, was identified as a synthetic target. The synthetic strategy used to access **1** was based on previous approaches with some minor modifications (Scheme 1). Thus, glycosylation of the known alcohol **3**²⁴ with the known thioglycoside donor **2**²⁵ gave the desired $\beta(1\rightarrow4)$ -linked disaccharide **4**. Selective removal of the Lev protection gave alcohol **5**, which was triflated and reacted with tetrabutylammonium acetate with sonication,²⁶ to give the *manno*-configured disaccharide **6**. Removal of the allyl protecting group gave the alcohol **7**, which was used as the acceptor for glycosylation with the known selectively protected $\alpha(1\rightarrow2)$ -linked disaccharide donor **8**²⁷ to give tetrasaccharide **9**. Regioselective reductive opening of the 4,6-benzylidene on **9** gave alcohol **10**, allowing access to the 6-hydroxyl group of the central branching mannose residue. A second glycosylation using $\alpha(1\rightarrow2)$ -linked donor **8** then gave the hexasaccharide **11**. Following removal of the phthalimide protecting group and immediate acetylation to give acetamide **12**, the two TIPS protecting groups were removed by treatment with BF_3 etherate, to give hexasaccharide diol **13**. Phosphitylation of **13** with excess dibenzyl diisopropylphosphoramidate and immediate oxidation by the addition of hydrogen peroxide then gave the *bis* phosphate ester **14**. Finally, global deprotection by Birch reduction gave the bis-phosphorylated hexasaccharide **1**.



Scheme 1. Synthesis of M6P-containing hexasaccharide **1** (a) TTBP, MeOTf, MS4Å, CH_2Cl_2 , 0 °C to r.t., 97%; (b) $\text{H}_2\text{NNH}_2 \cdot \text{AcOH}$, MeOH, CH_2Cl_2 , 98%; (c) (i) Tf_2O , pyridine, CH_2Cl_2 ; (ii) BuN_4AOAc , toluene, sonication, 84% over 2 steps; (d) $[\text{Ir}(\text{cod})(\text{PPh}_2\text{Me})_2]\text{PF}_6$, NIS, H_2O , THF, 91%; (e) **8**, NIS, TfOH, MS4Å, Et_2O , -20 °C, 74%; (f) Et_3SiH , PhBCl_2 , MS4Å, CH_2Cl_2 , -78 °C, 77%; (g) **8**, NIS, TfOH, MS4Å, Et_2O , -20 °C, 62%; (h) (i) ethylenediamine, MeOH, 70 °C, (ii) Ac_2O , pyridine, 68% over 2 steps; (i) $\text{BF}_3 \cdot \text{OEt}_2$, CH_2Cl_2 , 0 °C, 63%; (j) $(\text{BnO})_2\text{PNiPr}_2$, 1*H*-tetrazole, CH_2Cl_2 , then $\text{H}_2\text{O}_2(\text{aq})$, 92%; (k) Na, $\text{NH}_3(\text{l})$, THF, -78 °C, 61%.

Following production of the desired bis-phosphorylated oligosaccharide, attention turned to its conjugation to β -CD by use of our reported method for the *in situ* formation of glucosyl azides of unprotected oligosaccharides using 2-azido-1,3-dimethylimidazolium hexafluorophosphate (ADMP), and their immediate Cu-catalysed Huisgen cycloaddition to species



Scheme 3 Synthesis of fluorescent probes for *in vitro* studies of β -CD localisation in mammalian cells. (a) **20**, **21**, DMF, $\text{NaHCO}_3(\text{aq})$, rt, 16 h; (b) **22**, β -CD or **19**, H_2O , rt, 30 min.

Next, HEK293 cells were treated with both unmodified and modified β -CD complexes **22 \subset β -CD** and **22 \subset **19****, and compound **22** as control. Cultured cells were incubated with 5 μM solutions of these compounds for either 2 h or 4 h. Following incubation cells were washed with ice-cold phosphate buffered saline to remove non-endocytosed fluorescent materials, treated with LysoTrackerTM Yellow in order to visualise lysosomes, and Hoechst 33342 to stain the nuclei. Investigation by fluorescence microscopy produced a series of cellular images at different wavelengths, corresponding to Cy5 (red), LysoTracker (yellow), and the Hoechst nuclear stain (blue); merged images are shown in Fig 1A. Measurement of the corrected total cell fluorescence (CTCF) corresponding to Cy5 (red) for each experiment provided the data shown in Fig 1B.

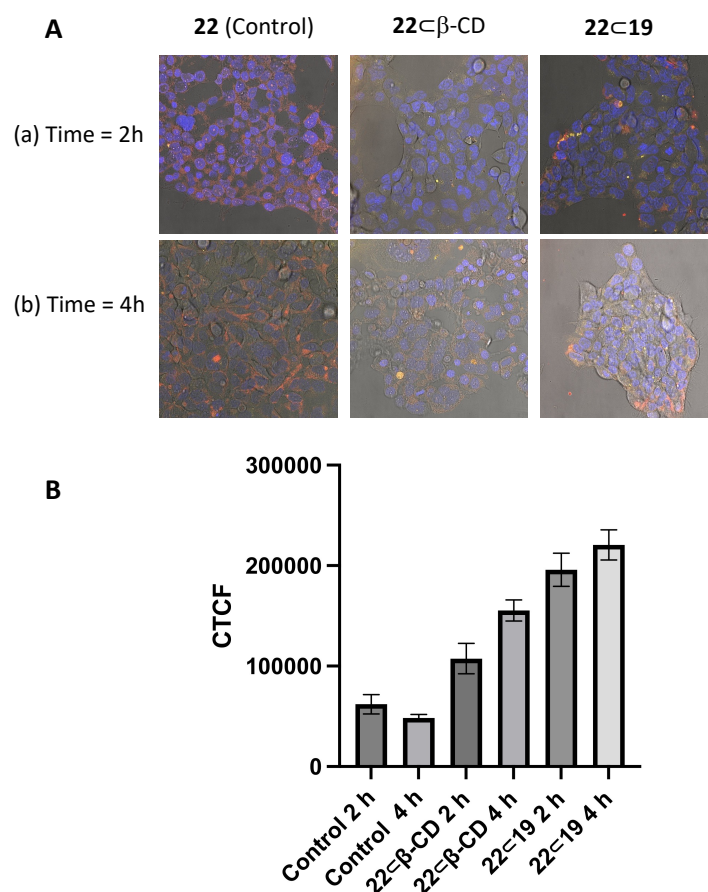


Figure 1. Fluorescence microscopy studies. **A**) HEK293 cells were incubated with 5 μ M with **22** (as control), **22 \subset β -CD**, or **22 \subset 19** for either (a) 2h or (b) 4 h. After washing, cells were treated with LysoTrackerTM Yellow and then Hoechst 33342. **B**) Graph shows corrected total cell fluorescence (CTCF) of Cy5 in cells; analysis was performed using ImageJ software. Mean \pm SD, n = 3.

After 2 h, only limited amounts of the unmodified **22 \subset β -CD** complex had been endocytosed. In contrast, after 2 h incubation fluorescence arising from the **22 \subset 19** complex was approximately two-fold higher. In both cases the red fluorescence was higher than the control (Fig 1B), indicating that complex formation with either modified or unmodified β -CD also increased the rate of endocytosis of **22**. After 4 h incubation, whilst more of the unmodified **22 \subset β -CD** complex had been endocytosed, the amount of red fluorescence observed from the **22 \subset 19** complex was still considerably higher. Experiments were repeated three times and similar results obtained.

Next the co-localisation of Cy5 and LysoTracker fluorescence was assessed (Fig. 2) by measurement of both Cy5 and LysoTracker fluorescence at varying positions along lines (marked as red in Fig. 2) across the cells. Firstly, no correlation between red and yellow fluorescence was observed for control **22** after either 2 h or 4 h.

After 2 h, unmodified **22 \subset β -CD** produced limited levels of red fluorescence, the location of which only weakly correlated with the lysosomes (Fig. 2A). In contrast, complex **22 \subset 19** was both more effectively endocytosed, and more effectively transported to the lysosome, as shown by excellent correlation between red and yellow fluorescence (Graph, Fig2A (iv)). After 4 h more of the unmodified **22 \subset β -CD** was endocytosed and some lysosomal transport was observed. However, endocytosis and lysosomal transport of complex **22 \subset 19** were still significantly higher. A second series of experiments was performed in HepG2 cells, and similar results were obtained (see Supporting Information; Figs S2 and S3).

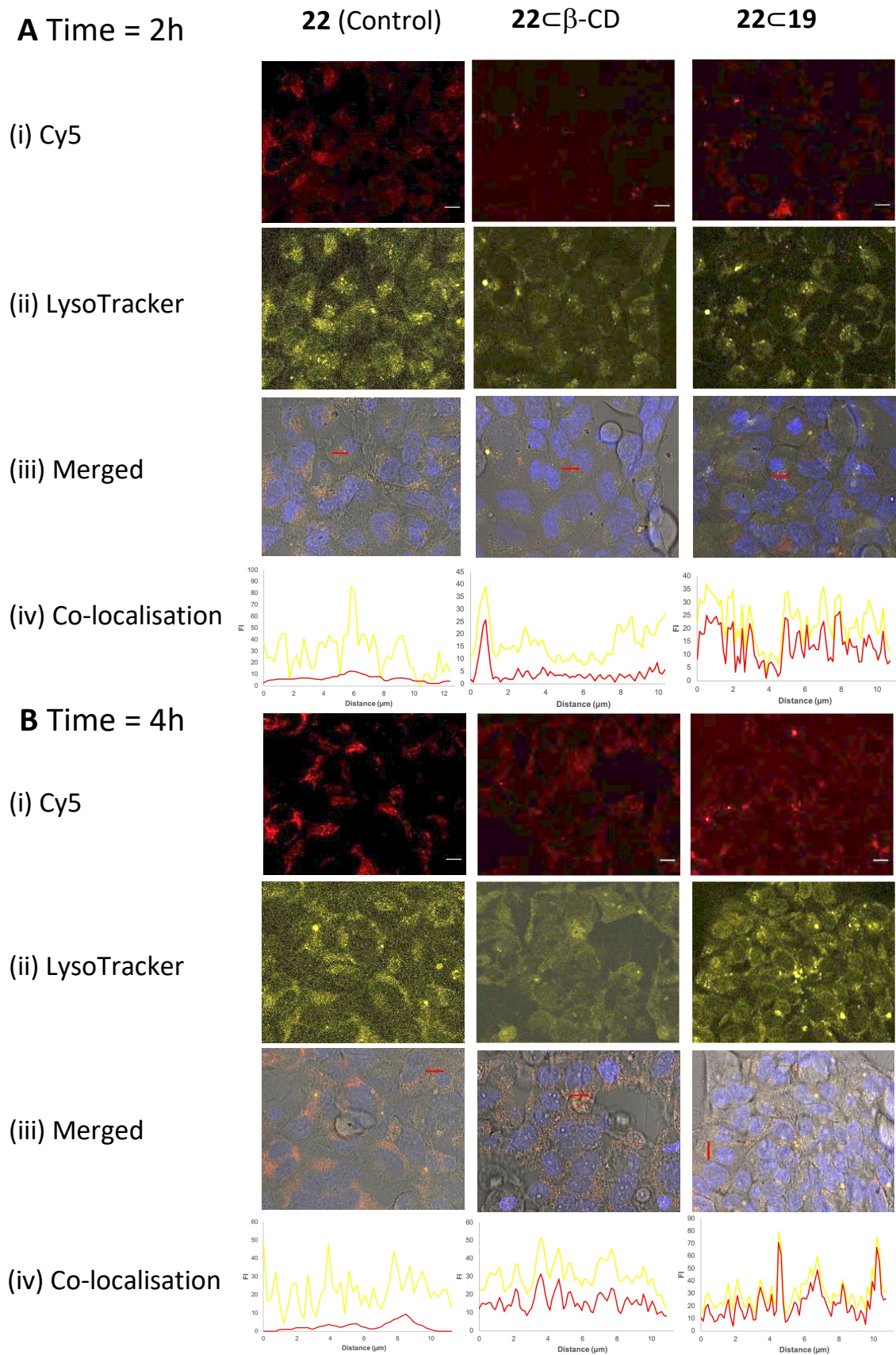


Figure 2. Cell images obtained after: **A**) 2 h incubation; **B**) 4 h incubation. (i) Cy5 (red), white scale bar = 10 μ m; (ii) LysoTrackerTM (yellow); (iii) merged with Hoechst 33342 (blue); (iv) graphs of fluorescence intensity (FI) of LysoTracker (yellow) and Cy5 (red) at positions along the red line across cells. Analysis was performed using ImageJ software.

It is known that β -CD undergoes both endocytosis and lysosomal transport, as demonstrated by previous studies and attempted applications for the treatment of lysosomal storage disorders and Stargardt's disease.¹¹⁻¹⁶ However, this study indicates that the attachment of a bis-M6P-containing glycan to β -CD not only increased the rate of endocytosis, but also of lysosomal transport. Presumably this arose as a result of interaction of the M6P residues attached to the β -CD with the CI-M6PR, though definitive evidence of this mechanism has not yet been obtained.

This work reports the first synthesis of a β -CD derivative decorated with a glycan that contains mannose-6-phosphate residues. The use of a functionalised adamantane derivative, comprising a Cy5 fluorescent label, allowed comparative *in vitro* studies of the localisation of both modified and unmodified β -CDs by way of the formation of fluorescent host-guest complexes. Fluorescence microscopy using mammalian cell lines demonstrated that the β -CD modified with the M6P-containing glycan was both more rapidly internalised into the cell and transported to the lysosome. These results indicate that M6P-glycan attachment augments the efficiency of trafficking of β -CD to the lysosome, which in turn may improve the efficacy of β -CD-based therapies for lysosomal storage disorders, such as Niemann-Pick type C, and other disease states which may be ameliorated by the reduction of an accumulated lipophilic metabolite in the lysosome. A logical next step is an *in vivo* study of the biological effects of applications of M6P-modified β -CD in appropriate disease models. Furthermore, the effectiveness of the host-guest complexes in these *in vitro* localization studies implies that different ligands, for example comprising other oligosaccharides, may be usefully attached to β -CD in order to assess the effects of such ligands on the rate of endocytosis and the destination of intracellular transport.

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Conflict of Interest

The authors declare no conflict of interest

- [1] A. Villiers *Compt. Rend. Acad. Sci* **1891**, *112*, 536–538.
- [2] T. Loftsson, M. E. Brewster, *J Pharm. Pharmacol.* **2010**, *62*, 1607–1621.
- [3] A. Gu, N. J. Wheate, *J. Inclu. Phenom. Macrocycl. Chem.* **2021**, *100*, 55–69.
- [4] T. Loftsson, P. Jarho, M. Måsson, T. Järvinen, *Expert Opin. Drug Deliv.* **2005**, *2*, 335–351.
- [5] M. E. Davis, M. E. Brewster, *Nat. Rev. Drug Discov.* **2004**, *3*, 1023–1035.
- [6] F. M. Platt, *Nat. Rev. Drug Discov.* **2017**, *17*, 133–150.
- [7] M. Beck, *Dev. Med. Child Neurol.* **2017**, *60*, 13–18.
- [8] (a) M. Solomon, S. Muro, *Adv. Drug Deliver. Rev.* **2017**, *118*, 109–134; (b) A. Jurecka, A. Tyłki-Szymańska, *Expert Opin. Orphan Drugs* **2015**, *3*, 293–305.
- [9] M. T. Vanier, *Orphanet J. Rare Dis.* **2010**, *5*, 16.
- [10] R. Zidovetzki, I. Levitan, *Biochim. Biophys. Acta* **2007**, *1768*, 1311–1324.
- [11] C. D. Davidson, N. F. Ali, M. C. Micsenyi, G. Stephney, S. Renault, K. Dobrenis, D. S. Ory, M. T. Vanier, S. U. Walkley, *PLoS ONE* **2009**, *4*, e6951.
- [12] A. I. Rosenbaum, G. Zhang, J. D. Warren, F. R. Maxfield, *Proc. Natl. Acad. Sci.* **2010**, *107*, 5477–5482.
- [13] E. A. Ottinger, M. L. Kao, N. Carrillo-Carrasco, N. Yanjanin, R. K. Shankar, M. Janssen, M. Brewster, I. Scott, X. Xu, J. Craddock, et al., *Curr. Top. Med. Chem.* **2014**, *14*, 330–339.
- [14] J. E. Megías-Vericat, A. García-Robles, M. J. Company-Albir, M. J. Fernández-Megía, F. C. Pérez-Miralles, E. López-Briz, B. Casanova, J. L. Poveda, *Neurol. Sci.* **2017**, *38*, 727–743.
- [15] P. Tanna, R. W. Strauss, K. Fujinami, M. Michaelides, *Br. J. Ophthalmol.* **2017**, *101*, 25–30.
- [16] M. M. Nociari, G. L. Lehmann, A. E. Perez Bay, R. A. Radu, Z. Jiang, S. Goicochea, R. Schreiner, J. D. Warren, J. Shan, S. Adam de Beaumais, et al., *Proc. Natl. Acad. Sci.* **2014**, *111*, E1402–8.
- [17] P. Ghosh, N. M. Dahms, S. Kornfeld, *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 202–212.
- [18] R. N. Bohnsack, X. Song, L. J. Olson, M. Kudo, R. R. Gotschall, W. M. Canfield, R. D. Cummings, D. F. Smith, N. M. Dahms, *J. Biol. Chem.* **2009**, *284*, 35215–35226.
- [19] N. M. Dahms, L. J. Olson, J.-J. P. Kim, *Glycobiology* **2008**, *18*, 664–678.
- [20] a) P. Priyanka, T. B. Parsons, A. Miller, F. M. Platt, A. J. Fairbanks, *Angew. Chem. Int. Ed.* **2016**, *55*, 5058–5061; b) T. Yamaguchi, M. N. Amin, C. Toonstra, L.-X. Wang, *J. Am. Chem. Soc.* **2016**, *138*, 12472–12485.
- [21] For some recent leading references see: a) J. Y. Hyun, S. Kim, H. S. Lee, I. Shin, *Cell Chem. Biol.* **2018**, *25*, 1255–1267.e8; b) I. Basile, A. Da Silva, K. El Cheikh, A. Godefroy, M. Daurat, A. Harmois, M. Perez, C. Caillaud, H.-V. Charbonné, B. Pau, et al., *J. Control Release* **2018**, *269*, 15–23; c) K. El Cheikh, I. Basile, A. Da Silva, C. Bernon, P. Cérutti, F. Salgues, M. Perez, M. Maynadier, M. Gary-Bobo, C. Caillaud, et al., *Angew. Chem. Int. Ed.* **2016**, *55*, 14774–14777.
- [22] Á. Martínez, C. Ortiz Mellet, J. M. García Fernández, *Chem. Soc. Rev.* **2013**, *42*, 4746–4773; G. Rivero-Barbarroja, J. M. Benito, C. Ortiz Mellet, J. M. García Fernández, *Nanomaterials* **2020**, *10*, 2517.
- [23] J. J. Distler, J. F. Guo, G. W. Jourdan, O. P. Srivastava, O. Hindsgaul, *J. Biol. Chem.* **1991**, *266*, 21687–21692.
- [24] T. Ogawa and S. Nakabayashi, *Carbohydr. Res.* **1981**, *97*, 81–86.
- [25] T. B. Parsons, J. W. B. Moir and A. J. Fairbanks, *Org. Biomol. Chem.* **2009**, *7*, 3128–3140.

- [26] A. Furstner, I. Konetzki, *Tetrahedron Lett.* **1998**, *39*, 5721–5724.
- [27] Y. Liu, Y. M. Chan, J. Wu, C. Chen, A. Benesi, J. Hu, Y. Wang and G. Chen, *ChemBioChem* **2011**, *12*, 685-690.
- [28] D. Lim, M. A. Brimble, R. Kowalczyk, A. J. A. Watson, A. J. Fairbanks, *Angew. Chem. Int. Ed.* **2014**, *53*, 11907–11911.
- [29] S. Guieu, M. Sollogoub, *Angew. Chem. Int. Ed.* **2008**, *47*, 7060-7063.
- [30] (a) T. Lecourt, A. J. Pearce, A. Herault, M. Sollogoub, P. Sinaÿ, *Chem. Eur. J.* 2004, *10*, 2960-2971; (b) S. Guieu, M. Sollogoub, *J. Org. Chem.* **2008**, *73*, 2819-2828; E. Zaborova, Y. Blériot, M. Sollogoub, *Tetrahedron Lett.* **2010**, *51*, 1254-1256.
- [31] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, *Tetrahedron Lett.* **1989**, *30*, 1927-1930.
- [32] A. P. Plazzo, C. T. Höfer, L. Jicsinszky, É. Fenyvesi, L. Szente, J. Schiller, A. Herrmann, P. Muller, *Chem. Phys. Lipids* **2012**, *165*, 505–511.
- [33] Z. Zhou, Z. Han, Z.-R. Lu, *Biomaterials* **2016**, *85*, 168–179.