

This is a repository copy of *Selective Fluorogenic β -Glucocerebrosidase Substrates for Convenient Analysis of Enzyme Activity in Cell and Tissue Homogenates*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/159502/>

Version: Accepted Version

Article:

Deen, Matthew C., Proceviat, Cameron, Shan, Xiaoyang et al. (4 more authors) (2020) Selective Fluorogenic β -Glucocerebrosidase Substrates for Convenient Analysis of Enzyme Activity in Cell and Tissue Homogenates. ACS Chemical Biology. ISSN 1554-8929

<https://doi.org/10.1021/acscchembio.9b01044>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

Selective Fluorogenic β -Glucocerebrosidase Substrates for Convenient Analysis of Enzyme Activity in Cell and Tissue Homogenates

Matthew C. Deen, Cameron Proceviat, Xiaoyang Shan, Liang Wu, David L. Shen, Gideon J. Davies, and David J. Vocadlo*



Cite This: <https://dx.doi.org/10.1021/acscchembio.9b01044>



Read Online

ACCESS |



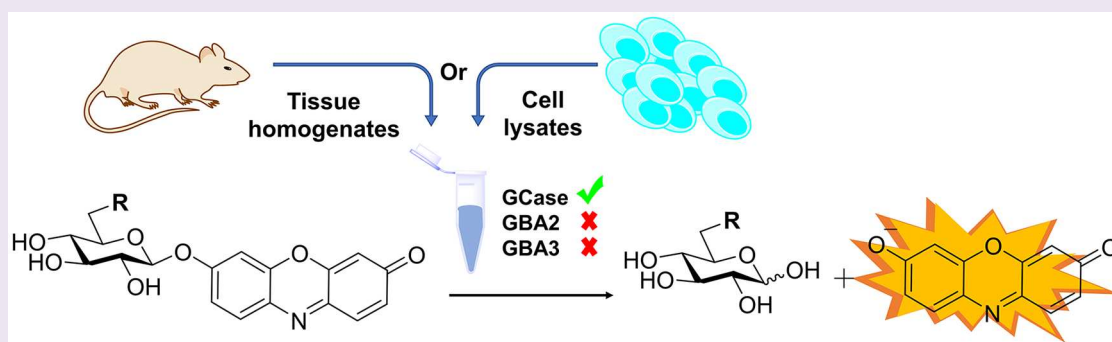
Metrics & More



Article Recommendations



Supporting Information



ABSTRACT: Within mammals, there are often several functionally related glycoside hydrolases, which makes monitoring their activities problematic. This problem is particularly acute for the enzyme β -glucocerebrosidase (GCCase), the malfunction of which is a key driver of Gaucher's disease (GD) and a major risk factor for Parkinson's disease (PD). Humans harbor two other functionally related β -glucosidases known as GBA2 and GBA3, and the currently used fluorogenic substrates are not selective, which has driven the use of complicated subtractive assays involving the use of detergents and inhibitors. Here we describe the preparation of fluorogenic substrates based on the widely used nonselective substrate resorufin β -D-glucopyranoside. Using recombinant enzymes, we show that these substrates are highly selective for GCCase. We also demonstrate their value through the analysis of GCCase activity in brain tissue homogenates from transgenic mice expressing mutant human GCCase and patient fibroblasts expressing mutant GCCase. This approach simplifies the analysis of cell and tissue homogenates and should facilitate the analysis of clinical and laboratory tissues and samples.

INTRODUCTION

β -Glucocerebrosidase (GCCase) is a member of lysosomal glycoside hydrolase family 30 (GH30)¹ encoded by the gene *GBA* and is responsible for the hydrolysis of the glycosidic linkage of the glycolipid glucosylceramide. Homozygous loss of function mutations in the *GBA* gene cause the lysosomal storage disease known as Gaucher's disease (GD),² which shows high variability in presentation and sometimes manifests neuronopathic symptoms.³ Heterozygous loss of function mutations in *GBA* are the greatest genetic risk factor for the development of Parkinson's disease (PD).⁴ Reduced GCCase activity correlates with increased aggregation of the protein α -synuclein, which forms the toxic fibrils that are a pathological hallmark of PD.⁵ Encouraging preclinical data from transgenic PD model mice have demonstrated that increasing GCCase activity hinders the progression of PD.^{6–8} Furthermore, GCCase modulators that enhance activity may reduce the extent of α -synuclein aggregation in patient cell lines.^{9,10} The robust data in the literature demonstrating a direct relationship between

GCCase and both GD and PD, coupled to its potential as a therapeutic target, has raised high interest in developing therapies that work to increase GCCase activity in brain.

One technical challenge within the field is the lack of simple and robust methods to assay GCCase activity in tissue samples. Measuring GCCase activity *in vitro* generally relies on the use of simple fluorogenic substrates such as resorufin β -D-glucopyranoside (Res-Glc, Figure 1)¹¹ or 4-methylumbelliferyl β -D-glucopyranoside (4-MU-Glc).^{5,6,8,9} The presence of functionally related β -glucosidases which also cleave these substrates including GBA2¹² (GH116) and GBA3¹³ (GH1), however,

Received: December 28, 2019

Accepted: February 28, 2020

Published: February 28, 2020



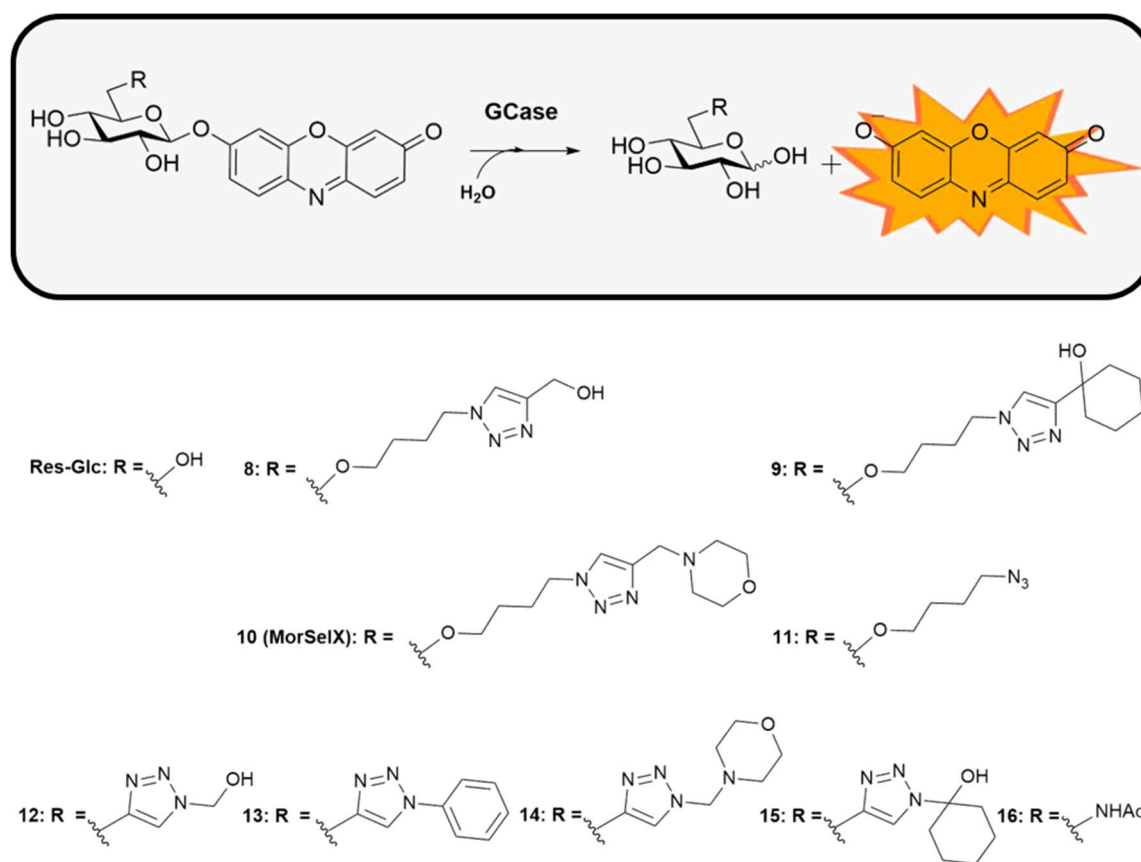


Figure 1. Candidate GCCase-selective substrates. GCCase-catalyzed hydrolysis of Res-Glc fluorogenic substrates to liberate resorufin (boxed) and the series of candidate GCCase-selective substrates designed for synthesis and testing.

complicates such assays. In efforts to circumvent this problem, subtractive assays using inhibitors such as conduritol B epoxide (CBE),^{5,8–10,14,15} which preferentially inactivates GCCase over GBA2 and GBA3, or miglustat, a GBA2-selective inhibitor,¹⁶ are commonly used. The more extensive manipulations required in such assays coupled to the use of CBE, which shows significant activity toward GBA2 at commonly used concentrations (>1 mM),¹⁷ compromise the accuracy of these measures.

The common use of such subtractive assays stems from a lack of selective substrates for GCCase over GBA2 and GBA3, and whereas GCCase is a prominent example, this shortage of enzyme-selective substrates is, with few exceptions,^{18,19} a problem that is common to most mammalian glycosidase hydrolases. Here we aimed to work toward addressing this problem, focusing on generating a GCCase-selective substrate and illustrating its utility in assaying enzymatic activity within brain tissue homogenates from transgenic model mice expressing a clinically relevant mutant form of human GCCase.

RESULTS AND DISCUSSION

In considering designs for selective substrates for GCCase, we were inspired by work from the Overkleeft group in which cyclophellitol, a pan-selective covalent β -glucosidase inactivator, was made selective for GCCase by appending a fluorophore to the pseudo C-6 position.²⁰ The tolerance of GCCase for modifications at the C-6 position was found to be a general approach to confer selectivity, as shown in subsequent work directed toward fluorescence-quenched substrates for this enzyme.²¹ Whereas it is useful for imaging GCCase activity in

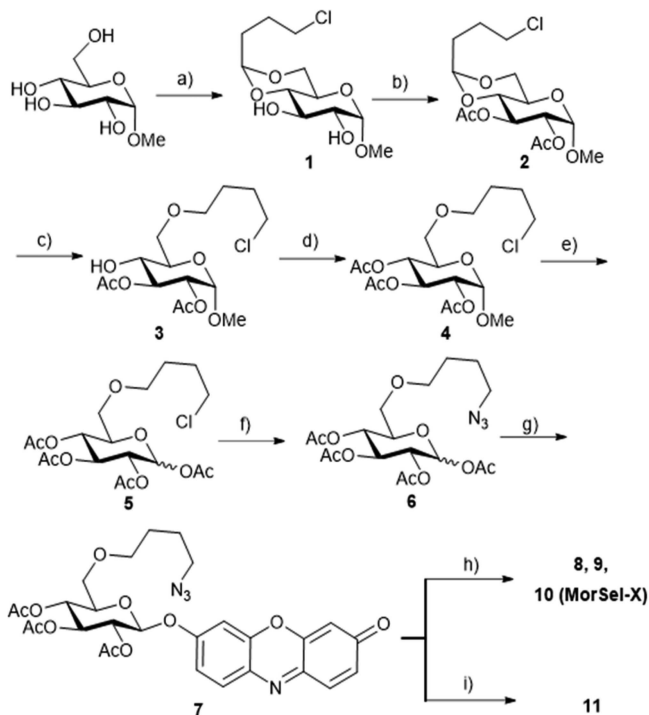
cells, this fluorescence-quenched substrate is only slowly processed by GCCase and is poorly suited to analyze GCCase activity in biological fluids or tissue homogenates. Given these considerations, we selected resorufin β -D-glucopyranoside as the starting point for a selective substrate because it offers several advantages over the more widely used 4-MU-Glc. In particular, the low-pH optimum of GCCase leaves resorufin ($pK_a = 5.8$) suitable for more precise continuous assays, whereas 4-methylumbelliferone (4-MU, $pK_a = 7.8$) requires the use of a stopped assay in which base is used to terminate the enzymatic reaction. Furthermore, resorufin was also attractive because the red-shifted excitation and emission (Ex: 572 nm, Em: 583 nm) helps to improve the sensitivity in cell lysates and tissue homogenates by reducing the background signal due to autofluorescence. We therefore designed and synthesized (Supporting Information, Scheme S1) a small panel of C-6 triazole-modified analogues of resorufin β -D-glucopyranoside (12–15), each having different hydrophobicities and steric bulk (Figure 1).

We next assayed these candidate substrates with recombinant GCCase and counterassayed them with GBA2 and GBA3. To our surprise, no enzymatic turnover was observed for any of the substrates against any of these three enzymes. We reasoned that C-6 modification in combination with the bulky aryl group of resorufin was simply too sterically demanding for the active site of GCCase. We therefore decided to test whether changing our C-6 conjugation strategy from a triazole linker to a simple amide linker might reduce the steric congestion and yield a viable GCCase substrate. Unfortunately, after the preparation of the C-6 acetamide (16; Scheme S1), we

found that it, too, was not turned over by GCase. We reasoned that perhaps further reducing the steric bulk of the C-6 linker by using a less demanding linker that also has more flexibility could solve this problem.

We therefore prepared O-6 alkylated derivatives by establishing a route that minimized the number of protecting group manipulations and permitted the installation of a 4-azidobutane group at this position using reductive alkylation chemistry (Scheme 1). Using methyl α -D-glucopyranoside as a

Scheme 1. Synthesis of Candidate O-6 Alkylated Res-Glc GCase-Selective Substrates^a



^aReagents and conditions: (a) chlorobutylaldehyde dimethyl acetal, CSA (cat.), 4 Å sieves, MeCN 70 °C, 70%; (b) Py., Ac₂O, 0 °C, quant.; (c) TMS, AlCl₃, DCM, -78 to 0 °C, 66%; (d) Py., Ac₂O, 0 °C, 93%; (e) H₂SO₄ (cat.), AcOH/Ac₂O (1:1), 85%; (f) NaN₃, DMF, 70 °C, 86%; (g) i, TMS-Br, ZnBr₂, DCM 0 °C, ii, resorufin, TBABr, NaOH, DCM/H₂O (1:1), 24% over two steps; (h) i, alkyne, sodium ascorbate, Cu(II)SO₄, DCM/H₂O (1:1), ii, K₂CO₃ (cat.), MeOH, 24–57% over two steps; and (i) K₂CO₃ (cat.), MeOH, 82%.

starting material, selective protection using 4-chlorobutanol dimethyl acetal afforded methyl 4,6-(4-chlorobutylidene)- α -D-glucopyranoside (**1**). The reductive ring opening of (**1**) repeatedly failed and required acetylation of the free hydroxyl groups to generate di-O-acetyl (**2**) prior to ring opening using AlCl₃ and tetramethyl disiloxane (TMS).²² The desired 6-O-chlorobutane (**3**) was the only regioisomer we observed. Acetylation of the free hydroxyl to give tri-O-acetyl (**4**), followed by acid-catalyzed hydrolysis and O-acetylation yielded tetra-O-acetyl (**5**). Halide displacement provided azide (**6**). The generation of the glycosyl bromide followed by the subsequent phase transfer glycosylation yielded our common intermediate resorufin 2,3,4-tri-O-acetyl-6-(4-azidobutane)-6-deoxy- β -D-glucopyranoside (**7**). Using copper-catalyzed azide alkyne cycloaddition (CuAAC) chemistry, we efficiently obtained a series of per-O-acetylated O-6 modified glucosides, which we deprotected using MeOH/K₂CO₃ to furnish the

target substrates (**8–10**). Likewise, compound **11** was obtained by de-O-acetylation of compound **7** using MeOH/K₂CO₃.

We were pleased to observe that GCase processed these compounds and established Michaelis–Menten parameters for each substrate (Table 1 and Figure S1). The large K_m values of

Table 1. Michaelis–Menten Parameters for Turnover of Res-Glc and C-6-Modified Res-Glc Analogues by β -Glucocerebrosidase

substrate	K_m (μ M) ^a	k_{cat} (s ⁻¹) ^a	k_{cat}/K_m (s ⁻¹ M ⁻¹)
Res-Glc	29 ± 3	1.5 ± 0.1	31 120 ± 50
8	n.d.	n.d.	3600 ± 100
9	200 ± 10	2.7 ± 0.1	16 600 ± 400
10 , MorSel-X	n.d.	n.d.	6900 ± 200
11	260 ± 30	14 ± 1	58 000 ± 3000

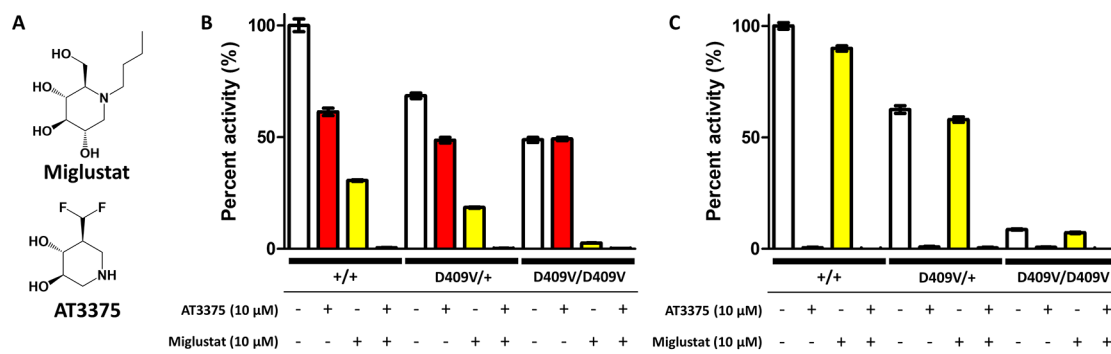
^an.d. = not determined

substrates **8** and **10** made it impractical to achieve the saturation of GCase, precluding the determination of complete Michaelis–Menten parameters. We were, however, able to determine k_{cat}/K_m values (Table 1) for all substrates, enabling their direct comparison. The second-order rate constants for these substrates were between 7 and 186% of the parent Res-Glc substrate, and the k_{cat} values were between 0.5 and 10 times higher than that measured for Res-Glc (Table 1). Having established that these substrates are efficiently hydrolyzed by GCase, we evaluated their selectivity with respect to GBA2 and GBA3. There was negligible turnover of any of these O-6-modified substrates by GBA2 (Table 2). This observation is consistent with the recent X-ray crystal structure of a bacterial homologue of GBA2, which reveals that the O-6 hydroxyl is involved in forming critical binding interactions within the active site.²³ For GBA3, which is known to be a promiscuous β -glucosidase,¹³ we observed the turnover of all substrates tested (Table 2). To quantify the selectivity, we compared the second-order rate constants obtained for each substrate with these enzymes and observed selectivity ranging from 8- to 49-fold for GCase over GBA3. Interestingly, the substrate selectivity appeared to be largely independent of the steric bulk because substrate **8**, which simply bears a primary alcohol, and the much larger cyclohexanol substrate **9** showed similar second-order rate constants. The morpholine derivative **10**, which we term MorSel-X (morpholine-selective substrate 10), which is positively charged at the pH of the assay, was most selective (49-fold). We judged that the level of selectivity of MorSel-X was adequate for the analysis of GCase in lysates, particularly because GBA3 is mostly present in the liver, kidney, intestine, and spleen,^{13,24} and set out to validate it as a potential tool compound for studying GCase activity in tissues.

Given the high interest in studying the role of GCase in PD and neuropathic forms of GD, the ability to conveniently monitor GCase activity in brain tissue lysates is of importance to the field. To demonstrate the ability of MorSel-X (**10**) to selectively measure GCase activity, we collected brain tissue from a transgenic Gaucher's mouse model (JAX no. 019106)²⁵ in which mouse *GBA* is replaced by mutant human *GBA* that expresses the D409V mutant form of human GCase, which has greatly reduced activity. This mouse model has been reliably used to study the relationship between GCase and PD and its GCase activity is well characterized.²⁶ We used MorSel-X and contrasted it with the commonly used nonselective substrate

Table 2. Comparison of the Second-Order Rate Constants for Processing of Candidate-Selective Substrates by Functionally Related Human β -Glucosidases

substrate	GCase k_{cat}/K_m ($\text{s}^{-1} \text{M}^{-1}$)	GBA2 k_{cat}/K_m ($\text{s}^{-1} \text{M}^{-1}$) ^a	GBA3 k_{cat}/K_m ($\text{s}^{-1} \text{M}^{-1}$)	selectivity for GCase
8	3600 \pm 100	3.8 \pm 0.5	390 \pm 60	9
9	16 600 \pm 400	1700 \pm 100	1710 \pm 80	9
10, MorSel-X	6900 \pm 200	n.d.	140 \pm 50	49
11	58 000 \pm 3000	110 \pm 50	7100 \pm 400	8

^an.d. = not determined**Figure 2.** Comparison of activity assay results obtained using Res-Glc with the subtractive β -glucosidase standard activity assay as compared with those obtained using the selective GCase substrate MorSel-X. Analysis of tissue homogenates from mice (+/+, D409V/+, D409V/D409V) in the presence of 0.16% (w/v) sodium deoxytaurocholate. Selective inhibitors miglustat and AT3375 were used to control for each of the human β -glucosidases (AT3375 for GCase, miglustat for GBA2, and a combination of both for GBA3). (A) Structures of the selective iminosugar inhibitors miglustat and AT3375. (B) Measurement of β -glucosidase activity in tissue homogenates using Res-Glc (100 μM). (C) Measurement of β -glucosidase activity using MorSel-X (100 μM). Error bars represent SEM for the mean values obtained over three replicates of each of three independent biological mouse brain samples for each of WT, heterozygote, and homozygote mice ($n = 3$ for each group).

Res-Glc by assaying β -glucosidase activity in brain tissue from age-matched homozygous (D409V/D409V), heterozygous (+/D409V), and wild type (WT) (+/+) mice having the same genetic background (Figure 2). Using selective inhibitors including AT3375 (Figure 2a), which is GCase-selective (GCase $\text{IC}_{50} = 43$ nM, GBA2 $\text{IC}_{50} = >100$ μM , Figure S2), and miglustat, a GBA2-selective inhibitor (GCase $K_i = 685$ μM , GBA2 $K_i = 326$ nM),²⁷ it was possible to parse out the contribution of GCase, GBA2, and GBA3 to the observed overall β -glucosidase activity. Using the nonselective Res-Glc substrate, we found that the majority of β -glucosidase activity in the mouse brain originates from GBA2, as the treatment with AT3375 (10 μM) results in a modest reduction of only 39% in activity (Figure 2b). Likewise, treatment with miglustat (10 μM) led to a 69% reduction in activity. Treatment with both selective inhibitors, to enable the observation of GBA3 activity, led to background levels of signal, which is consistent with the known absence of GBA3 in the brain based on the expression profiling of GBA3.²⁸ Using the current standard subtractive approach, we measured GCase activities of $60 \pm 1\%$ for the heterozygote and $8.5 \pm 0.3\%$ for the homozygote. We also assayed the homogenates using the more commonly used fluorogenic substrate 4-methylumbelliferyl β -D-glucopyranoside and closely recapitulated the literature values for GCase activity in brain homogenate from D409V/D409V mice, measuring $20.9 \pm 0.6\%$ as compared with the reported literature value of $22 \pm 4\%$ (Figure S3). In contrast, we found that when assaying homogenates treated with AT3375 (10 μM) using MorSel-X there was no detectable enzymatic turnover (Figure 2c). This result demonstrated that the selectivity we observed using recombinant enzymes translates to selectivity in the more complex environment of brain homogenates. Furthermore, the selectivity of MorSel-X for

GCase is independent of the presence of sodium deoxytaurocholate (Figure S4), which has been shown to modulate GCase and GBA2 activity.¹² Interestingly, when we treated homogenates with miglustat (10 μM), a 10–20% reduction in activity as measured using MorSel-X was observed. We note that Krschen et al. has reported similar levels of off-target inhibition of GCase by miglustat in lysates,¹² indicating that it may have some limitations as an inhibitor tool compound and further illustrating the complications associated with the current subtractive assays.

We next used MorSel-X to measure the amount of residual GCase activity in the mutant mouse lines as compared with the WT mice. We measured $63 \pm 3\%$ residual GCase activity in heterozygotes, which is in accord with literature reports,¹⁵ and $9 \pm 3\%$ in homozygotes. This latter value, in particular, contrasts with reported values for residual GCase activity in brain homogenates from D409V/D409V mice as compared with homogenates from WT mice, which have been reported as ranging from 19% to >30%.^{6,25,29} Interestingly, the $9 \pm 3\%$ residual activity we measured is in direct agreement with measurements of GCase activity in a wide range of other tissue types including lung, liver, spleen, and fibroblasts from D409V/D409V mice, where the measured activity ranges from $2.5 \pm 0.9\%$ to $6 \pm 3\%$.²⁵ We speculated that reports on the relatively high residual GCase activity in brains from D409V/D409V mice as compared with WT mice stem from inaccuracies arising due to the very high expression of GBA2 in brain,¹² which complicates the accurate measurements of low levels of residual GCase activity in the brain that exacerbates errors made when using subtractive assays with inhibitors.

To further test the utility of MorSel-X as a tool compound for selectively monitoring GCase activity, we evaluated the levels of GCase activity in GD patient fibroblasts. We assayed

fibroblast lysates from a Gaucher's patient (L444P/L444P, GM01260) in the same way as the mouse brain homogenates (Figure S5a). Notably, there is only low-level expression of GBA2 in fibroblasts.¹² The L444P mutation is particularly deleterious, and the residual activity in this patient cell line is reported to be 5%.³⁰ When assaying lysates with MorSel-X, we found a similarly low 2% residual activity (Figure S5b). This concordance demonstrated the ability of MorSel-X to accurately measure levels of residual GCCase in patient cell lines.

In conclusion, O-alkylation of the O-6 position of Glc-Res leads to fluorogenic substrates selective for GCCase over GBA2 and GBA3. The nature of the pendent group allows tuning of substrate selectivity, and we found that MorSel-X shows good selectivity for GCCase with a >100 times higher k_{cat}/K_m value as compared with GBA2 and 49 times higher value as compared with GBA3. This selectivity is preserved in more in complex tissue samples such as the brain. Therefore, the application of MorSel-X significantly simplifies the current approaches used to measure the GCCase activity and will facilitate research in the field focused on advancing knowledge regarding GCCase and GCCase-targeted therapeutics for PD and GD. Finally, we hope that the general approach used here to realize selective substrates for GCCase also provides a framework for the pursuit of selective substrates for other glycoside hydrolases as a means to facilitate research into this increasingly disease-relevant superfamily of enzymes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acscchembio.9b01044>.

Synthetic methods for all numbered compounds, *in vitro* assays of recombinant β -glucosidases (GCCase, GBA2, and GBA3), assays of β -glucosidase activities in homogenates of transgenic mouse brain tissues along with Michaelis–Menten kinetics for compounds 8–11 (GCCase, GBA2, and GBA3), IC_{50} curves of AT3375 against human β -glucosidases (GCCase, GBA2, and GBA3), activities of β -glucosidases in transgenic mouse brain homogenates as measured with 4MU-Glc, comparison of GCCase activity measurements using compound 10 in the presence or absence of sodium deoxytaurocholate, measurement of GCCase activity in human fibroblasts (WT and L444P/L444P) using compound 10, NMR spectra for compound 10, and an HPLC trace of compound 10 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

David J. Vocadlo – Department of Chemistry and Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada; orcid.org/0000-0001-6897-5558; Email: dvocadlo@sfu.ca

Authors

Matthew C. Deen – Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada
Cameron Proceviat – Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada
Xiaoyang Shan – Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Liang Wu – York Structural Biology Laboratory, Department of Chemistry, University of York, York YO10 5DD, United Kingdom

David L. Shen – Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Gideon J. Davies – York Structural Biology Laboratory, Department of Chemistry, University of York, York YO10 5DD, United Kingdom; orcid.org/0000-0002-7343-776X

Complete contact information is available at: <https://pubs.acs.org/10.1021/acscchembio.9b01044>

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.C.D is supported by a NSERC PGS-D scholarship. D.J.V. is supported by a Tier I Canada Research Chair in Chemical Glycobiology and an E.W.R. Steacie Memorial Fellowship. G.J.D. is supported by the Royal Society through the Ken Murray Research Professorship. Alice Shan is thanked for preliminary synthetic studies, and the Centre for High Throughput Chemical Biology (HTCB) at Simon Fraser University is thanked for access to facilities. Financial support was provided by grants from the Canadian Glycomics Network (RG-1) to D.J.V., the Michael J. Fox Foundation (MJFF) for Parkinson's Research to D.J.V., and the European Research Council ERC-2012-AdG-322942 "Glycopoise" to L.W and G.J.D.

■ REFERENCES

- (1) Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., and Henrissat, B. (2014) The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 42, D490–D495.
- (2) Danes, B. S., and Bearn, A. G. (1968) Gauchers disease: A genetic disease detected in skin fibroblast cultures. *Science* 161, 1347–1348.
- (3) Nagral, A. (2014) Gaucher disease. *J. Clin. Exp. Hepatol.* 4, 37–50.
- (4) Sidransky, E., Nalls, M. A., Aasly, J. O., Aharon-Peretz, J., Annesi, G., Barbosa, E. R., Bar-Shira, A., Berg, D., Bras, J., Brice, A., Chen, C.-M., Clark, L. N., Condroyer, C., De Marco, E. V., Dürr, A., Eblan, M. J., Fahn, S., Farrer, M. J., Fung, H.-C., Gan-Or, Z., Gasser, T., Gershoni-Baruch, R., Giladi, N., Griffith, A., Gurevich, T., Januario, C., Kropp, P., Lang, A. E., Lee-Chen, G.-J., Lesage, S., Marder, K., Mata, I. F., Mirelman, A., Mitsui, J., Mizuta, I., Nicoletti, G., Oliveira, C., Ottman, R., Orr-Urtreger, A., Pereira, L. V., Quattrone, A., Rogaeva, E., Rolfs, A., Rosenbaum, H., Rozenberg, R., Samii, A., Samadpour, T., Schulte, C., Sharma, M., Singleton, A., Spitz, M., Tan, E.-K., Tayebi, N., Toda, T., Troiano, A. R., Tsuji, S., Wittstock, M., Wolfsberg, T. G., Wu, Y.-R., Zabetian, C. P., Zhao, Y., and Ziegler, S. G. (2009) Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease. *N. Engl. J. Med.* 361, 1651–1661.
- (5) Mazzulli, J. R., Xu, Y. H., Sun, Y., Knight, A. L., McLean, P. J., Caldwell, G. A., Sidransky, E., Grabowski, G. A., and Krainc, D. (2011) Gaucher disease glucocerebrosidase and α -synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* 146, 37–52.
- (6) Sardi, S. P., Clarke, J., Viel, C., Chan, M., Tamsett, T. J., Treleaven, C. M., Bu, J., Sweet, L., Passini, M. A., Dodge, J. C., Yu, W. H., Sidman, R. L., Cheng, S. H., and Shihabuddin, L. S. (2013) Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies. *Proc. Natl. Acad. Sci. U. S. A.* 110, 3537–3542.
- (7) Sardi, S. P., Clarke, J., Kinnecom, C., Tamsett, T. J., Li, L., Stanek, L. M., Passini, M. A., Grabowski, G. A., Schlossmacher, M. G., Sidman, R. L., Cheng, S. H., and Shihabuddin, L. S. (2011) CNS

expression of glucocerebrosidase corrects α -synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy. *Proc. Natl. Acad. Sci. U. S. A.* 108, 12101–12106.

(8) Richter, F., Fleming, S. M., Watson, M., Lemesre, V., Pellegrino, L., Raney, B., Zhu, C., Mortazavi, F., Mulligan, C. K., Sioshansi, P. C., Hean, S., De La Rosa, K., Khanna, R., Flanagan, J., Lockhart, D. J., Wustman, B. A., Clark, S. W., and Chesselet, M. F. (2014) A GCase Chaperone Improves Motor Function in a Mouse Model of Synucleinopathy. *Neurotherapeutics* 11, 840–856.

(9) Mazzulli, J. R., Zunke, F., Tsunemi, T., Toker, N. J., Jeon, S., Burbulla, L. F., Patnaik, S., Sidransky, E., Marugan, J. J., Sue, C. M., and Krainc, D. (2016) Activation of β -Glucocerebrosidase Reduces Pathological α -Synuclein and Restores Lysosomal Function in Parkinson's Patient Midbrain Neurons. *J. Neurosci.* 36, 7693–7706.

(10) Aflaki, E., Borger, D. K., Moaven, N., Stubblefield, B. K., Rogers, S. A., Patnaik, S., Westbroek, W., Sullivan, P., Fujiwara, H., Lopez, G., Goldstein, D. S., Ory, D. S., Marugan, J., and Sidransky, E. (2016) iPSC-derived dopaminergic neurons from patients with Gaucher disease and Parkinsonism demonstrate the potential of a new glucocerebrosidase chaperone. *Mol. Genet. Metab.* 117, S15.

(11) Zheng, W., Padia, J., Urban, D. J., Jadhav, A., Goker-Alpan, O., Simeonov, A., Goldin, E., Auld, D., LaMarca, M. E., Inglesse, J., Austin, C. P., and Sidransky, E. (2007) Three classes of glucocerebrosidase inhibitors identified by quantitative high-throughput screening are chaperone leads for Gaucher disease. *Proc. Natl. Acad. Sci. U. S. A.* 104, 13192–7.

(12) Körschen, H. G., Yildiz, Y., Raju, D. N., Schonauer, S., Böningk, W., Jansen, V., Kremmer, E., Kaupp, U. B., and Wachten, D. (2013) The Non-lysosomal β -Glucosidase GBA2 Is a Non-integral Membrane-associated Protein at the Endoplasmic Reticulum (ER) and Golgi. *J. Biol. Chem.* 288, 3381–3393.

(13) Berrin, J., Czjzek, M., Kroon, P. A., Mclauchlan, W. R., Puigserver, A., Williamson, G., and Juge, N. (2003) Substrate (aglycone) specificity of human cytosolic β -glucosidase. *Biochem. J.* 373, 41–48.

(14) Aflaki, E., Borger, D. K., Moaven, N., Stubblefield, B. K., Rogers, S. A., Patnaik, S., Schoenen, F. J., Westbroek, W., Zheng, W., Sullivan, P., Fujiwara, H., Sidhu, R., Khaliq, Z. M., Lopez, G. J., Goldstein, D. S., Ory, D. S., Marugan, J., and Sidransky, E. (2016) A New Glucocerebrosidase Chaperone Reduces α -Synuclein and Glycolipid Levels in iPSC-Derived Dopaminergic Neurons from Patients with Gaucher Disease and Parkinsonism. *J. Neurosci.* 36, 7441–7452.

(15) Sidman, R. L., Cheng, S. H., Tamsett, T. J., Grabowski, G. A., Shihabuddin, L. S., Li, L., Stanek, L. M., Passini, M. A., Schlossmacher, M. G., Clarke, J., Sardi, S. P., and Kinnecom, C. (2011) CNS expression of glucocerebrosidase corrects α -synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy. *Proc. Natl. Acad. Sci. U. S. A.* 108, 12101–12106.

(16) Huebner, M., Moloney, E. B., van der Spoel, A. C., Priestman, D. A., Isacson, O., Hallett, P. J., and Platt, F. M. (2019) Reduced sphingolipid hydrolase activities, substrate accumulation and ganglioside decline in Parkinson's disease. *Mol. Neurodegener.* 14, 40.

(17) Kuo, C., Kallemeijn, W. W., Lelieveld, L. T., Mirzaian, M., Zoutendijk, I., Vardi, A., Futeran, A. H., Meijer, A. H., Spaink, H. P., Overkleeft, H. S., Aerts, J. M. F. G., and Artola, M. (2019) In vivo inactivation of glycosidases by conduritol B epoxide and cyclophellitol as revealed by activity-based protein profiling. *FEBS J.* 286, 584–600.

(18) Asanuma, D., Sakabe, M., Kamiya, M., Yamamoto, K., Hiratake, J., Ogawa, M., Kosaka, N., Choyke, P. L., Nagano, T., Kobayashi, H., and Urano, Y. (2015) Sensitive β -galactosidase-targeting fluorescence probe for visualizing small peritoneal metastatic tumours in vivo. *Nat. Commun.* 6, 6463.

(19) Cecioni, S., and Vocadlo, D. J. (2017) Carbohydrate Bis-acetal-Based Substrates as Tunable Fluorescence-Quenched Probes for Monitoring exo-Glycosidase Activity. *J. Am. Chem. Soc.* 139, 8392–8395.

(20) Witte, M. D., Kallemeijn, W. W., Aten, J., Li, K.-Y., Strijland, A., Donker-Koopman, W. E., van den Nieuwendijk, A. M. C. H.,

Bleijlevens, B., Kramer, G., Florea, B. I., Hooibrink, B., Hollak, C. E. M., Ottenhoff, R., Boot, R. G., van der Marel, G. A., Overkleeft, H. S., and Aerts, J. M. F. G. (2010) Ultrasensitive in situ visualization of active glucocerebrosidase molecules. *Nat. Chem. Biol.* 6, 907–913.

(21) Yadav, A. K., Shen, D. L., Shan, X., He, X., Kermode, A. R., and Vocadlo, D. J. (2015) Fluorescence-Quenched Substrates for Live Cell Imaging of Human Glucocerebrosidase Activity. *J. Am. Chem. Soc.* 137, 1181–1189.

(22) Zhang, Y. J., Dayoub, W., Chen, G. R., and Lemaire, M. (2012) TMDS as a dual-purpose reductant in the regioselective ring cleavage of hexopyranosyl acetals to ethers. *Eur. J. Org. Chem.* 2012, 1960–1966.

(23) Charoenwattanasatien, R., Pengthaisong, S., Breen, I., Mutoh, R., Sansanya, S., Hua, Y., Tankrathok, A., Wu, L., Songsirittitigul, C., Tanaka, H., Williams, S. J., Davies, G. J., Kurisu, G., and Cairns, J. R. K. (2016) Bacterial β -Glucosidase Reveals the Structural and Functional Basis of Genetic Defects in Human Glucocerebrosidase 2 (GBA2). *ACS Chem. Biol.* 11, 1891–1900.

(24) de Graaf, M., van Veen, C., van der Meulen-Muileman, I. H., Gerritsen, W. R., Pinedo, H. M., and Haisma, H. J. (2001) Cloning and characterization of human liver cytosolic β -glucosidase. *Biochem. J.* 356, 907–910.

(25) Xu, Y.-H., Quinn, B., Witte, D., and Grabowski, G. A. (2003) Viable Mouse Models of Acid β -Glucosidase Deficiency. *Am. J. Pathol.* 163, 2093–2101.

(26) Cullen, V., Sardi, S. P., Ng, J., Xu, Y. H., Sun, Y., Tomlinson, J. J., Kolodziej, P., Kahn, I., Saftig, P., Woulfe, J., Rochet, J. C., Glicksman, M. A., Cheng, S. H., Grabowski, G. A., Shihabuddin, L. S., and Schlossmacher, M. G. (2011) Acid β -glucosidase mutants linked to Gaucher disease, Parkinson disease, and Lewy body dementia alter α -synuclein processing. *Ann. Neurol.* 69, 940–953.

(27) Lahav, D., Liu, B., Van Den Berg, R. J. B. H. N., Van Den Nieuwendijk, A. M. C. H., Wennekes, T., Ghisaidoobe, A. T., Breen, I., Ferraz, M. J., Kuo, C. L., Wu, L., Geurink, P. P., Ova, H., Van Der Marel, G. A., Van Der Stelt, M., Boot, R. G., Davies, G. J., Aerts, J. M. F. G., and Overkleeft, H. S. (2017) A Fluorescence Polarization Activity-Based Protein Profiling Assay in the Discovery of Potent, Selective Inhibitors for Human Nonlysosomal Glucosylceramidase. *J. Am. Chem. Soc.* 139, 14192–14197.

(28) Yahata, K., Mori, K., Arai, H., Koide, S., Ogawa, Y., Mukoyama, M., Sugawara, A., Ozaki, S., Tanaka, I., Nabeshima, Y., and Nakao, K. (2000) Molecular cloning and expression of a novel klotho-related protein. *J. Mol. Med.* 78, 389–394.

(29) Xu, Y.-H., Reboulet, R., Quinn, B., Huelsken, J., Witte, D., and Grabowski, G. A. (2008) Dependence of reversibility and progression of mouse neuronopathic Gaucher disease on acid β -glucosidase residual activity levels. *Mol. Genet. Metab.* 94, 190–203.

(30) Sun, Y., Florer, J., Mayhew, C. N., Jia, Z., Zhao, Z., Xu, K., Ran, H., Liou, B., Zhang, W., Setchell, K. D. R., Gu, J., and Grabowski, G. A. (2015) Properties of Neurons Derived from Induced Pluripotent Stem Cells of Gaucher Disease Type 2 Patient Fibroblasts: Potential Role in Neuropathology. *PLoS One* 10, e0118771.