

This is a repository copy of *Carba-Cyclophellitols are Neutral Retaining Glucosidase Inhibitors*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/115949/>

Version: Accepted Version

Article:

Beenakker, Thomas J. M., Wander, Dennis, Offen, Wendy A. orcid.org/0000-0002-2758-4531 et al. (13 more authors) (2017) Carba-Cyclophellitols are Neutral Retaining Glucosidase Inhibitors. *Journal of the American Chemical Society*. 6534–6537. ISSN 1520-5126

<https://doi.org/10.1021/jacs.7b01773>

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.

Carba-Cyclophellitols are Neutral Retaining Glucosidase Inhibitors

Thomas J. M. Beenakker[†], Dennis P. A. Wander[†], Wendy A. Offen[§], Marta Artola[†], Lluís Raich[¶], Maria J. Ferraz[‡], Kah-Yee Li[†], Judith H. P. M. Houben[‡], Erwin R. van Rijssel[†], Thomas Hansen[†], Gijsbert A. van der Mare[†], Jeroen D. C. Codée[†], Johannes M. F. G. Aerts[‡], Carme Rovira^{¶,||}, Gideon J. Davies^{§,*} and Herman S. Overkleeft^{†,*}

[†]Department of Bio-organic Synthesis and [‡]Department of Medical Biochemistry, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2300 RA Leiden, The Netherlands.

[§]Department of Chemistry, University of York, Heslington, York, YO10 5DD, U.K.

[¶]Departament de Química Inorgànica i Orgànica (Secció de Química Orgànica) & Institut de Química Teòrica i Computacional (IQTCUB), Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain.

^{||}Institució Catalana de Recerca i Estudis Avançats (ICREA), 08020 Barcelona, Spain.

Supporting Information Placeholder

ABSTRACT: The conformational analysis of glycosidases affords a route into their specific inhibition through transition-state mimicry. Inspired by the rapid reaction rates of cyclophellitol and cyclophellitol aziridine, both covalent retaining β -glucosidase inhibitors, we postulated that the corresponding carba "cyclopropyl" analogue would be a potent retaining β -glucosidase inhibitor for those enzymes reacting through the ⁴H₃ transition-state conformation. *Ab initio* metadynamics simulation of the conformational free energy landscape for the cyclopropyl inhibitors show a strong bias for the ⁴H₃ conformation and carba-cyclophellitol with an *N*-(4-azidobutyl)carboxamide moiety proved to be a potent inhibitor (*K*_i of 8.2 nM) of the *Thermotoga maritima* TmGH1 β -glucosidase. 3-D structural analysis and comparison with unreacted epoxides shows that this compound indeed bind in ⁴H₃ conformation suggesting that conformational strain induced through a cyclopropyl unit may add to the armory of tight binding inhibitor designs.

The diverse conformational pathways of glycosidases^{1,2} (for example Figure 1A) coupled to their phenomenal transition-state stabilization³ offers a powerful route to selective enzyme inhibition. One of the main goals of the field, very rarely achieved, is to design and apply conformationally-restricted inhibitors in order to provide both potency and specificity; conformationally-biased inhibitors that target specific classes of glycoside hydrolase (GH) would be of considerable use as cellular and mechanistic probes and potential as starting points for therapeutic compounds. Cyclophellitol (**1**, Figure 1), isolated in 1990 from the mushroom *Phellinus sp.*⁴, is a potent mechanism-based inhibitor of retaining β -glucosidases. It finds primary use as a covalent inactivator of β -glucosidases.⁵ Cyclophellitol is a configurational analogue of β -glucopyranose, but its conformational behavior is different. Whereas β -glucopyranoses preferably adopt a ⁴C₁ conformation; the epoxide annulation in **1** likely enforces a preferred ⁴H₃ half-chair conformation onto the cyclitol moiety. Cyclophellitol (**1**) is thus a potential conformational analogue of the oxocarbenium ion transition-state during β -glucosidase-mediated hydrolysis of a β -glucosidic linkage.

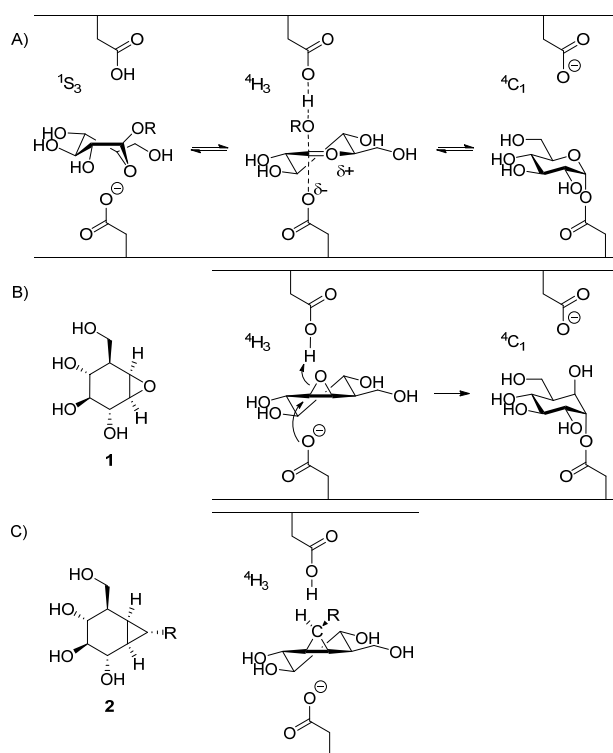


Figure 1. A) Mechanistic itinerary of retaining β -glucosidases. B) Structure of cyclophellitol (**1**) adopting a ⁴H₃ conformation and its proposed mechanism of binding. C) Structure of carba-cyclophellitol (**2**) in ⁴H₃ conformation.

Although the mode of action of **1** is covalent (Figure 1B) the potency and specificity of cyclophellitol (**1**) as a retaining β -glucosidase inhibitor and its mode of action (entering the enzyme active site as a ⁴H₃ half-chair transition-state analogue followed by S_N2 displacement of the epoxide heteroatom) led us to consider whether the corresponding carba analogue (that is, substitution

of the oxygen for carbon) would result in competitive inhibitors in which potency and potentially specificity would be accrued by virtue of partial transition-state mimicry (Figure 1C).

To test this hypothesis, a set of carba-cyclophellitols was designed. Here we present the synthesis of carba-cyclophellitols **3-5** (Figure 2), the quantum mechanical analysis of their favored conformation and their structural and inhibitory dissection towards β -glucosidases. Carba-cyclophellitols are shown to be low μ M inhibitors. Furthermore, exploiting the possibility of incorporating pseudo-axial R groups – consistent with the catalytic itinerary – that bearing a hydrophobic moiety at the terminal cyclopropyl carbon (**5**) was indeed a potent (low nM) inhibitor of a classical model β -glucosidase, namely *Thermotoga maritima* TmGH1.^{5,6} The crystal structure of TmGH1 containing carba-cyclophellitol **5** was determined, in comparison with an unreacted cyclophellitol derivative and, as predicted, both bind in ⁴H₃ conformation, which is the presumed transition state conformation during the TmGH1-catalysed hydrolysis of β -glucosidic linkages.

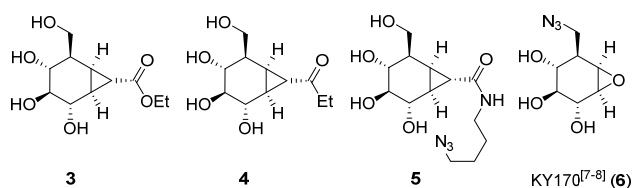
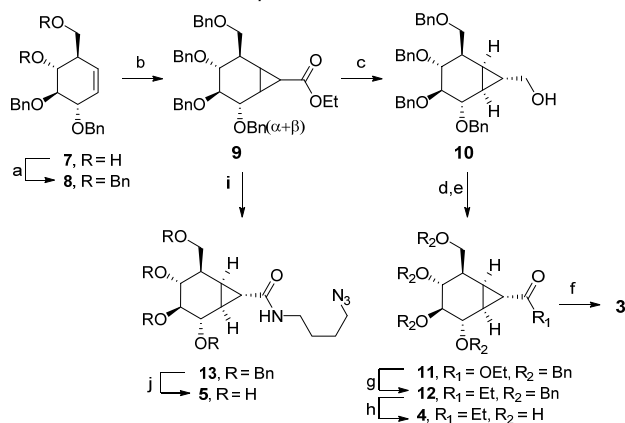


Figure 2. Structures of carba-cyclophellitols (**3-5**) and 8-azidocyclophellitol **6** (KY170^{7,8}).

The synthesis of compounds **3-5** commenced with the easy access of key intermediate **7**, which was obtained via the synthetic procedure described by the group of Madsen⁹ and optimized in our laboratory (Scheme 1).⁸ Global benzylation of **7** gave cyclohexene **8**, and cyclopropanation with ethyl diazoacetate (EDA)^{10,11} under the agency of Cu(acac)₂ resulted in the formation of product **9** as a mixture of α - and β -isomers (α/β , 2:1). After the reduction step¹² the β -isomer could be isolated by column chromatography to give alcohol **10**, which was oxidized and ensuing esterification yielded enantiomerically pure β -ester **11**. Sequential one-pot formation and Grignard addition onto the Weinreb amide yielded β -ketone **12**. Both benzyl-protected ester **11** and ketone **12** were subjected to palladium-catalyzed hydrogenolysis conditions in ethyl acetate and acetic acid (**11**) or in methanol (**12**) to obtain target compounds **3** and **4**. The mixture of α - and β -esters **9** was saponified and the resulting carboxylates condensed with 4-azidobutan-1-amine (see Supporting Information (SI)). The mixture of α - and β -amides was separated by preparative HPLC purification. Finally, the benzyl groups were removed in the presence of the azide with anhydrous BCl₃ in dichloromethane to afford β -amide **5**.



Scheme 1. Reagents and conditions. (a) BnBr, NaH, TBAI, DMF, 0 °C to rt., 24 h, 94%; (b) EDA, Cu(acac)₂, EtOAc, (35%, 2:1, as a mixture of α/β); (c) DIBAL, THF, 30 min at 0 °C and then 1 h at rt., 13%; (d) Jones reagent, acetone, 0 °C, 3 h, 53%; (e) EtOH, *N,N'*-diisopropylcarbodiimide, 4-dimethylaminopyridine, toluene, rt., 4 h, 62%; (f) Pd(OH)₂/C, H₂, EtOAc, AcOH, rt., overnight, 81%; (g) *N,O*-dimethylhydroxylamine hydrochloride, EtMgBr, THF, 48%; (h) Pd(OH)₂/C, H₂, MeOH, rt., overnight, (58%); (i) *i*) LiOH, MeOH, H₂O, rt., overnight; *ii*) 4-azidobutan-1-amine (see SI), DIPEA, HCTU, CH₂Cl₂, rt., overnight; (j) BCl₃, DCM, 99%.

After having carba-cyclopropane **3-5** in hand, their inhibition potency was studied in comparison with deoxynojirimycin (DNJ), a known competitive TmGH1 inhibitor and AMP-DNM (MZ-21), a known human retaining β -glucosidase inhibitor.¹³ Initial binding constant (K_i) values were determined on TmGH1 by monitoring the UV-absorbance of *p*-nitrophenolate from *p*-nitrophenyl β -D-glucopyranoside using the *Lineweaver–Burk* method. Carba-cyclophellitol **3** and **4** showed micromolar inhibition, consistent with our design strategy and similar to that displayed by the charged species DNJ, whereas **5** proved to be a strong reversible binding TmGH1 inhibitor with a K_i value of 8.2 nM, much more potent than DNJ¹⁴ and AMP-DNM (low micromolar) (Table 1 and supplementary Figure S4). We then explored the activity of compound **5** in human lysosomal retaining β -glucosidase, GBA1 (deficiency of which is causative of the human lysosomal storage disorder, Gaucher disease) with an apparent IC₅₀ of around 100 μ M. No apparent inhibition of the human lysosomal α -glucosidase, GAA (deficient in the human glycogen storage disease, Pompe disease) was observed at final concentrations of **5** up to 150 μ M. Thus, although less potent for GBA1 than for the bacterial enzyme tested, compound **5** appears to have selectivity for the human lysosomal β -glucosidase over the human lysosomal α -glucosidase, which is opposite of the selectivity observed for deoxynojirimycin (DNJ, Table 1).

Table 1. Apparent IC₅₀ values and inhibitory constants (K_i) for *in vitro* inhibition of α - and β -glucosidase activity by compounds **3-5, DNJ and AMP-DNM.**

Compound	TmGH1 ^[a]	GBA1 ^[b]	GAA ^[b]
	K_i ^[c]	App IC ₅₀	App IC ₅₀
3	22.3 μ M	>150 μ M	> 150 μ M
4	88.9 μ M	>150 μ M	> 150 μ M
5	8.20 nM	99 \pm 1.9 μ M	> 150 μ M
DNJ	2.50 μ M ^[d]	109 \pm 1.0 μ M ^[e]	1.5 μ M ^[g]
AMP-DNM (MZ-21)	4.97 μ M	156 \pm 16 nM ^[f]	0.4 μ M ^[g]

The assay was performed with (a) *p*-NPG or (b) 2,4-DNPG as substrate. (c) K_m TmGH1 = 0.24 mM. Values in agreement with literature: (d) K_i DNJ = 3.8 μ M in TmGH1¹⁴, (e) IC₅₀ DNJ = 250 μ M in GBA1¹⁵, (f) IC₅₀ AMP-DNM = 100-200 nM in GBA1^{15,16}. (g) values from reference.¹⁷ App: apparent.

Inspired by the low μ M to nM inhibition of TmGH1 by the carba-cyclopropanes, we sought to determine whether the cyclopropyl moiety indeed biased the conformation to ⁴H₃. We calculated the conformational free energy landscape (FEL) for generic cyclopropyl (**2**, R = H) by *ab initio* metadynamics (see SI), and the Cremer-Pople puckering coordinates θ and ϕ were used as collective variables, yielding a Mercator representation for the FEL (as used previously for diverse glycosidase inhibitors¹⁸⁻²⁰), Figure 3A. Compound **2** clearly favors the ⁴H₃ conformation *in vacuo*, with the flipped ³H₄ form in another local energy minimum. Subsequent to FEL calculation, we compared the experimental *J* values of several (cyclohexane) ring protons of compound **4** with their calculated counterparts, in which calculations were performed on

compound **4** in the 4H_3 conformation. Both sets of values are in good agreement, which underscores the notion that compound **4**, and by extension also the other compounds subject of this paper (whose proton NMR give broadened signals due to the amide present – see SI) do indeed adopt the 4H_3 conformation in solution.

Structural dissection of the inhibitory action of **5**, and the conceptual link through to cyclophellitol **1**, was achieved first by rapid soaking (as opposed to pre-incubation as used previously to trap the covalent adduct⁵) of crystals of *TmGH1* with cyclophellitol derivative KY170^{7,8} (**6**). Serendipitously, this indeed afforded the unreacted cyclophellitol KY170 in 4H_3 conformation, with the nucleophile poised to attack, Figure 3B, confirming our hypothesis that (unreacted) cyclophellitols adopt a transition-state like 4H_3 conformation. In order to dissect similar mimicry by carba-cyclopropane **5**, and confirm the FEL calculated by *ab initio* metadynamics, *TmGH1* crystals were soaked with carba-cyclophellitol **5** and the subsequently obtained structure was analyzed and solved with X-ray crystallography. The obtained electron density pattern clearly demonstrates the presence of carba-cyclophellitol **5** in the active site in 4H_3 conformation (Figure 3C; the butyl azide moiety is mobile and differently disordered in the structure and not shown for clarity).

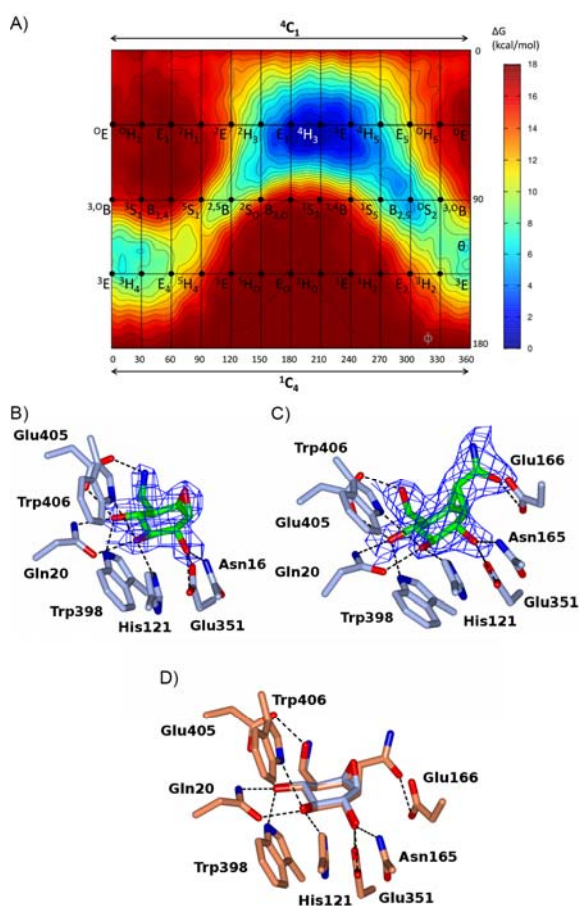


Figure 3. A) A mercator representation for the computed free energy landscape (FEL) of cyclopropyl (**2**, R = H) (θ and ϕ are given in degrees). B) Crystal structure of *TmGH1* in complex with unreacted KY170. C) Crystal structure of *TmGH1* in complex with carba-cyclophellitol **5**, showing the carba-cyclophellitol-CO-NH group. Electron density maps for both B and C are maximum likelihood / σ_A weighted $2F_{\text{obs}} - F_{\text{calc}}$ syntheses contoured at 1.4σ . D) Overlay of (B) in ice blue on (C) in coral.

Overlay of cyclophellitol derivative KY170 with carba-cyclophellitol **5** (Figure 3D) shows almost perfect coincidence of atomic position showing that, as suggested by the FEL, **5** is a permanent mimic of cyclophellitol posted in the active site prior to nucleophilic attack.

The improved binding of **5**, relative to **3** and **4** presumably stems from desolvation caused by the alkyl-azido “tail” sitting in the aglycone site. One of the design advantages of the carba-cyclopropanes is that any pendent R groups are disposed pseudo-axial to the sugar ring, consistent with the distortions seen during catalysis which presumably adds to their augmentation of binding. The 3-D structure with **5** confirms this and shows a lateral, anti-trajectory interaction of the catalytic amino acid Glu166 with the pseudo-axially disposed amide of **5**. There are 4 molecules of *TmGH1* in the crystallographically-observed asymmetric unit. Whilst they all show the R group axial, they all show different degrees of disorder of this alkyl region itself. In one molecule, there is essentially no electron density for the tail, whilst in two molecules the chain passes through the aglycon region (that is flanked by Val169 and Trp168 and 324) making non-specific interactions with this region. In the fourth molecule of the AU, the alkyl azido chain appears to follow two separate routes along each hydrophobic flank of the substrate binding cleft.

Bicyclic cyclopropyl glucosidase inhibitors, with the bridge between the “C6” and “O5” atoms were first proposed by Tanaka and co-workers²¹ and later developed in *galacto* configuration by Bennet and co-workers and found to be good α -glucosidase and galactosidase inhibitors, respectively.²² More recently, activated forms of these compounds have been used as covalent inhibitors.²³ In these cases the conformational restriction limits the accessible conformations to “off-pathway” 3H_2 and 2H_3 half-chairs²³ (or perhaps their related 1,4 boats) recently elegantly revealed by X-ray crystallography.²⁴ Further, Stick and Stubbs²⁵ synthesized a bicyclic cyclopropyl inhibitor with the bridge between the “anomeric” C1 carbon position and the “C2” atom with a millimolar K_i value. The carba-cyclophellitol derivatives presented here offer, by virtue of the advantage of their conformational restriction between the “O5” and “anomeric” C1 carbon position a potent inhibitor in which the conformational restraint is a glycosidase reaction coordinate relevant 4H_3 . Given the large number of glycosidase inhibitors in medical use, including those being developed as pharmacological chaperones and as diagnostic tools, the harnessing of appropriate conformational restraint, coupled to correct stereochemistry should add greatly to the enzymological, cellular and, ultimately, therapeutic toolbox.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, supporting figures and tables and 1H and ${}^{13}C$ NMR spectra (PDF).

The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

h.s.Overkleeft@lic.leidenuniv.nl
gideon.davies@york.ac.uk

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

We thank the Netherlands Organization for Scientific Research (NWO-CW, ChemThem grant to J.M.A. and H.S.O.), the European Research Council (ERC-2011-AdG-290836 “Chembiosphing”, to H.S.O., and ERC-2012-AdG-32294 “Glycopoise”, to G.J.D.), the Spanish Ministry of Economy and Competitiveness (CTQ2014-55174, to C.R.) and the Generalitat de Catalunya (2014SGR-987, to C.R.) for financial support. L.R. thanks the University of Barcelona for an APIF predoctoral fellowship. We gratefully acknowledge the computer resources at *MareNostrum* and the technical support provided by BSC-CNS (RES-QCM-2016-3-00017). We thank the European Synchrotron Radiation Facility at Grenoble for access to beamline ID23-2 and the Diamond Light Source for access to beamline IO2 (proposal number mx-13587) that contributed to the results presented here.

REFERENCES

- (1) Davies, G. J.; Planas, A.; Rovira, C. *Acc. Chem. Res.* **2012**, *45*, 308.
- (2) Speciale, G.; Thompson, A. J.; Davies, G. J.; Williams, S. J. *Curr. Opin. Struct. Biol.* **2014**, *28*, 1.
- (3) Wolfenden, R.; Snider, M. J. *Acc. Chem. Res.* **2001**, *34*, 938.
- (4) Atsumi, S.; Umezawa, K.; Iinuma, H.; Naganawa, H.; Nakamura, H.; Iitaka, Y.; Takeuchi, T. *J. Antibiot.* **1990**, *43*, 49.
- (5) Gloster, T. M.; Madsen, R.; Davies, G. J. *Org. Biomol. Chem.* **2007**, *5*, 444.
- (6) Gloster, T. M.; Meloncelli, P.; Stick, R. V.; Zechel, D.; Vasella, A.; Davies, G. J. *J. Am. Chem. Soc.* **2007**, *129*, 2345.
- (7) 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.; Aerts, J. M. F. G. *Nat. Chem. Biol.* **2010**, *6*, 907.
- (8) Li, K.-Y.; Jiang, J.; Witte, M. D.; Kallemeijn, W. W.; van den Elst, H.; Wong, C.-S.; Chander, S. D.; Hoogendoorn, S.; Beenakker, T. J. M.; Codée, J. D. C.; Aerts, J. M. F. G.; van der Marel, G. A.; Overkleeft, H. S. *Eur. J. Org. Chem.* **2014**, *2014*, 6030.
- (9) Hansen, F. G.; Bundgaard, E.; Madsen, R. *J. Org. Chem.* **2005**, *70*, 10139.
- (10) Ye, T.; McKervey, M. A. *Chem. Rev.* **1994**, *94*, 1091.
- (11) Caballero, A.; Prieto, A.; Diaz-Requejo, M. M.; Pérez, P. J. *Eur. J. Inorg. Chem.* **2009**, *2009*, 1137.
- (12) Zhou, S.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Drach, J. C.; Tamiya, S.; Mitsuya, H.; Zemlicka, J. *J. Med. Chem.* **2006**, *49*, 6120.
- (13) Overkleeft, H. S.; Renkema, G. H.; Neele, J.; Vianello, P.; Hung, I. O.; Strijland, A.; van der Burg, A. M.; Koomen, G. J.; Pandit, U. K.; Aerts, J. M. *J. Biol. Chem.* **1998**, *273*, 26522.
- (14) Zechel, D. L.; Boraston, A. B.; Gloster, T.; Boraston, C. M.; Macdonald, J. M.; Tilbrook, D. M. G.; Stick, R. V.; Davies, G. J. *J. Am. Chem. Soc.* **2003**, *125*, 14313.
- (15) Wennekes, T.; Meijer, A. J.; Groen, A. K.; Boot, R. G.; Groener, J. E.; van Eijk, M.; Ottenhoff, R.; Bijl, N.; Ghauharali, K.; Song, H.; O’Shea, T. J.; Liu, H.; Yew, N.; Copeland, D.; van den Berg, R. J.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. *J. Med. Chem.* **2010**, *53*, 689.
- (16) Ghisaidoobe, A. T.; van den Berg, R. J. B. H. N.; Butt, S. S.; Strijland, A.; Donker-Koopman, W. E.; Scheij, S.; van den Nieuwendijk, A. M. C. H.; Koomen, G.-J.; van Loevezijn, A.; Leemhuis, M.; Wennekes, T.; van der Stelt, M.; van der Marel, G. A.; van Boeckel, C. A. A.; Aerts, J. M. F. G.; Overkleeft, H. S. *J. Med. Chem.* **2014**, *57*, 9096.
- (17) Wennekes, T.; van den Berg, R. J. B. H. N.; Donker, W.; van der Marel, G. A.; Strijland, A.; Aerts, J. M. F. G.; Overkleeft, H. S. *J. Org. Chem.* **2007**, *72*, 1088.
- (18) Petricevic, M.; Sobala, L. F.; Fernandes, P. Z.; Raich, L.; Thompson, A. J.; Bernardo-Seisdedos, G.; Millet, O.; Zhu, S.; Sollogoub, M.; Jiménez-Barbero, J.; Rovira, C.; Davies, G. J.; Williams, S. J. *J. Am. Chem. Soc.* **2017**, *139*, 1089.
- (19) Thompson, A. J.; Dabin, J.; Iglesias-Fernández, J.; Ardèvol, A.; Dinev, Z.; Williams, S. J.; Bande, O.; Siriwardena, A.; Moreland, C.; Hu, T.-C.; Smith, D. K.; Gilbert, H. J.; Rovira, C.; Davies, G. J. *Angew. Chem. Int. Ed.* **2012**, *51*, 10997.
- (20) Williams, R. J.; Iglesias-Fernández, J.; Stepper, J.; Jackson, A.; Thompson, A. J.; Lowe, E. C.; White, J. M.; Gilbert, H. J.; Rovira, C.; Davies, G. J.; Williams, S. J. *Angew. Chem. Int. Ed.* **2013**, *53*, 1087.
- (21) Tanaka, K. S. E.; Winters, G. C.; Batchelor, R. J.; Einstein, F. W. B.; Bennet, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 998.
- (22) Wang, Y.; Bennet, A. J. *Org. Biomol. Chem.* **2007**, *5*, 1731.
- (23) Chakladar, S.; Wang, Y.; Clark, T.; Cheng, L.; Ko, S.; Vocolo, D. J.; Bennet, A. J. *Nat. Commun.* **2014**, *5*, 1.
- (24) Adamson, C.; Pengelly, R. J.; Shamsi Kazem Abadi, S.; Chakladar, S.; Draper, J.; Britton, R.; Gloster, T. M.; Bennet, A. J. *Angew. Chem. Int. Ed.* **2016**, *128*, 15202.
- (25) Stick, R. V.; Stubbs, K. A. *J. Carbohydr. Chem.* **2005**, *24*, 529.

Insert Table of Contents artwork here

