

Carba-cyclophellitols Are Neutral Retaining-Glucosidase Inhibitors

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Supporting Information

ABSTRACT: The conformational analysis of glycosidases affords a route to 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 4H_3 transition-state conformation. *Ab initio* metadynamics simulations of the conformational free energy landscape for the cyclopropyl inhibitors show a strong bias for the 4H_3 conformation, and carba-cyclophellitol, with an *N*-(4-azidobutyl)-carboxamide moiety, proved to be a potent inhibitor ($K_i = 8.2$ nM) of the *Thermotoga maritima* TmGH1 β -glucosidase. 3-D structural analysis and comparison with unreacted epoxides show that this compound indeed binds in the 4H_3 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 offer 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 with 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 prefer to adopt a 4C_1 conformation, the epoxide annulation in **1** likely enforces a preferred 4H_3 half-chair conformation onto the cyclitol moiety.

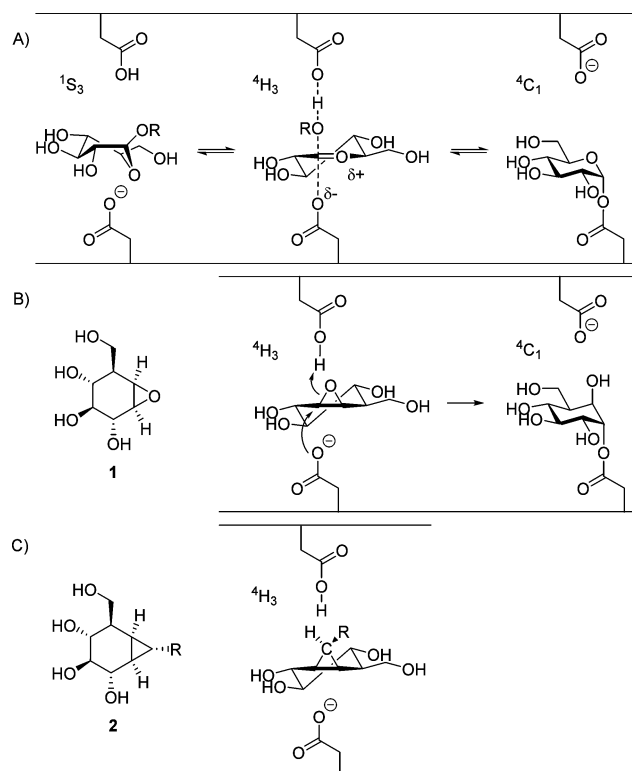


Figure 1. (A) Mechanistic itinerary of retaining β -glucosidases. (B) Structure of cyclophellitol (**1**) adopting a 4H_3 conformation and its proposed mechanism of binding. (C) Structure of carba-cyclophellitol (**2**) in 4H_3 conformation.

Cyclophellitol (**1**) is thus a potential conformational analogue of the oxocarbenium ion transition-state during β -glucosidase-mediated hydrolysis of a β -glucosidic linkage.

Although the mode of action of **1** is covalent (Figure 1B), its potency and specificity as a retaining β -glucosidase inhibitor

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and its mode of action (entering the enzyme active site as a ${}^4\text{H}_3$ half-chair transition-state analogue followed by $\text{S}_{\text{N}}2$ 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

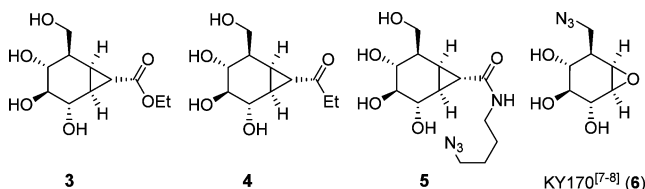


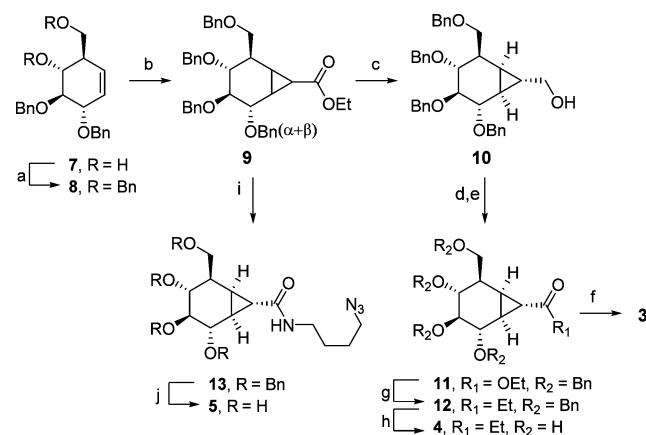
Figure 2. Structures of carba-cyclophellitols 3–5 and 8-azidocyclophellitol (6, KY170^{7,8}).

favored conformation, and their structural and inhibitory dissection toward β -glucosidases. Carba-cyclophellitols are shown to be low μM inhibitors. Furthermore, exploiting the possibility of incorporating pseudoaxial 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 and compared with that of an unreacted cyclophellitol derivative; as predicted, both bind in ${}^4\text{H}_3$ conformation, which is the presumed transition-state conformation during the TmGH1-catalyzed hydrolysis of β -glucosidic linkages.

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 $\text{Cu}(\text{acac})_2$ 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 were 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 BCl_3 in dichloromethane to afford β -amide 5.

Having carba-cyclopropane 3–5 in hand, we studied their inhibition potency 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

Scheme 1. The synthesis of carba-cyclophellitols 3–5^a



^aReagents and conditions: (a) BnBr, NaH, TBAI, DMF, 0 °C to rt, 24 h, 94%; (b) EDA, $\text{Cu}(\text{acac})_2$, 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) $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , EtOAc, AcOH, rt, overnight, 81%; (g) *N,O*-dimethylhydroxylamine hydrochloride, EtMgBr, THF, 48%; (h) $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , MeOH, rt, overnight, (58%); (i) *i*) LiOH, MeOH, H_2O , rt, overnight; *ii*) 4-azidobutan-1-amine (see SI), DIPEA, HCTU, CH_2Cl_2 , rt, overnight; (j) BCl_3 , DCM, 99%.

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

Table 1. Apparent IC_{50} 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	K_i^a		
	TmGH1 ^b	GBA1 ^c	GAA ^c
3	22.3 μM	>150 μM	>150 μM
4	88.9 μM	>150 μM	>150 μM
5	8.20 nM	99 \pm 1.9 μM	>150 μM
DNJ	2.50 $\mu\text{M}^{d,e}$	109 \pm 1.0 μM^e	1.5 $\mu\text{M}^{e,f}$
AMP-DNM (MZ-21)	4.97 μM	156 \pm 16 nM ^f	0.4 $\mu\text{M}^{e,g}$

^a K_m TmGH1 = 0.24 mM. ^bThe assay was performed with *p*-NPG as substrate. ^cThe assay was performed with 2,4-DNPG as substrate. Values in agreement with literature. ^d K_i DNJ = 3.8 μM in TmGH1.¹⁴ ^e IC_{50} DNJ = 250 μM in GBA1.¹⁵ ^f IC_{50} AMP-DNM = 100–200 nM in GBA1.^{15,16} ^gValues from ref 17. App: apparent.

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 $\text{IC}_{50} \approx 100 \mu\text{M}$. 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 μM . 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 DNJ (Table 1).

correct stereochemistry, should add greatly to the enzymological, cellular, and, ultimately, therapeutic toolbox.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b01773.

Primary NMR data files for 3–5, 8, 10–14 (ZIP)

Experimental procedures, Figures S1–S5 and Table S1, and ^1H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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