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A Convenient Approach to Stereoisomeric Iminocyclitols: Generation of Potent Brain-Permeable OGA Inhibitors

Milan Bergeron-Brlek, Jake Goodwin-Tindall, Nevena Cekic, Christian Roth, Wesley F. Zandberg, Xiaoyang Shan, Vimal Varghese, Sherry Chan, Gideon J. Davies, David J. Vocadlo, and Robert Britton**

Abstract: *Pyrrolidine-based iminocyclitols are a promising class of glycosidase inhibitors. Reported herein is a convenient epimerization strategy that provides direct access to a range of stereoisomeric iminocyclitol inhibitors of O-GlcNAcase (OGA), the enzyme responsible for catalyzing removal of O-GlcNAc from nucleocytoplasmic proteins. Structural details regarding the binding of these inhibitors to a bacterial homologue of OGA reveal the basis for potency. These compounds are orally available and permeate into rodent brain to increase O-GlcNAc, and should prove useful tools for studying the role of OGA in health and disease.*

The glycosylation of serine and threonine residues with O-linked *N*-acetylglucosamine (O-GlcNAc)[1] is a conserved protein modification which occurs at high levels in the brains of eukaryotes.[2] O-GlcNAc is installed by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA, a family GH84 glycoside hydrolase).[3] This modification has been found to hinder protein phosphorylation, including on the microtubule-associated protein tau.[4] Notably, the progression of Alzheimer's disease (AD) is closely associated with hyperphosphorylation and subsequent aggregation of tau,[5] and decreased levels of O-GlcNAc have been found in the brains of AD patients.[4c,6] It has also been shown that O-GlcNAcylation of tau[4c] hinders its phosphorylation and aggregation in vitro,[6b,7] thus suggesting a protective role for this modification. Moreover, maintaining high levels of O-GlcNAc in the brain by using small-molecule inhibitors of OGA blocks tau hyperphosphorylation, aggregation, and neurodegeneration in various transgenic mouse models of AD.[7,8] Not surprisingly, the potential to block AD progression by increasing levels of O-GlcNAc has stimulated interest in identifying potent, brain penetrant, and selective inhibitors of OGA.[4d,9]

[*] M. Bergeron-Brlek, Dr. J. Goodwin-Tindall, N. Cekic, Dr. W. F. Zandberg, Dr. X. Shan, Dr. V. Varghese, Prof. D. J. Vocadlo, Prof. R. Britton
Department of Chemistry, Simon Fraser University
Burnaby, British Columbia (Canada)
E-mail: dvocadlo@sfu.ca
rbritton@sfu.ca
Dr. X. Shan, Prof. D. J. Vocadlo
Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia (Canada)
Dr. C. Roth, S. Chan, Prof. G. J. Davies
Department of Chemistry, University of York, York (UK)

Toward this goal, a number of pyrrolidine-based iminosugars have demonstrated significant inhibitory activity toward various glycoside hydrolases,[10] including GH20 β-hexosaminidases as well as the functionally related GH84 OGA.[11] For example, Wong and co-workers have demonstrated that the C2-epimeric pyrrolidines **1** and **2** (Figure 1)[12]

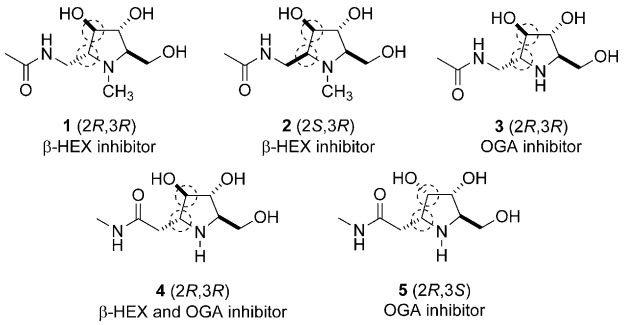
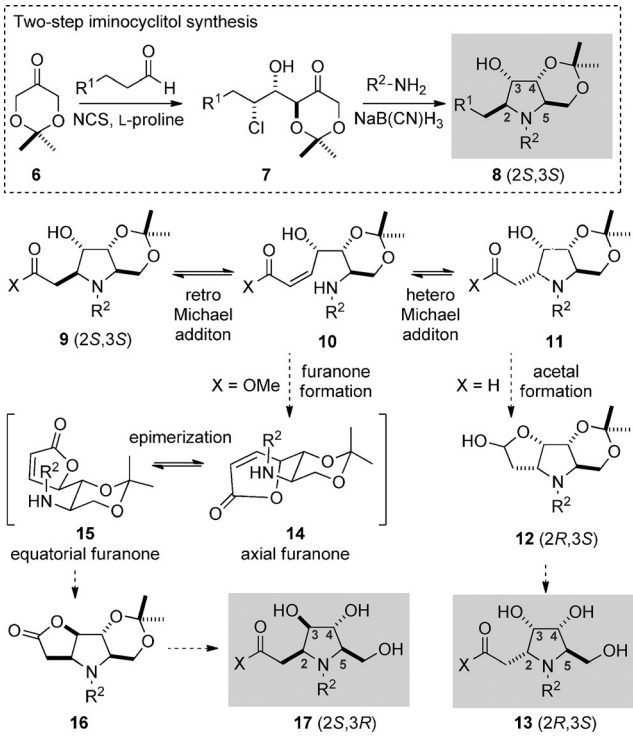


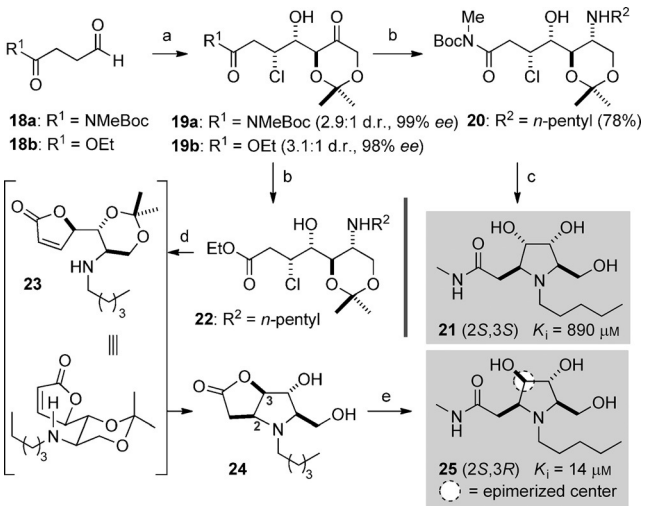
Figure 1. Structures of iminocyclitol OGA and β-HEX inhibitors.

are both potent inhibitors of GH20 β-hexosaminidases (β-Hex), and the structurally related iminocyclitol **3**, as well as the configurationally unique reverse amides **4** and **5**, and analogues, have appeared in the patent literature as inhibitors of OGA.[11d] Considering the profound effects iminocyclitol configuration has on both the potency and selectivity of these glycoside hydrolase inhibitors, access to stereoisomers and an improved understanding of their binding interactions with OGA is critical to advancing this class of inhibitor for treatment of AD.

Despite significant therapeutic potential, the synthesis of polyhydroxypyrrolidine iminosugars is often complicated by a reliance on carbohydrate starting materials and elaborate protecting/functional-group interconversion strategies.[10b,13,14] These challenges are manifested in the synthesis of configurationally distinct iminocyclitols (e.g., **2**, **4**, and **5**), where each target requires a unique carbohydrate building block. Recently, we reported a convenient two-step iminocyclitol synthesis (see inset, Scheme 1) and demonstrated this process in the preparation of a small library of imino C-nucleoside analogues.[15] Given the notable activity of the iminocyclitols **4** and **5** as OGA inhibitors, we were interested in adapting this synthesis for the preparation of related iminocyclitols possessing the relative stereochemistry of the general structure **9**. Moreover, we envisioned that a series of retro-Michael (*r*M) hetero-Michael (*h*M) reactions involving **9** could be stereochemically tuneable through deliberate



Scheme 1. A two-step synthesis of ribose-configured iminocyclitols **8** and a unified strategy to access the C2/C3 stereoisomeric iminocyclitols **13** and **17**.

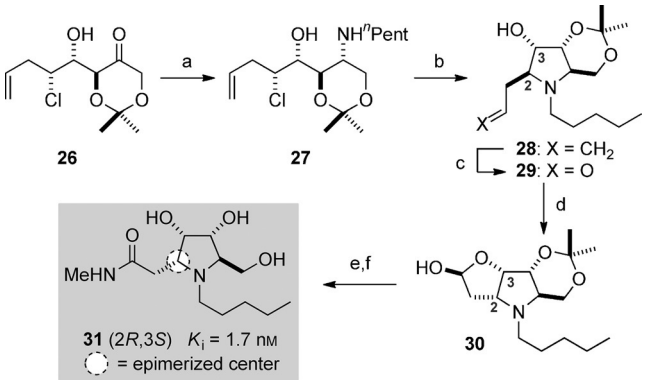


Scheme 2. Synthesis of the C3-epimeric iminocyclitols **21** and **25**. Reaction conditions: a) **6**, NCS, (*S*)-proline, CH_2Cl_2, RT, 24 h (55%); b) *n*-pentylamine, AcOH, THF, RT, 1 h then $NaB(CN)H_3$, 1 h (78%); c) 1. $NaHCO_3$, PhMe, 105 °C, 24 h, (54%); 2. MeOH, TFA/CH_2Cl_2 (1:1), RT, 1 h (79%); d) MeOH, 120 °C, 4 h (55%); e) $MeNH_2$ (40% in H_2O), RT, 1 h (88%). Boc = *tert*-butoxycarbonyl, NCS = *N*-chlorosuccinimide, TFA = trifluoroacetic acid.

choice of the acyl group, and thus afford access to diastereomers at both C2 and C3 to probe the relationship between stereochemistry and OGA inhibition. For example, *r*M reaction of amides or esters (e.g., **9**, X = OR/NR_2) could lead sequentially to the enoate **10** and furanone **14**. A subsequent *h*M reaction through the equilibrating and epimeric furanone **15**, in which the furanone is situated pseudo-equatorially, would afford the lactone **16** and ultimately the C3-epimeric iminocyclitol **17**. Alternatively, a *r*M*h*M reaction involving the corresponding aldehyde (e.g., **9**, X = H) should lead to an equilibrating mixture of 2*S* and 2*R* epimers **9** and **11**, respectively.[16] As only the 2,3-*cis*-configured diastereomer **11** is capable of forming the hemiacetal **12**, this process would provide straightforward access to the C2 epimeric iminocyclitol **13**. Here we report concise syntheses of a series of novel polyhydroxypyrrolidinyl acetamides through the realization of these two complementary *r*M*h*M epimerization strategies. By exploiting this convenient process we also report on a potent, selective, and brain penetrant OGA inhibitor.

As outlined in Scheme 2, exploiting our one-pot proline-catalyzed α-chlorination DKR aldol reaction[17] with the readily available aldehydes **18a** and **18b**,[18] we first targeted the functionalized iminoribitol **21**. Despite the potential for β-elimination, each of these reactions afforded the expected *syn*-chlorohydrin as the major product in good yield, diastereoselectivity, and enantioselectivity. While reductive amination of the aldol adduct **19a** did not lead directly to the iminoribitol **21**, the intermediate amino chlorohydrin **20** could be readily cyclized by simply heating in toluene with $NaHCO_3$.[15] Removal of both the acetonide and Boc protecting groups required brief treatment with acid and delivered **21** in excellent overall yield. Though this remarkably efficient (four-step) synthesis of **21** could be readily adapted for the preparation of structural analogues for medicinal chemistry purposes, this diastereomer proved to be a weak inhibitor of human OGA (hOGA) with a K_i value of 890 μM (see the Supporting Information).

We therefore investigated the *r*M*h*M reaction sequence, shown in Scheme 1, in an effort to access the corresponding 3*R*-diastereomer **25**. Unfortunately, under a variety of reaction conditions, **21** proved incapable of engaging in *r*M reactions. Anticipating that the increased acidity of the α-protons in the corresponding ester would improve the likelihood of *r*M processes, we targeted the equivalent polyhydroxypyrrolidinyl acetate. As detailed in Scheme 2, we were delighted to find that reductive amination of ester **19b** and subsequent heating in MeOH resulted directly in the formation of the lactone **24**, in which the C3 stereocenter had been inverted relative to that in **21**. The selective formation of **24** and realization of this unique C3-epimerization process presumably involves formation of rapidly equilibrating furanone diastereomers (e.g., **14** and **15**; Scheme 1). The subsequent *h*M reaction then occurs through a pseudo-chair conformation in which the furanone is situated pseudo-equatorially with respect to the forming pyrrolidine. Notably, small amounts of the C2/C3-bis-epimeric lactone (not shown), presumably derived from cyclization through a pseudo-axially oriented furanone (e.g., **14**, Scheme 1), were also produced in this reaction (d.r. ≈ 12:1). However, when purified samples of these lactones were re-subjected to the reaction conditions neither underwent further epimerization, thus indicating a kinetic preference for the formation of **24**. Exploiting this facile C3-epimerization strategy, **25** was made readily avail-



Scheme 3. Synthesis of the C2-epimeric iminocyclitol **31**. Reaction conditions: a) *n*-pentylamine, AcOH, THF, RT, 1 h then NaB(CN)H$_3$, 1 h (87%); b) NaHCO$_3$, PhMe, 105 °C, 24 h (88%); c) 1. AD-mix-β, acetone/H$_2$O (1:1), 4 °C, 24 h; 2. NaIO$_4$, EtOH/H$_2$O (2:1), RT, 1 h, d) silica gel, CHCl$_3$, RT, 16 h (62% from **28**) e) TEMPO, DIB, CH$_2$Cl$_2$, RT, 16 h (66%); f) HCl (cat.), MeOH, RT, 1 h then MeNH$_2$ (40 wt% in H$_2$O), RT, 1 h (95%). DIB = (diacetoxyiodo)benzene, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxyl, THF = tetrahydrofuran.

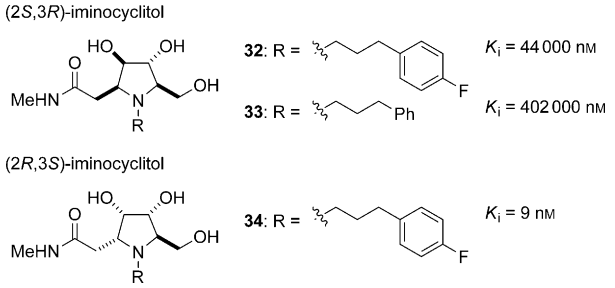


Figure 2. The iminocyclitols **32**–**34** and their inhibitory activity towards hOGA.

able in four steps from the aldehyde **19b**. Notably, the 2*S*-epimer **25** proved to be a significantly improved inhibitor of hOGA when compared with **21**, exhibiting a K_i value of 14(±3) μM.

Finally, we targeted the aldehyde **29** as an intermediate that could be used to explore the C2-epimerization strategy outlined in Scheme 1. Toward this goal, reductive amination of the ketochlorohydrin **26** (available in one step from 4-pentenal)[17] afforded the aminochlorohydrin **27**, which was cyclized to the pyrrolidine **28** after brief heating in toluene with NaHCO$_3$ (Scheme 3). The required aldehyde **29** was readily accessed by oxidative cleavage of the alkene function in **28**. We were delighted then to find that simply exposing **29** to silica gel in chloroform[16] resulted in complete conversion into the C2-epimeric lactol **30**. Conversion of this latter material into the amide **31** then involved a straightforward sequence of reactions including oxidation to the corresponding lactone and brief exposure to acid and then methylamine. The iminocyclitol **31** proved to be a potent inhibitor of OGA with a K_i value of 1.7(±0.3) nM.

With concise synthetic routes to **21**, **25**, and **31** established, we rapidly assembled a small collection of analogues (**32**–**34**; see the Supporting Information for details). As summarized in Figure 2, the most potent inhibitors of hOGA among this series of stereoisomeric iminocyclitols are the (2*R*,3*S*)-configured congeners **31** and **34**.

Further, we sought to understand the molecular basis for the discrepancy in potencies observed toward hOGA by obtaining structures of several of these compounds bound to the active site of a GH84 homologue of hOGA from the bacterium *Bacteroides thetaiotamicron*. This enzyme, *Bt*GH84, is capable of removing O-GlcNAc from modified proteins and has an active site which comprises residues which are conserved with hOGA.[19] Structures, obtained by soaking several of these compounds into apo-crystals of *Bt*GH84, revealed the critical interactions for each diastereomer (Figure 3). For binding of all the compounds, movements of amino-acid side-chains or the main chain are not observed, thus indicating a rigid active site which avoids compensatory changes on protein geometry to accommodate the different stereochemistry of the inhibitors. All four compounds formed a network of multiple interactions with BtGH84. However,

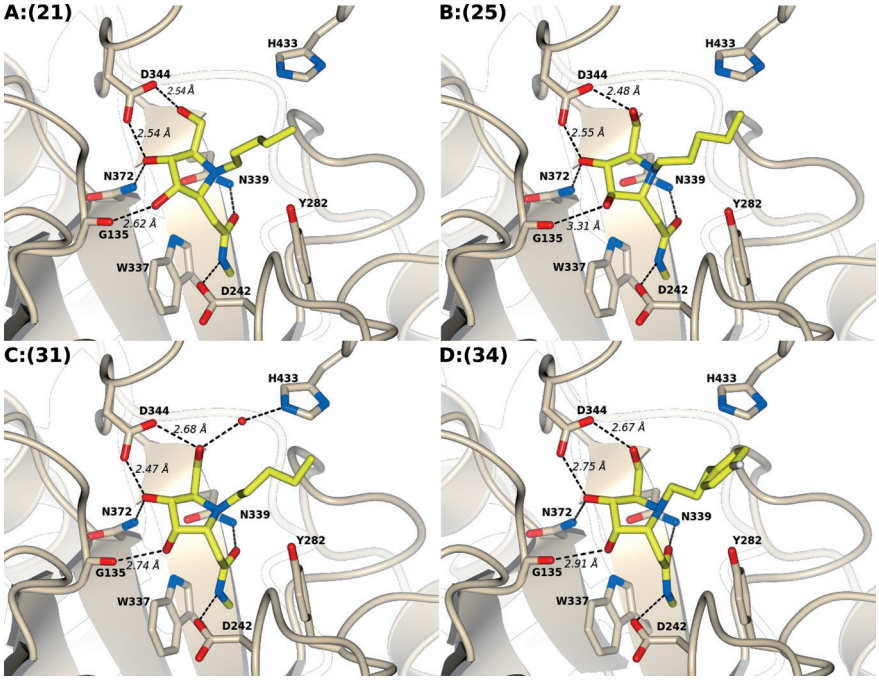


Figure 3. Structural analysis of a series of OGA inhibitors in complex with the *h*OGA homologue *Bt*GH84. A) Iminocyclitol **21**. B) Iminocyclitol **25**. C) Iminocyclitol **31**. D) Iminocyclitol **34**. The enzyme is shown in beige, the ligand is shown in yellow, and hydrogen bonds are indicated by dashed black lines. The length of the hydrogen bonds may contribute to the differences in affinity.

relative stereochemistry was a critical determinant of hydrogen-bond lengths. For example, in the complex with **21** (2*S*,3*S*-configuration), the position of the *N*-acyl moiety leads to three close contacts, two with D344 and one with the main chain oxygen atom of G135. This position potentially yields more repulsive interactions between the protein and the inhibitor. Better hydrogen-bond geometry was observed for **25** (2*S*,3*R*-configuration) primarily reflecting improved interactions between the 3*R*-hydroxy group and the carbonyl of G135. In the complex with **31** and **34** all hydrogen bonds adopt nearly optimal geometries in respect to their length and angle, thus explaining the high affinity of both compounds. Such variations in binding of diastereomeric diol inhibitors were also observed for HIV-1 protease, where a repulsive interaction between a hydroxy group and the carbonyl of a glycine was noted.[20]

Given the potency of the (2*R*,3*S*)-configured iminocyclitols, we were interested in evaluating the potential value of such compounds in vivo. Further, considering the potent inhibition of hOGA in cells by PUGNAc[21] and GlcNAcstatin C,[9d] both of which bear a phenyl moiety positioned several atoms away from the pseudo-sugar ring, we selected **34** (Figure 2) for further studies [$K_i = 9(\pm 2)$ nM]. As depicted in Figure 3 (panel D), the X-ray structure of **34** complexed with *Bt*GH84 is consistent with that of the configurationally related **31**. We next determined the pharmacokinetic properties of **34** in plasma and brains of mice and found it shows good bioavailability and distribution as well as ability to cross the blood-brain-barrier (see Figures S1 and S2 in the Supporting Information) Analysis of sagittal brain tissue sections obtained from mice at various times following oral dosing by gavage reveals that O-GlcNAc levels are qualitatively increased within the brain in various regions, including cortex and hippocampus, as compared to control mice (Figure 4). Immunoblot analysis of mouse brain tissue homogenates obtained from mice 16 hours post dosing with **34** shows quantitative increases in O-GlcNAc levels within brain (Figure 4).

In summary, we describe a unique and convenient synthetic approach to access a series of diastereomeric pyrrolidine iminocylitol inhibitors of hOGA. The most potent of these compounds are single-digit nanomolar inhibitors of hOGA. Structural analysis of selected diastereomers in complex with the bacterial hOGA homologue, *Bt*GH84, revealed critical hydrogen bonds engaging D344 and G135, and offer a rationale for the preference for the (2*R*,3*S*)-configured iminocyclitols **31** and **34**. Furthermore, we found a member among this class of compounds to be both orally bioavailable and brain penetrant in mice, with treatment leading to sustained increases in global O-GlcNAcylation. Given the ease of synthetic access to significant quantities of **34**, we anticipate that **34** may prove to be a useful research tool for probing the functional role of O-GlcNAc in vivo, including within the brain. Further, the ability to access diastereomeric pyrrolidines may be useful for the study of other glycoside hydrolases and structural variation could also enable the identification of still more potent compounds having significantly improved properties.

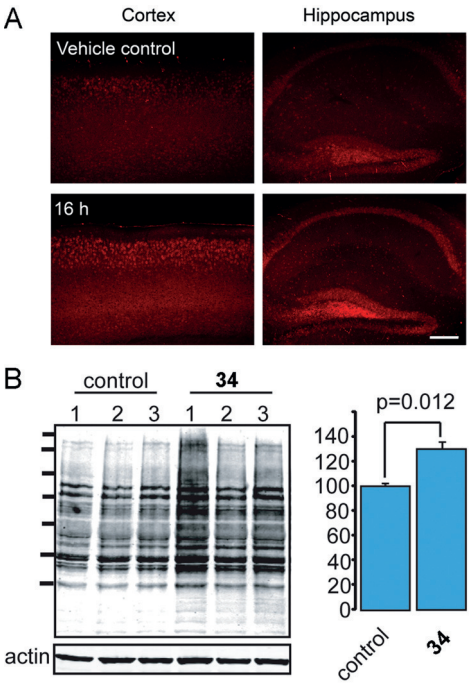


Figure 4. Acute oral gavage dosing of C57/BL6 mice with 200 mg kg^{-1} of **34** increases global O-GlcNAc levels in the brain. A) Analysis of O-GlcNAc immunoreactivity within saggital section of the cortex and hippocampus from treated mice is higher relative to control mice. Scale bar indicates 200 μm. B) Immunoblot analysis of brain tissue lysates shows global O-GlcNAc levels, as detected by the antibody CTD110.6, are increased in treated mice compared to control mice. Immunoblot of actin shows equivalent loading of lysates. $n = 3$; $P = 0.012$ (two-tailed unpaired *t*-test); error bars indicate $\pm$ s.e.m.

Acknowledgments

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