A divergent synthesis of 2-acyl derivatives of PUGNAc yields selective inhibitors of *O*-GlcNAcase†

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A divergent route facilitating the rapid synthesis of a series of *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (PUGNAc)-based inhibitors, bearing different *N*-acyl groups has been developed. All compounds of this series are inhibitors of both human *O*-GlcNAcase and human β-hexosaminidase, yet some effectively exploit differences between the active site architectures of these two human enzymes which render them selective for *O*-GlcNAcase. Such inhibitors may be valuable tools in dissecting the role of the *O*-GlcNAc post-translational modification at the cellular and organismal level since these compounds may have different pharmacokinetic properties when compared to other inhibitors of β-*N*-acetyl-glucosaminidases.

Introduction

Humans have three genes encoding for enzymes that remove *N*-acetyl-D-glucosamine residues from glycoconjugates. The first of these, *O*-glycoprotein 2-acetamido-2-deoxy-β-D-glucopyranosidase (*O*-GlcNAcase), is a member of family 84‡ of the glycoside hydrolases.[1,2] This enzyme acts to hydrolyse *O*-GlcNAc off serine and threonine residues of modified proteins.[3] The post-translational modification of nucleocytoplasmic proteins with 2-acetamido-2-deoxy-β-D-glucopyranoside (*O*-GlcNAc) is found in every tissue of multi-cellular eukaryotes that have been investigated to date.[4] *O*-GlcNAc mediates the function of many cellular proteins including, for example, those involved in cellular signaling,[5] proteasomal degradation,[6] and transcription.[7–10] Consistent with the presence of *O*-GlcNAc on many intracellular proteins, this enzyme appears to have a role in the etiology of several diseases including cancer,[11] Alzheimers,[12–14] and type II diabetes.[5,15]

HEXA and *HEXB* are the two other genes that encode enzymes catalyzing the hydrolytic cleavage of terminal *N*-acetyl-D-glucosamine from glycoconjugates. The two gene products of *HEXA* and *HEXB* yield predominantly two dimeric isozymes. The heterodimeric isozyme, hexosaminidase A, is composed of an α- and a β-subunit. The heterodimeric isozyme, hexosaminidase B, is composed of two β-subunits. Both of these enzymes are classified as members of family 20 of the glycoside hydrolases and are normally localised within lysosomes. Dysfunction of either of these enzymes results in the accumulation of gangliosides and other glycoconjugates within lysosomes causing the inheritable neurodegenerative disorders known as Tay-Sachs and Sandhoff diseases.[16]

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‡ For the classification of glycoside hydrolases, see refs 1 and 2 and the CAZY database (available at http://afmb.cnrs-mrs.fr/CAZY/).

As a result of the biological importance of these β-*N*-acetyl-glucosaminidases, the design of small molecule inhibitors[17–21] has received considerable attention both as tools for establishing their role in biological processes[22] as well as in potential therapeutic applications.[19,23] A major challenge in developing inhibitors for blocking the function of mammalian glycosidases, including *O*-GlcNAcase, is the large number of functionally related enzymes present in tissues of higher eukaryotes. Accordingly, the use of non-selective inhibitors in studying the cellular and organismal physiological role of one particular enzyme is complicated because complex phenotypes arise from the concomitant inhibition of such functionally related enzymes.

Many inhibitors of β-*N*-acetyl-glucosaminidases are known, but two of the most potent and best characterized are NAG-thiazoline **1** and PUGNAc **2**. 2′-Methyl-α-D-glucopyrano-[2,1-*d*]-Δ2′-thiazoline (NAG-thiazoline) **1**, has been found to be a potent inhibitor of family 20 hexosaminidases,[20,24] and more recently, the family 84 *O*-GlcNAcases.[21] The potent inhibition of these enzymes by NAG-thiazoline **1** is consistent with these enzymes sharing a common catalytic mechanism involving anchimeric assistance from the 2-acetamido group (Fig. 1). Support for such a catalytic mechanism operating for the family 20 β-*N*-acetyl-glucosaminidases is supported by both kinetic and structural studies.[20,24] Macauley *et al.* have shown that the family 84 β-*N*-acetyl-glucosaminidases also use anchimeric assistance through kinetic analyses of human *O*-GlcNAcase.[21] The potent inhibition by NAG-thiazoline **1** is presumably due its similarity to the oxazoline or oxazolinium ion intermediate or a closely derived transition state. Despite its potency, a downside to using NAG-thiazoline **1** in a complex biological context, is that it lacks selectivity and therefore perturbs multiple cellular processes.

O-(2-Acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (PUGNAc)[18,28] **2** is another compound that suffers from the same problem of lack of selectivity, yet has enjoyed use as an inhibitor of both human *O*-GlcNAcase[3,26] and the family 20 human β-hexosaminidases.[25] This molecule, developed by Vasella and coworkers, was found to be a potent competitive inhibitor of the β-*N*-acetyl-glucosaminidases from

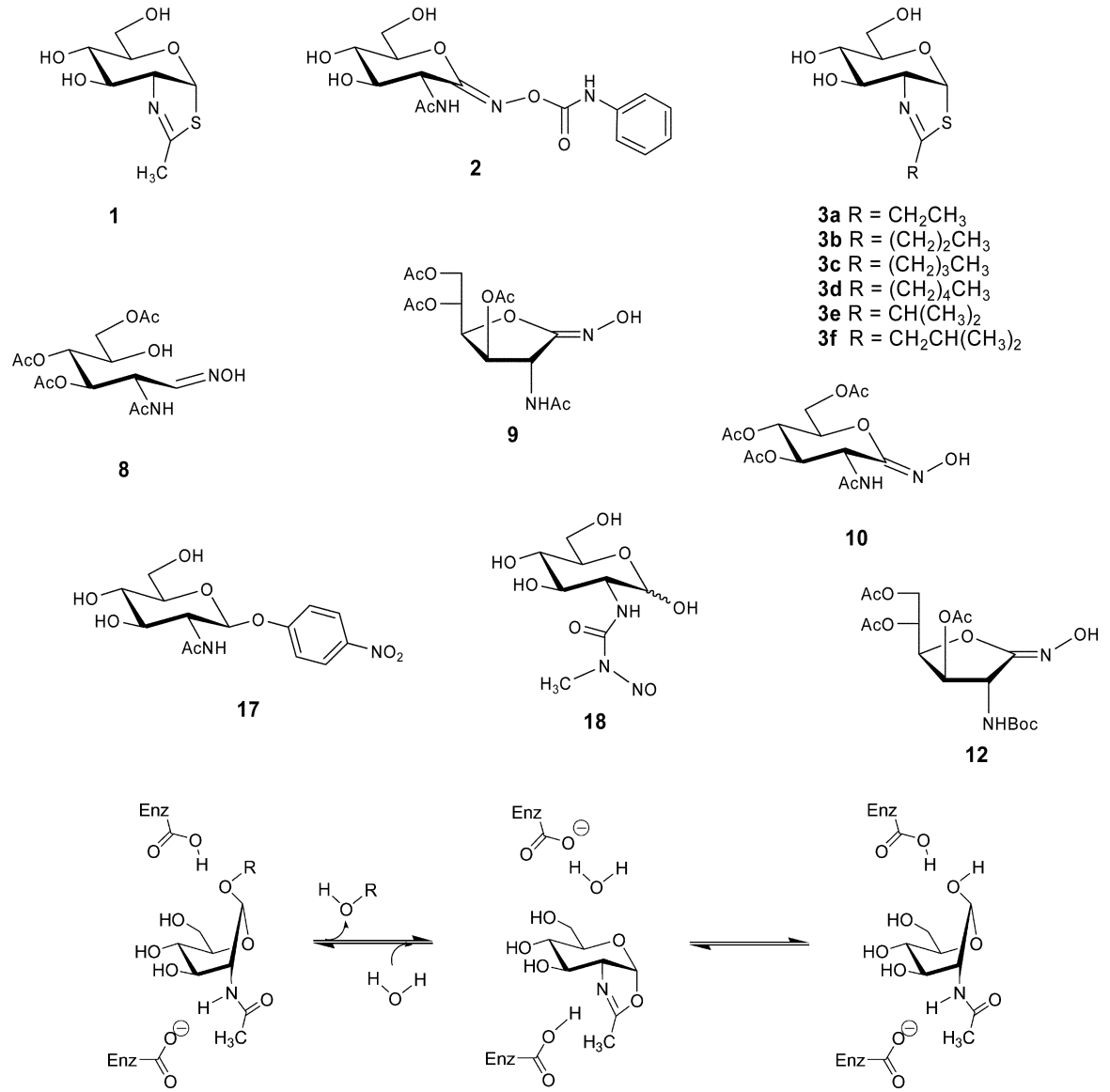


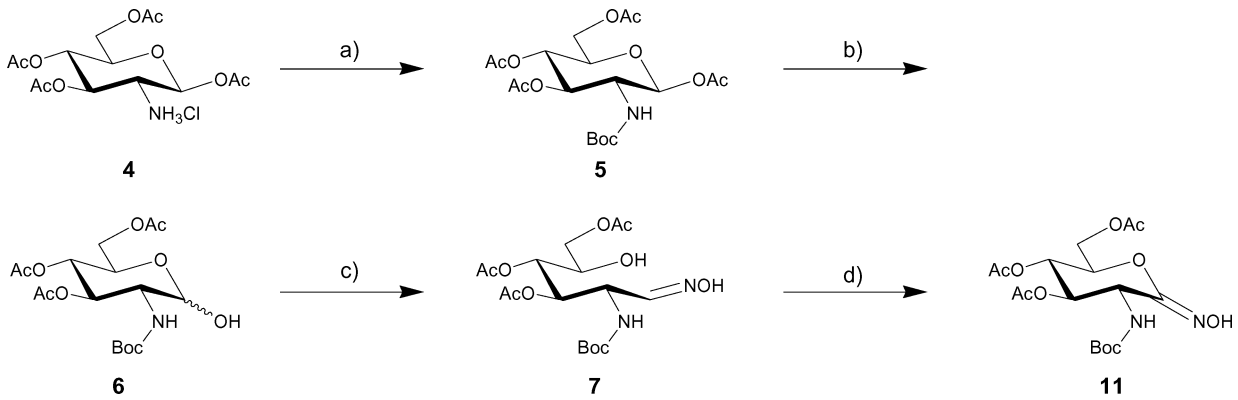
Fig. 1 The catalytic mechanism of family 20 and 84 β-*N*-acetyl-glucosaminidases. The mechanism involves anchimeric assistance from the 2-acetamido group to form an oxazoline intermediate.

Canavalia ensiformis, *Mucor rouxii*, and the β-hexosaminidase from bovine kidney.[18] One rationale for the potency of PUGNAc **2** toward both the family 84 *O*-GlcNAcases and the family 20 β-hexosaminidases is the sp^2 hybridisation of the anomeric carbon. This sp^2 hybridisation is thought to mimic the geometry of the dissociative transition states of most glycosidases and is accordingly a common design feature of many glycosidase inhibitors.[27] Indeed, the inhibitory potency of PUGNAc **2** has prompted the suggestion that it is a transition state analogue,[18] although this assertion waits to be rigourously substantiated.

To generate more useful probes of *O*-GlcNAcase and circumvent the problems associated with the concomitant inhibition of the lysosomal β-hexosaminidases, Macauley *et al.* recently prepared a series of thiazoline inhibitors **3a–f**.[21] Several of these compounds proved both potent and highly selective for human *O*-GlcNAcase over human β-hexosaminidase. As well, some of these inhibitors were shown to rapidly block *O*-GlcNAcase function in cultured cells resulting in rapid and marked increases in cellular *O*-GlcNAc levels. Accordingly, these molecules are superior candidates for studying the cellular and physiological processes involving the *O*-GlcNAc post-translational modification.[21] Based on these observations we envisaged that making modifications in the pendant *N*-acyl chain of PUGNAc **2**, might yield an additional set of potent and selective inhibitors for *O*-GlcNAcase based on a different inhibitor scaffold. Such inhibitors may have different pharmacokinetic properties and be valuable tools in dissecting the role of the *O*-GlcNAc post-translational modification at the cellular and organismal level.

Results and discussion

Both previous syntheses of PUGNAc **2** described in the literature[28,29] use *N*-acetyl-D-glucosamine as a starting material. To prepare the series of compounds we desired, we envisaged



Scheme 1 (a) Boc_2O, Et_3N, THF; (b) $(NH_4)_2CO_3$, THF, MeOH; (c) $NH_2OH.HCl$, C_5H_5N, MeOH; (d) DBU, NCS, CH_2Cl_2.

that a divergent synthesis would facilitate their rapid preparation. Accordingly, *N*-acetyl-D-glucosamine is not a viable starting material since we aimed to generate a panel of compounds with various *N*-acyl groups. Despite this limitation, we expected that the general synthetic route of Mohan and Vasella,[29] using *N*-acetyl-D-glucosamine to prepare **2**, could be adapted to our needs since it is a much improved and convenient approach when compared to the first report outlining the synthesis of PUGNAc **2**.[28]

Due to the nature of the synthesis described by Mohan and Vasella, which primarily involves base-catalyzed reactions we felt that the *tert*-butoxycarbonyl group (Boc) would be stable throughout the synthesis. We also expected that this group could be easily removed in the presence of all the other functionalities at the end of the sequence. This common intermediate obtained at a late stage in the synthesis should be amenable to rapid diversification.

Starting from the readily accessible hydrochloride **4**,[30] the Boc group was introduced to protect the amine moiety to give the tetraacetate **5**[31](Scheme 1). With the tetraacetate **5** in hand, selective de-*O*-acetylation using $(NH_4)_2CO_3$ afforded the hemiacetal **6** in excellent yield. Treating this hemiacetal **6** with $NH_2OH.HCl$ yields the crude *E* and *Z* oximes **7** in good yield. The next reaction, an oxidative ring closure, has been reported by Mohan and Vasella to be quite temperamental. When oxidising the oxime **8** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and *N*-chlorosuccinimide (NCS) careful control of the temperature of the reaction mixture during the addition of the NCS was critical to avoid formation of the undesired 1,4-lactone oxime **9**.[29] Consistent with these observations several trial reactions with **8** show that the temperature is indeed critical to successfully produce the 1,5-lactone oxime **10** over the undesired 1,4-lactone oxime **9**. Indeed, at slightly elevated temperatures (−20 °C) only the 1,4-lactone oxime **9** is obtained.

Treating the oxime **7** with DBU/NCS under the literature conditions[29] provided us with a mixture of the desired 1,5-lactone **11** and the unwanted 1,4-lactone oxime **12** in a 4 : 1 ratio. We found, however, that having NCS already in solution with **7** and only then adding DBU to the mixture furnished exclusively **11**, albeit in a somewhat lower yield. One complication encountered here is that succinimide, a by-product of these reactions, is difficult to separate from **11**. Furthermore, spectral analysis of **11** is complicated due to broadening of the signals obtained in the ^1H NMR spectrum.

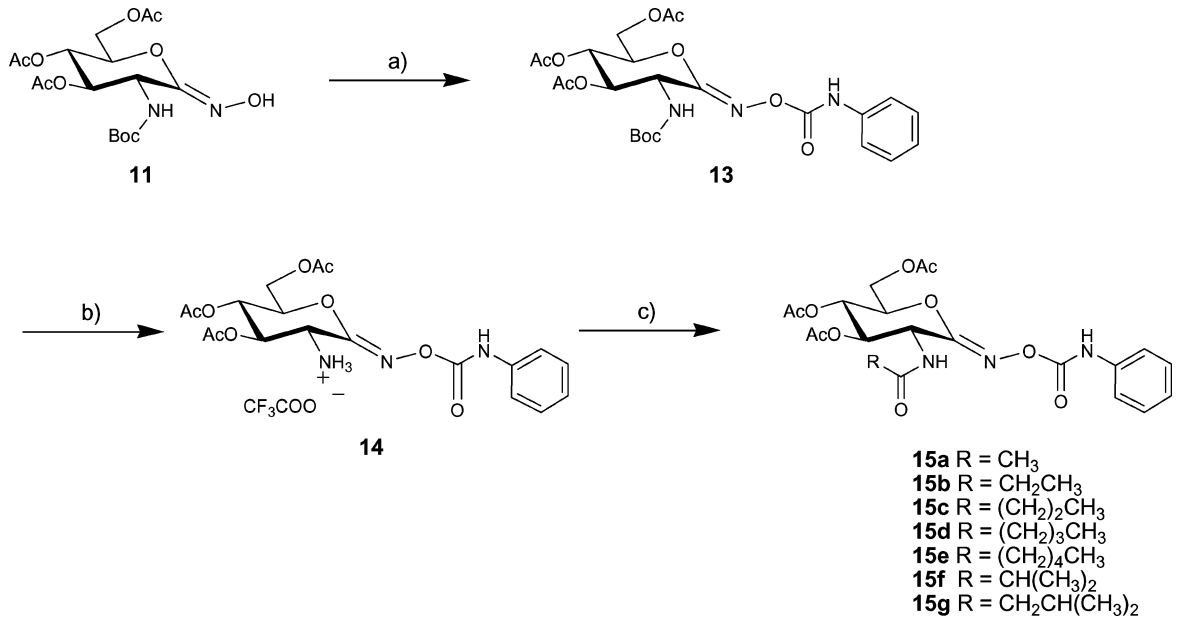
Nonetheless, treating impure hydroximolactone **11** with phenyl isocyanate yields pure carbamate **13** in good yield after purification by flash chromatography (Scheme 2). At this stage of the synthesis we expected that even though **13** contains two carbamate groups, the phenyl carbamate would be more stable to acidic conditions unlike the Boc-carbamate. Indeed, the Boc protecting group is smoothly removed using anhydrous trifluoroacetic acid in dichloromethane. Treating the resulting crude amine salt **14** with the appropriate acyl chloride yields amides **15a–g** in good overall yields. Detailed analyses of **15a** and **15b** support the identities of this entire *N*-acylated series of compounds. Subsequent de-*O*-acetylation of **15a** with saturated ammonia in methanol affords PUGNAc **2** in good yield (Scheme 3). **16a** was obtained from **15b** in a similar fashion. To highlight the ease of these conversions, the common intermediate, **14** can be treated with a range of acyl chlorides to provide **15c–g** as crude products. Immediate de-*O*-acetylation of these crude intermediates readily furnishes the triols **16b–f**, in good yield.

Inhibition studies

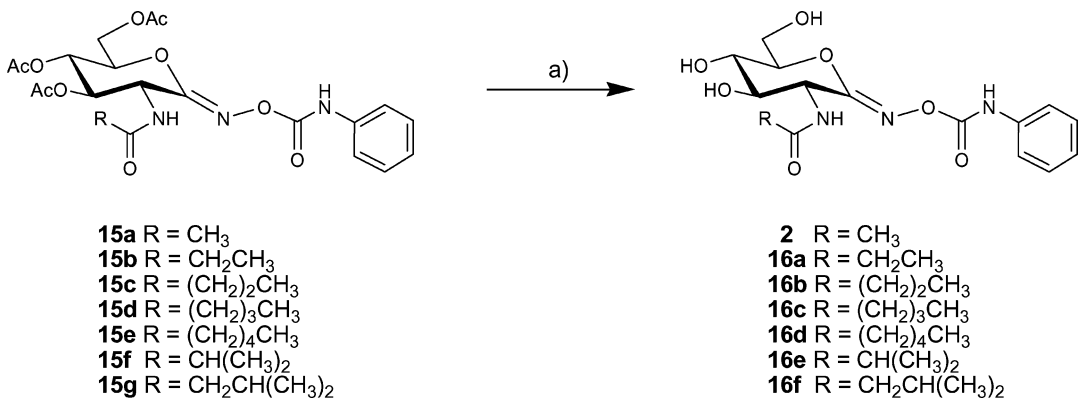
It has been previously established that PUGNAc **2** is a potent competitive inhibitor of both *O*-GlcNAcase[3,26] and β-hexosaminidase.[18,25] For the human enzymes the respective K_I values are 46 nM and 36 nM.[3,21] As described above, selective inhibitors of these enzymes are of interest and we therefore evaluated the selectivity of putative inhibitors **16a–f**. Using *p*NP-GlcNAc **17** as a substrate we found that these compounds are indeed inhibitors of both human *O*-GlcNAcase and human β-hexosaminidase (Table 1).

Analysis of the inhibition of human β-hexosaminidase reveals that increasing the chain length of the *N*-acyl group results in a marked decrease in the potency of these inhibitors (Table 1). The inclusion of only one methylene group (compound **16a**) results in a 33-fold increase in the K_I value for β-hexosaminidase as compared to the parent PUGNAc **2**. Additional increases in chain length lead to still greater increases in K_I values. Furthermore, as we hoped, *O*-GlcNAcase tolerates increases in chain length better than β-hexosaminidase (Table 1).

Interestingly, for these two human enzymes, direct comparison of the K_I values of the previously prepared NAG-thiazoline derivatives, **3a–f**,[21] reveals that the modified thiazolines are better inhibitors than the corresponding PUGNAc-based compounds, **16a–f**. These observations point to the importance of the position of the acyl group of the inhibitor and potentially, the hybridisation



Scheme 2 (a) PhNCO, Et_3N, THF; (b) CF_3COOH, CH_2Cl_2; (c) RC(O)Cl, C_5H_5N.



Scheme 3 (a) NH_3, MeOH.

Table 1 Inhibition constants and selectivities of inhibitors for *O*-GlcNAcase and β-hexosaminidase

Compound	*O*-GlcNAcase K_I/μM	β-hexosaminidase K_I/μM	Selectivity ratio (β-hexosaminidase K_I/*O*-GlcNAcase K_I)
2	0.046[3,21]	0.036[21]	1
16a	1.2	1.2	1
16b	2.4	26	11
16c	40	220	6
16d	210	> 900	≥5
16e	9	20	2
16f	190	> 1200	≥6

of the anomeric carbon. The thiazolines, **1** and **3a–f**, with a sp^3 hybridised anomeric carbon, comprise a bicyclic scaffold that restricts movement of the acyl chain as compared to the acyl chain on the corresponding PUGNAc derivatives, **2** and **16a–f**, that have a sp^2 hybridised anomeric carbon. As well, the precise positioning of the side chain within the active sites must vary between these two sets of compounds. Together, these two factors must contribute to both the overall somewhat poorer inhibition and lesser selectivity of the PUGNAc-derived compounds compared to the thiazoline derivatives. Consistent with these observations is that streptozotocin (STZ) **18**, a poor inhibitor of *O*-GlcNAcase (K_I = 1.5 mM),[21,32,33] also has a bulky, freely rotating acyl chain and shows moderate selectivity for *O*-GlcNAcase over β-hexosaminidase.[21,32,33]

These differences between the thiazoline-based compounds, **1** and **3a–f**, and the PUGNAc derivatives, **2** and **16a–f**, may stem from the former compounds emulating the presumed bicyclic-like transition state involved in the catalytic mechanism of *O*-GlcNAcase[21] and β-hexosaminidase.[20,24,34,35] In contrast, the position of the *N*-acyl group of the PUGNAc analogues and

STZ may resemble that of the natural substrate *N*-acetyl-D-glucopyranoside. The different hybridisation of the anomeric carbons of the PUGNAc and thiazoline derivatives may also contribute to the precise positioning of these *N*-acyl groups. Further crystallographic and kinetic studies defining the molecular basis accounting for these differences between NAG-thiazoline **1** and PUGNAc **2** would be of considerable interest.

Conclusions

We have devised a divergent route that enables the rapid synthesis of a series of PUGNAc-based inhibitors bearing different *N*-acyl groups. All of the prepared compounds were inhibitors of both human *O*-GlcNAcase and human β-hexosaminidase. These compounds, however, exploit differences in active site architectures between these two enzymes, which results in them being selective for *O*-GlcNAcase. This observation is fairly consistent with what has been reported for a set of thiazoline analogues.[21] Despite the somewhat surprising lower selectivity of these PUGNAc analogues as compared to the thiazoline derivatives, these compounds may have different pharmacokinetic properties owing to their different scaffold. Accordingly, these PUGNAc derivatives may prove to be valuable tools for dissecting the role of the *O*-GlcNAc post-translational modification at the cellular and organismal level. Indeed, elaboration of other glycosidase inhibitors has yielded clinically useful compounds targeting entirely different enzymes.[36] As well, using the strategy outlined here, systematic elaboration of other β-*N*-acetyl-glucosaminidase inhibitor scaffolds[37,38] may also yield selective inhibitors for human *O*-GlcNAcase.

Experimental

General

All solvents were dried prior to use. Synthetic reactions were monitored by TLC using Merck Kieselgel 60 F_{254} aluminium-backed sheets. Compounds were detected by charring with a 10% concentrated sulfuric acid in ethanol solution and heating. Flash chromatography under a positive pressure was performed with Merck Kieselgel 60 (230–400 mesh) using specified eluants. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX400 at 400 MHz (100 MHz for ^{13}C) or a Varian AS500 Unity Innova spectrometer at 500 MHz (125 MHz for ^{13}C) (chemical shifts quoted relative to $CDCl_3$ or CD_3OD where appropriate). Elemental analyses of all synthesized compounds used in enzyme assays were performed at the Simon Fraser University or the University of British Columbia Analytical Facility.

Kinetic analysis of *O*-GlcNAcase and *β*-hexosaminidase

All assays were carried out in triplicate at 37 °C for 30 min by using a stopped assay procedure in which the enzymatic reactions (50 μL) were quenched by the addition of a 4-fold excess (200 μL) of quenching buffer (200 mM glycine, pH 10.75). Assays were initiated by the careful addition, *via* pipette, of enzyme (5 μL), and in all cases the final pH of the resulting quenched solution was greater than 10. Time-dependent assay of *O*-GlcNAcase and β-hexosaminidase revealed that enzymes were stable in their respective buffers over the period of the assay: 50 mM NaH_2PO_4, 100 mM NaCl, 0.1% BSA, pH 6.5 and 50 mM citrate, 100 mM NaCl, 0.1% BSA, pH 4.25. The progress of the reaction at the end of 30 min was determined by measuring the extent of 4-nitrophenol liberated as determined by UV measurements at 400 nm using a 96-well plate (Sarstedt) and 96-well plate reader (Molecular Devices). Human placental β-hexosaminidase was purchased from Sigma (lot043K3783), and *O*-GlcNAcase was overexpressed and purified freshly, prior to use.[39] *O*-GlcNAcase and β-hexosaminidase were used in the inhibition assays at a concentration (μg μL^{-1}) of 0.0406 and 0.012, respectively using substrate **17** at a concentration of 0.5 mM. All inhibitors were tested at seven concentrations ranging from three times to 1/3 K_I, with the exception of the assay of inhibitor **16d** with β-hexosaminidase, where such high concentrations of inhibitor could not be reached because of its high K_I value. K_I values were determined by linear regression of data from Dixon plots.

(*E*)- and (*Z*)-3,4,6-Tri-*O*-acetyl-2-tert-butoxycarbonamido-2-deoxy-D-glucose oxime (7). Hydroxylamine hydrochloride (3.2 g, 46 mmol) was added to the hemiacetal **6**[30] (12 g, 31 mmol) and pyridine (6.3 mL, 77 mmol) in MeOH (200 mL) and the resulting solution stirred at reflux (2 h). The solution was concentrated and co-evaporated with toluene (2 × 20 mL). The residue was taken up in EtOAc and washed with water (2 × 50 mL), brine (50 mL), dried ($MgSO_4$), filtered and concentrated to give the presumed oxime **7** (9.5 g). The residue was used without further purification.

3,4,6-Tri-*O*-acetyl-2-tert-butoxycarbonamido-2-deoxy-D-glucono-hydroximo-1,5-lactone (11). (a) 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.98 mL, 6.6 mmol) was added to the crude oxime **7** (2.5 g, 5.9 mmol) in CH_2Cl_2 (60 mL) at −45 °C and the mixture stirred (5 min). *N*-chlorosuccinimide (0.87 g, 6.5 mmol) was then added to the solution in such a way that the temperature did not go above −40 °C and the resulting mixture was allowed to stir for 30 min at this temperature and then was allowed to warm to room temperature over two h. The mixture was quenched with water and diluted with EtOAc (100 mL). The organic layer was separated and washed with water (2 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$) filtered and concentrated. Flash chromatography of the resultant residue (EtOAc/hexanes 2 : 3) gave the title compound **11** as a colourless oil (1.7 g, 68%). The ^{1}H and ^{13}C NMR spectra appeared to show the compound of interest but was contaminated with the 1,4-lactone oxime **12** and succinimide.

(b) 1,8-Diazabicyclo[5.4.0]undec-7-ene (3.0 mL, 20 mmol) was added to the oxime **7** (7.7 g, 18 mmol) and *N*-chlorosuccinimide (2.7 g, 20 mmol) in CH_2Cl_2 (110 mL) at −45 °C in such a way that the temperature did not go above −40 °C and the resulting mixture was allowed to stir for 30 min at this temperature and then was allowed to warm to room temperature over two h. The mixture subsequently treated as in (a) to give the title compound **11** (4 g, 52%§). R_f 0.16 (EtOAc/hexane 1 : 1); δ_H (500 MHz, CD_3OD): 9.12 (*br* s, 1H, NOH), 5.33–5.18 (m, 3H, H3, H4, NH), 4.60–4.52 (m, 1H, H2), 4.33 (dd, J = 3.5, 12.5, 1H, H6), 4.33 (dd, J = 2.0, 1H, H6), 4.19 (ddd, J = 9.5, 1H, H5), 2.08 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.40 (s, 9H, $C(CH_3)_3$). δ_C (125 MHz, CD_3OD): 171.5, 170.5, 170.3, 169.0 (4C, C=O), 150.7 (C1), 76.5, 72.4, 67.3 (3C, C3, C4, C5), 61.5 (C6), 50.8 (C2), 29.6 ($C(CH_3)_3$), 28.2 ($C(CH_3)_3$), 20.7, 20.6, 20.5 (3C, CH_3).

§ The quoted yield does not include the 5% (w/w) of succinimide in the ^{1}H and ^{13}C NMR spectra.

***O*-(3,4,6-Tri-*O*-acetyl-2-tert-butoxycarbonamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (13).** Phenyl isocyanate (0.5 mL, 3.7 mmol) was added to the lactone **11** (1.3 g, 3.1 mmol) and Et_3N (1.3 mL, 9.3 mmol) in THF (50 mL) and the solution stirred (r.t., 3 h). Concentration followed by flash chromatography of the resultant residue (EtOAc/hexanes 1:4) yielded the carbamate **13** as a colourless oil (1.2 g, 71%). R_f 0.65 (EtOAc/hexane 1:1); δ_H (500 MHz, $CDCl_3$): 7.83 (*br* s, 1H, PhN*H*), 7.42 (m, 2H, Ar), 7.32 (m, 2H, Ar), 7.11 (m, 1H, Ar), 5.38–5.30 (m, 2H, H3, H4), 5.18 (*br* s, 1H, NH), 4.62 (m, 1H, H2), 4.45–4.40 (m, 2H, H5, H6), 4.31 (dd, $J = 3.5$, 13.5, 1H, H6), 2.12 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 1.44 (s, 9H, $C(CH_3)_3$). δ_C (125 MHz, $CDCl_3$): 171.1, 170.3, 170.0, 169.1 (4C, C=O), 155.0 (*C*ONHPh), 151.4 (C1), 136.8, 129.1, 124.2, 119.4 (4C, Ar), 77.2, 71.0, 67.2 (3C, C3, C4, C5), 61.2 (C6), 51.1 (C2), 30.6 ($C(CH_3)_3$), 28.2 ($C(CH_3)_3$), (20.7, 20.6, 20.5 (3C, CH_3); Anal. calcd for $C_{24}H_{31}N_3O_{11}$: C, 53.63; H, 5.81; N, 7.82. Found: C, 53.72; H, 5.78; N, 7.78%.

General procedure for the preparation of the *O*-(3,4,6-tri-*O*-acetyl-2-acylamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamates (15a–g). Trifluoroacetic acid (13 mmol) was added to the carbamate **13** (1 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the solution stirred (2 h). Pyridine (200 mmol) was then slowly added to the solution and the resulting mixture left to stand (0 °C, 10 min). The appropriate acyl chloride (3 mmol) was then added at 0 °C and the solution allowed to stand at 4 °C overnight. Concentration of the mixture gave a yellowish residue which was dissolved in EtOAc (30 mL) and washed with (2 × 20 mL), brine (1 × 20 mL), dried ($MgSO_4$) filtered and concentrated. For the presumed intermediate tri-*O*-acetates **15c–g** these were carried through without further purification. Flash chromatography of the residues presumably **15a** and **15b** (EtOAc/hexanes 1 : 1) gave the desired acyl derivatives **15a** and **15b** in yields of 48% and 42% respectively.

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (15a).** Gave ^1H and ^{13}C NMR spectra consistent with that found in the literature.[27] (48%) R_f 0.2 (EtOAc/hexane 7 : 3).

***O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-propamido-D-glucopyranosylidene)amino *N*-phenylcarbamate (15b).** (42%) R_f 0.11 (EtOAc/hexane 1 : 1); δ_H (500 MHz, $CDCl_3$): 7.71 (*br* s, 1H, PhN*H*), 7.41 (m, 2H, Ar), 7.32 (m, 2H, Ar), 7.11 (m, 1H, Ar), 6.59 (d, $J = 7.5$, 1H, NH), 5.40 (dd, $J = 9.0$, 1H, H3), 5.35 (dd, $J = 9.0$, 1H, H4), 4.89 (dd, 1H, H2), 4.53 (ddd, $J = 3.0$, 4.0, 1H, H5), 4.43 (dd, $J =$ 13.0, 1H, H6), 4.32 (dd, 1H, H6), 2.22 (q, $J = 7.5$, CH_2), 2.13 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 1.14 (t, 3H, CH_3). δ_C (125 MHz, $CDCl_3$): 174.2, 170.4, 170.2, 169.1 (4C, C=O), 155.1 (*C*ONHPh), 151.7 (C1), 136.8, 129.2, 124.3, 119.2 (4C, Ar), 77.2, 71.2, 67.1 (3C, C3, C4, C5), 61.2 (C6), 49.6 (C2), 29.4 (CH_2), 20.7, 20.6, 20.5 (3C, $COCH_3$), 9.7 (CH_3).

General procedure for the preparation of the *O*-(2-acylamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamates 2, 16a–f. A saturated solution of ammonia in MeOH (2 mL) was added to the carbamate (0.3 mmol) in MeOH (10 mL) and the solution left to stand (r.t., 2 h). Concentration followed by flash chromatography of the residue (MeOH/EtOAc 3 : 97) gave the desired triols **2**, **16a–f** in yields ranging from 21% to 32%.

***O*-(2-Acetamido-2-deoxy-D-glucopyranosylidene)amino *N*-phenylcarbamate (2).** (32%) Gave ^1H and ^{13}C NMR spectra consistent with that found in the literature.[27] R_f 0.15 (MeOH/EtOAc 1 : 19).

***O*-(2-Deoxy-2-propamido-D-glucopyranosylidene)amino *N*-phenylcarbamate (16a).** (32%) R_f 0.11 (MeOH/EtOAc 1 : 19); δ_H (500 MHz, CD_3OD): 7.41 (m, 2H, Ar), 7.26 (m, 2H, Ar), 7.03 (m, 1H, Ar), 4.54 (m, 1H, H2), 3.96–3.93 (m, 2H, H5, H6), 3.83 (dd, $J = 4.0$, 12.5, 1H, H6), 3.75–3.71 (m, 2H, H3, H4), 2.31 (q, $J = 7.5$, CH_2), 1.16 (t, 3H, CH_3); δ_C (125 MHz, CD_3OD): 177.5 (C=O), 159.5 (*C*ONHPh), 154.7 (C1), 139.3, 129.9, 124.8, 120.3 (4C, Ar), 84.1, 74.4, 69.9 (3C, C3, C4, C5), 61.8 (C6), 52.9 (C2), 30.2 (CH_2), 10.2 (CH_3); Anal. calcd for $C_{16}H_{21}N_3O_7.H_2O$: C, 50.00; H, 5.77; N, 10.93. Found: C, 50.02; H, 5.78; N, 10.76%.

***O*-(2-Deoxy-2-butamido-D-glucopyranosylidene)amino *N*-phenylcarbamate (16b).** (26%) R_f 0.26 (MeOH/EtOAc 1 : 19); δ_H (500 MHz, CD_3OD): 7.41 (m, 2H, Ar), 7.27 (m, 2H, Ar), 7.03 (m, 1H, Ar), 4.54 (m, 1H, H2), 3.94–3.92 (m, 2H, H5, H6), 3.83 (dd, $J = 4.5$, 13.0, 1H, H6), 3.74–3.70 (m, 2H, H3, H4), 2.26 (t, $J =$ 6.0, $COCH_2$), 1.66 (m, 2H, CH_2CH_3), 0.96 (t, $J = 7.0$, 3H, CH_3); δ_C (100 MHz, CD_3OD): 176.7 (C=O), 159.5 (*C*ONHPh), 154.7 (C1), 139.3, 130.0, 124.8, 120.3 (4C, Ar), 84.1, 74.5, 69.9 (3C, C3, C4, C5), 61.8 (C6), 52.9 (C2), 39.1 (CH_2), 20.3 (CH_2), 14.1 (CH_3); Anal. calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02. Found: C, 53.52; H, 6.09; N, 10.90%.

***O*-(2-Deoxy-2-valeramido-D-glucopyranosylidene)amino *N*-phenylcarbamate (16c).** (23%) R_f 0.43 (MeOH/EtOAc 1 : 19); δ_H (400 MHz, CD_3OD): 7.45 (m, 2H, Ar), 7.30 (m, 2H, Ar), 7.03 (m, 1H, Ar), 4.58 (m, 1H, H2), 3.99–3.94 (m, 2H, H5, H6), 3.87 (dd, $J = 4.0$, 12.4, 1H, H6), 3.79–3.75 (m, 2H, H3, H4), 2.33 (t, $J =$ 7.2, $COCH_2$), 1.64 (m, 2H, CH_2CH_2), 1.40 (m, 2H, CH_2CH_2), 0.92 (t, $J = 7.2$, 3H, CH_3); δ_C (100 MHz, CD_3OD): 176.8 (C=O), 159.5 (*C*ONHPh), 154.7 (C1), 139.3, 130.0, 124.8, 120.4 (4C, Ar), 84.1, 74.3, 69.9 (3C, C3, C4, C5), 61.8 (C6), 52.9 (C2), 37.0 (CH_2), 29.0 (CH_2), 23.5 (CH_2), 14.2 (CH_3); Anal. calcd for $C_{18}H_{25}N_3O_7.2H_2O$: C, 50.11; H, 6.34; N, 9.79. Found: C, 50.25; H, 6.04; N, 9.94%.

***O*-(2-Deoxy-2-hexamido-D-glucopyranosylidene)amino *N*-phenylcarbamate 16d.** (26%) R_f 0.48 (MeOH/EtOAc 1 : 19); δ_H (500 MHz, CD_3OD): 7.43 (m, 2H, Ar), 7.28 (m, 2H, Ar), 7.05 (m, 1H, Ar), 4.55 (m, 1H, H2), 3.97–3.92 (m, 2H, H5, H6), 3.85 (dd, $J = 4.0$, 12.5, 1H, H6), 3.77–3.72 (m, 2H, H3, H4), 2.30 (t, $J = 7.5$, $COCH_2$), 1.66 (m, 2H, $COCH_2CH_2$), 1.36–1.27 (m, 4H, CH_2CH_2), 0.87 (t, $J = 6.5$, 3H, CH_3); δ_C (125 MHz, CD_3OD): 176.8 (C=O), 159.5 (*C*ONHPh), 154.7 (C1), 139.3, 130.0, 124.8, 120.4 (4C, Ar), 84.1, 74.3, 69.9 (3C, C3, C4, C5), 61.8 (C6), 52.9 (C2), 37.2 (CH_2), 32.6 (CH_2), 26.6 (CH_2), 23.5 (CH_2), 14.3 (CH_3); Anal. calcd for $C_{19}H_{27}N_3O_7$: C, 55.74; H, 6.65; N, 10.26. Found: C, 55.55; H, 6.86; N, 9.99%.

***O*-(2-Deoxy-2-isobutamido-D-glucopyranosylidene)amino *N*-phenylcarbamate (16e).** (29%) R_f 0.22 (MeOH/EtOAc 1 : 19); δ_H (500 MHz, CD_3OD): 7.42 (m, 2H, Ar), 7.27 (m, 2H, Ar), 7.03 (m, 1H, Ar), 4.52 (m, 1H, H2), 3.96–3.92 (m, 2H, H5, H6), 3.83 (dd, $J = 4.5$, 12.5, 1H, H6), 3.77–3.71 (m, 2H, H3, H4), 2.54 (m, $J = 7.0$, CH), 1.16 (d, 3H, CH_3), 1.14 (d, 3H, CH_3); δ_C (125 MHz, CD_3OD): 180.6 (C=O), 159.4 (*C*ONHPh), 154.7 (C1), 139.3, 130.1, 124.8, 120.4 (4C, Ar), 84.1, 74.3, 69.9 (3C, C3, C4, C5), 61.8 (C6), 52.8

(C2), 36.4 (CH), 19.9, 19.8 (2C, CH_3); Anal. calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02. Found: C, 53.17; H, 6.18; N, 10.87%.

***O*-(2-Deoxy-2-isovaleramido-D-glucopyranosylidene)amino *N*-phenylcarbamate (16f).** (23%) R_f 0.47 (MeOH/EtOAc 1 : 19); δ_H (400 MHz, CD_3OD): 7.44 (m, 2H, Ar), 7.30 (m, 2H, Ar), 7.06 (m, 1H, Ar), 4.59 (m, 1H, H2), 3.98–3.95 (m, 2H, H5, H6), 3.86 (dd, $J = 4.4$, 12.8, 1H, H6), 3.77–3.74 (m, 2H, H3, H4), 2.17 (d, $J = 7.5$, $COCH_2$), 2.12, (m, 1H, $CH(CH_3)_2$), 0.98 (m, 6H, CH_3); δ_C (100 MHz, CD_3OD): 176.0 (C=O), 159.5 (*C*ONHPh), 154.7 (C1), 139.3, 130.0, 124.8, 120.4 (4C, Ar), 84.1, 74.5, 69.9 (3C, C3, C4, C5), 61.8 (C6), 52.9 (C2), 46.5 (CH_2), 27.5 ($CH(CH_3)_2$), 23.0, 22.9 (2C, CH_3); Anal. calcd for $C_{18}H_{25}N_3O_7$: C, 54.68; H 6.37; N, 10.63. Found: C, 54.33; H, 6.48; N, 10.56%.

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