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Synthesis of pyrrolidine-based analogues of 2-acetamidosugars as N-acetyl glucosaminidase inhibitors

Anh Tuan Tran, Bo Luo, Jagadeesh Yerri, Nicolas Aubeger, Jérôme Désiré, Shinpei Nakagawa, Atsushi Kato, Yongmin Zhang, Yves Blériot, * Matthieu Sollogoub

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Abstract. A ring-contraction strategy applied to β-azido,γ-hydroxyazepanes yielded after functional group manipulation new tetrahydroxylated pyrrolidines displaying an acetamido moiety, one of these iminosugars demonstrating low micromolar inhibition on N-acetylglucosaminidases.

Keywords. Iminosugars, Ring-contraction, Glycosidase inhibitors, Pyrrolidines

Highlights.
-A new family of amino-functionalised pyrrolidines was synthesized.
-A ring-contraction of β-azido,γ-hydroxyazepanes was used.
-A low micromolar inhibitor of β-N-acetyhexosaminidase identified.
Polyhydroxylated pyrrolidines\textsuperscript{1,2} are well-established powerful glycosidase inhibitors, even though their analogy with hexopyranoses, and therefore the structural basis of their inhibition, are less straightforward than for the corresponding piperidines.\textsuperscript{3} Hexosaminidases are a very important class of glycosidases that cleave the pyranosidic N-acetyl-D-glucosamine unit from glycoconjugates. Several pyrrolidines bearing an acetamide group have been reported as potent hexosaminidases inhibitors. Interestingly, only one naturally occurring acetamido-containing pyrrolidine was isolated so far: Pochonicine.\textsuperscript{4-6} The main classes of synthetic nitrogen functionalized polyhydroxylated pyrrolidines are represented in Figure 1, the most studied one being the 2,5-dideoxy-2,5-imino-hexitols \textbf{A}\textsuperscript{7,21} but other scaffolds such as \textbf{B},\textsuperscript{22-24} \textbf{C},\textsuperscript{25-26} \textbf{D},\textsuperscript{27} \textbf{E}\textsuperscript{28} and \textbf{F}\textsuperscript{8} have also been prepared. It is rather striking that structure \textbf{G}, which can be seen as a combination of \textbf{A} and \textbf{E} possessing as many hydroxyl groups as the hexosaminidase substrate and product, has never been synthesized and assessed as a hexosaminidase inhibitor. The present study reports the synthesis and hexosaminidase inhibitory evaluation of molecules derived from scaffold \textbf{G}. (Figure 1)
In the course of our studies aimed at the synthesis of GlcNAc-like piperidine homoisomimosugars exploiting a ring-contraction methodology, a 2,3-trans-2-hydroxy,3-azido-azepane was required and obtained from the unsaturated 7-membered ring 1. The obvious synthetic route transits via the formation of an epoxide, followed by its azidolysis. We observed that it was possible to operate the epoxidation with some degree of stereocontrol to afford either epoxides 2 or 3 as the main products. These latter could then be opened using sodium azide to give, in both cases, a significant amount of the 2-azido derivatives 4 and 6 together with the desired 3-azido compounds 5 and 7. (Scheme 1)
Scheme 1: Synthesis of azidoazepanes 4-7. Reagents and conditions: a) Oxone, D-epoxone, NaHCO₃, 2: 54%, b) Oxone, CF₃COCH₃, NaHCO₃, 2: 29%, 3: 51%; c) NaN₃, NH₄Cl, DMF/H₂O, 90°C

Compounds 4 and 6 are also suitable candidates for a ring contraction reaction to give pyrrolidine derivatives through a γ-aminoalcohol rearrangement. Hence, we decided to apply the TFAA-mediated ring contraction conditions developed by Cossy to β-azidoazepanes 4 and 6 that were first converted into the N-benzyl derivatives 8 (80%) and 13 (68%) respectively, using TFA followed by N-benzylation (BnBr, K₂CO₃). Their ring contraction with TFAA furnished the azidopyrrolidines 9 (93%) and 14 (86%) respectively in good yield. Reduction of the azide moiety (PPh₃, THF/H₂O) followed by N-acetylation was achieved to provide the acetamide 10 (80%) and 15 (60%) respectively. Final O-deacetylation followed by hydrogenolysis yielded the target pyrrolidines 11 (95%) and 16 (88%). Compound 9 was also directly submitted to the action of hydrogen in the presence of Pd/C to give the diamine 12 in 95% yield as its hydrochloride salt (Scheme 2).
Scheme 2: Synthesis of NHAc derived pyrrolidines 11 and 16. Reagents and conditions: a) i) TFA, DCM; ii) BnBr, K$_2$CO$_3$, EtOAc/H$_2$O; b) i) Trifluoroacetic anhydride (TFAA), Et$_3$N, toluene, reflux, ii) 10% aq. NaOH; c) i) PPh$_3$, THF/H$_2$O, 80°C, ii) Pyridine, Ac$_2$O; d) i) Et$_3$N, MeOH, H$_2$O, ii) H$_2$, Pd/C, MeOH, HCl; e) H$_2$, Pd/C, MeOH, HCl

The ring contraction reaction is initiated by the esterification of the free hydroxyl group in azepane 13 to give intermediate H, in which the amine displaces this leaving group to produce the fused pyrrolidine-azetidinium ion I. Nucleophilic ring opening at the less hindered carbon affords pyrrolidine J which leads to the five-membered iminosugar 14 upon saponification. The stereochemistry of the ring-contracted product is the one expected by this mechanism as attested by the NOE cross-correlation between H-3 and H-5 on 14. (Scheme 3)
The three pyrrolidines 11, 12 and 16 were assayed as inhibitors of a panel of hexosaminidases and β-glucuronidases. Iminosugar 11 is a moderate inhibitor of β-N-acetylglucosaminidases with IC$_{50}$ in the high micromolar range. The present work revealed that inversion of C-1 side chain in 11 to give 16 significantly enhanced its inhibition potency against these enzymes, pyrrolidine 16 demonstrating potent Jack bean β-N-acetylglucosaminidase inhibition, with a IC$_{50}$ value of 3.4 µM. In contrast, replacement of the acetamide group by an amine as in 12 is detrimental to hexosaminidase inhibition (Table 1). It is noteworthy that pyrrolidine 11 showed low micromolar inhibition against bovine liver and *E. coli* β-glucuronidase, with IC$_{50}$ values of 26 and 15 µM, respectively. Previous study suggested that β-glucuronidase recognized uronic acid and carboxylic acid part is required for tight binding.$^{37,38}$ Thus, pyrrolidine 11 is an interesting case for β-glucuronidase inhibition.
Table 1. Concentration of iminosugars giving 50 % inhibition of various glycosidases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC_{50} (µM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>β-N-Acetylgalactosaminidase</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>241</td>
<td>NI (0%) 323</td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>181</td>
<td>NI (0%) 31</td>
</tr>
<tr>
<td>HL60</td>
<td>538</td>
<td>NI (6.6%) 18</td>
</tr>
<tr>
<td>Human placenta</td>
<td>597</td>
<td>NI (0%) 15</td>
</tr>
<tr>
<td>Jack bean</td>
<td>61</td>
<td>NI (7.3%) 3.4</td>
</tr>
<tr>
<td>α-N-Acetylgalactosaminidase</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Chicken liver</td>
<td>NI (26.2%)</td>
<td>NI (3.3%) NI (7.2%)</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine liver</td>
<td>26</td>
<td>NI (0%) NI (48.5%)</td>
</tr>
<tr>
<td>E.coli</td>
<td>15</td>
<td>NI (17.8%) 145</td>
</tr>
</tbody>
</table>

^aNI : No inhibition (less than 50% inhibition at 1000 µM).
^b(   ) : inhibition % at 1000 µM

In conclusion, a ring-contraction methodology applied to seven-membered iminosugars bearing an azido group in β position furnished a low micromolar hexosaminidase inhibitor after conversion of the azide function into an acetamide and final deprotection. This work complements previous work on the conversion of polyhydroxylated azepanes into six-membered NHAc-homoiminosugars.

1. Experimental

1.1 Material and methods

All commercial reagents were used as supplied. Solvents (DMF, THF) were distilled under anhydrous conditions. TLC plates (Macherey-Nagel, ALUGRAM® SIL G/UV_{254}, 0.2 mm silica gel 60 Å) were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of 3 g of phosphomolybdic acid in 100 mL of ethanol followed by heating with a heat gun. Flash column
chromatography was performed using Macherey-Nagel silica gel 60 (15-40 µm). NMR experiments were recorded with a Bruker AM-400 spectrometer at 400 MHz for $^1$H nuclei and at 100 MHz for $^{13}$C nuclei. The chemical shifts are expressed in part per million (ppm) using residual CHCl$_3$ signal as internal reference ($\delta(^1H) = 7.26$ ppm and $\delta(^{13}C) = 77.16$ ppm) and the coupling constant $J$ in hertz (Hz). NMR multiplicities are reported using the following abbreviations: b = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. HRMS were recorded on a Bruker microTOF spectrometer, using Tuning-Mix as reference. Optical rotations were measured on a Perkin–Elmer 341 digital polarimeter or a Jasco P-2000 polarimeter with a path length of 1 dm.

1.2 tert-butyl (2R,3R,4R,5R,6S)-6-azido-3,4-bis(benzyl oxy)-2-((benzyl oxy)methyl)-5-hydroxyazepane-1-carboxylate (4)

Known epoxide $^2$ (465 mg, 0.853 mmol) was dissolved in a DMF/H$_2$O mixture (9.0/1.0 mL), then NaN$_3$ (277 mg, 4.26 mmol) and NH$_4$Cl (226 mg, 4.26 mmol) were added. The resulting mixture was stirred at 90 ºC for 3 days. After being cooled to room temperature, EtOAc and H$_2$O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried over MgSO$_4$, filtered and evaporated. The residue was purified by flash chromatography (Cy/EtOAc: 9/1) to give 4 (285 mg, 57%) as colorless oil and 5 (160 mg, 32%) $\alpha_D$ +18.6 ($c = 1.0$, CHCl$_3$) $^1$H NMR (400 MHz, CDCl$_3$, 2 rotamers): 7.39-7.26 (m, 26H, H$_{ar}$), 7.23-7.22 (m, 4H, H$_{ar}$), 4.81 (d, 1H, $^2J = 11.5$ Hz, CH$_2$Ph), 4.75 (d, 1H, $^2J = 12.0$ Hz, CH$_2$Ph), 4.69-4.59 (m, 4H, CH$_2$Ph), 4.49-4.40 (m, 6H, CH$_2$Ph), 4.02-3.91 (m, 6H, H$_8a$, H$_2$, H$_3$, H$_3'$, H$_5$, H$_5$), 3.87-3.53 (m, 12H, H$_8b$, H$_8a'$, H$_8b'$, H$_2'$, H$_4$, H$_4'$, H$_6$, H$_6'$, H$_7a$, H$_7b$, H$_7a'$, H$_7b'$), 2.57 (bs, 0.8H, OH), 2.52 (bs, 0.8H, OH'), 1.49 (s, 9H, CH$_3$, Boc), 1.41 (s, 9H, CH$_3$, Boc); $^{13}$C NMR (100 MHz, CDCl$_3$, 2 rotamers): $\delta$ 155.4, 155.1 (CO, Boc), 138.3, 138.1, 138.0, 138.0, 137.9, 137.8 (C$_{ipso}$) 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5 (CH$_{ar}$), 81.8, 81.4 (C$_4$, C$_4'$), 80.3, 80.2 (C(CH$_3$)$_3$, Boc) 74.4, 74.3 (C$_3$, C$_3'$), 74.0, 74.0, 73.4, 72.9 (2C), 72.8 (CH$_2$Ph), 72.5, 72.4 (C$_5$, C$_5'$), 69.5, 69.0 (C$_8$, C$_8'$), 63.1, 62.4 (C$_6$, C$_6'$), 58.9 (2C, C$_2$, C$_2'$), 45.1, 43.7 (C$_7$, C$_7'$), 28.3, 28.2 (CH$_3$, Boc); ESI-HRMS calcd. for C$_{33}$H$_{40}$N$_4$NaO$_6$ [M+Na$^+$]: 611.2846, found 611.2840.
1.3 (3S,4R,5R,6R,7R)-3-azido-1-benzyl-5,6-bis(benzyloxy)-7-((benzyl-oxymethyl)azepan-4-ol (8)

To a solution of 4 (46 mg, 0.078 mmol) in CH$_2$Cl$_2$ (2.0 mL) was added trifluoroacetic acid (2.0 mL) and the solution was stirred at room temperature for 1 hour. The solvents were evaporated and co-evaporated with toluene to remove completely the TFA. The obtained residue was dissolved in a mixture of EtOAc/H$_2$O (5.0/0.5 mL) and BnBr (13 µL, 0.101 mmol), K$_2$CO$_3$ (32 mg, 0.234 mmol) were added respectively. The mixture was refluxed for 18h. After being cooled to room temperature, H$_2$O and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc. Then the organic layer was dried over MgSO$_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Cy/EtOAc: 8.5/1.5) to give 8 as colorless oil (36 mg, 80%). [$\alpha$]$_D$ +19.0 (c = 1.0, CHCl$_3$); $^1$H (400 MHz, CDCl$_3$): 7.37-7.19 (m, 20H, H$_{ar}$), 4.74 (d, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 4.69 (d, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 4.54 d, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 4.41 (s, 2H, 2xCH$_2$Ph), 4.36 (d, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 4.10 (ddd, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 4.05 (dd, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 4.01 (d, 1H, $^2$J = 11.5 Hz, CH$_2$Ph), 3.92 (d, 1H, $^2$J = 11.5 Hz, NCH$_2$Ph), 3.82-3.75 (m, 2H, H$_3$, H$_6$), 3.68 (dd, 1H, $^2$J = 11.5 Hz, NCH$_2$Ph), 3.33 (dd, 1H, $^2$J = 11.5 Hz, H$_{7a}$), 3.03 (dt, 1H, $^2$J = 11.5 Hz, H$_{7a}$), 2.69 (dd, 1H, $^2$J = 11.5 Hz, H$_{7b}$), 2.55 (d, 1H, $^2$J = 11.5 Hz, H$_{7b}$), 2.29 (d, 1H, $^2$J = 11.5 Hz, H$_{7b}$), 1.44 (s, 2H, NCH$_2$Ph), 1.30 (s, 2H, NCH$_2$Ph), 0.96 (s, 2H, NCH$_2$Ph), 0.82 (s, 2H, NCH$_2$Ph), 0.60 (s, 2H, NCH$_2$Ph); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 140.1, 138.3, 138.2, 138.1 (C$_{ipso}$), 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.0 (CH$_{ar}$), 82.1 (C$_4$), 76.6 (C$_3$), 73.8 (CH$_2$Ph), 73.2 (2C, C$_5$, CH$_2$Ph), 72.7 (CH$_2$Ph), 68.9 (C$_8$), 63.9 (C$_6$), 63.5 (C$_2$), 57.2 (NCH$_2$Ph), 51.6 (C$_7$); ESI- HRMS calcd. for C$_{35}$H$_{39}$N$_4$O$_4$ [M+H]$^+$: 579.2971, found 579.2975

1.4 (R)-2-azido-2-((2S,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyl-oxymethyl)pyrrolidin-2-yl)ethan-1-ol (9)

To a solution of 8 (60 mg, 0.104 mmol) in toluene (1.0 mL) were added trifluoroacetic anhydride (28 µL, 0.194 mmol) and Et$_3$N (26 µL, 0.194 mmol). The obtained solution was refluxed for 3h and cooled to room temperature. A solution of
NaOH (10%, 5 mL) was added and the mixture was stirred for 30 minutes. EtOAc and H$_2$O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried on Na$_2$SO$_4$, filtered and evaporated. The obtained crude was purified by flash chromatography (Cy/EtOAc: 9/1) to give compound 9 (55 mg, 92%). $[\alpha]_D +1.3$ (c = 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): δ 7.38-7.27 (m, 18H, H$_{ar}$), 7.18-7.17 (m, 2H, H$_{ar}$), 4.60 (d, 1H, $^2$J = 12.0 Hz, CH$_2$Ph), 4.56 (d, 1H, $^2$J = 12.0 Hz, CH$_2$Ph), 4.53 (s, 2H, CH$_2$Ph), 4.27 (s, 2H, CH$_2$Ph), 4.15-4.09 (m, 2H, H$_3$, H$_4$), 4.07 (d, 1H, $^2$J = 14.0 Hz, NCH$_2$Ph), 3.45 (t, 1H, $^J$H$_5$-$^J$H$_4$ = $^J$H$_5$-$^J$H$_6$ = 6.5 Hz, H$_5$), 3.36-3.31 (m, 1H, H$_8$), 3.18-3.12 (m, 2H, H$_{8a}$, H$_2$); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 138.2, 138.1, 138.1, 137.4 (C$_{ipso}$), 129.4, 128.4, 128.2, 127.7, 127.7, 127.5, 127.5, 127.4 (CH$_{ar}$), 83.5 (C$_4$), 81.5 (C$_3$), 72.8, 72.4, 71.7 (CH$_2$Ph), 69.8 (C$_8$), 67.3 (C$_5$), 66.4 (C$_2$), 63.6 (C$_7$), 62.6 (C$_6$), 61.5 (NCH$_2$Ph); ESI-HRMS calcd. for C$_{35}$H$_{39}$N$_4$O$_4$ [M+H]$^+$: 579.2971, found 579.2947.

1.5 (R)-2-acetamido-2-((2R,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethyl acetate (10)

To a solution of azide 9 (28 mg, 0.044 mmol) in THF/H$_2$O (2.0 mL/1.0mL) was added Ph$_3$P (35 mg, 0.132 mmol) and the resulting solution was stirred at 65 °C for 2h. The solution was cooled to room temperature, solvents were evaporated and the crude was dried for 2h under reduced pressure. The residue was dissolved in pyridine (2.0 mL) and Ac$_2$O (1.0 mL) was added at 0°C. The resulting solution was then stirred for 12 h at room temperature. Pyridine and Ac$_2$O were removed by evaporation and co-evaporation with toluene (5 x 3 mL). The residue was purified by flash chromatography (cyclohexane/AcOEt: 6/4) to give 10 (22 mg, 76%). $[\alpha]_D$ -22.9 (c = 1.05, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): 7.36-7.22 (m, 16H, H$_{ar}$), 7.19-7.15 (m, 4H, H$_{ar}$), 4.73-4.67 (m, 1H, H$_6$), 4.62 (d, 1H, $^2$J = 12.0 Hz, CH$_2$Ph), 4.51 (d, 1H, $^2$J = 12.0 Hz, CH$_2$Ph), 4.41 (d, 1H, $^2$J = 10.5 Hz, CH$_2$Ph), 4.34 (d, 1H, $^2$J = 12.0 Hz, CH$_2$Ph), 4.31(d, 1H, $^2$J = 10.5 Hz, CH$_2$Ph), 4.19 (d, 1H, $^2$J = 12.0 Hz, CH$_2$Ph), 4.15 (dd, 1H, $^J$H$_{7a}$-$^J$H$_6$ = 6.5 Hz, $^J$H$_{7a}$-$^J$H$_{7b}$ = 10.5 Hz, H$_7$), 4.11 (dd, 1H, $^J$H$_{7b}$-$^J$H$_6$ = 6.5 Hz, $^J$H$_{7b}$-$^J$H$_{7a}$ = 10.5 Hz, H$_7$), 4.02-3.98 (m, 3H, H$_3$, H$_4$, NCH$_2$Ph), 3.55 (d, 1H, $^2$J = 13.0 Hz, NCH$_2$Ph), 3.27 (t, 1H, $^J$H$_5$-$^J$H$_4$ = $^J$H$_5$-$^J$H$_6$ = 4.5 Hz, H$_5$), 3.20-3.14 (m, 2H, H$_8$), 2.91 (dd, $^J$H$_{8a}$-$^J$H$_8b$ = 10.0 Hz, $^J$H$_{8b}$-$^J$H$_8a$ = 16.0 Hz, H$_8$), 2.05 (s, 3H, CH$_3$, Ac), 1.77 (s, 3H, CH$_3$,
A solution of 10 (20 mg, 0.314 mmol) in MeOH/H₂O/Et₃N (4/0.5/0.5 mL) was stirred for 18h at room temperature. The solvents were evaporated and co-evaporated three times with toluene. The obtained residue was dissolved in MeOH (2 mL) and aqueous HCl (1M, 0.2 mL) was added under argon. After addition of Pd/C (10%, 20 mg), the argon was removed. The H₂ was introduced and the mixture was bubbled for 5 minutes. After stirring the solution for 24 under H₂ atmosphere, the mixture was filtered on micro-filter (0.3 µm). The solvent was evaporated to give compound 11 (6 mg, 82%) as a white solid. [α]¹⁸_D +22.7 (c = 0.5, MeOH); ¹H NMR (400 MHz, D₂O): 4.46 (dt, 1H, \( J_{H6-H7a} = J_{H6-H7b} = 5.5 \text{ Hz}, J_{H6-H5} = 10.5 \text{ Hz}, H_6 \)), 4.23 (d, 1H, \( J_{H4-H5} = 2.5 \text{ Hz}, H_4 \)), 4.13 (dd, 1H, \( J_{H3-H4} = 1.0 \text{ Hz}, J_{H3-H2} = 2.0 \text{ Hz}, H_3 \)), 4.01 (dd, 1H, \( J_{H8a-H2} = 5.0 \text{ Hz}, J_{H8a-H8b} = 12.0 \text{ Hz}, H_8a \)), 3.95-3.87 (m, 2H, \( H_8b \), \( H_5 \)), 3.84 (dd, 1H, \( J_{H7a-H6} = 5.5 \text{ Hz}, J_{H7b-H7a} = 12.0 \text{ Hz}, H_7a \)), 3.77 (dd, 1H, \( J_{H7b-H6} = 5.5 \text{ Hz}, J_{H7a-H7b} = 12.0 \text{ Hz}, H_7b \)), 3.71 (ddd, 1H, \( J_{H2-H3} = 2.0 \text{ Hz}, J_{H2-H8a} = 5.0 \text{ Hz}, J_{H2-H8b} = 8.0 \text{ Hz}, H_2 \)), 2.08 (s, 3H, CH₃, Ac); ¹³C NMR (100 MHz, D₂O): δ 174.6 (CO), 75.0 (C₄), 74.9 (C₃), 69.0 (C₂), 61.9 (C₅), 61.1 (C₇), 59.6 (C₆), 47.4 (C₆), 21.9 (CH₃, Ac); ESI-HRMS calcd. for C₉H₁₉N₂O₅ [M+H]⁺: 235.1294, found 235.1297.

1.6 N-((R)-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)-2-hydroxyethyl)acetamide (11)
1.8 tert-butyl (2R,3R,4R,5S,6R)-6-azido-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-hydroxyazepane-1-carboxylate (6)

To a solution of known epoxide 3\textsuperscript{33} (160 mg, 0.294 mmol) in a mixture of DMF/H\textsubscript{2}O (2.9/0.3 mL) was added NaN\textsubscript{3} (88 mg, 1.358 mmol) followed by NH\textsubscript{4}Cl (53 mg, 1.358 mmol). The mixture was stirred for 28 h at 90 °C. EtOAc (50 mL) and H\textsubscript{2}O (50 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were dried on MgSO\textsubscript{4}, filtered and evaporated. The residue was purified by flash chromatography (Cy/ EtOAc: 96/4 - 95/5) to give compound 7\textsuperscript{6} (90 mg, 52%) and 6 (70 mg, 40%) as a pale yellow oil. 

[α]\textsubscript{D} +63.7 (c = 0.2, MeOH); \textsuperscript{1}H RMN (400 MHz, D\textsubscript{2}O) δ 4.41 (dd, 1H, J\textsubscript{H4-H3} = 1.0 Hz, J\textsubscript{H5-H4} = 3.0 Hz, H\textsubscript{4}); 4.22 (dd, 1H, J\textsubscript{H3-H4} = 1.0 Hz, J\textsubscript{H3-H2} = 2.5 Hz, H\textsubscript{3}); 4.16 (dd, J\textsubscript{H5-H4} = 3.0 Hz, J\textsubscript{H5-H6} = 9.0 Hz, H\textsubscript{5}); 4.07-4.00 (m, 3H, H\textsubscript{8a}, H\textsubscript{6}, H\textsubscript{7a}); 3.94-3.89 (m, 2H, H\textsubscript{8b}, H\textsubscript{7b}); (ddd, 1H, J\textsubscript{H2-H3} = 2.5 Hz, J\textsubscript{H2-H8a} = 4.5 Hz, J\textsubscript{H2-H8b} = 8.5 Hz, H\textsubscript{2}); \textsuperscript{13}C RMN (100 MHz, D\textsubscript{2}O) δ 75.6 (C\textsubscript{3}), 74.1 (C\textsubscript{4}), 68.8 (C\textsubscript{2}), 59.5 (2C, C\textsubscript{5}, C\textsubscript{7}), 59.0 (C\textsubscript{8}), 48.6 (C\textsubscript{6}), HRMS calcd. for C\textsubscript{7}H\textsubscript{17}N\textsubscript{2}O\textsubscript{4}: [MH]\textsuperscript{+}: 193.1188 found 193.1191.

1.9 (3R,4S,5R,6R,7R)-3-azido-1-benzyl-5,6-bis(benzyloxy)-7-((benzyloxy)methyl)azepan-4-ol (13)
To a solution of 6 (42 mg, 0.071 mmol) in CH$_2$Cl$_2$ (4 mL) was added trifluoroacetic acid (2 mL) and the obtained solution was stirred at room temperature for 1h. The solution was evaporated and co-evaporated with toluene (3x5 mL). The residue was dissolved in EtOAc/H$_2$O (4/0.4 mL) and K$_2$CO$_3$ (49 mg, 0.355 mmol), BnBr (13 µL, 0.107 mmol) were added respectively. The resulting mixture was stirred at 80 °C for 16 h and cooled to room temperature. EtOAc and H$_2$O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried on Na$_2$SO$_4$, filtered and evaporated. The obtained crude was purified by flash chromatography to give 13 (28 mg, 68%). $[\alpha]_D^+43.7$ (c = 1.0, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.40-7.26 (m, 18H, H$_{ar}$), 7.20-7.18 (m, 2H, H$_{ar}$), 5.06 (d, 1H, $^2$J = 11.0 Hz, CH$_2$Ph), 4.93 (d, 1H, $^2$J = 11.0 Hz, CH$_2$Ph), 4.63 (d, 1H, $^2$J = 11.0 Hz, CH$_2$Ph), 4.47-4.40 (m, 3H, CH$_2$Ph), 3.95-3.88 (m, 2H, H$_4$, NCH$_2$Ph), 3.77 (d, 1H, $^2$J = 13.0 Hz, NCH$_2$Ph), 3.10-3.63 (m, 2H, H$_8a$, H$_3$), 3.02 (d, 1H, $^2$J = 11.5 Hz, H$_7a$), 2.90 (dt, 1H, $^2$J = 11.5 Hz, H$_7b$), 2.63 (dd, 1H, $^2$J = 9.0 Hz, H$_2$), 2.50 (m, 2H, H$_7$), 1.22 (m, 18H, CH$_3$) $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 139.1, 138.3, 138.1, 138.0 (C$_{ipso}$), 128.8, 128.6, 128.4, 128.0, 127.7, 127.7, 127.4 (CH$_{ar}$), 83.2 (C$_4$), 79.2 (C$_3$), 78.1 (C$_2$), 76.1, 75.3, 73.2 (CH$_2$Ph), 67.8 (C$_8$), 64.4 (C$_6$), 63.7 (C$_2$), 59.6 (NCH$_2$Ph), 48.9 (C$_7$); ESI-HRMS calcd. for C$_{35}$H$_{39}$N$_4$O$_4$ [M+H]$^+$: 579.2971, found 579.2952.

1.10  

(S)-2-azido-2-((2R,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethan-1-ol (14)

To a solution of 13 (28 mg, 0.048 mmol) in toluene (0.5 mL) were added trifluoroacetic anhydride (14 µL, 0.1 mmol) and Et$_3$N (13 µL, 0.097 mmol). The obtained solution was refluxed for 3 h and cooled to room temperature. A solution of NaOH (10%, 2 mL) was added and the mixture was stirred for 30 minutes. AcOEt and H$_2$O were added and the layers were separated. The aqueous layer was extracted twice with AcOEt and the combined organic layers were dried on Na$_2$SO$_4$, filtered and evaporated. The obtained crude was purified by flash chromatography (Cyclohexane/AcOEt: 95/5) to give compound 14 (24 mg, 86%). $[\alpha]_D^{19}$ +16.8 (c = 0.5, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.38-7.22 (m, 20H, H$_{ar}$), 4.56 (d, 1H, $^2$J
= 12.0 Hz, CH$_2$Ph), 4.50-4.43 (m, 5H, CH$_2$Ph), 4.16 (dd, 1H, J$_{H4-H3}$ = 2.0 Hz, J$_{H4-H5}$ = 4.0 Hz, H$_a$), 4.10 (t, 1H, J$_{H3-H2}$ = J$_{H3-H4}$ = 2.0 Hz, H$_3$), 4.05 (d, 1H, $^2$J = 14.0 Hz, NCH$_2$Ph), 3.86 (td, 1H, J$_{H6-H5}$ = 4.0 Hz, J$_{H6-H7a}$ = J$_{H6-H7b}$ = 6.5 Hz, H$_6$), 3.76 (d, 1H, $^2$J = 14.0 Hz, NCH$_2$Ph), 3.68-3.56 (m, 4H, H$_8$a, H$_8$b, H$_7$a, H$_7$b), 3.46 (td, 1H, J$_{H2-H3}$ = 2.0 Hz, J$_{H2-H8a}$ = J$_{H2-H8b}$ = 6.0 Hz, H$_2$), 3.27 (t, 1H, J$_{H5-H4}$ = J$_{H5-H6}$ = 4.0 Hz, H$_5$), 3.07 (s, 0.9 H, OH); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 138.2, 138.0, 137.9, 137.6 (C$_{ipso}$), 128.5, 128.4, 128.3, 127.9, 127.9, 127.7, 127.6, 127.6, 127.1 (CH$_{ar}$), 85.7 (C$_4$), 83.6 (C$_3$), 73.2, 71.7, 71.4 (CH$_2$Ph), 68.9 (C$_3$), 66.6 (C$_8$), 63.2 (C$_2$), 62.9 (C$_7$), 62.0 (C$_6$), 51.9 (NCH$_2$Ph); ESI-HRMS calcd. for C$_{35}$H$_{39}$N$_4$O$_4$ [M+H$^+$]: 579.2971, found 579.2977.

1.11 (S)-2-acetamido-2-((2S,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethyl acetate (15)

To a solution of azide 14 (24 mg, 0.042 mmol) in THF/H$_2$O (2.0 mL/1.0mL) was added Ph$_3$P (32 mg, 0.125 mmol) and the resulting solution was stirred at 65 °C for 2 h. The solution was cooled to room temperature, solvents were evaporated and the reaction crude was dried for 2 h under reduced pressure. The residue was dissolved in pyridine (2.0 mL) and Ac$_2$O (1.0 mL) was added at 0°C. The resulting solution was then stirred for 12 h at room temperature. Pyridine and Ac$_2$O were removed by evaporation and co-evaporation with toluene (5 x 3 mL). The residue was purified by flash chromatography (Cy/EtOAc: 6.5/3.5) to give 15 as a white solid (16 mg, 60%). [$\alpha$]$_D$-12.3 (c = 0.5, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): 7.36-7.26 (m, 20H, H$_{ar}$), 6.67 (d, 1H, J$_{NH-H6}$ = 6.5 Hz, NHAc), 4.56 (d, 1H, $^2$J = 11.0 Hz, CH$_2$Ph), 4.50-4.44 (m, 5H, CH$_2$Ph), 4.32-4.27 (m, 1H, H$_6$), 4.14 (dd, 1H, J$_{H7a-H6}$ = 5.0 Hz, J$_{H7a-H7b}$ = 11.0 Hz, H$_7$a), 4.08 (bs, 1H, H$_3$), 3.99 (dd, 1H, J$_{H7b-H6}$ = 7.5 Hz, J$_{H7b-H7a}$ = 11.0 Hz, H$_7$b), 3.94 (bs, 1H, H$_4$), 3.90 (d, 1H, $^2$J = 14.5 Hz, NCH$_2$Ph), 3.81 (d, 1H, $^2$J = 14.5 Hz, NCH$_2$Ph), 3.71 (dd, 1H, J$_{H8a-H2}$ = 4.5 Hz, J$_{H8a-H8b}$ = 9.0 Hz, H$_8$a), 3.54 (t, 1H, J$_{H8b-H2}$ = J$_{H8b-H8a}$ = 9.0 Hz, H$_8$b), 3.50 (dd, 1H, J$_{H2-H8a}$ = 4.5 Hz, J$_{H2-H8b}$ = 9.0 Hz, H$_2$), 3.40-3.39 (m, 1H, H$_5$), 1.97 (s, 3H, CH$_3$, Ac), 1.56 (s, 3H, CH$_3$, Ac); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 170.7, 170.5 (CO), 139.0, 138.2, 137.6, 137.1 (C$_{ipso}$), 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.6, 127.0 (CH$_{ar}$), 83.9 (C$_4$), 82.6 (C$_3$), 73.3, 71.6, 71.4 (CH$_2$Ph), 68.0 (C$_8$) 66.7 (C$_5$), 64.2 (C$_2$), 63.7
(C₇), 50.7 ((NCH₂Ph), 47.0 (C₆), 22.8, 20.7 (CH₃, Ac); ESI-HRMS calcd. for C₃₉H₄₅N₂O₆ [M+H]⁺: 637.3278 found 637.3281.

1.12 N-((S)-1-((2S,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)-2-hydroxyethyl)acetamide (16)

A solution of 15 (8 mg, 0.013 mmol) in MeOH/H₂O/Et₃N (2/0.25/0.25 mL) was stirred for 18 h at room temperature. The solvents were evaporated and co-evaporated three times with toluene. The obtained residue was dissolved in MeOH (1 mL) and aqueous HCl (1M, 0.1 mL) was added under argon. After adding Pd/C (10%, 10 mg), the argon was removed. The H₂ was introduced and the mixture was bubbled for 5 minutes. After stirring the solution for 24 under H₂ atmosphere, the mixture was filtered on micro-filter (0.3 µm). The solvent was evaporated to give compound 16 (3 mg, 88%). [α]²²D = +18.3 (c = 0.16, MeOH); ¹H NMR (400 MHz, D₂O): 4.44 (q, 1H, J₆-H₇a = J₆-H₇b = J₆-H₅ = 5.5 Hz, H₆), 4.20 (dd, 1H, J₄-H₃ = 6.5 Hz, J₄-H₅ = 8.0 Hz, H₄), 4.14 (dd, 1H, J₃-H₄ = 6.5 Hz, J₃-H₂ = 8.0 Hz, H₃), 3.98 (dd, 1H, J₈a-H₂ = 3.5 Hz, J₈a-H₈b = 12.5 Hz, H₈a), 3.91 (dd, 1H, J₈b-H₈a = 5.5 Hz, J₈b-H₈b = 12.5 Hz, H₈b) 3.84 (2d, 2H, J = 5.5 Hz, H₇a, H₇b), 3.78 (dd, 1H, J₅-H₆ = 5.5 Hz, J₅-H₄ = 8.0 Hz, H₅), 3.63 (ddd, 1H, J₄-H₂ = 3.5 Hz, J₄-H₈b = 5.5 Hz, J₄-H₃ = 8.0 Hz, H₂), 2.14 (s, 3H, CH₃, Ac); ¹³C NMR (100 MHz, D₂O): δ 175.9 (CO), 74.8 (C₄), 74.3 (C₃), 62.7 (C₂), 61.7 (C₅), 60.5 (C₇), 57.5 (C₈), 51.0 (C₆), 21.8 (CH₃, Ac); ESI-HRMS calcd. for C₉H₁₆N₂O₄ [M+Na]⁺: 257.1113, found 257.1108.

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References


Highlights.

- A new family of amino-functionalised pyrolidines was synthesized.
- Ring-contraction of β-azido,γ-hydroxyazepanes was used.
- A low micromolar inhibitor of β-N-acetyleneosaminidase identified.
SUPPLEMENTARY INFORMATION

Synthesis of pyrrolidine-based analogues of 2-acetamidosugars as N-acetyl glucosaminidase inhibitors

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