Note

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

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A B S T R A C T
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.

In the course of our studies aimed at the synthesis of GlcNAc-like piperidine homoiminosugars exploiting a ring-contraction methodology, 2,3-trans-2-hydroxy-3-azido-azepane was required and obtained from the unsaturated 7-membered ring 1.13 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.13 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 73 (Scheme 1).

Compounds 4 and 6 33 are also suitable candidates for a ring contraction reaction 34 to give pyrrolidine derivatives 35 through a γ-aminoalcohol rearrangement. Hence, we decided to apply the TFAA-mediated ring contraction conditions developed by Cossy 36 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 (BrN, 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%).

Polyhydroxylated pyrrolidines1,2 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.3 Hexosaminidases are a very important class of glycosidases that cleave the N-acetyl derivative of a carbohydrate residue of a glycoprotein. The mode of inhibition of these enzymes by sugar and sugar analogues has been extensively investigated.4

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.4

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.4 The main classes of synthetic nitrogen functionalized polyhydroxylated pyrrolidines are represented in Fig. 1, the most studied one being the 2,5-dideoxy-2,5-imino-hexitols A–E but other scaffolds such as B, C, D, E and F have also been prepared. It is rather striking that structure G, which can be seen as a combination of A and 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 G (Fig. 1).
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).

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-acetylgalactosaminidases with IC₅₀ 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-acetylgalactosaminidase inhibition, with an IC₅₀ 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 Escherichia coli β-glucuronidase, with IC₅₀ 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. Thus, pyrrolidine 11 is an interesting case for β-glucuronidase inhibition.
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 (Merck, ALUGRAM® SIL G/UV254, 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 1H nuclei and at 100 MHz for 13C nuclei. The chemical shifts are expressed in part per million (ppm) using residual CHCl3 signal as internal reference (δ(1H)=7.26 ppm and δ(13C)=77.16 ppm) and the coupling constant J in Hertz (Hz). NMR multiplicities are reported using the following abbreviations: b=broad, s=singulet, d=doublet, t=triplet, q=quartet, 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(benzyloxy)-2-((benzoyloxy)methyl)-5-hydroxyazepane-1-carboxylate (4)

Known epoxide 2 (465 mg, 0.853 mmol) was dissolved in a DMF/H2O mixture (9.0/1.0 mL), then NaN3 (277 mg, 4.26 mmol) and NH4Cl (226 mg, 4.26 mmol) were added. The resulting mixture was stirred at 90 °C for 3 days. After being cooled to room temperature, EtOAc and H2O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried over MgSO4, filtered and evaporated. The residue was purified by flash chromatography (Cy/EtOAc: 9:1) to give 4 (285 mg, 57%) as colourless oil and 5 (160 mg, 32%).

\[ \left\{ \begin{array}{l}
\delta_{19}^\text{H} = 18.6 (c 1.0, \text{CHCl}_3), \\
\delta_{13}^\text{C} = 77.6 (\text{CHCl}_3)
\end{array} \right. \]

The NMR data are consistent with the proposed structure. Further details on the synthesis and characterization are provided in the Supporting Information.
43.7 \((C_7, C_7)\), 28.3, 28.2 \((CH_3, Boc)\); ESI-HRMS calcd for \(C_{33}H_{40}N_4NaO_6\) \([M+Na]^+\): 611.2846, found 611.2840.

1.3. (3S,4R,5R,6R,7R)-3-Azido-1-benzyl-5,6-bis(benzyloxy)-7-((benzyloxy)methyl)azepan-4-ol (8)

To a solution of 4 (46 mg, 0.078 mmol) in \(CH_2Cl_2\) (2.0 mL) was added trifluoroacetic acid (2.0 mL) and the solution was stirred at room temperature for 1 h. 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_2O (5:0) 0.5 mL) and BnBr (13 \(\mu L, 0.101 \text{ mmol}\), K_2CO_3 (32 mg, 0.234 mmol) were added respectively. The mixture was refluxed for 18 h. After being cooled to room temperature, H_2O 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

Table 1
Concentration of iminosugars giving 50% inhibition of various glycosidases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC_{50} (\mu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-N-Acetylgalactosaminidase</td>
<td></td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>241</td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>181</td>
</tr>
<tr>
<td>HL60</td>
<td>538</td>
</tr>
<tr>
<td>Human placenta</td>
<td>597</td>
</tr>
<tr>
<td>Jack bean</td>
<td>61</td>
</tr>
<tr>
<td>(\beta)-N-Acetylglucosaminidase</td>
<td></td>
</tr>
<tr>
<td>Chicken liver</td>
<td>NI (26.2%)</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>26</td>
</tr>
<tr>
<td>E. coli</td>
<td>15</td>
</tr>
</tbody>
</table>

\(^a\) NI: No inhibition (less than 50% inhibition at 1000 \(\mu M\)).

\(^b\) (\(\cdot\)): inhibition \% at 1000 \(\mu M\).
and concentrated under reduced pressure. The residue was purified by flash chromatography (Cy/EtOAc: 8.5/1.5) to give 8 as colourless oil (36 mg, 80%). \[\text{[33x700]} \]

The resulting solution was then stirred for 12 h at room temperature. The solution was filtered and evaporated. The residue was puriﬁed by flash chromatography (Cy/CHCl3: 80/20) to give compound 9 (55 mg, 92%). \[\text{[33x700]} \]

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 Et3N (26 μL, 0.194 mmol). The obtained solution was reﬂuxed for 3 h and cooled to room temperature. A solution of NaOH (10%, 5 mL) was added and the mixture was stirred for 30 min. EtOAc and H2O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried on Na2SO4, ﬁltered and evaporated. The obtained crude was puriﬁed by flash chromatography (Cy/CH2Cl2: 10/1) to give compound 10 (20 mg, 0.044 mmol) in THF/H2O (2.0 mL/1.0 mL) was added Ph3P (35 mg, 0.132 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 crude was dried for 2 h under reduced pressure. The residue was dissolved in pyridine (2.0 mL) and Ac2O (1.0 mL) was added to 0 °C. The resulting solution was then stirred for 12 h at room temperature. Pyridine and Ac2O were removed by evaporation and co-evaporation with toluene (5×3 mL). The residue was puriﬁed by flash chromatography (cyclohexane/AcOEt: 6/4) to give 10 (22 mg, 76%). \[\text{[33x700]} \]
To a solution of 6 (42 mg, 0.071 mmol) in CH₂Cl₂ (4 mL) was added trifluoroacetic acid (2 mL) and the solution was stirred at room temperature for 1 h. The solution was evaporated and co-evaporated with toluene (3:5 mL). The residue was dissolved in EtOAc/H₂O (4:0.4 mL) and K₂CO₃ (49 mg, 0.355 mmol) and the mixture was stirred for 3 h and cooled to room temperature. EtoAc and H₂O were added and the layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layers were dried on Na₂SO₄ filtered and evaporated. The obtained crude was purified by flash chromatography to give 13 (28 mg, 68%). [α]D = +43.7 (c 10.0, CHCl₃). H1 NMR (400 MHz, CDCl₃): 7.40–7.26 (m, 18H, Har), 7.20–7.18 (m, 2H, Har), 5.06 (d, 1H, J = 11.0 Hz, CH₂Ph), 4.93 (d, 1H, J = 11.0 Hz, CH₂Ph), 4.63 (t, 1H, J = 11.0 Hz, H8a), 4.47–4.40 (m, 3H, CH₂Ph), 3.95–3.88 (m, 2H, H4), 3.77 (d, 1H, J = 13.0 Hz, H7a), 3.10–3.63 (m, 2H, H8a), 3.61 (dd, 1H, J = 3.5 Hz, H18b), 3.53 (t, 1H, J = 4.5 Hz, J = 8.0 Hz, H18c), 3.31 (s, 1H, OH), 3.21 (dd, 1H, J = 11.5 Hz, H12c), 3.12 (dd, 1H, J = 11.5 Hz, J = 16.5 Hz), 2.90 (dt, 1H, J = 12.0 Hz, J = 12.0 Hz, H2), 2.63 (dd, 1H, J = 16.0 Hz, J = 14.0 Hz, H9b), 13C NMR (100 MHz, CDCl₃): δ 139.1, 133.8, 138.1, 138.0 (C₉), 128.8, 126.8, 128.4, 128.4, 128.1, 128.0, 127.7, 127.7, 127.4 (CH₃), 83.2 (C₄), 79.2 (C₁), 761.7, 75.3, 73.2 (CH₂Ph), 67.8 (C₈), 64.4 (C₆), 63.7 (C₉), 59.6 (CH₂Ph), 48.9 (C₃); ESI-HRMS calcld for C₃5H₆N₂O₄ [M+Na]+: 579.2971, found 579.2952.

1.10. (S)-2-Azido-2-((2S,3R,4SR,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 Et₃N (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 min. AcOEt and H₂O were added and the layers were separated. The aqueous layer was extracted twice with AcOEt and the combined organic layers were dried on Na₂SO₄ filtered and evaporated. The obtained crude was purified by flash chromatography (Cylohexane/AcOEt: 95:5) to give compound 14 (24 mg, 86%). [α]D = +16.8 (c 0.5, CHCl₃). H1 NMR (400 MHz, CDCl₃): δ 7.38–7.22 (m, 20H, HAr), 4.56 (d, 1H, J = 12.0 Hz, CH₂Ph), 4.50–4.43 (m, 5H, CH₂Ph), 4.16 (dd, 1H, J = 14.0 Hz, H4), 4.10 (t, 1H, J = 12.0 Hz, H5), 2.00 (H₂, J = 9.5 Hz, H3), 1.79 (m, 1H, J = 12.0 Hz, H9b), 1.45 (m, 1H, J = 14.0 Hz, NCH₂Ph), 1.36 (m, 1H, J = 14.0 Hz, NCH₂Ph), 0.86–0.36 (m, 4H, H8a, H8b, H9a, H9b), 3.21 (m, 1H, J = 14.0 Hz, H12), 3.09 (s, 0.9H, OH). 13C NMR (100 MHz, CDCl₃): δ 138.2, 138.0, 137.9, 137.6 (C₅₋₉), 128.5, 128.4, 128.3, 128.3, 127.9, 127.9, 127.6, 127.6, 127.1 (CH₂), 85.7 (C₄), 83.6 (C₃), 73.2, 71.7, 71.4 (CH₂Ph), 68.9 (C₈), 66.5 (C₉), 63.2 (C₆), 62.9 (C₅), 62.0 (C₉), 51.9 (NCH₂Ph); ESI-HRMS calcld for C₃₃H₃₈N₄O₆ [M+H]+: 579.2971, found 579.2977.
Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.carres.2015.02.014.