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New 1-amino-1-deoxy- and 2-amino-2-deoxy-polyhydroxyazepanes: synthesis and inhibition of glycosidases

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Abstract—Eight new seven-membered ring iminoalditols, displaying an amino group and a hydroxymethyl group on the ring, have been synthesized from D-arabinose via epoxidation of a protected azacycloheptene and subsequent nucleophilic opening. Three of them show a potent glycosidase inhibition on amyloglucosidase and, to a lesser extend, on α-L-fucosidase.

1. Introduction

The quest for potent and selective glycosidase inhibitors has emerged from their therapeutic potential;[1] several glycosidase inhibitors are being currently tested or approved in the treatment of diabetes,[2] Gaucher's disease,[3] HIV infection,[4] viral infection[5] or cancer.[6] Some of them have also been used as chemical probes, in combination with protein crystallography and kinetics studies, to provide new insights into glycosidase mechanism.[7] Extensive synthetic work has led to the design of many five- and six-membered ring azasugars,[8] which mimic the ring size of their parent sugar, but only a few higher homologues with seven-[9] or eight-membered rings[10] have been synthesized so far. As part of an ongoing project on the design of new carbohydrates mimetics, we have already reported the synthesis, conformational study and the biological evaluation of D-*gluco*-, D-*manno*-[11,12] and D-*galacto*-like[13] 1,6-dideoxy-1,6-iminoheptitols (Fig. 1), some of which display potent glycosidase inhibition.

These seven-membered ring compounds possess an extra hydroxymethyl group in order to closely mimic the parent sugar and can also be considered as higher homologues of nojirimycin. Insertion of a methylene group between the nitrogen atom and the pseudoanomeric hydroxyl group ensures chemical stability unlike nojirimycin. We anticipated that the relative flexibility of such structures associated with the unusual spatial distribution of the hydroxyl groups might generate an atypical inhibition profile for these molecules. This was indeed the case with the α-D-*gluco* analogue, which was found to be a rather potent and selective green coffee bean α-galactosidase inhibitor (K_i 2.2 μM),[1] the α-D-*galacto* analogue being almost inactive.[13] Other compounds were also recently reported by Dhivale et al.[14] All these compounds were obtained through the *syn*-dihydroxylation of the C=C bond of a fully protected azacycloheptene. We have now explored the epoxidation of this C=C bond and its subsequent opening with sodium azide to access 1,2-*trans*-hydroxylamino derivatives. This 1,2-*trans*-hydroxylamino motif displayed on an azepane ring is found in balanol, a fungal metabolite

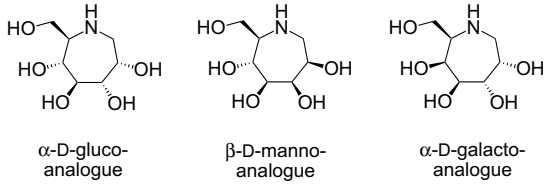

Figure 1. Structure of 1,6-dideoxy-1,6-iminoheptitols previously reported.

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produced by *Verticillium balanoides* with potent protein kinase C inhibitory properties.[15] Constrained diamine systems continue to attract synthetic interest due to their potential as therapeutic agents while 3-aminoazepane derivatives have been developed as antitumour chiral *cis*-platin analogues.[16] Polyhydroxylated azepanes with an extra amino group on the ring have been reported before by Farr et al.[17] and Wang et al.[18] but unfortunately Farr's compound was not active on jack bean mannosidase while no inhibition data were reported for Wang's molecule. Another compound of this type has been described by Depezay et al. who used the azepane ring as a scaffold to design non-peptide mimics of somatostatin.[19] Herein, we report the synthesis and biological evaluation of a series of new 1,6-dideoxy-1,6-iminoheptitols displaying an amino group at the pseudoanomeric or at the pseudo C-2 position of the ring (Fig. 2). For clarity reasons, the numbering used for these compounds follows the numbering of the parent pyranoside to emphasize the analogy between the azepanes and the corresponding monosaccharides.

2. Results and discussion

Our strategy takes advantage of our previously reported azacycloheptenes **9** and **10** and is based on the ring opening of the corresponding epoxides with sodium azide followed by subsequent hydrogenation. Treatment of compound **9** with *m*-CPBA in dichloromethane afforded the separable epoxides **11** (29% yield) and **12** (53% yield) in a 5/3 ratio in favour of compound **12**. Subsequent ring opening of epoxides **11** and **12** with sodium azide in the presence of ammonium chloride[20] gave in high yield the 1-azido-1-deoxy and 2-azido-2-deoxy derivatives **13** (34% yield), **14** (55% yield) and **15** (28% yield), **16** (57% yield). Final hydrogenolysis converted the azido group into an amino group and quantitatively afforded the corresponding 1-amino-1-deoxy- and 2-amino-2-deoxy-polyhydroxylated azepanes **1**–**4** (Scheme 1).

The same sequence was uneventfully applied to the diastereomeric azacycloheptene **10** to afford the corresponding azepanes **5**–**8**. Treatment of compound **10** with

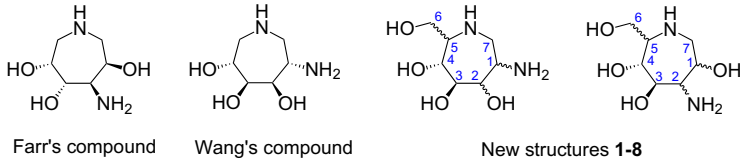
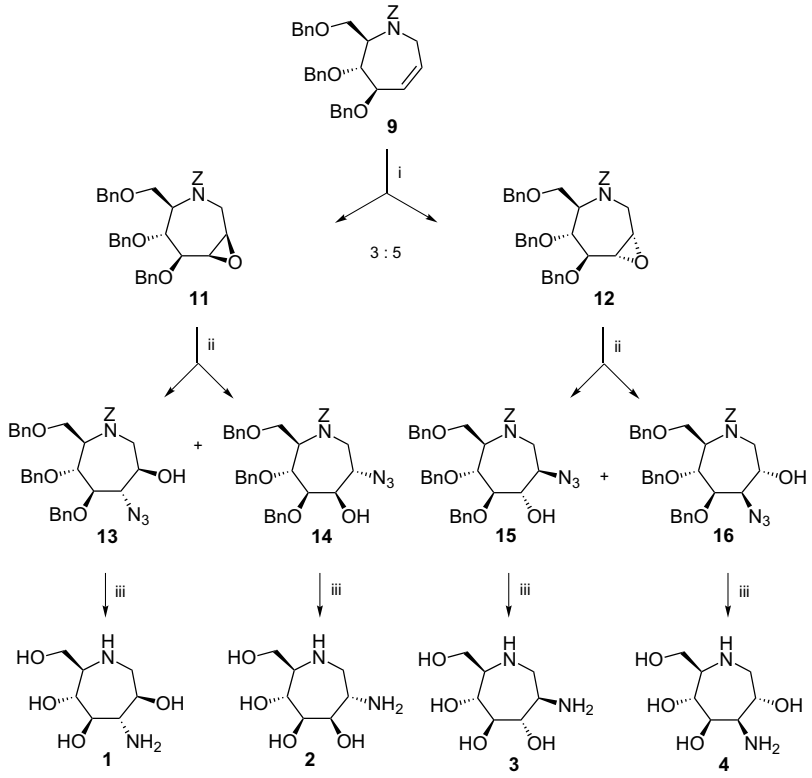


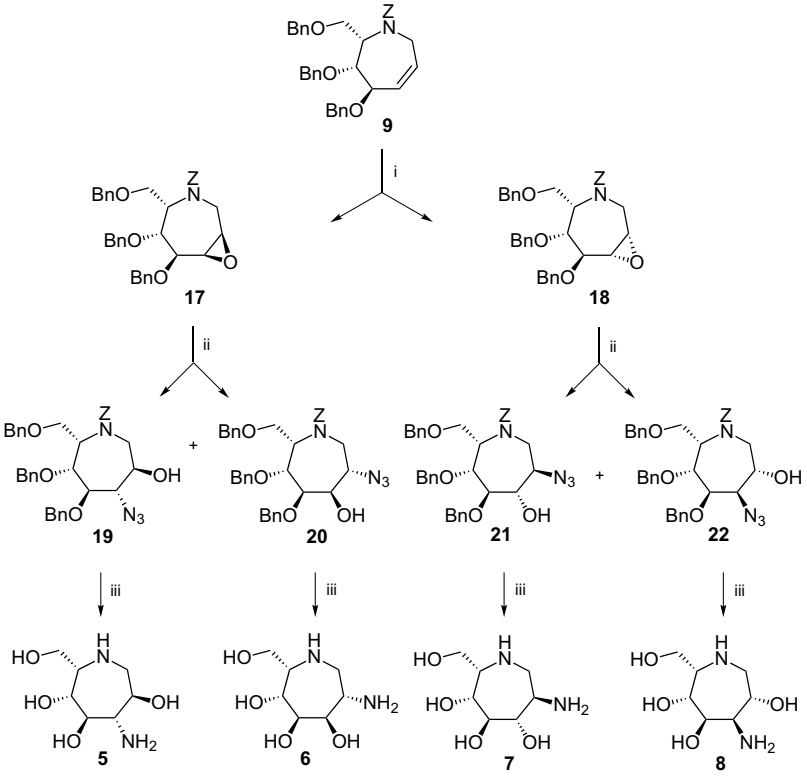
Figure 2. Analogy between Farr et al. and Wang co-workers compounds and the amino-1,6-dideoxy-1,6-iminoheptitols **1**–**8** described herein.



Scheme 1. Synthesis of aminopolyhydroxyazepanes **1**–**4**. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, 82% yield; (ii) NaN_3, NH_4Cl, DMF/H_2O, 90 °C, 85–89% yield; (iii) H_2, 10% Pd/C AcOH quant.

m-CPBA in dichloromethane afforded the separable epoxides **17** (42% yield) and **18** (42% yield). Subsequent ring opening of epoxides **17** and **18** with sodium azide in the presence of ammonium chloride gave the 1-azido-1-deoxy- and 2-azido-2-deoxy-derivatives **19** (64% yield), **20** (29% yield) and **21** (23% yield), **22** (69% yield) in very high yield. Final hydrogenolysis afforded quantitatively the corresponding 1-amino-1-deoxy- and 2-amino-2-deoxy-polyhydroxylated azepanes **5**–**8** (Scheme 2).

The NMR analysis of all the *Z*-protected compounds was found problematic due to the presence of rotamers generated from the π-bonding between the nitrogen and carbonyl group of the benzyloxycarbonyl group. The stereochemistry at positions 1 and 2 of compounds **1**–**8** was thus obtained from the analysis of their respective NMR spectra (Fig. 3) while the structure of compound **7** was deduced from the structures of other compounds.[21]



Scheme 2. Synthesis of aminopolyhydroxyazepanes **5**–**8**. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, 84% yield; (ii) NaN_3, NH_4Cl, DMF/H_2O, 90 °C, 92% yield; (iii) H_2, 10% Pd/C AcOH quant.

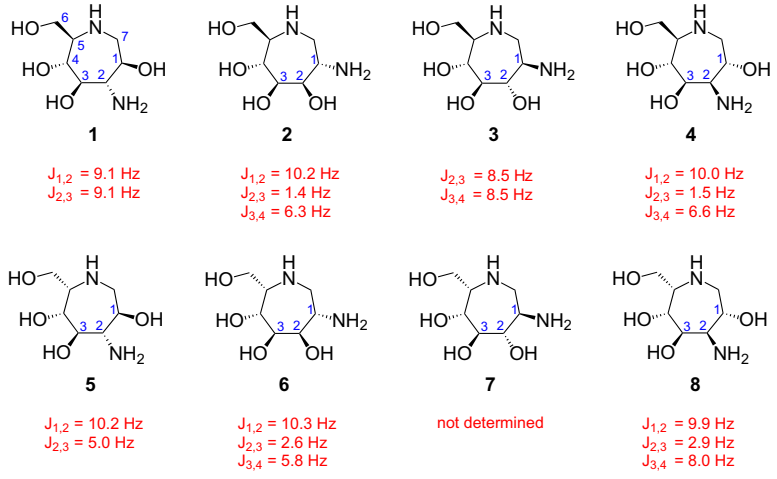
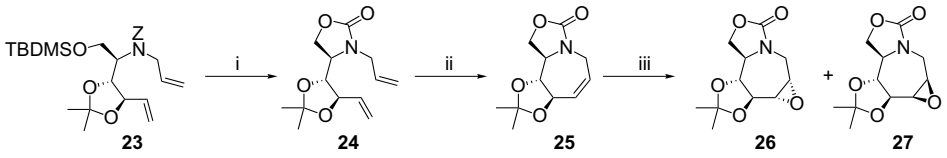


Figure 3. Important ^3J coupling constants for compounds **1**–**8**.



Scheme 3. Access to epoxides **26** and **27**. Reagents and conditions: (i) TBAF, THF, 99% yield; (ii) Grubbs' catalyst, CH_2Cl_2, 96%; *m*-CPBA, CH_2Cl_2, 75%.

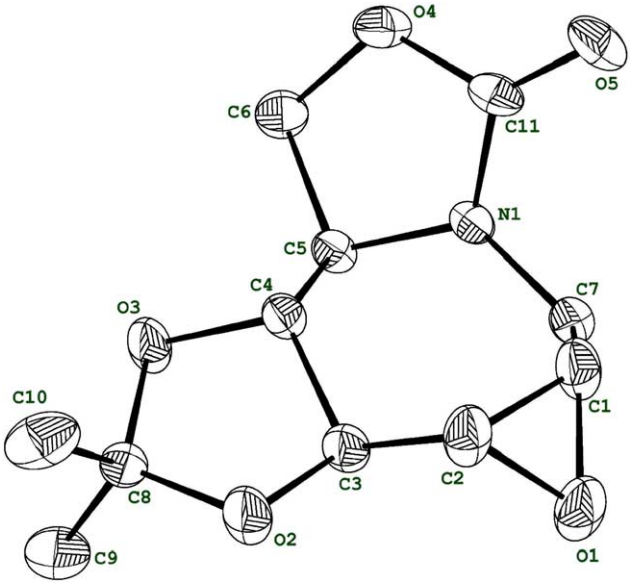


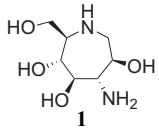
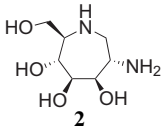
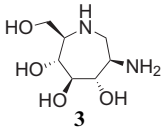
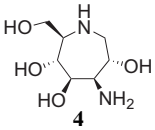
Figure 4. X-ray structure of epoxide **26**.

We were also interested in applying our sequence to orthogonally protected azacycloheptenes in order to enable a future regioselective modification of virtually each hydroxyl group present on the ring. This access to such azacycloheptenes has already been described.[13] Therefore the readily available diene **23** was first quantitatively converted to the bicyclic oxazolidinone **24** by treatment with TBAF in THF. This conformationally locked oxazolidinone was subjected to RCM using Grubb's catalyst to afford the corresponding tricyclic azacycloheptene **25** in excellent yield (96%). Treatment of **25** with *m*-CPBA in dichloromethane afforded epoxide **26** (53% yield) *trans* to the oxazolidinone ring along with diastereomeric epoxide **27** (22% yield) (Scheme 3). The structure of epoxide **26** was confirmed by X-ray analysis (Fig. 4).[22] Work is currently in progress to explore the regioselective opening of epoxides **26** and **27** with several nucleophiles.

2.1. Inhibition on glycosidases

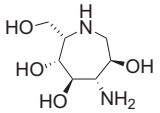
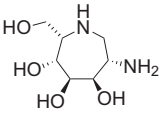
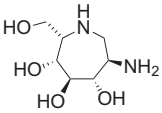
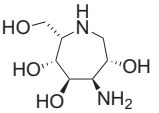
The eight iminoheptitols have been assayed for their inhibitory activity towards 24 commercially available glycosidases.[23] They did not inhibit the following enzymes at 1 mM: α-galactosidase from *E. coli*, β-galactosidases from *E. coli*, *Aspergillus niger* and *Aspergillus oryzae*, α-glucosidases from rice, β-glucosidases from almonds and *Saccharomyces cerevisiae* β-mannosidase from *Helix pomatia*, β-xylosidase from *A. niger*, α-*N*-acetylglucosaminidase from chicken liver, β-*N*-acetylglucosaminidases from jack bean and bovine epididymis A and B. For other enzymes the results are shown in Tables 1 and 2.

Table 1. Inhibitory activity of compounds **1**, **2**, **3** and **4**

Compound/enzyme	 **1**	 **2**	 **3**	 **4**
α-L-*Fucosidase* Bovine epididymis	42%	82% (**100 μM**)	76%	25%
α-*Galactosidase* Coffee beans	NI	30%	40%	NI
β-*Galactosidase* Bovine liver	76%	39%	22%	62%
α-*Glucosidase* Yeast	35%	30%	34%	21%
Amyloglucosidase				
Aspergillus niger	95% (**105 μM**)	82%	85% (**152 μM**)	56%
Rhizopus mould	92% (**143 μM**)	83%	85% (**245 μM**)	57%
α-*Mannosidase*				
Jack bean	NI	33%	30%	NI
Almonds	NI	24%	24%	NI

Percentage of inhibition at 1 mM concentration, IC_{50} (in brackets), optimal pH, 35 °C, NI = no inhibition at 1 mM concentration of the inhibitor.

Table 2. Inhibitory activity of compounds **5**, **6**, **7** and **8**

Compound/enzyme	 **5**	 **6**	 **7**	 **8**
α-L-*Fucosidase* Bovine epididymis	57%	67%	48%	NI
α-*Galactosidase* Coffee beans	NI	NI	49%	NI
β-*Galactosidase* Bovine liver	31%	28%	39%	23%

Percentage of inhibition at 1 mM concentration, IC_{50} (in brackets), optimal pH, 35 °C, NI = no inhibition at 1 mM concentration of the inhibitor.

Compounds **1**–**8** display a profile of inhibition very different from the ones obtained with our previously reported polyhydroxylated azepanes.[11] In fact none of them significantly inhibit α-galactosidase from coffee beans or β-galactosidase from bovine liver, the two enzymes, which were strongly inactivated before. Introduction of an amino group on the ring combined with a *trans* relationship of the OH and NH_2 groups located at positions 1 and 2 of the azepanes greatly modifies the inhibition profile of these molecules. Furthermore none of the enzymes inhibited with this new series of compounds were significantly inactivated with the previously reported compounds. We saw that compounds **1**–**4** with an (*R*)-configured hydroxymethyl group were better inhibitors than compounds **5**–**8**, which displayed an (*S*)-configured CH_2OH group, a trend already observed with previously reported polyhydroxylated azepanes. Compounds **1** and **3** show the best results and display a rather good and selective inhibition of mixed type on amyloglucosidase from *A. niger* and *Rhizopus* mould. These enzymes hydrolyze both 1,4- and 1,6-α-glucosidic linkages[24] and thus constitute very important industrial enzymes used to convert starch to glucose.[25] Interestingly, compound **1**, a 2-amino-2-deoxy-β-D-*gluco* analogue and compound **3**, a 1-amino-1-deoxy-β-D-*gluco* analogue, both inhibit an α-glucosidase despite a β-pseudoanomeric substituent. These results can be explained by an unusual conformation imposed by the seven-membered ring, an explanation already invoked and proven valid in the case of an α-D-*gluco* analogue displaying good inhibition on α-galactosidase from coffee beans.[13] To our knowledge, these are the first examples of polyhydroxylated azepanes inhibiting amylogluco-sidase. Compound **2**, a 1-amino-1-deoxy-α-D-*manno* analogue, whilst displaying a poor inhibition on α-mannosidase, is a moderate inhibitor of α-L-fucosidase from bovine epididymis (IC_{50} 100 μM) being less potent than other reported polyhydroxylated azepanes.[9f,l,26] Work is currently in progress to rationalize these biological results.

3. Conclusion

We have synthesized eight new seven-membered ring iminoheptitols **1**–**8** displaying an amino group and an extra hydroxymethyl group on the ring starting from our previously reported azacycloheptenes **9** and **10**. Compounds **1** and **3**, displaying, respectively, a 2-amino-2-deoxy-β-D-*gluco* and a 1-amino-1-deoxy-β-D-*gluco* configuration, inhibit amyloglucosidase, while compound **2** with a 1-amino-1-deoxy-α-D-*manno* configuration inhibits an α-L-fucosidase in the micromolar range. The introduction of an amino group either at the pseudoanomeric position or at the C-2 position of the ring combined with the *trans* relationship between the substituents at positions C-1 and C-2 generates a completely new inhibition profile for these compounds. Work is currently in progress to synthesize the 1,2-*trans*-diols and the *N*-acetyl derivatives at positions 1 and 2, which should hopefully inhibit other enzymes, such as hexosaminidases, as reported by Wong et al. for other polyhydroxylated azepanes.[26]

Acknowledgements

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21. Selective data for compounds **1**–**8** and **26**:
Compound **1**: $[\alpha]_D$ = +5 (*c* 0.24, CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.26 (ddd, 1H, *J* = 4.0 Hz, *J* = 6.4 Hz, *J* = 9.1 Hz, H-1), 4.04 (dd, 1H, *J* = 3.5 Hz, *J* = 12.5 Hz, H-6a), 3.90 (dd, 1H, *J* = 6.7 Hz, *J* = 12.5 Hz, H-6b), 3.80 (m, 2H, H-3, H-4), 3.52 (dd, 1H, *J* = 4.0 Hz, *J* = 14.6 Hz, H-7a), 3.47 (dd, 1H, *J* = 6.4 Hz, *J* = 14.6 Hz, H-7b), 3.37 (t, 1H, *J* = 9.1 Hz, H-2), 3.36 (ddd, 1H, *J* = 3.5 Hz, *J* = 6.7 Hz, *J* = 8.9 Hz, H-5); ^{13}C NMR (D_2O, 100 MHz): 70.17 (C-3), 69.74 (C-4), 64.70 (C-1), 61.20 (C-5), 59.22 (C-6), 57.83 (C-2), 48.39 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1192.
Compound **2**: $[\alpha]_D$ = +27 (*c* 0.58, CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.33 (dd, 1H, *J* = 1.4 Hz, *J* = 6.3 Hz, H-3), 4.28 (dd, 1H, *J* = 1.4 Hz, *J* = 10.2 Hz, H-2), 4.10 (dd, 1H, *J* = 3.3 Hz, *J* = 6.3 Hz, H-4), 3.95 (dd, 1H, *J* = 4.3 Hz, *J* = 12.2 Hz, H-6a), 3.90 (ddd, 1H, *J* = 4.1 Hz, *J* = 10.2 Hz, *J* = 12.0 Hz, H-1), 3.81 (dd, 1H, *J* = 9.0 Hz, *J* = 12.2 Hz, H-6b), 3.78 (dd, 1H, *J* = 4.1 Hz, *J* = 13.7 Hz, H-7a), 3.46 (ddd, 1H, *J* = 3.3 Hz, *J* = 4.3 Hz, *J* = 9.0 Hz, H-5), 3.37 (dd, 1H, *J* = 12.0 Hz, *J* = 13.7 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 74.59 (C-3), 68.68 (C-2), 67.17 (C-5), 67.04 (C-4), 61.28 (C-6), 49.62 (C-1), 44.13 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1185.
Compound **3**: $[\alpha]_D$ = −1 (*c* 0.3, CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.00 (dd, 1H, *J* = 3.7 Hz, *J* = 12.5 Hz, H-6a), 3.94 (dd, 1H, *J* = 6.5 Hz,*J* = 12.5 Hz, H-6b), 3.85 (t, 1H, *J* = 8.5 Hz, H-4), 3.78 (m, 2H, H-1, H-2), 3.69 (m, 2H, H-3, H-7a), 3.51 (ddd, 1H, *J* = 3.7 Hz, *J* = 6.5 Hz, *J* = 8.5 Hz, H-5), 3.49 (dd, 1H, *J* = 9.3 Hz, *J* = 14.3 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 76.20 (C-3), 72.23 (C-2), 68.06 (C-4), 59.70 (C-5), 58.72 (C-6), 49.06 (C-1), 42.31 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1193.
Compound **4**: $[\alpha]_D$ = +32 (*c* 0.54, CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.41 (dd, 1H, *J* = 1.5 Hz, *J* = 6.6 Hz, H-3), 4.32 (ddd, 1H, *J* = 4.3 Hz, *J* = 10.8 Hz, *J* = 14.6 Hz, H-1), 4.15 (dd, 1H, *J* = 2.7 Hz, *J* = 6.7 Hz, H-4), 3.93 (dd, 1H, *J* = 4.6 Hz, *J* = 12.2 Hz, H-6a), 3.79 (dd, 1H, *J* = 9.2 Hz, *J* = 12.2 Hz, H-6b), 3.80 (dd, 1H, *J* = 1.5 Hz, *J* = 10.0 Hz, H-2), 3.63 (dd, 1H, *J* = 4.3 Hz, *J* = 13.6 Hz, H-7a), 3.50 (ddd, 1H, *J* = 2.7 Hz, *J* = 4.6 Hz, *J* = 9.2 Hz, H-5), 3.28 (dd, 1H, *J* = 11.1 Hz, *J* = 13.6 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 69.52 (C-3), 67.26 (C-4), 66.28 (C-5), 64.87 (C-1), 61.36 (C-6), 55.12 (C-2), 47.93 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1189.
Compound **5**: $[\alpha]_D$ = −9 (*c* 0.42, CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.42 (dt, 1H, *J* = 2.6 Hz, *J* = 10.1 Hz, H-1), 4.11 (m, 2H, H-3, H-4), 3.84 (dd, 1H, *J* = 5.1 Hz, *J* = 11.8 Hz, H-6a), 3.76 (dd, 1H, *J* = 8.8 Hz, *J* = 11.8 Hz, H-6b), 3.66 (app. dd, 1H, *J* = 5.1 Hz, *J* = 8.8 Hz, H-5), 3.52 (dd, 1H, *J* = 2.5 Hz, *J* = 13.5 Hz, H-7a), 3.42 (dd, 1H, *J* = 5.0 Hz, *J* = 10.1 Hz, H-2), 3.31 (dd, 1H, *J* = 10.4 Hz, *J* = 13.5 Hz, H-7b); ^{13}C NMR (D_2O,100 MHz): 71.28 (C-3), 69.00 (C-4), 64.34 (C-1), 61.13 (C-2), 60.62 (C-6), 57.64 (C-5), 49.60 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1190.
Compound **6**: $[\alpha]_D$ = +12 (*c* 1, CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.21 (dd, 1H, *J* = 2.6 Hz, *J* = 5.9 Hz, H-3), 4.13 (dd, 1H, *J* = 2.6 Hz, *J* = 10.3 Hz, H-2), 4.09 (d, 1H, *J* = 5.9 Hz, H-4), 3.88 (dt, 1H, *J* = 4.0 Hz, *J* = 10.3 Hz, H-1), 3.84 (m, 2H, H-6a, H-6b), 3.76 (m, 1H, H-5), 3.65 (dd, 1H, *J* = 3.8 Hz, *J* = 13.8 Hz, H-7a), 3.54 (dd, 1H, *J* = 9.7 Hz, *J* = 13.8 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 71.35 (C-3), 69.26 (C-2), 66.97 (C-4), 60.55 (C-6), 55.95 (C-5), 46.83 (C-1), 43.35 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1189.
Compound **7** could not be isolated as a pure product and was contaminated with a trace of a monobenzylated

derivative: ^{1}H NMR (D_2O, 400 MHz): 4.10–4.03 (m, 3H, H-1, H-2, H-3), 3.90–3.75 (m, 3H, H-4, H-6a, H-6b), 3.71–3.66 (m, 2H, H-5, H-7a), 3.43 (dd, 1H, H-7b), ^{13}C NMR (D_2O, 100 MHz): 76.25, 76.05, 68.42, 58.36, 49.43 (C-1, C-2, C-3, C-4, C-5), 60.75 (C-6) 45.18 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%).

Compound **8**: $[\alpha]_D$ = +5 (*c* 1, CH_3OH);^{1}H NMR (D_2O, 400 MHz): 4.31 (dd, 1H, *J* = 3.4 Hz, *J* = 9.4 Hz, H-1), 4.28 (dd, 1H, *J* = 3.1 Hz, *J* = 6.1 Hz, H-3), 4.13 (d, 1H, *J* = 6.1 Hz, H-4), 3.85 (m, 2H, H-6a, H-6b), 3.80 (m, 1H, H-5), 3.72 (dd, 1H, *J* = 2.9 Hz, *J* = 9.9 Hz, H-2), 3.55 (dd, 1H, *J* = 3.4 Hz, *J* = 13.6 Hz, H-7a), 3.45 (dd, 1H, *J* = 9.2 Hz, *J* = 13.6 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 67.57 (C-3, C-4), 62.31 (C-1), 60.77 (C-6), 56.15 (C-2), 55.58 (C-5), 48.24 (C-7); *m/z* (CI, NH_3): 193 ($M+H^+$, 100%); HRMS (CI, NH_3): calcd for $C_7H_{17}O_4N_2$ ($M+H^+$): 193.1188, found 193.1192.

Compound **26**: $[\alpha]_D$ = +21 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): 4.57 (dd, 1H, *J* = 6.3 Hz, *J* = 15.3 Hz, H-7a), 4.52 (dd, 1H, *J* = 7.7 Hz, *J* = 9.2 Hz, H-6a), 4.31 (d, 1H, *J* = 5.0 Hz, *J* = 9.2 Hz, H-6b), 3.79 (dd, 1H, *J* = 5.5 Hz, *J* = 9.2 Hz, H-3), 3.75 (t, 1H, *J* = 8.5 Hz, H-4), 3.70 (dt, 1H, *J* = 5.0 Hz, *J* = 8.5 Hz, H-5), 3.40 (ddd, 1H, *J* = 4.8 Hz, *J* = 6.3 Hz, *J* = 7.8 Hz, H-1), 3.23 (t, 1H, *J* = 4.8 Hz, H-2), 2.74 (dd, 1H, *J* = 7.8 Hz, *J* = 15.3 Hz, H-7b), 1.50 (s, 3H, CH_3), 1.43 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 157.21 (C=O), 112.07 ($C(CH_3)_2$), 80.44 (C-3), 77.63 (C-4), 65.80 (C-6), 59.58 (C-5), 53.84 (C-2), 49.64 (C-1), 45.39 (C-7), 26.85 (CH_3), 26.79 (CH_3); *m/z* (CI, NH_3): 259 ($M + NH_4^+$, 100%).

22. Selected crystal structure data for **26**; crystal system orthorhombic; space group $P2_12_12_1$; $Z = 4$; cell parameters: a = 5.8997(9), b = 12.970(2), c = 15.2263(18), $\alpha = 90$, $\beta = 90$, $\gamma = 90$; radiation (Mo Kα) λ = 0.71073 Å; 155 variables for 1947 reflections; final $R = 0.0699$, $R_W = 0.0761$; Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 255152. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44 1223/336 033; E-mail: deposit@ccdc.cam.ac.uk.
23. A known protocol was applied: Saul, R.; Chambers, J. P.; Molyneux, R. J.; Elbein, A. D. *Arch. Biochem. Biophys.* **1983**, *221*, 593; Brandi, A.; Cicchi, S.; Cordero, F. M.; Frignoli, B.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem.* **1995**, *60*, 6806–6812, We verified that the delay of inhibitor/enzyme incubation did not affect the inhibition measurements. Under standard conditions, optimal inhibitory activities were measured after 5 min of incubation.
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