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Design and synthesis of acetamido tri- and tetra-hydroxyazepanes: Potent and selective β-*N*-acetylhexosaminidase inhibitors

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ABSTRACT

A series of seven-membered iminosugars bearing an acetamido group β- or γ- to the endocyclic nitrogen have been synthesized via simple transformations of previously described polysubstituted azepanes. These tetra- and trihydroxylated acetamido azepanes are ring homologues of 2-acetamido-1,2-dideoxy-glyconojirimycins and 2-acetamido-1-*N*-iminosugars respectively. Screening of these azepanes towards a range of commercially available glycosidases demonstrated their potential as selective and potent hexosaminidase inhibitors with K_i's in the submicromolar range. A correlation between the relative configuration of the azepanes and their ability to inactivate hexosaminidases was also observed for the first time for this class of compounds with one notable exception for the most potent compound.

1. Introduction

N-Acetyl-β-hexosaminidases (EC 3.2.1.52) belong to the group of lysosomal hydrolases and catalyze the hydrolysis of non-reducing terminal *N*-acetyl-β-D-glucosamine and *N*-acetyl-β-D-galactosamine units in glycoproteins, GM_2-gangliosides and glycosami- noglycans (GAG) including chondroitin 4-sulfate, chondroitin 6-sulfate, hyaluronic acid, keratan sulfate and dermatan sulfate.[1] *N*-Acetyl-β-hexosaminidases are of particular interest as therapeutic targets since they are the main GAG-degrading glycosidases present in the synovial fluid of patients diagnosed with osteoarthritis and are directly involved in the cartilage matrix degradation.[2] As a consequence, potent inhibitors of *N*-acetyl-β-hexosaminidases appear as new drug candidates for the treatment of osteoarthritis. Additionally, the deficiency of these enzymes is responsible for some lysosomal storage diseases including Sanfilippo,[3] Tay-Sachs and Sandhoff[4] pathologies in which neuronal accumulation of gangliosides leads to the CNS degeneration. At the molecular level, *N*-acetyl-β-hexosaminidases cleave the terminal *N*-acetyl-β-D-glucosamine unit via a retaining double displacement mechanism[5] involving the anchimeric assistance of the acetamido group.

The most effective molecules reported to date aimed at inhibiting glycosidases are iminosugars in which the endocyclic oxygen or the anomeric carbon of the sugar substrate has been replaced by a nitrogen atom. These iminosugars, protonated at physiological pH, have been devised as stable analogues of the oxacarbenium-like TS.[6] In the case of hexosaminidases, it has been shown that the presence of an *N*-acyl moiety in the sugar mimic is compulsory to obtain strong inhibitors[7] and a vast array of five and six-membered iminosugars have been reported as efficient competitive *N*-acetyl-β-hexosaminidase inactivators. Wong has shown that pyrrolidines such as **1** were good candidates by virtue of their resemblance with the substrate, the conformation of the five-membered ring mimicking the half-chair conformation of the TS.[2] Pyrrolidine **2**[8] as well as piperidines **3**[9] and **4**[10] also displayed potent inhibition towards *N*-acetyl-β-hexosaminidase. In the last decade, mimicry of the carbocationic character of the oxacarbenium-like TS has led the development of 1-*N*-azasugars including *gem*-diamine **5**[11] which proved to be a good albeit unstable inhibitor.[12] Recently, Noort et al. reported piperidine **6** displaying a chemically stable homologated acetamido group as a potent and selective human spleen lysosomal β-hexosaminidase inhibitor (Fig. 1).[13]

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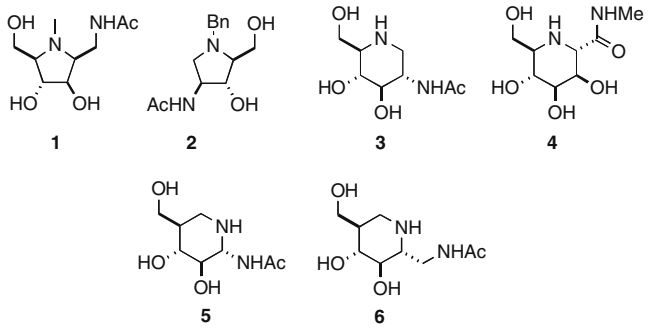


Figure 1. Structures of potent five- and six-membered hexosaminidase inhibitors **1**–**6**.

2. Results and discussion

A structurally distinct class of glycosidase inhibitors is constituted by seven-membered iminosugars.[14] Their ring flexibility has been put forward to suggest that they could more easily match the proposed half-chair or boat conformation of the transition state compared to pyrrolidine and piperidine derivatives and get insights into the substrate distorsion during processing of the glycosidic bond by glycosidases.[15] We[16] and others[17] have previously reported synthetic routes to polyhydroxylated azepanes which can be seen as ring homologues of nojirimycin,[18] several of which inhibiting glycosidases in the micromolar range. However, to the best of our knowledge, only two azepanes **7**[19] and **8**[20] have been reported that mimic *N*-acetylglucosamine. We present herein the synthesis and biological evaluation towards hexosaminidases of a series of tri- and tetrahydroxylated acetamido azepanes **A** and **B**, respectively (Fig. 2).

2.1. Synthesis of tetrahydroxy acetamido azepanes

We have previously described the synthesis of partially protected β- and γ-azido azepanes **9**–**13** from D-arabinose via epoxidation and azide-based oxirane opening of a trisubstituted azacycloheptene.[21] Azide reduction, N-acetylation of the free primary amine with acetic anhydride followed by hydrogenolysis under mild acidic conditions afforded the corresponding tetrahydroxylated acetamido azepanes **14**–**18** in 37–65% yield over three steps which can be seen as 2-acetamido-1,2-dideoxy-nojirimycin homologues (Scheme 1).[22]

2.2. Synthesis of trihydroxyacetamido azepanes

We have also reported the synthesis of noeuromycin ring homologues in which a β-hydroxyazepane intermediate **19** was obtained.[23] An acetamide group could be easily introduced by substitution of the free hydroxyl group in **19** to access a 3-acetamido-4,5,6-trihydroxy azepane displaying the relevant D-*gluco* like configuration. While activation of the free alcohol with triflic anhydride was found problematic leading to a bicyclic compound resulting probably from the intramolecular displacement of the triflate by the carbamate group, switching to the mesylate was successful allowing its displacement with sodium azide to yield the azide **20** in 80% yield over two steps. Reduction with triphenylphosphine followed by N-acetylation with acetic anhydride gave the acetamide **21** in 72% yield over two steps. Final hydrogenolysis under mild acidic conditions afforded the trihydroxylated acetamido azepane **22** in quantitative yield. In order to check whether hexosaminidases can discriminate between the various stereochemistries displayed by the azepane ring, the synthesis of a D-*manno*-like configured acetamido azepane was also achieved. Inversion of the free OH group in **19** under Mitsunobu conditions yielded the diastereomeric β-hydroxy azepane **23** in 63% yield. The same mesylation/azide displacement/reduction/acetylation/deprotection sequence was applied to sequentially furnish the azide **24**, the acetamide **25** and the target azepane **26**, respectively (Scheme 2).

3. Glycosidase inhibition

Azepanes **14–18**, **22** and **26** were assayed towards a range of commercially available glycosidases including β-*N*-acetyl glucosaminidases from Jack bean and bovine liver (Table 1).[24] At 1 mM concentration, they did not inhibit α-galactosidase from coffee bean and β-galactosidases from *Escherichia coli* and *Aspergillus oryzae,* β-glucosidase from almonds, β-mannosidase from snail and β-xylosidase from *A. oryzae*.

All the azepanes synthesized are potent competitive hexosaminidase inhibitors with K_i ranging from 30 μM to 50 nM, similar potencies being observed for the two sources of hexosaminidases. Importance of the presence of an acetamide group to target *N*-acetylglucosaminidases is illustrated here as the corresponding amines proved not to be inhibitors of *N*-acetylglucosaminidases.[21] Concerning the tetrahydroxylated acetamido azepanes **14–18**, unlike previously described pentahydroxylated azepanes,[17] a rather good correlation between the relative configuration of the azepane and the inhibitory potency towards the hexosaminidases is observed. The β-D-*gluco* like azepane **14**, which perfectly matches the β-glucosaminidase substrate configuration is more potent than the α-L-*ido*-like azepane **16** and the α-D-*manno*-like azepane **18**. Positioning of the acetamido group at the relevant γ position relative to the endocyclic nitrogen is also important, as the β-acetamido derivative **17** is the less potent inhibitor of the series. The SAR observed for this family of compounds can be tentatively explained by the presence of the extra acetamido group. Compounds **14–18** bear two nitrogen-containing groups, the ring amine and the NHAc group which, when interacting both with key amino acids in the hexosaminidase active site, might restrain the positioning of the azepane scaffold in the active site. Nevertheless and surprisingly, while its configuration does not match three out of the five stereocentres of the hexosaminidase substrate, the β-L-*gulo*-like azepane **15** (K_i 50 nM) is the most potent inhibitor in the azepane series and competes well with piperidines and pyrrolidines based hexosaminidase inhibitors. The high flexibility of the azepane ring might account for the potency of azepane **15** and suggests an atypical binding in the active site which is currently under investigation.

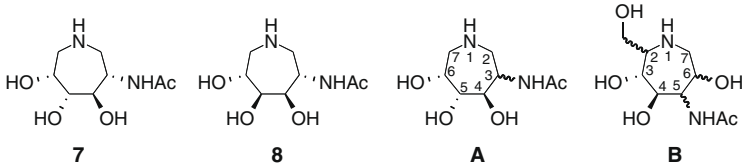
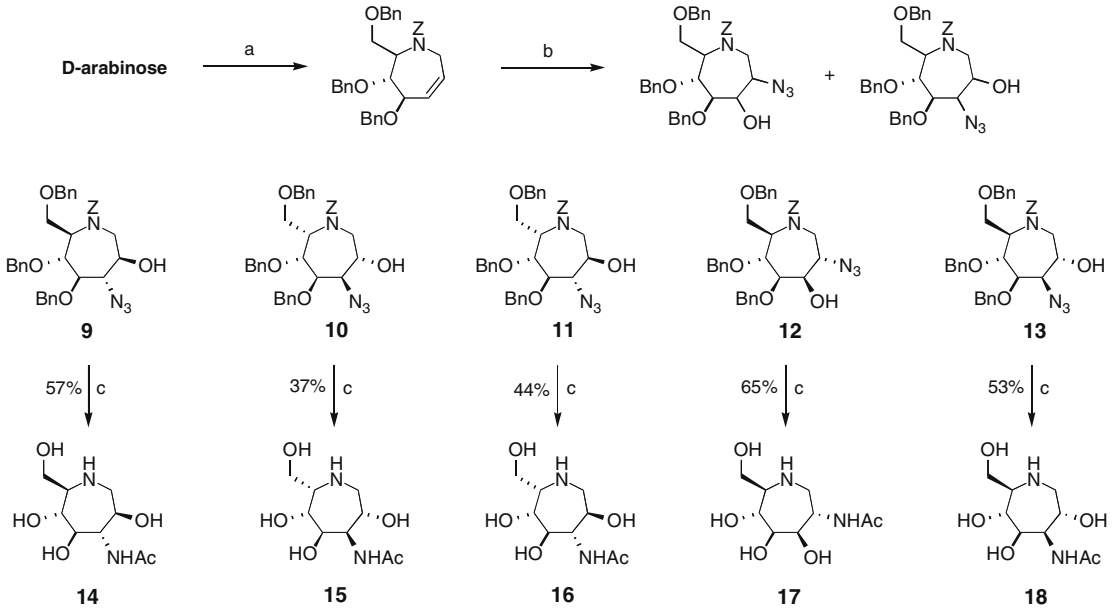
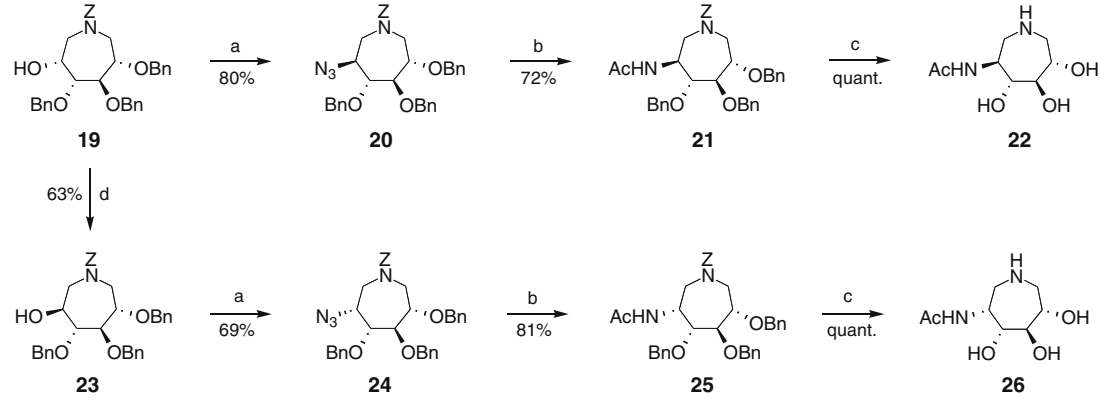


Figure 2. Structures of azepanes **7**, **8**, **A** and **B**.



Scheme 1. Reagents and conditions: (a) Ref. 16a; (b) Ref. 21; (c) (i) PPh_3, THF/H_2O; (ii) Ac_2O, $KHCO_3$, $AcOEt/H_2O$; (iii) H_2, 10% Pd/C, CH_3OH, 1 M aq HCl.



Scheme 2. Reagents and conditions: (a) MsCl, DMAP, pyridine then NaN_3, DMF, 90 °C; (b) PPh_3, THF/H_2O, 50 °C then Ac_2O, pyridine; (c) H_2, 10% Pd/C, CH_3OH, 1 M aq HCl; (d) PPh_3, DEAD, PhCOOH, THF then NaOMe, MeOH.

Regarding trihydroxylated acetamido azepanes **22** and **26**, and as previously observed in the case of noeuromycin analogs,[23] matching the substrate configuration is necessary to gain inhibitory potency as the D-*gluco*-like azepane **22** (K_i 0.4 μM) is more potent than the D-*manno*-like azepane **26** and the L-*ido*-like azepane **7** reported by Wong which is surprisingly only a poor hexosaminidase inhibitor (22% of inhibition at 240 μM concentration of inhibitor).

4. Conclusion

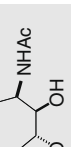
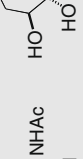
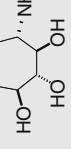
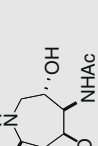
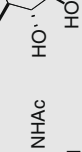
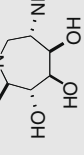
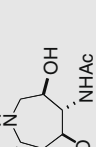
In conclusion, a series of tri- and tetrahydroxylated acetamido azepanes has been synthesized to target *N*-acetylglucosaminidases. All the azepanes described are selective hexosaminidase inhibitors with K_i in the micromolar range. Interestingly, a fairly good fitting is observed between their relative configuration and their inhibitory potency. This makes them useful scaffolds to further explore their use as *N*-acetylglycosaminidase inhibitors and as chemical chaperones for pathologies in which hexosaminidases are involved including Tay-Sachs, Sandhoff and Sanfilippo diseases.[25] In these pathologies, a genetic defect results in the misfolding of a lysosomal β-hexosaminidase, which is not further maturated and accumulates in the ER prior to degradation. An emerging and promising therapy to treat such diseases derives from the recent chemical chaperone approach[26] and would consist in administrating competitive *N*-acetyl-β-hexosaminidase inhibitors at low intracellular concentrations that would act as chemical chaperones to help the mutant enzyme to fold properly avoiding its pre-lysosomal degradation. Noteworthy and intriguingly, the best inhibitor reported herein displays a relative configuration away from the corresponding substrate configuration indicative of its peculiar binding in the hexosaminidase active site, which is currently under investigation.

5. Experimental

5.1. General methods

Melting points were determined with a Büchi B-510 capillary apparatus and are uncorrected. Optical rotations were measured at 20 ± 2 °C with a Perkin–Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell. Mass spectra (CI (ammonia)) were ob-

Table 1
Inhibitory activity of compounds **14–18**, **22**, **26** towards glycosidases

Inhibitor/enzyme	 **14**	 **15**	 **16**	 **17**	 **18**	 **22**	 **26**
Bovine epididymis α-L-fucosidase	NI	NI	NI	NI	NI	28%	NI
Yeast α-glucosidase	NI	NI	18%	NI	NI	NI	NI
Rice α-glucosidase	NI	NI	NI	NI	NI	NI	NI
Aspergillus niger amylo glucosidase	NI	NI	NI	NI	NI	19%	NI
Jack bean α-mannosidase	25%	NI	NI	NI	NI	NI	NI
Jack bean β-*N*-acetyl glucosaminidase	98% (0.5)	100% (0.05)	91% (8.9)	88% (30)	98% (3)	99% (0.4)	93%
Bovine liver β-*N*-acetyl glucosaminidase	99% (0.6)	99% (0.4)	40%	74%	93% (26)	99% (0.7)	68%

% of inhibition at 1 mM concentration of inhibitor, *K*$_i$ in brackets in μM.

tained with a JMS-700 spectrometer. ^{1}H NMR spectra were recorded at 400 MHz with a Brüker DRX 400 for solns in $CDCl_3$ or D_2O at room temperature. Assignments were confirmed by COSY experiments. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), br s (broad singlet), etc. ^{13}C NMR spectra were recorded at 100.6 MHz with a Brüker DRX 400 spectrometer. Assignments were confirmed by J-mod technique, HMQC and HMBC. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of Silica Gel 60 F_{254} (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with H_2SO_4 10% in EtOH or with 0.2% w/v cerium sulfate and 5% ammonium molybdate in 2 M H_2SO_4. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

All compounds are numbered following IUPAC rules (see Fig. 2). Compounds **20, 21, 23, 24, 25** in which the ring nitrogen is protected with a Z group appear as a mixture of two rotamers by NMR, presumably due to the π bonding in the amide bond.

5.1.1. General procedure for the conversion of azido azepanes 9–13 into tetrahydroxylated acetamido azepanes 14–18

Azido azepane **9** (90 mg, 0.144 mmol) was dissolved in a 10:1 THF/H_2O mixture (1 mL) and PPh_3 (95 mg, 0.36 mmol) was added. The reaction mixture was stirred at rt for 10 h and then at 60 °C for 2 h. The reaction mixture was then partitioned between ethyl acetate and water and the organic layer was separated, dried ($MgSO_4$) and concentrated to afford the crude amine which was used directly. The crude amine was dissolved in a 10:1 ethyl acetate/H_2O mixture (1 mL) and $KHCO_3$ (54 mg) was added followed by Ac_2O (50 μL). The reaction mixture was stirred for 2 h, diluted with ethyl acetate (5 mL) and the organic layer was separated, dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (cyclohexane/ethyl acetate 1:3) afforded the corresponding acetamido azepane (46 mg, 50% yield) as an oil. To a solution of acetamido azepane (22 mg, 0.030 mmol) in CH_3OH (3 mL) was added 10% Pd/C (20 mg) and a 1 M HCl aq solution (40 μL). The solution was degased three times and air was replaced by H_2. After stirring 10 h at rt, the mixture was filtered through a Rotilabo® Nylon 0.45 μm filter eluted with CH_3OH, and concentrated to afford the azepane **14** (7 mg, quantitative yield) as a colorless oil.

5.1.2. Spectroscopic data for (3*R*,4*S*,5*R*,6*R*,7*R*),*N*-(3,5,6-trihydroxy-7-hydroxymethyl-azepan-4-yl)-acetamide 14

$[\alpha]_D$ = +4.5 (*c* 0.2, CH_3OH); ^{1}H NMR (400 MHz, D_2O): δ = 4.11 (dt, ^3J(6,7a) = 2.7 Hz, ^3J(5,6) = ^3J(6,7b) = 6.5 Hz, 1H, H-6), 4.05 (dd, ^3J(8a,2) = 3.5 Hz, ^3J(8a,8b) = 12.5 Hz, 1H, H-8a), 3.96 (dd, ^3J(5,6) = 6.5 Hz, ^3J(4,5) = 8.6 Hz, 1H, H-5), 3.91 (dd, ^3J(8b,2) = 9.3 Hz, ^3J(8b,8a) = 12.5 Hz, 1H, H-8b), 3.85 (dd, ^3J(2,3) = 7.3 Hz, ^3J(3,4) = 8.6 Hz, 1H, H-3), 3.72 (t, ^3J(4,5) = ^3J(3,4) = 8.6 Hz, 1H, H-4), 3.41–3.38 (m, 2H, H-7a, H-7b), 3.32 (ddd, ^3J(2,8a) = 3.5 Hz, ^3J(2,3) = 7.3 Hz, ^3J(2,8b) = 9.3 Hz, 1H, H-3), 2.05 (s, 3H, $COCH_3$); ^{13}C NMR (100.6 MHz, D_2O): δ = 172.9 (C=O), 73.0 (C-4), 69.4 (C-3), 66.8 (C-6), 61.9 (C-2), 59.6 (C-8), 57.9 (C-5), 47.7 (C-7), 22.4 (CH_3CO); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_9H_{19}O_5N_2$: 235.1294; found: 235.1289.

5.1.3. Spectroscopic data for (3*S*,4*R*,5*R*,6*R*,7*S*),*N*-(3,5,6-trihydroxy-7-hydroxymethyl-azepan-4-yl)-acetamide 15

$[\alpha]_D$ = +10 (*c* 0.2, CH_3OH); ^{1}H NMR (400 MHz, D_2O): δ = 4.31 (dd, ^3J(4,5) = 2.3 Hz, ^3J(5,6) = 10.6 Hz, 1H, H-5), 4.12–4.05 (m, 1H, H-6), 4.01 (dd, ^3J(2,3) = 1.5 Hz, ^3J(3,4) = 7.1 Hz, 1H, H-3), 3.97 (dd, ^3J(4,5) = 2.3 Hz, ^3J(3,4) = 7.1 Hz, 1H, H-4), 3.74–3.69 (m, 2H, H-8a, H-8b), 3.54–3.50 (m, 1H, H-2), 3.36 (dd, ^3J(7a,6) = 4.3 Hz, ^3J(7a,7b) = 15.6 Hz, 1H, H-7a), 3.27 (dd, ^3J(7b,6) = 7.8 Hz, ^3J(7a,7b) = 15.6 Hz, 1H, H-7b), 1.67 (s, 3H, $COCH_3$); ^{13}C NMR

(100.6 MHz, D_2O) δ = 173.9 (C=O), 71.1 (C-4), 67.4 (C-3), 65.1 (C-6), 60.0 (C-8), 57.8 (C-2), 53.7 (C-5), 49.3 (C-7), 22.0 ($COCH_3$); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_9H_{19}O_5N_2$: 235.1294; found: 235.1286.

5.1.4. Spectroscopic data for (3*R*,4*S*,5*R*,6*R*,7*S*),*N*-(3,5,6-trihydroxy-7-hydroxymethyl-azepan-4-yl)-acetamide 16

$[\alpha]_D$ = +8.5 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, D_2O): δ = 4.00 (dt, $^3J(6,7a)$ = 2.3 Hz, $^3J(5,6) = {}^3J(6,7b)$ = 9.8 Hz, 1H, H-6), 3.86 (app. d, $^3J(3,4)$ = 5.6 Hz, 1H, H-3), 3.79–3.74 (m, 2H, H-4, H-5), 3.60–3.47 (m, 3H, H-2, H-8a, H-8b), 3.21 (dd, $^3J(7a,6)$ = 2.3 Hz, $^3J(7a,7b)$ = 13.6 Hz, 1H, H-7a), 3.12 (dd, $^3J(7b,6)$ = 9.8 Hz, $^3J(7b,7a)$ = 13.6 Hz, 1H, H-7b), 1.79 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, D_2O): δ = 173.0 (C=O), 72.9 (C-4), 68.5 (C-3), 67.4 (C-6), 60.4 (C-8), 60.2 (C-5), 57.0 (C-2), 48.4 (C-7), 21.0 ($COCH_3$); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_9H_{19}O_5N_2$: 235.1294; found: 235.1291.

5.1.5. Spectroscopic data for (3*S*,4*R*,5*R*,6*R*,7*R*),*N*-(4,5,6-trihydroxy-7-hydroxymethyl-azepan-3-yl)-acetamide 17

$[\alpha]_D$ = +62.8 (*c* 1.3, CH_3OH); ^1H NMR (400 MHz, D_2O): δ = 4.42 (ddd, $^3J(6,7a)$ = 4.8 Hz, $^3J(5,6)$ = 9.6 Hz, $^3J(6,7b)$ = 11.2 Hz, 1H, H-6), 4.30 (dd, $^3J(4,5)$ = 1.3 Hz, $^3J(3,4)$ = 6.7 Hz, 1H, H-4), 4.19 (dd, $^3J(4,5)$ = 1.3 Hz, $^3J(5,6)$ = 9.6 Hz, 1H, H-5), 4.10 (dd, $^3J(2,3)$ = 3.9 Hz, $^3J(3,4)$ = 6.7 Hz, 1H, H-3), 3.97 (dd, $^3J(2,8a)$ = 4.4 Hz, $^3J(8a,8b)$ = 12.3 Hz, 1H, H-8a), 3.83 (dd, $^3J(2,8b)$ = 8.5 Hz, $^3J(8a,8b)$ = 12.3 Hz, 1H, H-8b), 3.57 (dd, $^3J(6,7a)$ = 4.8 Hz, $^3J(7a,7b)$ = 13.8 Hz, 1H, H-7a), 3.40 (m, $^3J(2,3)$ = 3.9 Hz, $^3J(2,8a)$ = 4.4 Hz, $^3J(2,8b)$ = 8.5 Hz, 1H, H-2), 3.12 (dd, $^3J(6,7b)$ = 11.2 Hz, $^3J(7a,7b)$ = 13.8 Hz, 1H, H-7b), 1.89 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, D_2O): δ = 174.8 (C=O), 74.6 (C-4), 70.3 (C-5), 67.2 (C-3), 66.6 (C-2), 61.2 (C-8), 48.7 (C-6), 46.0 (C-7), 22.4 ($COCH_3$); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_9H_{19}O_5N_2$: 235.1294; found: 235.1292.

5.1.6. Spectroscopic data for (3*S*,4*R*,5*R*,6*R*,7*R*),*N*-(3,5,6-trihydroxy-7-hydroxymethyl-azepan-4-yl)-acetamide 18

$[\alpha]_D$ = +72.7 (*c* 1.3, CH_3OH); ^1H NMR (400 MHz, D_2O): δ = 4.39 (dd, $^3J(4,5)$ = 1.6 Hz, $^3J(5,6)$ = 9.6 Hz, 1H, H-5), 4.21 (ddd, $^3J(6,7a)$ = 4.1 Hz, $^3J(1,7b)$ = 10.6 Hz, $^3J(5,6)$ = 9.6 Hz, 1H, H-6), 4.18 (dd, $^3J(3,4)$ = 6.6 Hz, $^3J(4,5)$ = 1.6 Hz, 1H, H-4), 4.08 (dd, $^3J(2,3)$ = 3.0 Hz, $^3J(3,4)$ = 6.6 Hz, 1H, H-3), 3.96 (dd, $^3J(8a,2)$ = 4.5 Hz, $^3J(8a,8b)$ = 12.2 Hz, 1H, H-8a), 3.83 (dd, $^3J(8b,2)$ = 9.0 Hz, $^3J(8a,8b)$ = 12.2 Hz, 1H, H-8b), 3.62 (dd, $^3J(6,7b)$ = 4.1 Hz, $^3J(7a,7b)$ = 13.6 Hz, 1H, H-7b), 3.49 (ddd, $^3J(2,3)$ = 3.0 Hz, $^3J(2,8a)$ = 4.5 Hz, $^3J(2,8b)$ = 9.0 Hz, 1H, H-2), 3.24 (dd, $^3J(7a,6)$ = 10.6 Hz, $^3J(7a,7b)$ = 13.6 Hz, 1H, H-7a); ^{13}C NMR (100.6 MHz, D_2O): δ = 174.5 (C=O), 72.9 (C-4), 67.6 (C-3), 66.3 (C-6), 66.1 (C-2), 61.4 (C-8), 53.8 (C-5), 48.0 (C-7), 22.4 ($COCH_3$); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_9H_{19}O_5N_2$: 235.1294; found: 235.1290.

5.1.7. (3*S*,4*R*,5*R*,6*S*) 3-Azido-4,5,6-tris-benzyloxy-azepane-1-carboxylic acid benzyl ester 20

Methanesulfonyl chloride (17.4 μL, 0.23 mmol) was added dropwise at 0 °C under argon to a solution of alcohol **19** (41 mg, 72.2 μmol) and a catalytic amount of DMAP (3.5 mg, 29 μmol) in dry pyridine (1 mL). The ice bath was removed and the reaction mixture was stirred for 2 h at rt, co-evaporated with toluene and concentrated. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with a 1 M HCl aq solution (5 mL). The organic layer was then dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude mesylate was used directly without further purification. To a solution of crude mesylate in DMF (2 mL) was added NaN_3 (28 mg, 0.43 mmol) and the reaction mixture was heated at 90 °C for 60 h. The solvent was removed and the residue was dissolved in CH_2Cl_2 (15 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. Purification by flash column chromatography (cyclohexane/EtOAc 1:10) afforded the azido azepane **20** (29 mg, 80%) as an oil. $[\alpha]_D$ = +5.8 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.29–7.05 (m, 40H, aromatic H), 5.14–5.01 (m, 4H, 4 × NCOOCHPh), 4.71 (d, *J* = 12.0 Hz, 1H, CHPh), 4.54–4.42 (m, 9H, 9 × CHPh), 4.39 (d, *J* = 12.0 Hz, 1H, CHPh), 4.34 (d, *J* = 12.0 Hz, 1H, CHPh), 3.91 (dt, $^3J(6,7a)$ = 2.0 Hz, $^3J(6,5) = {}^3J(6,7b)$ = 8.0 Hz, 1H, H-6), 3.85 (dt, $^3J(6',7'a)$ = 1.6 Hz, $^3J(6',5') = {}^3J(6',7'b)$ = 7.6 Hz, 1H, H-6′), 3.80–3.67 (m, 5H, H-2a, H-3′, H-4, H-4′, H-2′a), 3.65–3.60 (m, 3H, H-7′a, H-7a, H-3), 3.58–3.55 (m, 2H, H-5, H-5′), 3.50–3.45 (m, 3H, H-2b, H-2′b, H-7b), 3.39 (dd, $^3J(7'b,6')$ = 7.6 Hz, $^3J(7'b,7'a)$ = 14.8 Hz, 1H, H-7′b); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.2, 156.1 (2 × C=O), 138.4, 138.1, 137.9, 137.8, 137.7, 137.6, 136.5, 136.4 (8 × *Cipso*), 128.6–127.4 (40 × aromatic CH), 83.0, 82.4 (C-5′, C-5), 81.7, 81.1 (C-4, C-4′), 79.4, 78.5 (C-3, C-3′), 73.3, 73.1, 73.0, 72.9, 71.7, 71.6 (6 × CH_2Ph), 67.8, 67.5 (2 × $NCOOCH_2Ph$), 64.0, 63.3 (C-6′, C-6), 46.1, 46.0 (C-7, C-7′), 44.8, 44.4 (C-2′, C-2); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_{35}H_{37}O_5N_4$: 593.2764; found: 593.2763.

5.1.8. (3*S*,4*R*,5*R*,6*S*) 3-Acetylamino-4,5,6-tris-benzyloxy-azepane-1-carboxylic acid benzyl ester 21

Triphenyl phosphine (16 mg, 61 μmol) was added to a solution of the azido azepane **20** (24 mg, 0.040 mmol) in a 3:1 mixture of THF–H_2O (2 mL) under argon. The reaction mixture was stirred at 50 °C for 18 h, by which time TLC revealed no trace of starting material. The reaction mixture was concentrated under reduced pressure and the crude amine was directly engaged in the next step. The crude amine was dissolved in anhydrous pyridine (1.5 mL) and acetic anhydride (0.5 mL) was added under argon. The reaction mixture was stirred at rt for 2 h and was then co-evaporated with toluene and concentrated. Purification by flash column chromatography (cyclohexane/ethyl acetate, 10:1 then 3:1 then 1:1) afforded the corresponding acetamido azepane **21** (18 mg, 72%) as a colorless oil. $[\alpha]_D$ = −1.2 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.08 (m, 30 H, aromatic H), 6.57–6.55 (m, 2H, NH, NH'), 5.07–4.98 (m, 4H, 4 × NCOOCHPh), 4.71 (d, *J* = 11.7 Hz, 1H, CHPh), 4.59–4.24 (m, 10 H, 4 × CH_2Ph, H-6, H-6′), 4.20–4.12 (m, 3 H, H-2a, H-7a, H-7′a), 4.00–3.87 (m, 5 H, H-2′a, H-4, H-4′, H-3, H-5′), 3.80–3.72 (m, 2 H, H-3′, H-5), 3.29–3.21 (m, 4H, H-2b, H-2′b, H-7b, H-7′b), 1.68 (s, 3H, CH_3CO), 1.36 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 169.7 (2 × C=O, Ac), 156.5, 156.2 (2 × C=O, Z), 138.2, 138.1, 137.7, 137.5, 137.4, 137.3, 136.6, 136.5 (8 × *Cipso*), 128.6–127.6 (40 × aromatic CH), 83.7, 83.4 (C-4, C-4′), 81.3, 81.1 (C-3′, C-3), 76.0, 75.8 (C-5, C-5′), 72.7, 72.5, 72.3, 71.9, 71.8 (6 × CH_2Ph), 67.5, 67.2 (2 × $NCOOCH_2Ph$), 51.9, 51.5 (C-6′, C-6), 47.8 (C-2′ or C-7′), 47.7 (C-2′ or C-7′), 47.6 (C-2 or C-7), 46.8 (C-2 or C-7), 22.8 (2 × CH_3CO); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{41}O_6N_2$: 609.2965; found: 609.2961.

5.1.9. (3*S*,4*R*,5*R*,6*S*),*N*-(4,5,6-Trihydroxy-azepan-3-yl)-acetamide 22

To a solution of the protected acetamido azepane **21** (18 mg, 0.030 mmol) in CH_3OH (3 mL) was added 10% Pd/C (20 mg) and a 1 M HCl aq solution (30 μL). The solution was degased three times and air was replaced by H_2. After stirring for 7 h at rt, the reaction mixture was filtered through a Rotilabo® Nylon 0.45 μm filter eluted with CH_3OH, and concentrated to afford the acetamido azepane **22** (6.1 mg, quantitative yield) as a colorless oil. $[\alpha]_D$ = +14.8 (*c* 0.4, CH_3OH); ^1H NMR (400 MHz, D_2O): δ= 4.18 (dt, $^3J(3,2a)$ = 2.7 Hz, $^3J(3,4) = {}^3J(3,2b)$ = 9.7 Hz, 1H, H-3), 4.03 (dt, $^3J(6,7a)$ = 2.4 Hz, $^3J(5,6) = {}^3J(6,7b)$ = 6.6 Hz, 1H, H-6), 3.76 (t, $^3J(5,6) = {}^3J(4,5)$ = 6.6 Hz, 1H, H-5), 3.65 (dd, $^3J(4,5)$ = 6.6 Hz, $^3J(3,4)$ = 9.7 Hz, 1H, H-4), 3.33–3.22 (m, 3H, H-2a, H-2b, H-7a), 3.15–3.07 (m, 1H, H-7b), 1.95 (s, 3H, CH_3CO); ^{13}C NMR

(100 MHz, D_2O): δ= 174.0 (C=O), 75.9 (C-5), 74.8 (C-4), 67.6 (C-6), 49.4 (C-3), 46.5 (C-2 or C-7), 46.1 (C-2 or C-7), 21.9 ($COCH_3$); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_8H_{17}O_4N_2$: 205.1188; found: 205.1183.

5.1.10. (3*S*,4*R*,5*R*,6*R*) 4,5,6-Tris-benzyloxy-3-hydroxy-azepane-1-carboxylic acid benzyl ester 23

To a stirred solution of alcohol **19** (133 mg, 0.23 mmol), triphenyl phosphine (307 mg, 1.17 mmol), and benzoic acid (143 mg, 1.17 mmol) in dry THF (4.5 mL) were added. To this reaction mixture a solution of DEAD (180 μL, 1.17 mmol) was slowly added at 0 °C under argon. The resulting yellow solution was stirred at rt for 5 h. Solvent was then removed under reduced pressure and the resulting residue was purified by flash column chromatography (cyclohexane/ethyl acetate 10:1 then 5:1) to afford the benzoyl derivative (106 mg, 66%) as a colorless oil. A 0.1 M NaOMe solution was added dropwise to a solution of benzoyl derivative (106 mg, 0.16 mmol) in MeOH (2.5 mL) in order to reach pH= 9–10. After stirring 4 h at rt the reaction mixture was stirred at 40 °C for 10 h. The reaction mixture was then neutralized by stirring with acidic Amberlite IR 120 (H^+) resin for 30 min, filtered and concentrated under reduced pressure. Purification by flash column chromatography (cyclohexane/ethyl acetate 3:1) afforded alcohol **23** (84 mg, 94%) as a colorless oil. $[\alpha]_D$ = +6.2 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.17 (m, 40H, aromatic H), 5.22–5.11 (m, 4H, 4 × NCOOCHPh), 4.82–4.75 (m, 2H, 2 × CHPh), 4.74 (d, *J* = 12.4 Hz, 1H, CHPh), 4.66–4.57 (m, 5H, 5 × CHPh), 4.52–4.43 (m, 4H, 4 × CHPh), 4.06 (dt, ^3J(3,2b) = 2.7 Hz, ^3J(3,2a) = ^3J(3,4) = 6.8 Hz, 1H, H-3), 3.98 (dt, ^3J(3′,2′b) = 2.9 Hz, ^3J(3′,2′a) = ^3J(3′,4′) = 6.5 Hz, 1H, H-3′), 3.85–3.68 (m, 8H, H-2a, H-3′, H-7a, H-4, H-6, H-6′, H-7′a, H-4′, H-2′a), 3.66–3.62 (m, 2H, H-5, H-5′), 3.56 (dd, ^3J(2′b,3′) = 2.9 Hz, ^3J(2′b,2′a) = 14.5 Hz, 1H, H-2′b), 3.51 (dd, ^3J(2b,3) = 2.7 Hz, ^3J(2b,2a) = 14.5 Hz, 1H, H-2b), 3.47–3.32 (m, 2H, H-7b, H-7′b); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 156.5, 156.3 (2 × C=O), 138.3, 138.1, 137.9, 137.8, 137.7, 137.6, 136.7, 136.4 (8 × *Cipso*), 128.5–127.5 (40 × aromatic CH), 83.5, 83.2 (C-4, C-4′), 81.4, 81.0 (C-5, C-5′), 80.7, 79.6 (C-6, C-6′), 73.7, 73.6, 73.5, 71.9, 71.8 (6 × CH_2Ph), 71.1, 71.0 (C-3, C-3′), 67.7, 67.3 (2 × NCOOCH$_2$Ph), 49.2, 49.1 (C-2, C-2′), 46.0 (C-7, C-7′); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_{35}H_{38}O_6N$: 568.2699; found: 568.2704.

5.1.11. (3*R*,4*R*,5*R*,6*S*) 3-Azido-4,5,6-tris-benzyloxy-azepane-1-carboxylic acid benzyl ester 24

Methanesulfonyl chloride (31.4 μL, 0.42 mmol) was added dropwise at 0 °C under argon to a solution of alcohol **23** (74 mg, 130 μmol) and a catalytic amount of DMAP (6.4 mg, 52 μmol) in dry pyridine (2 mL). The ice bath was removed and the reaction mixture was stirred for 3 h at rt, co-evaporated with toluene and concentrated. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with a 1 M HCl aq solution (5 mL). The organic layer was then dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude mesylate was used directly without further purification. To a solution of crude mesylate in DMF (3 mL) was added NaN_3 (49 mg, 0.72 mmol) and the reaction mixture was heated at 90 °C for 96 h. The solvent was removed and the residue was dissolved in CH_2Cl_2 (25 mL) and washed successively with water (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. Purification by flash column chromatography (cyclohexane/ethyl acetate 1:10 then 1:6) afforded the azido azepane **24** (53 mg, 69%) as an oil. $[\alpha]_D$ = −23.9 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.27–7.09 (m, 40H, aromatic H), 5.05–4.94 (m, 4H, 4 × NCOOCHPh), 4.64 (d, *J* = 10.8 Hz, 1H, CHPh), 4.62–4.59 (m, 2H, 2 × CHPh), 4.52–4.25 (m, 9H, 9 × CHPh), 4.17–4.09 (m, 2H, H-2a, H-7a), 3.98 (dd, ^3J(2′a,3′) = 4.8 Hz, ^3J(2′a,2′b) = 13.8 Hz, 1H, H-2′a), 3.93–3.87 (m, 5H, H-4, H-5, H-5′, H-6, H-7′a), 3.80 (dd, ^3J(4′,3′) = 3.3 Hz, ^3J(4′,5′) = 6.0 Hz, 1H, H-4′), 3.75 (ddd, ^3J(3,2a) = 1.2 Hz, ^3J(3,4) = 4.7 Hz, ^3J(3,2b) = 10.2 Hz, 1H, H-3), 3.67–3.62 (m, 2H, H-3′, H-6′), 3.30–3.16 (m, 4H, H-2b, H-2′b, H-7b, H-7′b); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 155.5, 155.4 (2 × C=O), 138.2, 138.1, 137.7, 137.6, 137.5, 137.4, 136.5, 136.4 (8 × *Cipso*), 128.5–127.6 (40 × aromatic CH), 80.3, 80.1 (C-5, C-5′), 80.0, 79.4 (C-4, C-4′), 79.1, 78.9 (C-6, C-6′), 73.0, 72.9, 72.4, 72.1, 71.8 (6 × CH_2Ph), 67.5, 67.3 (2 × NCOOCH$_2$Ph), 58.2, 58.2 (C-3, C-3′), 48.0, 47.2 (C-7, C-7′), 46.5, 46.4 (C-2, C-2′); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_{35}H_{37}O_5N_4$: 593.2764; found: 593.2759.

5.1.12. (3*R*,4*R*,5*R*,6*S*) 3-Acetylamino-4,5,6-tris-benzyloxy-azepane-1-carboxylic acid benzyl ester 25

Triphenyl phosphine (16 mg, 61 μmol) was added to a solution of the azido azepane **24** (25 mg, 0.040 mmol) in a 3:1 mixture of THF–H_2O (2 mL) under argon. The reaction mixture was stirred at 50 °C for 18 h, by which time TLC revealed no trace of starting material. The reaction mixture was concentrated under reduced pressure and the crude amine was directly engaged in the next step. The crude amine was dissolved in anhydrous pyridine (1.5 mL) and acetic anhydride (0.5 mL) was added under argon. The reaction mixture was stirred at rt for 2 h and was then co-evaporated with toluene and concentrated. Purification by flash column chromatography (cyclohexane/ethyl acetate, 10:1 then 3:1 then 1:1) afforded the corresponding acetamido azepane **25** (20 mg, 81%) as a colorless oil. $[\alpha]_D$ = −5.2 (*c* 0.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.19 (m, 40 H, aromatic H), 6.26 (d, $^3J_{NH,3}$ = 9.5 Hz, 1H, NH), 5.96 (d, $^3J_{NH',3'}$ = 9.5 Hz, 1H, NH'), 5.19 (d, *J* = 12.5 Hz, 1H, NCOOCHPh), 5.15 (d, *J* = 12.5 Hz, 1H, NCOOCHPh), 5.07 (d, *J* = 12.5 Hz, 1H, NCOOCHPh), 5.06 (d, *J* = 12.5 Hz, 1H, NCOOCHPh), 4.76 (d, *J* = 10.9 Hz, 1H, CHPh), 4.71–4.62 (m, 4 H, 2 × CH_2Ph, H-3, H-3′), 4.56–4.47 (m, 8 H, 8 × CHPh), 4.44 (d, *J* = 11.6 Hz, 1H, CHPh), 4.39 (d, *J* = 11.5 Hz, 1H, CHPh), 4.03 (dd, ^3J(7a,6) = 7.9 Hz, ^3J(7a,7b) = 14.1 Hz, 1H, H-7a), 3.92–3.87 (m, 4 H, H-4, H-4′, H-6, H-7′a), 3.83–3.71 (m, 5H, H-2a, H-2′a, H-5, H-5′, H-6′), 3.67–3.55 (m, 3H, H-2′b, H-7b, H-7′b), 3.43 (dd, ^3J(2b,3) = 2.5 Hz, ^3J(2b,2a) = 14.1 Hz, 1H, H-2b), 1.71 (s, 3H, CH_3CO), 1.53 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.5, 169.2 (2 × C=O, CH_3CO), 156.2, 155.9 (2 × C=O, Z), 137.9, 137.8, 137.7, 137.6, 136.6, 136.5 (8 × *Cipso*), 128.5–127.6 (40 × aromatic CH), 82.8, 81.7 (C-5, C-5′), 81.5, 80.2 (C-4, C-4′), 78.5, 77.3 (C-6′, C-6), 72.5, 72.3, 72.2, 72.1, 71.9, 71.8 (6 × CH_2Ph), 67.5, 67.2 (2 × NCOOCH$_2$Ph), 48.1, 48.0 (C-2, C-2′), 47.2, 47.0 (C-3, C-3′), 46.5, 46.4 (C-7′, C-7), 23.2, 22.9 (2 × CH_3CO); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{41}O_6N_2$: 609.2965; found: 609.2957.

5.1.13. Synthesis of (3*R*,4*R*,5*R*,6*S*),*N*-(4,5,6-trihydroxy-azepan-3-yl)-acetamide 26

To a solution of the protected acetamido azepane **25** (18 mg, 0.030 mmol) in CH_3OH (3 mL) was added 10% Pd/C (20 mg) and a 1 M HCl aq solution (30 μL). The solution was degased three times and air was replaced by H_2. After stirring for 7 h at rt, the reaction mixture was filtered through a Rotilabo® Nylon 0.45 μm filter eluted with CH_3OH, and concentrated to afford the acetamido azepane **26** (6.1 mg, quantitative yield) as a colorless oil. $[\alpha]_D$ = +9.8 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, D_2O): δ = 4.41 (app dd, ^3J(3,2b) = 3.9 Hz, ^3J(3,2a) = 8.7 Hz, 1H, H-3), 3.98 (app dd, ^3J(5,6) = 4.7 Hz, ^3J(6,7a) = 8.0 Hz, 1H, H-6), 3.88 (br d, ^3J(4,5) = 4.7 Hz, H-4), 3.80 (t, ^3J(4,5) = ^3J(5,6) = 4.7 Hz, 1H, H-5), 3.35–3.24 (m, 3H, H-7a, H-7b, H-2a), 3.16 (dd, ^3J(2b,3) = 3.9 Hz, ^3J(2b,2a) = 13.3 Hz, H-2b), 1.91 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, D_2O): δ = 173.5 (C=O), 75.0 (C-4), 73.7 (C-5), 68.9 (C-6), 46.1 (C-3), 45.1 (C-7), 43.9 (C-2), 21.8 (CH_3CO); HRMS (CIMS) *m/z*: $[M+H]^+$ calcd for $C_8H_{17}O_4N_2$: 205.1188; found: 205.1192.

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References and notes

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