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Selective trihydroxylated azepane inhibitors of NagZ, a glycosidase involved in *Pseudomonas aeruginosa* resistance to β-lactam antibiotics†

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The synthesis of a series of D-*gluco*-like configured 4,5,6-trihydroxyazepanes bearing a triazole, a sulfonamide or a fluorinated acetamide moiety at C-3 is described. These synthetic derivatives have been tested for their ability to selectively inhibit the muropeptide recycling glucosaminidase NagZ and to thereby increase sensitivity of *Pseudomonas aeruginosa* to β-lactams, a pathway with substantial therapeutic potential. While introduction of triazole and sulfamide groups failed to lead to glucosaminidase inhibitors, the $NHCOCF_3$ analog proved to be a selective inhibitor of NagZ over other glucosaminidases including human *O*-GlcNAcase and lysosomal hexosaminidases HexA and B.

Introduction

β-Lactam antibiotics are the mainstay treatment for bacterial infections, constituting over 50% of all antibiotics in clinical use. Their therapeutic efficacy however, is being steadily eroded by the increasing prevalence of bacterial resistance mechanisms.[1] A formidable mechanism of β-lactam resistance in Gram-negative bacteria is the production of the inducible AmpC β-lactamase,[2] an enzyme that deactivates most β-lactams, including penicillins, cephems and monobactams. AmpC is produced by *Pseudomonas aeruginosa*, a pathogen that is the leading cause of chronic lung infections and mortality among people with cystic fibrosis, and a major cause of severe hospital-acquired infections. AmpC induction depends on the activity of the highly conserved peptidoglycan (PG) recycling pathway of Gram-negative bacteria.[3] β-Lactams activate this pathway, and during prolonged therapy, mutations that cause constitutive AmpC hyperproduction inevitably arise in *P. aeruginosa*. These mutations confer resistance to the majority of β-lactams and is the foremost cause of the therapeutic failure of β-lactams against *P. aeruginosa*. Recently, the AmpC inhibitor avibactam in combination with ceftazidime has been clinically approved and works effectively against *P. aeruginosa*.[4] Most clinically available β-lactamase inhibitors, however, are ineffective against AmpC-mediated resistance and direct targeting of AmpC could lead to the rapid evolution of resistance. Accordingly, new strategies to indirectly block AmpC activity are a topic of considerable interest.[5]

One other potential strategy to fight inducible AmpC would be to inhibit one step in the peptidoglycan pathway by targeting the cytosolic glycoside hydrolase (GH) NagZ with small molecule inhibitors. NagZ is a member of CAZy family GH3[6] that catalyzes the hydrolysis of cytosolic GlcNAc (1–4)-1,6-anhydroMurNAc-peptide to yield free GlcNAc and anhMurNAc-peptide (Fig. 1b), which is the ligand that induces transcrip-

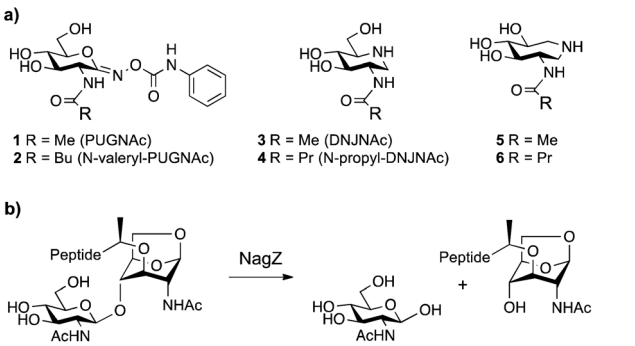

Fig. 1 (a) Structure of NagZ inhibitors **1–6**; (b) action of NagZ on peptidoglycan cell wall fragments.

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tion of AmpC. These two recycling fragments are ultimately recycled as precursors in central metabolism and PG biosynthesis. NagZ uses a two-step, double displacement mechanism involving the formation and breakdown of a covalent glycosyl–enzyme intermediate.[7–9] Significantly, the 2-acetamido group of the GlcNAc residue of the substrate is not involved in catalysis. Guided by these detailed structural and mechanistic studies of NagZ, several inhibitors including compounds **1–6** [10–14] (Fig. 1a) have been pursued as a strategy to block AmpC production and shown to potentiate the efficacy of co-administered β-lactams against *P. aeruginosa*.[15] Co-administration of the inhibitors with frontline β-lactam ceftazidime significantly reduces the minimal inhibitory concentration (MIC) of these β-lactams against AmpC hyperproducing mutants of *P. aeruginosa*, thereby demonstrating that our approach can revitalize the usefulness of these antibiotics against the pathogen. Interestingly, it has also been shown that genetic inactivation of NagZ suppress the emergence of highly resistant AmpC hyper producing mutants,[16] suggesting that NagZ inhibitors may also suppress the development of β-lactam resistance.

While being a submicromolar inhibitor of NagZ, iminosugar **5** (AzeNAc) proved to be also a potent inhibitor of functionally related human glycosidase *O*-GlcNAcase (OGA)[17] and lysosomal β-hexosaminidase HexA. Examination of the active sites of these enzymes that belong to the GH84 and GH20 families respectively revealed that the pocket interacting with the 2-acetamido group differs.[18] In this work, we have explored this structural discrepancy and incorporated *N*-acyl substituents of various sizes as well as acetamide bio-isosteric substituents with the aim of improving the selectivity and potency of this seven-membered ring scaffold towards NagZ enzymes.

Results and discussion

Several groups have shown that precise structural modifications of *N*-acetyl-D-glucosamine-based iminosugars can bias their inhibition profile toward a specific *N*-acetyl-D-glucosaminidase of therapeutic interest.[19–22] In the case of NagZ, selectivity can be achieved through modification of the acetamide moiety. Increasing the alkyl chain length up to 3 or 4 carbons provides potent and selective molecules. When applied to AzeNAc **5**, such modification led to inhibitors such as **6** that were more selective toward NagZ over OGA but also less potent.[14] The NagZ-azepane **6** crystal structure[23] revealed the presence of an extensive pocket with space available to accommodate modified acetamide moieties. Therefore, we postulated that replacement of the acetamide group by bio-isosteric functionalities could be tolerated by the enzyme and might generate more potent and selective inhibitors. We first focused on triazole and sulfonamide groups that have proven useful as replacements for acetamide groups in medicinal chemistry.[24] Moreover, the enzymatic pocket is surrounded by three histidine residues that might accommodate functions with distinct electronic properties. Therefore, as a more subtle modification, we also examined fluorinated acyl moieties that had previously proved beneficial in the case of an iminosugar targeting heparanase, another GlcNAc processing glycosidase.[25] To validate such structural modifications, docking experiments were first conducted using the crystal structure of the 1.8 Å resolution complex of *Burkholderia cenocepacia* NagZ (*Bc*NagZ) (PDB 4MSS) from which the AzeNAc inhibitor **5** has been removed. The *in silico* experiments were performed using GOLD software[26] (Fig. 2).

Except for the hydroxyl group beta to the nitrogen, a fairly good superimposition between the docked analogues bearing a methyltriazole, methylsulfonamide or fluorinated acetamide and the parent iminosugar is observed, which encouraged us to pursue their synthesis.

Synthesis

The known azidoazepane **7** [27] available in 11 steps from β-D-glucose pentaacetate was used as common precursor for all the syntheses described in this work.

Synthesis of triazole derivatives. The Cu(I) catalysed 1,3-dipolar cycloaddition of azides and terminal alkynes is the most popular strategy to access 1,2,3-triazoles. However, low molecular weight alkynes, typically C2 to C5 derivatives that are of interest to us, are generally under-represented as substrates in CuAAC reactions due to their volatility. Preliminary

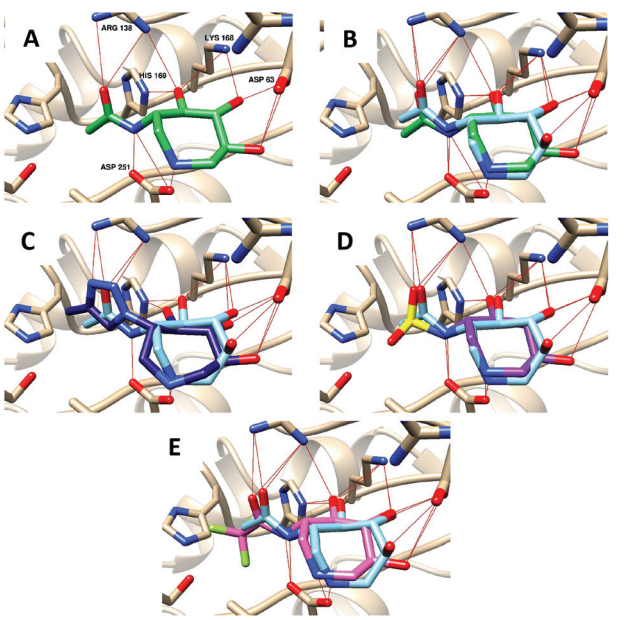
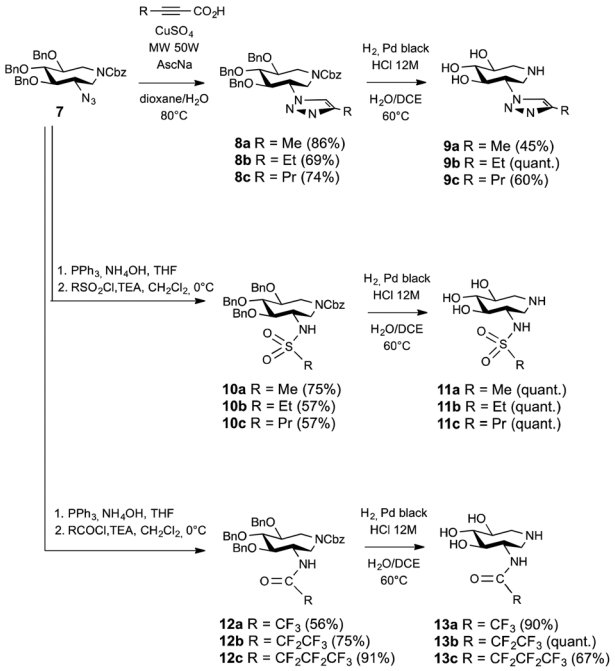


Fig. 2 A: Docking conformation of *Bc*NagZ/azepane **5** (green sticks). B: Superimposition of crystal structure (light blue sticks) and docked structure of *Bc*NagZ/azepane **5**. C: Superimposition of crystal structure of *Bc*NagZ/azepane **5** and methyltriazolo azepane **9a**. D: Superimposition of crystal structure of *Bc*NagZ/azepane **5** and methylsulfonamido azepane **11a**. E: Superimposition of crystal structure of *Bc*NagZ/azepane **5** and trifluoroacetamido azepane **13a**. Non carbon atoms are colored by type (O: red; N: dark blue; F: green; S: yellow). The blue structure represents the 1.8 Å resolution crystal structure of **5** bound to *Bc*NagZ (PDB ID: 4MSS).

trials were disappointing and confirmed this practical issue. Using Williams' one-pot strategy using non-volatile TMS-alkynes[28] proved unsuccessful with ethynyltrimethylsilane in our case. We were more successful with Kolarovič's tandem catalysis protocol based on the decarboxylative coupling of alkynoic acids followed by 1,3-dipolar cycloaddition of azides.[29] Using this procedure, azidoazepane **7** was efficiently clicked to butynoic, pentynoic and hexynoic acids in the presence of $CuSO_4$ and sodium ascorbate under microwave activation at 80 °C to produce the 1,4-disubstituted triazoles **8a–c** in 69–86% yield. Final hydrogenolysis using Pd black in a dichloroethane/water mixture under acidic conditions furnished the target triazolo-azepanes **9a–c**.

Synthesis of sulfonamide derivatives. A Staudinger-mediated azide reduction of azepane **7** using supported triphenylphosphine, followed by direct sulfonylation of the crude amine with the appropriate alkylsulfonyl chlorides afforded in satisfactory yields (57–75%) the corresponding sulfonamido-azepanes **10a–c** that were uneventfully and quantitatively deprotected by hydrogenolysis to yield the target sulfonamides **11a–c**.

Synthesis of fluoroalkyl derivatives. The length of the acetamido alkyl chain has been shown to be important for discrimination between therapeutically relevant β-*N*-acetyl-D-glucosaminidases OGA, HexA and NagZ. This difference stems from the shape of their specific binding pocket that accommodates the NHAc group. We reasoned that introduction of a fluorinated alkyl chain might be beneficial to selectively target NagZ for electronic reasons according to the histidine environment of the NagZ active site. To this end, the crude amine resulting from azide reduction was acylated with perfluorinated alkyl chains of one, two or three carbons to furnish the corresponding amides **12a–c** in good yield (56–91%). Their subsequent hydrogenolysis produced the target azepanes **13a–c** (Scheme 1).



Scheme 1 Synthesis of trihydroxylated azepanes bearing a triazole **9a–c**, a sulfonamide **11a–c** or a fluorinated acetamide **13a–c** moiety at C-3.

Biological evaluation of the polyhydroxylated azepanes

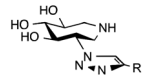
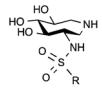
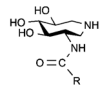
Inhibition of glycosidases. As a preliminary screen, polyhydroxylated azepanes **9a–c**, **11a–c**, **13a–c** were evaluated as inhibitors of a collection of twenty glycosidases, including four hexosaminidases. Interestingly, none of these derivatives was shown to inhibit hexosaminidases at 1 mM concentration (Table 1). The triazole derivative **9c** (R = Pr) weakly inhibited yeast α-glucosidase and *E. coli* β-glucuronidase, respectively. The sulfonamide analogs **11b** (R = Et) and **11c** (R = Pr) were identified as weak inhibitors of rice α-glucosidase and rat intestinal maltase as well as α- and β-galactosidases. In contrast, the fluoroalkyl derivatives **13b** (R = Et) and **13c** (R = Pr) showed weak but selective inhibition of rat intestinal cellobiase. The propensity of these azepanes to mainly inhibit enzymes that process D-glucose-configured substrates is in accord with the *gluco*-like configuration of the azepane scaffold. The fact that derivatives **9c**, **11b** and **11c** weakly inhibit glucosidases but not hexosaminidases suggests that the sulfonamide and triazole motifs are not recognized by these hexosaminidases as surrogates of the acetamido group. The library of derivatives was next assayed on the human relevant NagZ, OGA and a preparation of isolated lysosomal β-hexosaminidases HexA and B (HexAB) enzymes.

Inhibition of NagZ, OGA and HexAB. To analyse the relative ability of the azepanes to inhibit NagZ, OGA, and HexAB we used a continuous assay using the chromogenic substrate *para*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (*p*NP-GlcNAc). We first selected a representative compound of each set of azepanes, namely the methyl analogs **9a**, **11a** and **13a**. We found that replacing the *N*-acyl group by a triazole or sulfonamide moiety leads to a complete loss of inhibition against the three enzymes (Table 2, ESI Fig. S1†). Nevertheless, introduction of a $NHCOCF_3$ group (**13a**) provided a selective and competitive NagZ inhibitor (K_i = 5.7 ± 0.5 μM, ESI Fig. S2†) while longer $NHCOCF_2CF_3$ (**13b**) and $NHCOCF_2CF_2CF_3$ (**13c**) groups completely abolished NagZ inhibition. The substitution of hydrogen for fluorine is a monovalent isosteric replacement used widely in medicinal chemistry. This is due to the similar steric parameters for the hydrogen and fluorine atoms (van der Waals radii 1.2 and 1.35 Å). Fluorine, however, exerts strong field and inductive effects on nearby atoms. Therefore, the observed selectivity of **13a** for NagZ may stem from subtle steric or electronic effects.

Potentiation of the efficacy of ceftazidime

Since azepane **13a** displayed fair potency and selectivity toward NagZ, its ability to block NagZ in bacteria and attenuate inducible AmpC-mediated β-lactam resistance within a relevant bacterial model representative of clinically occurring *P. aeruginosa* was evaluated. While **13a** had no effect on its

Table 1 Concentration of polyhydroxylated azepanes giving 50% inhibition of various glycosidases

										
	R=	Me	Et	Pr	Me	Et	Pr	CF_3	CF_2CF_3	$CF_2CF_2CF_3$
Enzyme		9a	9b	9c	11a	11b	11c	13a	13b	13c
α-Glucosidase										
Rice		NI[a] (16.8%)[b]	NI (19.5%)	NI (29.7%)	NI (33.3%)	307	383	NI[a] (8.6%)[b]	NI (46.5%)	NI (0.5%)
Yeast		NI (9.4%)	NI (41.1%)	130	NI (9.8%)	NI (14.7%)	NI (6.0%)	NI (2.1%)	NI (11.4%)	NI (7.7%)
Rat intestinal maltase		NI (10.6%)	NI (14.6%)	NI (11.8%)	NI (15.0%)	814	NI (48.8%)	NI (5.3%)	NI (21.6%)	NI (6.1%)
β-Glucosidase										
Almond		NI (5.2%)	NI (10.3%)	NI (25.0%)	NI (2.9%)	NI (5.51%)	NI (5.9%)	NI (49.6%)	NI (0%)	NI (36.4%)
Bovine liver		NI (20.3%)	NI (29.6%)	NI (24.8%)	NI (28.3%)	NI (46.2%)	NI (38.4%)	111	NI (17.6%)	NI (47.7%)
Rat intestinal cellobiase		NI (36.2%)	NI (32.9%)	NI (40.4%)	NI (9.3%)	NI (8.2%)	NI (9.3%)	35	165	380
α-Galactosidase										
Coffee beans		NI (3.5%)	NI (49.4%)	NI (38.2%)	NI (46.6%)	502	529	NI (1.2%)	NI (0%)	NI (3.5%)
β-Galactosidase										
Bovine liver		NI (14.2%)	NI (34.0%)	NI (39.2%)	NI (41.6%)	503	627	47	NI (31.8%)	NI (46.3%)
α-Mannosidase										
Jack bean		NI (0%)	NI (0%)	NI (2.9%)	NI (0 %)	NI (0%)	NI (3.8%)	NI (16.6%)	NI (0.2%)	NI (5.5%)
β-Mannosidase										
Snail		NI (1.6%)	NI (0.61%)	NI (1.1%)	NI (1.8%)	NI (0%)	NI (0.92%)	NI (0%)	NI (0%)	NI (2.0%)
α-L-Rhamnosidase										
Penicillium decumbens		NI (0%)	NI (5.1%)	NI (2.9%)	NI (1.2%)	NI (5.1%)	NI (5.5%)	NI (39.7%)	NI (0%)	NI (2.0%)
α-L-Fucosidase										
Bovine kidney		NI (6.8%)	NI (4.4%)	NI (9.9%)	NI (3.6%)	NI (6.3%)	NI (11.8%)	NI (22.0%)	NI (0%)	NI (11.2%)
β-Glucuronidase										
E.coli		NI (12.6%)	NI (24.1%)	278	NI (15.7%)	NI (20.4%)	NI (38.4%)	NI (18.2%)	NI (14.6%)	NI (13.5%)
Bovine liver		NI (0%)	NI (4.1%)	NI (7.5%)	NI (5.9%)	NI (0%)	NI (1.5%)	NI (0%)	NI (0%)	NI (0%)
α,α-Trehalase										
Porcine kidney		NI (1.0%)	NI (0%)	NI (5.2%)	NI (0%)	NI (0%)	NI (0%)	NI (1.0%)	NI (0%)	NI (0%)
Amyloglucosidase										
Rhizopus sp.		NI (1.2%)	NI (0%)	NI (0%)	NI (4.0%)	NI (0%)	NI (0.2%)	NI (1.7%)	NI (0%)	NI (0%)
β-*N*-Acetylglucosaminidase										
Bovine kidney		NI (6.4%)	NI (O%)	NI (0%)	NI (1.7%)	NI (32.8%)	NI (7.6%)	NI (10.6%)	NI (0%)	NI (4.3%)
HL60		NI (30.3%)	NI (7.1%)	NI (8.4%)	NI (5.2%)	NI (32.0%)	NI (0%)	NI (34.3%)	NI (0%)	NI (13.8%)
Jack bean		NI (0%)	NI (7.8%)	NI (3.2%)	NI (0%)	NI (0%)	NI (O %)	NI (5.6%)	NI (0%)	NI (6.3%)
α-*N*-Acetylgalactosaminidase										
Chicken liver		NI (0%)	NI (3.7%)	NI (0%)	NI (0%)	NI (40.8%)	NI (5.3%)	NI (0%)	NI (0%)	NI (0%)

[a] NI: No inhibition (less than 50% inhibition at 1000 μM). [b] ( ): Inhibition % at 1000 μM.

Table 2 Inhibition of *Salmonella typhimurium* NagZ, human OGA, and human HexAB by azepane inhibitors

Compound	*O*-GlcNAcase K_i (μM)	NagZ K_i (μM)	HexAB K_i (μM)
5	0.7 ± 0.1[a]	0.4 ± 0.2[a]	3.6 ± 0.9[a]
6	47 ± 3.0[a]	7.4 ± 0.5[a]	NI
9a	NI	NI	NI
11a	NI	NI	NI
13a	NI	5.7 ± 0.5	NI
13b	NI	NI	NI

[a] Results determined previously.[14] NI: IC_{50} experiments yielded no significant inhibition at 0.46 mM concentration (see ESI Fig. S1).

Table 3 Susceptibility of PAΔ*dacB* to β-lactam antibiotic ceftazidime in the presence or absence of 1 mM azepane

Cefatzidime MIC[a] (μg ml^{-1})			
Strain			
PAΔ*dacB*	− Azepane	+ Azepane **13a**	+ Azepane **13c**
	32	24	32

[a] MICs were determined by the broth microdilution method using cation adjusted Mueller–Hinton media according to CLSI guidelines. Measurements were performed in duplicate.

own on bacterial growth, use of 1 mM of **13a** lowered the minimum inhibitory concentration (MIC) of *P. aeruginosa* Δ*dacB* by 25% from 32 μg ml^{-1} ceftazidime in the absence of inhibitor to 24 μg ml^{-1} (Table 3). In contrast, the more lipophilic derivative **13c** had no effect on ceftazidime efficacy.

Conclusions

A series of analogs of AzeNAc **5**, a submicromolar β-*N*-acetyl-D-glucosaminidase inhibitor, modified at the acetamide moiety, have been synthesized in order to improve its selectivity and potency toward NagZ, a key glycosidase involved in the inducible expression of AmpC β-lactamase that confers resistance to β-lactams antibiotics in many Gram negative bacteria. Interestingly, introduction of fluorinated acetamide moiety generated a selective albeit less potent NagZ inhibitor. This result suggests that introduction of a fluorinated acetamide group in GlcNAc-derived iminosugars represents a useful alternative to the NHAc bulk modification strategy to discriminate hexosaminidases that process GlcNAc conjugates. Work is in progress in our laboratory to further explore this finding.

Experimental

General methods

All commercial reagents were used as supplied. TLC plates were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of phosphomolybdic acid in ethanol (3 g per 100 mL) followed by heating with a heat gun. Flash column chromatography was performed using silica gel 60 (Macherey–Nagel 60, 15–40 μm) or carried on a combiflash® R_f automated apparatus (Teledyne-Isco) using columns specified in each protocol. NMR experiments were recorded with a 400 Bruker spectrometer at 400 MHz for ^{1}H, 376 MHz for ^{19}F and 100 MHz for ^{13}C nuclei. The chemical shifts are expressed in part per million (ppm) relative to TMS (δ = 0 ppm) and the coupling constant J in hertz (Hz). When spectra were recorded in D_2O, a drop of acetone or methanol was added as an internal reference for calibration. NMR multiplicities are reported using the following abbreviations: b = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. HRMS were obtained with a Q-TOF spectrometer from the Mass Spectrometry Service (IC2MP, UMR CNRS 7285-Poitiers University, France). Optical rotations were measured using a Modular Circular Polarimeter MCP100 (Anton Paar).

General procedure A for click-chemistry reactions: To a solution of azido azepane **7** in a dioxane/H_2O (4 : 1, 24 mL mmol^{-1}) mixture in a vial were added $CuSO_4$, $5H_2O$ (0.6 eq.) and sodium ascorbate (5.0 eq.). The alkyne (20.0 eq.) was then added and the vial was closed with a pierced rubber septum. The reaction mixture was heated at 50 °C under MW (50 W) during 1.5 h, by which time a TLC indicated total conversion of the starting material. The mixture was diluted with EtOAc, washed successively with $NaHCO_3$ sat. and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The desired product was obtained after purification over a flash chromatography column.

General procedure B for the transformation of azido azepane **7** into amido or sulfonamido azepanes: To a solution of azido azepane **7** in THF (16.7 mL mmol^{-1}) were added a solution of NH_4OH (28%) and triphenylphosphine polymer bound (3 mmol g^{-1}, 2.0 eq.). The resulting mixture was heated at 45 °C for 24 h, by which time a TLC indicated total consumption of the starting material. The mixture was filtered over a pad of Celite®, eluted with MeOH and concentrated *in vacuo* to give the amino azepane derivative as a colorless oil in a quantitative yield.

To a solution of the crude amine in dry DCM (9.4 mL mmol^{-1}) under Ar was added Et_3N (3.1 eq.) and the reaction mixture was cooled at 0 °C. The electrophile (1.5 eq.) was then added dropwise and the mixture was stirred for 1 h at 0 °C, by which time a TLC revealed no trace of the starting material. The mixture was diluted with DCM, washed with HCl 1 M, neutralized with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The desired product was obtained after purification over a flash chromatography column.

General procedure C for deprotections by hydrogenolysis: To a solution of the starting material in dichloroethane (52 mL mmol^{-1}) under Ar were added pure H_2O (52 mL mmol^{-1}), HCl 12 M (1.0 eq.) and Pd black (starting material mass × 2). The reaction mixture was stirred at 60 °C overnight under H_2 atmosphere, filtered over a pad of Celite® and eluted with MeOH. After concentration *in vacuo* the desired product was obtained without any further purification unless otherwise specified.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3-azido-4,5,6-tris(benzyloxy) azepane-1-carboxylate (7).[27] R_f = 0.53 (DCM/MeOH 95 : 5); $[\alpha]_D^{20}$ = +5.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.37–7.11 (20 H, m, CHAr), 5.21–5.08 (2 H, m, CH_2Cbz), 4.80–4.77 (0.5 H, m, CH_2OBn), 4.61–4.40 (5.5 H, m, CH_2OBn), 3.98 (0.5 H, dt, $J_{3,2}$ = 7.7 Hz, $J_{3,4}$ = 2.0 Hz, H3), 3.92 (0.5 H, dt, $J_{3,2}$ = 7.7 Hz, $J_{3,4}$ = 2.0 Hz, H3), 3.86–3.53 (6.5 H, m, 1.5 H2, 1 H4, 1 H5, 1 H6, 1 H7), 3.46 (0.5 H, dd, $J_{2,2'}$ = 14.7 Hz, $J_{2,3}$ = 8 Hz, H2); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.3, 156.2 (CO), 138.4, 138.2, 137.9, 137.7, 136.6, 136.4 (Cipso), 128.7–127.5 (CHAr), 83.0, 82.4, 81.7, 81.1, 79.5, 78.6 (C4, C5, C6), 73.4, 73.2, 73.0, 71.8, 71.7 (CH_2OBn), 67.9, 67.6 (CH_2Cbz), 64.1, 63.4 (C3), 46.1, 46.0 (C2), 44.9, 44.5 (C7); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_{35}H_{37}O_5N_4$: 593.2764, found: 593.2763.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(4-methyl-1*H*-1,2,3-triazol-1-yl)azepane-1-carboxylate (8a). Compound **7** (100 mg, 0.17 mmol) was reacted with butynoic acid (286 mg, 3.4 mmol) following general procedure A. The crude was purified over a flash chromatography column (Redisep® 4 g, EtOAc/PE 0 : 1 to 8 : 2) to give **8a** (93 mg, 86%) as a colorless and transparent oil. R_f = 0.71 (EtOAc/PE 4 : 6); $[\alpha]_D^{20}$ = +28.7° (*c* 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.34 (0.5 H, s, CHTria), 7.30–7.06 (18 H, m, CHAr), 6.98–6.89 (2 H, m, CHAr), 6.58 (0.5 H, s, CHTria), 5.16 (0.5 H, d, J = 12.0 Hz, CH_2Cbz), 5.06 (0.5 H, d, J = 12.3 Hz, CH_2Cbz), 4.98 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 4.86 (0.5 H, d, J = 12.0 Hz, CH_2Cbz), 4.78 (0.5 H, td, $J_{6,5}$ = 9.4 Hz, $J_{6,7}$ = 1.5 Hz, H6), 4.70 (0.5 H, d, J = 12.2 Hz, CH_2OBn), 4.62 (0.5 H, td, $J_{6,5}$ = 9.4 Hz, $J_{6,7}$ = 1.0 Hz, H6), 4.49 (1 H, d, J = 11.7 Hz, CH_2OBn), 4.44 (1 H, d, J = 7.9 Hz, CH_2OBn), 4.36 (0.5 H, dd, $J_{5,6}$ = 9.4 Hz, $J_{5,4}$ = 2.1 Hz, H5), 4.33–4.26 (2 H, m, 0.5 H2, 0.5 H7′, 0.5 H5, 0.5 CH_2OBn), 4.22 (0.5 H, d, J = 11.2 Hz, CH_2OBn), 4.19–4.14 (0.5 H, m, CH_2OBn), 4.12–3.95 (2.5 H, m, 0.5 H2′, 2 CH_2OBn), 3.89–3.79 (1.5 H, m, 1 H4, 0.5 H7), 3.75 (0.5 H, s, H3), 3.67–3.56 (1.5 H, m, 0.5 H7′, 0.5 H7, 0.5 H3), 3.37 (0.5 H, d, J = 14.2 Hz, 0.5 H2′), 3.29 (0.5 H, d, J = 14.4 Hz, 0.5 H2), 2.25 (1.5 H, s, Me), 2.13 (1.5 H, s, Me); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.6, 156.0 (CO), 142.4, 142.2 (CTria), 138.2, 137.9, 137.5, 137.4, 137.3, 137.3, 136.2 (Cipso), 128.9–126.9 (CHAr), 122.9, 122.5 (CHTria), 83.3, 83.1 (C5), 78.6, 78.5 (C4), 76.1 (C3), 73.2, 72.8, 72.4, 72.2, 71.4, 71.3 (CH_2OBn), 67.6, 67.4 (CH_2Cbz), 64.9, 64.2 (C6), 48.0, 47.5 (C7), 44.3, 43.4 (C2), 10.7, 10.6 (Me); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_{38}H_{41}N_4O_5$: 633.3071, found: 633.3088.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(4-ethyl-1*H*-1,2,3-triazol-1-yl)azepane-1-carboxylate (8b). Compound **7** (112 mg, 0.19 mmol) was reacted with pentynoic acid (373 mg, 3.8 mmol) following general procedure A. The crude was purified over a flash chromatography column (Redisep® 4 g, EtOAc/PE 0 : 1 to 1 : 0) to give **8b** (85 mg, 69%) as a colorless and transparent oil. R_f = 0.15 (EtOAc/PE 2 : 8); $[\alpha]_D^{20}$ = +2.1° (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.49 (0.5 H, s, CHTria), 7.44–7.19 (18 H, m, CHAr), 7.11–6.99 (2 H, m, CHAr), 6.80 (0.5 H, s, CHTria), 5.31 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 5.20 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 5.12 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 4.99 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 4.92 (0.5 H, t, $J_{6,5}$ = 9.5 Hz, H6), 4.84 (0.5 H, d, J = 12.3 Hz, CH_2OBn), 4.77 (0.5 H, t, J = 8.9 Hz, H6), 4.63 (1 H, d, J = 11.6 Hz, CH_2OBn), 4.58 (1 H, d, J = 9.5 Hz, CH_2OBn), 4.50 (0.5 H, dd, $J_{5,6}$ = 9.4 Hz, $J_{5,4}$ = 2.2 Hz, H5), 4.47–4.40 (1.5 H, m, 0.5 H2, 0.5 H7′, 0.5 H5), 4.35 (0.5 H, d, J = 11.2 Hz, CH_2OBn), 4.31 (0.5 H, d, J = 11.3 Hz, CH_2OBn), 4.27–4.10 (2 H, m, 0.5 H2′, 1.5 CH_2OBn), 4.05–3.95 (1.5 H, m, 0.5 H7, 1 H4), 3.89 (0.5 H, t, J = 5.3 Hz, H3), 3.82–3.70 (1.5 H, m, 0.5 H7′, 0.5 H7, 0.5 H3), 3.51 (0.5 H, d, J = 14.1 Hz, H2), 3.43 (0.5 H, d, J = 14.5 Hz, H2), 2.80 (1 H, q, J = 7.6 Hz, CH_2Et), 2.69 (1 H, q, J = 7.6 Hz, CH_2Et), 1.33 (1.5 H, t, J = 7.6 Hz, CH_3Et), 1.22 (1.5 H, t, J = 7.6 Hz, CH_3Et); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.6, 156.0 (CO), 148.9, 148.7 (CTria), 138.2, 137.9, 137.4, 137.4, 137.3, 137.3, 136.2 (Cipso), 128.8–126.9 (CHAr), 122.1, 121.6 (CHTria), 83.5, 83.4 (C5), 78.6, 78.5 (C4), 76.1 (C3), 73.3, 72.9, 72.4, 72.2, 71.4, 71.4 (CH_2OBn), 67.6, 67.4 (CH_2Cbz), 64.9, 64.2 (C6), 48.0, 47.4 (C7), 44.3, 43.5 (C2), 18.9, 18.8 (CH_2Et), 13.6, 13.6 (CH_3Et); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_{39}H_{42}N_4O_5$: 647.3228, found: 647.3225.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(4-propyl-1*H*-1,2,3-triazol-1-yl)azepane-1-carboxylate (8c). Compound **7** (63 mg, 0.11 mmol) was reacted with hexynoic acid (249.0 µL, 2.2 mmol) following general procedure A. The crude was purified over a flash chromatography column (Interchim® 50 µm 4 g, EtOAc/PE 0 : 1 to 6 : 4) to give **8c** (54 mg, 74%) as a colorless and transparent oil. R_f = 0.77 (EtOAc/PE 4 : 6); $[\alpha]_D^{20}$ = +16.6° (*c* 9.1, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.48 (0.5 H, s, CHTria), 7.40–7.19 (18 H, m, CHAr), 7.10–6.99 (2 H, m, CHAr), 6.79 (0.5 H, s, CHTria), 5.29 (0.5 H, d, J = 12.0 Hz, CH_2Cbz), 5.18 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 5.10 (0.5 H, d, J = 12.2 Hz, CH_2Cbz), 4.97 (0.5 H, d, J = 12.0 Hz, CH_2Cbz), 4.91 (0.5 H, t, $J_{6,5}$ = 9.1 Hz, H6), 4.82 (0.5 H, d, J = 12.2 Hz, CH_2OBn), 4.75 (0.5 H, t, $J_{6,5}$ = 9.1 Hz, H6), 4.61 (1 H, d, J = 11.9 Hz, CH_2OBn), 4.56 (1 H, d, J = 8.4 Hz, CH_2OBn), 4.49 (0.5 H, dd, $J_{5,6}$ = 9.2 Hz, $J_{5,4}$ = 1.9 Hz, H5), 4.46–4.39 (2 H, m, 0.5 H7′, 0.5 H2, 0.5 H5, 0.5 CH_2OBn), 4.33 (0.5 H, d, J = 11.2 Hz, CH_2OBn), 4.28 (0.5 H, d, J = 11.3 Hz, CH_2OBn), 4.24–4.10 (2.5 H, m, 2 CH_2OBn, 0.5 H2′), 4.02–3.93 (1.5 H, m, 0.5 H7, 1 H4), 3.87 (0.5 H, t, J = 5.3 Hz, H3), 3.80–3.69 (1.5 H, m, 0.5 H7′, 0.5 H7, 0.5 H3), 3.49 (0.5 H, d, J = 14.2 Hz, 0.5 H2′), 3.42 (0.5 H, d, J = 14.5 Hz, 0.5 H2), 2.73 (1 H, t, J = 7.5 Hz, CH_2CH$_2$CH$_3$), 2.61 (1 H, t, J = 7.3 Hz, CH_2CH$_2$CH$_3$), 1.72 (1 H, sxt, J = 7.4 Hz, CH$_2$CH_2CH$_3$), 1.61 (1 H, sxt, J = 7.3 Hz, CH$_2$CH_2CH$_3$), 1.00 (1.5 H, t, J = 7.3 Hz, CH$_2$CH$_2$CH_3), 0.95 (1.5 H, t, J = 7.3 Hz, CH$_2$CH$_2$CH_3); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.6, 156.1 (CO), 147.4, 147.2 (CTria), 138.2, 137.9, 137.5, 137.5, 137.4, 137.3, 136.2 (Cipso), 128.6–127.2 (CHAr), 122.4, 122.0 (CHTria), 83.5, 83.3 (C5), 78.7, 78.6 (C4), 76.1 (C3), 73.3, 72.9, 72.4, 72.2, 71.4, 71.4 (CH_2OBn), 67.6, 67.5 (CH_2Cbz), 65.0, 64.2 (C6), 48.0, 47.5 (C7), 44.3, 43.5 (C2), 27.6, 27.5 (CH_2Pr), 22.6, 22.6 (CH_2Pr), 13.8, 13.7 (CH_3Pr); HRMS

(ESI+) *m/z*: $[M + H]^+$ calcd for $C_{40}H_{44}N_4O_5$: 661.3385, found: 661.3380.

(3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(4-methyl-1*H*-1,2,3-triazol-1-yl) azepanium chloride (9a). Compound **8a** (53 mg, 0.084 mmol) was deprotected following general procedure C. The crude was purified over a flash chromatography column (DCM/EtOH 96° 7 : 3) to give **9a** (10 mg, 45%) as a colorless and transparent oil. R_f = 0.21 (DCM/EtOH 96°7 : 3); $[\alpha]_D^{20}$ = +0.9° (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 7.81 (1 H, s, CHTria), 5.18 (1 H, td, $J_{6,7}$ = 9.8 Hz, $J_{6,7'}$ = 1.8 Hz, H6), 4.28 (1 H, t, $J_{3,2'}$ = 6.3 Hz, H3), 4.16 (1 H, dd, $J_{5,6}$ = 9.7 Hz, $J_{5,4}$ = 5.0 Hz, H5), 4.02 (1 H, t, $J_{4,5}$ = 5.4 Hz, H4), 3.81 (1 H, dd, $J_{7,7'}$ = 13.9 Hz, $J_{7,6}$ = 10.0 Hz, H7), 3.67 (1 H, dd, $J_{7',7}$ = 13.8 Hz, $J_{7',6}$ = 2.0 Hz, H7′), 3.55 (1 H, d, $J_{2,2'}$ = 13.9 Hz, H2), 3.46 (1 H, dd, $J_{2',2}$ = 14.0 Hz, $J_{2',3}$ = 6.5 Hz, H2′), 2.31 (3 H, s, Me); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 144.1 (CTria), 123.8 (CHTria), 76.5 (C5), 75.4 (C4), 67.3 (C3), 59.8 (C6), 46.2 (C7), 46.0 (C2), 9.6 (Me); HRMS (ESI+) *m/z*: $[M + H]^+$ calcd for $C_9H_{17}N_4O_3$: 229.1295, found: 229.1303.

(3*S*,4*R*,5*R*,6*S*)-3-(4-Ethyl-1*H*-1,2,3-triazol-1-yl)-4,5,6-trihydroxy-azepanium chloride (9b). Compound **8b** (87 mg, 0.13 mmol) was deprotected following general procedure C to give **9b** (32 mg) in a quantitative yield as a colorless and transparent oil. R_f = 0.67 (DCM/MeOH 7 : 3); $[\alpha]_D^{20}$ = −1.2° (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 8.35 (1 H, s, CHTria), 5.43 (1 H, td, $J_{3,4}$ = 9.8 Hz, $J_{3,2'}$ = 2.4 Hz, H3), 4.32 (1 H, td, $J_{6,5}$ = 6.2 Hz, $J_{6,7}$ = 1.5 Hz, H6), 4.20 (1 H, dd, $J_{4,3}$ = 9.8 Hz, $J_{4,5}$ = 4.7 Hz, H4), 4.08 (1 H, dd, $J_{5,6}$ = 6.2 Hz, $J_{5,4}$ = 4.8 Hz, H5), 3.91 (1 H, dd, $J_{2,2'}$ = 14.1 Hz, $J_{2,3}$ = 10.0 Hz, H2), 3.80 (1 H, dd, $J_{2',2}$ = 13.9 Hz, $J_{2',3}$ = 2.0 Hz, H2′), 3.58 (1 H, dd, $J_{7,7'}$ = 14.1 Hz, $J_{7,6}$ = 1.6 Hz, H7), 3.49 (1 H, dd, $J_{7',7}$ = 14.1 Hz, $J_{7',6}$ = 6.3 Hz, H7′), 2.88 (2 H, q, *J* = 7.6 Hz, CH_2Et), 1.31 (3 H, t, *J* = 7.6 Hz, CH_3Et); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 148.5 (CTria), 127.6 (CHTria), 77.9 (C4), 76.6 (C5), 68.6 (C6), 63.5 (C3), 47.4 (C7), 46.9 (C2), 18.4 (CH_2Et), 13.3 (CH_3Et); HRMS (ESI+) *m/z*: $[M + H]^+$ calcd for $C_{10}H_{18}N_4O_3$: 243.1452, found: 243.1458.

(3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(4-propyl-1*H*-1,2,3-triazol-1-yl) azepanium chloride (9c). Compound **8c** (53 mg, 0.08 mmol) was deprotected using general procedure C. The crude was purified over a flash chromatography column (DCM/EtOH 96° 7 : 3) to give **9c** (14 mg, 60%) as a colorless and transparent oil. R_f = 0.38 (DCM/EtOH 96°7 : 3); $[\alpha]_D^{20}$ = −1.4° (*c* 1.5, MeOH); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 7.95 (1 H, s, CHTria), 5.24 (1 H, td, $J_{6,7}$ = 9.8 Hz, $J_{6,7'}$ = 2.3 Hz, H6), 4.30 (1 H, td, $J_{3,2}$ = 6.2 Hz, $J_{3,2'}$ = 1.5 Hz, H3), 4.17 (1 H, dd, $J_{5,6}$ = 9.8 Hz, $J_{5,4}$ = 5.0 Hz, H5), 4.04 (1 H, dd, $J_{4,3}$ = 6.1 Hz, $J_{4,5}$ = 5.1 Hz, H4), 3.84 (1 H, dd, $J_{7,7'}$ = 13.9 Hz, $J_{7,6}$ = 10.0 Hz, H7), 3.70 (1 H, dd, $J_{7',7}$ = 13.9 Hz, $J_{7',6}$ = 1.9 Hz, H7′), 3.57 (1 H, dd, $J_{2',2}$ = 13.9 Hz, $J_{2',3}$ = 1.5 Hz, H2′), 3.48 (1 H, dd, $J_{2,2'}$ = 14.0 Hz, $J_{2,3}$ = 6.5 Hz, H2), 2.71 (2 H, t, *J* = 7.4 Hz, C*H*$_2$CH$_2$CH$_3$), 1.67 (2 H, sxt, *J* = 7.4 Hz, CH$_2$C*H*$_2$CH$_3$), 0.90 (3 H, t, *J* = 7.4 Hz, CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 148.6 (CTria), 124.6 (CHTria), 77.1 (C5), 75.9 (C4), 67.8 (C3), 60.8 (C6), 46.6 (C7), 46.5 (C2), 26.8 (*C*H$_2$CH$_2$CH$_3$), 22.5 (CH$_2$*C*H$_2$CH$_3$), 13.3 (CH$_2$CH$_2$*C*H$_3$); HRMS (ESI+) *m/z*: $[M + H]^+$ calcd for $C_{11}H_{21}N_4O_3$: 257.1608, found: 257.1615.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(methylsulfonamido) azepane-1-carboxylate (10a). Compound **7** (70 mg, 0.12 mmol) was reacted following general procedure B (acylation step using MsCl (14.0 μL, 0.18 mmol)). A purification over a flash chromatography column (Interchim® 50 μm 4 g, EtOAc/PE 0 : 1 to 3 : 7) afforded **10a** (58 mg, 75%, 2 steps) as a colorless and transparent oil. R_f = 0.65 (EtOAc/PE 4 : 6); $[\alpha]_D^{20}$ = −3.7° (*c* 6.3, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.51–7.26 (19 H, m, CHAr), 7.20 (1 H, m, CHAr), 5.62 (0.5 H, d, $J_{NH,6}$ = 6.7 Hz, NH), 5.53 (0.5 H, d, $J_{NH,6}$ = 6.6 Hz, NH), 5.23–5.08 (2 H, m, CH_2Cbz), 4.81 (0.5 H, d, *J* = 11.7 Hz, CH_2OBn), 4.68–4.42 (5.5 H, m, CH_2OBn), 4.21 (1 H, td, $J_{7',7}$ = 14.0 Hz, *J* = 3.4 Hz, H7′), 4.15–4.11 (0.5 H, m, H6), 4.10 (0.5 H, m, H7), 4.06–4.02 (0.5 H, m, H4), 4.01–3.94 (2 H, m, 0.5 H3, 0.5 H4, 0.5 H5, 0.5 H2′), 3.90 (1 H, m, 0.5 H5, 0.5 H6), 3.79 (0.5 H, dt, *J* = 9.9 Hz, *J* = 3.9 Hz, H3), 3.54–3.47 (0.5 H, m, H2), 3.45–3.35 (1.5 H, m, 0.5 H7, 0.5 H2′, 0.5 H2), 3.04 (1.5 H, s, Me), 2.57 (1.5 H, s, Me); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.2, 155.9 (CO), 138.0, 137.9, 137.1, 137.1, 137.0, 136.9, 136.2, 136.1 (Cipso), 129.3–126.5 (CHAr), 82.9, 82.6 (C4), 81.9, 80.8 (C3), 77.5, 77.1 (C5), 72.9, 72.9, 72.5, 72.4, 71.8, 71.7 (CH_2OBn), 67.6, 67.6 (CH_2Cbz), 54.9, 54.7 (C6), 48.0 (C2), 47.2 (C7), 46.9 (C7 or C2), 46.6 (C2 or C7), 42.4, 41.2 (Me); HRMS (ESI+) *m/z*: $[M + Na]^+$ calcd for $C_{36}H_{40}N_2NaO_7S$: 667.2448, found: 667.2466.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(ethylsulfonamido)azepane-1-carboxylate (10b). Compound **7** (70 mg, 0.12 mmol) was reacted following general procedure B (acylation step using $ClSO_2Et$ (17.0 μL, 0.18 mmol)). A purification over a flash chromatography column (Interchim® 15 μm 4 g, EtOAc/PE 0 : 1 to 2 : 8) afforded **10b** (45 mg, 57%, 2 steps) as a colorless and transparent oil. R_f = 0.27 (EtOAc/PE 2 : 8); $[\alpha]_D^{20}$ = −0.69° (*c* 5.8, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.43–7.22 (19 H, m, CHAr), 7.19–7.13 (1 H, m, CHAr), 5.43 (0.5 H, d, $J_{NH,6}$ = 6.6 Hz, NH), 5.37 (0.5 H, d, $J_{NH,6}$ = 7.3 Hz, NH), 5.20–5.14 (0.5 H, m, CH_2Cbz), 5.12 (1 H, d, *J* = 2.2 Hz, CH_2Cbz), 5.10–5.04 (0.5 H, m, CH_2Cbz), 4.77 (0.5 H, d, *J* = 11.6 Hz, CH_2OBn), 4.66–4.36 (5.5 H, m, CH_2OBn), 4.19 (0.5 H, t, $J_{7,6}$ = 4.4 Hz, H7), 4.15 (0.5 H, t, $J_{7,6}$ = 4.1 Hz, H7), 4.12–3.86 (4 H, m, 0.5 H3, 1 H4, 1 H5, 0.5 H6, 0.5 H7′, 0.5 H2′), 3.83 (0.5 H, d, *J* = 6.9 Hz, H6), 3.75 (0.5 H, dt, *J* = 9.9 Hz, *J* = 3.9 Hz, H3), 3.46 (0.5 H, dd, $J_{2,2'}$ = 14.6 Hz, $J_{2,3}$ = 2.1 Hz, H2), 3.41–3.31 (1.5 H, m, 0.5 H2′, 0.5 H2, 0.5 H7′), 3.15–3.02 (1 H, m, CH_2Et), 2.66 (1 H, q, *J* = 7.4 Hz, CH_2Et), 1.31 (1.5H, t, *J* = 7.4 Hz, CH_3Et), 1.05 (1.5 H, t, *J* = 7.4 Hz, CH_3Et); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.2, 156.0 (COCbz), 138.0, 137.9, 137.2, 137.2, 137.0, 136.3, 136.2 (Cipso), 129.3–126.6 (CHAr), 83.0 (C5), 82.6 (C4), 82.0 (C5), 81.0 (C3), 72.9, 72.9, 72.5, 72.3, 71.8, 71.7 (CH_2OBn), 67.7, 67.6 (CH_2Cbz), 54.9, 54.7 (C6), 48.7 (CH_2Et), 48.2 (C2), 47.9 (CH_2Et), 47.4 (C2 or C7), 46.9 (C2 or C7), 46.6 (C2 or C7), 8.1, 7.9 (CH_3Et); HRMS (ESI+) *m/z*: $[M + Na]^+$ calcd for $C_{37}H_{42}N_2NaO_7S$: 681.2605, found: 681.2596.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(propylsulfonamido)azepane-1-carboxylate (10c). Compound **7** (60 mg, 0.11 mmol) was reacted following general procedure B (acylation step using $PrSO_2Cl$ (19.0 μL, 0.17 mmol)). A purification

over a flash chromatography column (Interchim® 50 μm 4 g, EtOAc/PE 0 : 1 to 2 : 8) afforded **10c** (42 mg, 57%, 2 steps) as a colorless and transparent oil. R_f = 0.41 (EtOAc/PE 2 : 8); $[\alpha]_D^{20}$ = +4.6° (*c* 4.7, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 7.41–7.24 (19 H, m, CHAr), 7.21–7.12 (1 H, m, CHAr), 5.44 (0.5 H, d, $J_{NH,6}$ = 6.6 Hz, NH), 5.37 (0.5 H, d, $J_{NH,6}$ = 7.3 Hz, NH), 5.22–5.18 (0.5 H, m, CH_2Cbz), 5.13 (1 H, d, *J* = 1.5 Hz, CH_2Cbz), 5.10–5.05 (0.5 H, m, CH_2Cbz), 4.79 (0.5 H, d, *J* = 11.7 Hz, CH_2OBn), 4.68–4.37 (5.5 H, m, CH_2OBn), 4.23–4.14 (1 H, m, H7′), 4.14–4.08 (0.5 H, dd, *J* = 14.8 Hz, *J* = 3.1 Hz, H7), 4.06 (0.5 H, m, H6), 4.04–3.99 (0.5 H, m, H4), 3.99–3.91 (1.5 H, m, 0.5 H3, 0.5 H4, 0.5 H5), 3.90–3.84 (0.5 H, m, H5), 3.80 (0.5 H, t, $J_{6,NH}$ = 6.2 Hz, H6), 3.76 (0.5 H, dt, *J* = 6.0 Hz, *J* = 3.9 Hz, H3), 3.48 (1 H, dd, *J* = 14.5 Hz, *J* = 1.9 Hz, H2), 3.42–3.32 (1.5 H, m, 0.5 H7, 1 H2′), 3.12–2.97 (1 H, m, CH_2Pr), 2.67–2.59 (1 H, m, C*H*$_2$CH$_2$CH$_3$), 1.79 (1 H, sxt, *J* = 7.5 Hz, CH$_2$C*H*$_2$CH$_3$), 1.57 (1 H, sxt, *J* = 7.5 Hz, CH$_2$C*H*$_2$CH$_3$), 1.02 (1.5 H, t, *J* = 7.4 Hz, CH$_2$CH$_2$C*H*$_3$), 0.82 (1.5 H, t, *J* = 7.4 Hz, CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.2, 156.0 (CO), 138.0, 137.9, 137.2, 137.1, 137.0, 136.3 (Cipso), 128.5–127.5 (CHAr), 83.0, 82.6 (C4), 82.0, 81.0 (C3), 77.4, 77.2 (C5), 72.9, 72.9, 72.4, 72.3, 71.8, 71.6 (CH_2OBn), 67.6, 67.5 (CH_2Cbz), 56.0, 55.1 (CH_2Pr), 54.9, 54.6 (C6), 48.3 (C2), 47.5 (C7), 46.9 (C2), 46.6 (C7), 17.3, 17.0 (CH_2Pr), 12.8, 12.7 (CH_3Pr); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_{38}H_{45}N_2O_7S$: 673.2942, found: 673.2955.

(3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(methylsulfonamido)azepanium chloride (11a). Compound **10a** (63 mg, 0.098 mmol) was deprotected following general procedure C to give **11a** (27 mg) in a quantitative yield as a colorless and transparent oil. R_f = 0.20 (DCM/MeOH 8 : 2); $[\alpha]_D^{20}$ = +24.1° (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.16 (1 H, td, *J* = 6.3 Hz, $J_{6,7}$ = 2.0 Hz, H6), 3.92 (1 H, dd, $J_{5,6}$ = 6.5 Hz, $J_{5,4}$ = 5.1 Hz, H5), 3.90 (1 H, td, $J_{3,2'}$ = 9.8 Hz, $J_{3,2}$ = 2.2 Hz, H3), 3.66 (1 H, dd, $J_{4,3}$ = 9.2 Hz, $J_{4,5}$ = 5.1 Hz, H4), 3.50 (1 H, dd, $J_{2,2'}$ = 13.9 Hz, $J_{2,3}$ = 2.2 Hz, H2), 3.39 (1 H, dd, $J_{7,7'}$ = 13.9 Hz, $J_{7,6}$ = 2.1 Hz, H7), 3.32 (1 H, dd, $J_{7',7}$ = 13.9 Hz, $J_{7',6}$ = 6.3 Hz, H7′), 3.27 (1 H, dd, $J_{2',2}$ = 13.9 Hz, $J_{2',3}$ = 10.0 Hz, H2′), 3.14 (3 H, s, Me); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 76.4 (C5), 75.9 (C4), 68.0 (C3), 54.2 (C6), 48.5 (C7), 46.3 (C2), 40.9 (Me); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_7H_{16}N_2O_5S$: 241.0853, found: 241.0857.

(3*S*,4*R*,5*R*,6*S*)-3-(Ethylsulfonamido)-4,5,6-trihydroxyazepanium chloride (11b). Compound **10b** (47 mg, 0.083 mmol) was deprotected following general procedure C to give **11b** (21 mg) in a quantitative yield as a colorless and transparent oil. R_f = 0.37 (DCM/MeOH 7 : 3); $[\alpha]_D^{20}$ = +37.3° (*c* 0.3, MeOH); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.18 (1 H, td, *J* = 6.3 Hz, $J_{3,2}$ = 1.9 Hz, H3), 3.93 (1 H, dd, $J_{4,3}$ = 6.5 Hz, $J_{4,5}$ = 5.1 Hz, H4), 3.88 (1 H, td, $J_{6,5}$ = 9.8 Hz, $J_{6,7}$ = 2.4 Hz, H6), 3.68 (1 H, dd, $J_{5,6}$ = 9.2 Hz, $J_{5,4}$ = 5.1 Hz, H5), 3.51 (1 H, dd, $J_{2,2'}$ = 13.8 Hz, $J_{2,3}$ = 1.9 Hz, H2), 3.40 (1 H, dd, $J_{7,7'}$ = 14.1 Hz, $J_{7,6}$ = 2.1 Hz, H7), 3.36–3.20 (4 H, m, H7′, H2′, CH_2Et), 1.33 (3 H, t, *J* = 7.3 Hz, CH_3Et); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 76.5 (C5), 75.8 (C4), 67.9 (C3), 54.2 (C6), 48.7 (C7), 47.8 (CH_2Et), 46.3 (C2), 7.8 (CH_3Et); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_8H_{18}N_2O_5S$: 255.1009, found: 255.1007.

(3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(propylsulfonamido)azepanium chloride (11c). Compound **10c** (47 mg, 0.07 mmol) was deprotected following general procedure C to give **11c** (21 mg) in a quantitative yield as a colorless and transparent oil. R_f = 0.25 (DCM/MeOH 8 : 2); $[\alpha]_D^{20}$ = +27.6° (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.17 (1 H, td, $J_{6,5}$ = 6.3 Hz, *J* = 1.9 Hz, H6), 3.92 (1 H, dd, $J_{5,6}$ = 6.5 Hz, $J_{5,4}$ = 5.1 Hz, H5), 3.87 (1 H, td, $J_{3,4}$ = 9.8 Hz, $J_{3,2'}$ = 2.2 Hz, H3), 3.66 (1 H, dd, $J_{4,3}$ = 9.2 Hz, $J_{4,5}$ = 5.0 Hz, H4), 3.49 (1 H, dd, $J_{2',2}$ = 13.7 Hz, $J_{2',3}$ = 2.1 Hz, H2′), 3.41–3.36 (1 H, m, H7′), 3.35–3.26 (2 H, m, H7, H2), 3.26–3.20 (2 H, m, SO$_2$C*H*$_2$CH$_2$CH$_3$), 1.86–1.73 (2 H, m, SO$_2$CH$_2$C*H*$_2$CH$_3$), 1.00 (3 H, t, *J* = 7.5 Hz, SO$_2$CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 76.5 (C4), 75.8 (C5), 68.0 (C6), 54.8 (CH_2Pr), 54.3 (C3), 48.7 (C2), 46.3 (C7), 17.4 (CH_2Pr), 12.6 (CH_3Pr); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_9H_{20}N_2O_5S$: 269.1166, found: 269.1171.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(2,2,2-trifluoroacetamido)azepane-1-carboxylate (12a). Compound **7** (58 mg, 0.10 mmol) was reacted following general procedure B (acylation step using $(CF_3CO)_2O$ (21.0 μL, 0.15 mmol)). A purification over a flash chromatography column (Interchim® 15 μm 4 g, EtOAc/PE 0 : 1 to 1 : 9) afforded **12a** (37 mg, 56%, 2 steps) as a colorless and transparent oil. R_f = 0.84 (EtOAc/PE 3 : 7); $[\alpha]_D^{20}$ = +21.0° (*c* 4.7, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 8.04 (0.5 H, d, $J_{NH,6}$ = 6.9 Hz, NH), 7.99 (0.5 H, d, $J_{NH,6}$ = 7.0 Hz, NH), 7.43–7.28 (19 H, m, CHAr), 7.26–7.20 (1 H, m, CHAr), 7.14–7.07 (1 H, m, CHAr), 5.31 (0.5 H, d, *J* = 12.3 Hz, CH_2Cbz), 5.23 (0.5 H, d, *J* = 12.2 Hz, CH_2Cbz), 5.04 (0.5 H, d, *J* = 12.3 Hz, CH_2Cbz), 4.83 (0.5 H, d, *J* = 12.2 Hz, CH_2Cbz), 4.80–4.74 (0.5 H, m, CH_2OBn), 4.70–4.62 (1.5 H, m, CH_2OBn), 4.60–4.51 (1.5 H, m, CH_2OBn), 4.50–4.44 (0.5 H, m, CH_2OBn), 4.42–4.32 (2.5 H, m, 0.5 H2′, 0.5 H2, 1 CH_2OBn, 0.5 H6), 4.32–4.23 (2 H, m, 0.5 H7, 1 CH_2OBn, 0.5 H6), 4.17–4.08 (1.5 H, m, 0.5 H7′, 0.5 H4, 0.5 H3), 4.03–3.96 (1 H, m, 0.5 H4, 0.5 H5), 3.91 (0.5 H, t, *J* = 4.8 Hz, H5), 3.86 (0.5 H, ddd, *J* = 10.2 Hz, *J* = 5.4 Hz, *J* = 2.4 Hz, H3), 3.44–3.29 (2 H, m, 0.5 H7′, 0.5 H7, 0.5 H2′, 0.5 H2); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 156.8, 156.7 (NHCO), 156.6 (COCbz), 156.5, 156.3 (NHCO), 155.9 (COCbz), 137.9, 137.8, 137.2, 137.0, 136.6, 136.4, 136.3, 136.1 (Cipso), 128.9–127.4 (CHAr), 116.9, 114.1 (CF_3), 82.6, 81.8 (C4), 80.1 (C3), 75.6, 75.4 (C5), 72.8, 72.6, 72.5, 72.4, 71.9, 71.7 (CH_2OBn), 67.5, 67.3 (CH_2Cbz), 53.1, 52.6 (C6), 48.5, 48.1 (C7), 47.5, 46.4 (C2); ^{19}F{^{1}H} NMR (376 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = −76.26 (s), −76.35 (s); HRMS (ESI+) *m*/*z*: $[M + Na]^+$ calcd for $C_{37}H_{37}F_3N_2NaO_6$: 685.2496, found: 685.2500.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(2,2,3,3,3-pentafluoropropanamido) azepane-1-carboxylate (12b). Compound **7** (70 mg, 0.12 mmol) was reacted following general procedure B (acylation step using $(CF_3CF_2CO)_2O$ (36.0 μL, 0.18 mmol)). A purification over a flash chromatography column (Interchim® 15 μm 4 g, EtOAc/PE 0 : 1 to 1 : 9) afforded **12b** (64 mg, 75%, 2 steps) as a colorless and transparent oil. R_f = 0.22 (EtOAc/PE

1 : 9); $[\alpha]_D^{20}$ = +33.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 8.12 (0.5 H, d, $J_{NH,6}$ = 6.7 Hz, NH), 8.09 (0.5 H, d, $J_{NH,6}$ = 7.0 Hz, NH), 7.42–7.26 (19 H, m, CHAr), 7.21 (1 H, s, CHAr), 7.14–7.09 (1 H, m, CHAr), 5.34 (0.5 H, d, *J* = 12.3 Hz, CH_2Cbz), 5.21 (0.5 H, d, *J* = 12.2 Hz, CH_2Cbz), 5.06 (0.5 H, d, *J* = 12.2 Hz, CH_2Cbz), 4.84–4.73 (1 H, m, 0.5 CH_2OBn, 0.5 CH_2Cbz), 4.68–4.60 (1.5 H, m, CH_2OBn), 4.59–4.50 (1.5 H, m, CH_2OBn), 4.48–4.43 (0.5 H, m, CH_2OBn), 4.42–4.23 (3.5 H, m, 0.5 H7, 0.5 H2′, 0.5 H2, 1 H6, 1 CH_2OBn), 4.15–4.04 (1 H, m, 0.5 H7′, 0.5 H3), 4.02–3.98 (1 H, m, H4), 3.96 (0.5 H, t, *J* = 5.0 Hz, H5), 3.89 (0.5 H, t, *J* = 5.0 Hz, H5), 3.84 (0.5 H, ddd, *J* = 10.2 Hz, *J* = 5.1 Hz, *J* = 2.9 Hz, H3), 3.44–3.28 (2 H, m, 0.5 H2′, 0.5 H2, 0.5 H7′, 0.5 H7); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 157.4, 157.2, 157.1, 156.9, 156.9 (NHCO), 156.5, 156.0 (COCbz), 137.9, 137.8, 137.2, 137.0, 136.6, 136.4, 136.3, 136.2 (Cipso), 128.8–127.4 (CHAr), 82.5, 81.8 (C4), 80.3 (C3), 75.9, 75.6 (C5), 72.9, 72.6, 72.5, 72.5, 71.9, 71.7 (CH_2OBn), 67.5, 67.3 (CH_2Cbz), 53.2, 52.7 (C6), 48.2, 47.8 (C7), 47.3, 46.3 (C2); ^{19}F{^{1}H} NMR (376 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = −82.77 (s), −82.93 (s), −123.17 (s), −123.23 (dd, *J* = 374.7 Hz, *J* = 273.8 Hz); HRMS (ESI+) *m*/*z*: $[M + Na]^+$ calcd for $C_{38}H_{37}F_5N_2O_6$: 735.2464, found: 735.2456.

Benzyl (3*S*,4*R*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-(2,2,3,3,4,4,4-heptafluorobutanamido) azepane-1-carboxylate (12c). Compound **7** (60 mg, 0.11 mmol) was reacted following general procedure B (acylation step using $(CF_3(CF_2)_2CO)_2O$ (41.0 μL, 0.17 mmol)). A purification over a flash chromatography column (Interchim® 50 μm 4 g, EtOAc/PE 0 : 1 to 1 : 9) afforded **12c** (76 mg, 91%, 2 steps) as a colorless and transparent oil. R_f = 0.31 (EtOAc/PE 1 : 9); $[\alpha]_D^{20}$ = +27.7° (*c* 5.9, $CHCl_3$); ^{1}H NMR (400 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 8.13 (0.5 H, d, $J_{NH,6}$ = 6.9 Hz, NH), 8.09 (0.5 H, d, $J_{NH,6}$ = 7.0 Hz, NH), 7.42–7.28 (19 H, m, CHAr), 7.26–7.20 (1 H, m, CHAr), 7.14–7.08 (1 H, m, CHAr), 5.36 (0.5 H, d, *J* = 12.5 Hz, CH_2Cbz), 5.23 (0.5 H, d, *J* = 12.2 Hz, CH_2Cbz), 5.06 (0.5 H, d, *J* = 12.2 Hz, CH_2Cbz), 4.81 (0.5 H, d, *J* = 12.3 Hz, CH_2Cbz), 4.78 (0.5 H, d, *J* = 11.6 Hz, CH_2OBn), 4.71–4.62 (1.5 H, m, CH_2OBn), 4.60–4.52 (1.5 H, m, CH_2OBn), 4.47 (0.5 H, d, *J* = 11.9 Hz, CH_2OBn), 4.42–4.26 (3.5 H, m, 0.5 H2′, 0.5 H2, 1 CH_2OBn, 1 H6, 0.5 H7), 4.17–4.06 (1 H, m, 0.5 H3, 0.5 H7′), 4.04–4.00 (0.5 H, m, H4), 3.97 (0.5 H, t, *J* = 5.0 Hz, H5), 3.91 (0.5 H, t, *J* = 4.9 Hz, H5), 3.87 (0.5 H, ddd, *J* = 10.2 Hz, *J* = 5.2 Hz, *J* = 2.9 Hz, H3), 3.44–3.33 (2 H, m, 0.5 H7′, 0.5 H7, 0.5 H2′, 0.5 H2); ^{13}C NMR (100 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = 157.2, 157.0, 156.9, 156.6 (NHCO weak signal), 156.4, 155.9 (COCbz), 137.9, 137.8, 137.2, 137.0, 136.5, 136.4, 136.3, 136.2 (Cipso), 129.0–127.2 (CHAr), 82.4, 81.7 (C4), 80.2, 80.1 (C3), 75.9, 75.7 (C5), 72.8, 72.6, 72.5, 72.5, 71.8, 71.7 (CH_2OBn), 67.5, 67.3 (CH_2Cbz), 53.3, 52.9 (C6), 48.2, 47.8 (C7), 47.4, 46.4 (C2); ^{19}F{^{1}H} NMR (376 MHz, chloroform-d_1): (presence of 2 rotamers) δ (ppm) = −80.48 (q, *J* = 8.2 Hz), −120.69 (s), −120.78 (ddq, *J* = 441.4 Hz, *J* = 277.9 Hz, *J* = 9.5 Hz), −126.83 (s), −126.97 (s); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_{39}H_{37}F_7N_2O_6$: 763.2613, found: 763.2625.

(3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(2,2,2-trifluoroacetamido) azepanium chloride (13a). Compound **12a** (40 mg, 0.06 mmol) was deprotected using general procedure C. The crude was purified over a flash chromatography column (DCM/EtOH 96°7 : 3) to give **13a** (16 mg, 90%) as a colorless oil. R_f = 0.54 (DCM/EtOH 96°7 : 3); $[\alpha]_D^{20}$ = +18.9° (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, methanol-d_4): δ (ppm) = 4.33 (1 H, dt, $J_{3,4}$ = 9.2 Hz, $J_{3,2}$ = 6.1 Hz, H3), 3.99 (1 H, td, $J_{6,7}$ = 6.2 Hz, $J_{6,7'}$ = 1.9 Hz, H6), 3.86 (1 H, t, *J* = 5.5 Hz, H5), 3.76 (1 H, dd, $J_{4,3}$ = 9.2 Hz, $J_{4,5}$ = 4.9 Hz, H4), 3.28 (1 H, d, $J_{7',6}$ = 2.0 Hz, H7′), 3.17 (2 H, d, $J_{2,3}$ = 6.0 Hz, H2), 3.11 (1 H, dd, *J* = 13.6 Hz, $J_{7,6}$ = 6.0 Hz, H7); ^{13}C NMR (100 MHz, methanol-d_4): δ (ppm) = 158.8 (q, *J* = 36.8 Hz, CO), 117.4 (q, *J* = 286.8 Hz, CF_3), 77.0 (C5), 76.7 (C4), 71.0 (C6), 53.7 (C3), 48.8 (C2, C7); ^{19}F{^{1}H} NMR (376 MHz, methanol-d_4): δ (ppm) = −75.85 (s); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_8H_{14}F_3N_2O_4$: 259.0900, found: 259.0902.

(3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(2,2,3,3,3-pentafluoropropanamido)azepanium chloride (13b). Compound **12b** (59 mg, 0.083 mmol) was deprotected using general procedure C to give **13b** (26 mg) in a quantitative yield as a colorless and transparent oil. R_f = 0.46 (DCM/EtOH 96°7 : 3); $[\alpha]_D^{20}$ = +22.7° (*c* 0.3, MeOH); ^{1}H NMR (400 MHz, D_2O): (presence of 2 rotamers) δ (ppm) = 4.49 (0.5 H, td, *J* = 10.0 Hz, *J* = 2.6 Hz, H6), 4.19–4.13 (1 H, m, H3), 3.93–3.85 (1.5 H, m, 0.5 H6, 1 H4), 3.83–3.76 (1 H, m, H5), 3.64 (1 H, dd, $J_{2,2'}$ = 13.8 Hz, $J_{2,3}$ = 2.2 Hz, H2), 3.45–3.28 (3 H, m, H2′, H7); ^{13}C NMR (100 MHz, D_2O): (presence of 2 rotamers) δ (ppm) = 159.8 (t, *J* = 26.4 Hz, CO), 76.7, 76.6 (C4), 75.6, 75.1 (C5), 67.8, 67.6 (C3), 50.1, 49.8 (C6), 46.5, 46.4 (C7), 44.9 (C2); ^{19}F{^{1}H} NMR (376 MHz, D_2O): (presence of 2 rotamers) δ (ppm) = −83.43 to −82.86 (3 F, m), −123.16 to −122.72 (2 F, m); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_9H_{14}F_5N_2O_4$: 309.0868, found: 309.0865.

(3*S*,4*R*,5*R*,6*S*)-3-(2,2,3,3,4,4,4-Heptafluorobutanamido)-4,5,6-trihydroxyazepanium chloride (13c). Compound **12c** (37 mg, 0.049 mmol) was deprotected using general procedure C. The crude was purified over a flash chromatography column (DCM/EtOH 96°7 : 3) to give **13c** (13 mg, 67%) as a colorless and transparent oil. R_f = 0.56 (DCM/EtOH 96°7 : 3); $[\alpha]_D^{20}$ = +6.3° (*c* 0.9, H_2O); ^{1}H NMR (400 MHz, D_2O): δ (ppm) = 4.19–4.13 (1 H, m, H6), 3.94–3.84 (2 H, m, H5, H3), 3.80 (1 H, dd, *J* = 10.1 Hz, *J* = 5.3 Hz, H4), 3.65 (1 H, dd, $J_{7,7'}$ = 13.9 Hz, $J_{7,6}$ = 2.1 Hz, H7), 3.45–3.31 (3 H, m, H2, H7′); ^{13}C NMR (100 MHz, D_2O): δ (ppm) = 159.6 (t, *J* = 26.4 Hz, CO), 76.7 (C5), 74.9 (C4), 69.1 (C6), 51.5 (C3), 47.5 (C7 or C2), 47.3 (C2 or C7); ^{19}F{^{1}H} NMR (376 MHz, D_2O): δ (ppm) = −80.69 to −80.87 (3 F, m), −121.26 (2 F, m), −127.41 (2 F, br. s); HRMS (ESI+) *m*/*z*: $[M + H]^+$ calcd for $C_{10}H_{14}F_7N_2O_4$: 359.0836, found: 359.0828.

Docking

Docking calculations for NagZ were performed with GOLD using the structure 4MSS[14] and a binding site defined as a 15 Å radius sphere centered on the NZ atom of Lys170. GoldScore was used as a scoring function, and all other parameters had default values.

Biological assays

Glycosidase inhibition profiling was performed using appropriate *p*-nitrophenyl glycosides as substrates at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

Kinetic analysis of OGA, NagZ and HexAB inhibition

The *Salmonella typhimurium* NagZ and human OGA proteins were purified as previously described.[30,31] Human placental HexAB was obtained as a mixture of HexA and HexB isozymes from Sigma (product number, A6152). Initial rate experiments were carried out in 384 well assay plates (Corning, #3702) at 25 °C. The assay buffer for OGA and NagZ was PBS (pH 7.4), whereas the HexAB assay buffer consisted of (50 mM sodium citrate pH 5, 0.1% Triton X-100). A continuous assay was developed in which all reactions were initiated *via* the addition of *p*NP-GlcNAc substrate. The assay contained 100 nM NagZ, 30 nM hOGA, or 200 nM HexAB in a final volume of 50 μL for K_i determination. The reaction progress was monitored continuously at a wavelength of 405 nm using a SpectraMax i3x multimode plate reader from Molecular Devices. Reaction velocities were assessed by linear regression of the progress curves over a 10 min period. The amount of product liberated was assessed using a standard curve for *p*NP in PBS buffer. For IC_{50} experiments (see Fig. S1†), $2K_M$ of *p*NP-GlcNAc substrate was used (hOGA; 200 μM, NagZ; 120 μM, HexAB; 300 μM). For K_i experiments, a total of 6–8 inhibitor concentrations were tested, ranging from 1/6 to 80 times the K_i value. The mode of inhibition was assessed using Lineweaver–Burk plots, and the data was globally fit to a competitive inhibition model to determine the K_i. Curve fitting was performed using GraphPad Prism, and error bars correspond to S.D. from quadruplicate reads. Data is displayed in Table 2, and representative K_i and LB-plots for **13a** mediated NagZ inhibition are shown in ESI Fig. S2.†

Minimum inhibitory concentration assay (MIC)

MICs were performed in duplicate using the broth microdilution method (Clinical and Laboratory Standards Institute) in a 96-well plate with appropriate serial dilutions of ceftazidime in 100 μl of cation-adjusted Mueller–Hinton broth (MHB) containing a final concentration of 1 mM **13a** or **13c**. To account for the DMSO used to dissolve **13a** and **13b**, an equivalent quantity of DMSO was added to MHB in control samples (DMSO control). Wells were inoculated with 100 μl of ~10^5 cells of *P. aeruginosa* Δ*dacB* grown to an optical density (OD_{600}) of ~0.5. MICs were determined after incubation of the cells for 18 h at 37 °C in a shaker incubator.

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