Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7807

www.rsc.org/obc

PAPER

Synthesis of *uronic*-Noeurostegine – a potent bacterial β-glucuronidase inhibitor†‡

Tina S. Rasmussen,[a] Heidi Koldsø,[a,b] Shinpei Nakagawa,[c] Atsushi Kato,[c] Birgit Schiøtt[a,b] and Henrik H. Jensen*[a]

Received 28th June 2011, Accepted 11th August 2011
DOI: 10.1039/c1ob06038d

Inhibition of β-glucuronidases has recently been shown to be useful in alleviating drug toxicity for common colon cancer chemotherapeutic CPT-11 (also called Irinotecan). We have prepared a new compound of the nortropane-type, *uronic*-Noeurostegine, and demonstrated that this is a competitive and potent *E. coli* β-glucuronidase inhibitor, while inhibition of the mammalian β-glucuronidase from bovine liver was found to be less significant. Although not intended, two other compounds having *N*-ethyl and *N*-(4-hydroxybutyl) substituents were also prepared in this study due to the sluggish debenzylation in the final step. The *N*-substituents are believed to come from reaction with the solvents used being ethanol and THF, respectively. These compounds also inhibited the two β-glucuronidases albeit to a lesser extent compared to the parent compound. Noeurostegine and the three *uronic*-noeurostegines were additionally evaluated as inhibitors against a wide panel of glycosidases with the former showing potent inhibition of rat intestinal lactase and trehalase, whereas the latter was found to be inactive.

Introduction

The chemistry literature holds numerous examples of how structural inspiration from naturally occurring compounds has lead the way to the design and synthesis of new biologically active species. This indeed also holds true with regards to how iminosugars like 1-deoxynojirimycin (**1**) and its analogues[1] have led to more potent synthetic variants like isofagomine (**2**),[2] azafagomines (**3**)[3] and noeuromycin (**4**)[4] (Fig. 1). The glycosidase inhibition by compounds of these iminosugar/azasugar families are of significant interest due to their potential use in treatment of a variety of diseases like cancer, diabetes and viral infections including HIV.[5]

Recently, we drew inspiration from the naturally occurring, stable nortropane hemiaminal calystegine B_2 (**5**)[6] to prepare a hybrid of this with the compound noeuromycin (**4**). We christened this new stable and structurally intermediary compound noeurostegine (**6**)[7] and demonstrated its potent inhibition of almond and *Thermotoga maritima* β-glucosidase and furthermore found it as a potentially valuable compound against Gaucher's disease.[8] Here we describe the synthesis and glucuronidase inhibitory effects from the first stable hemiaminal possessing a carboxylic acid functionality (**7**) (Fig. 1).

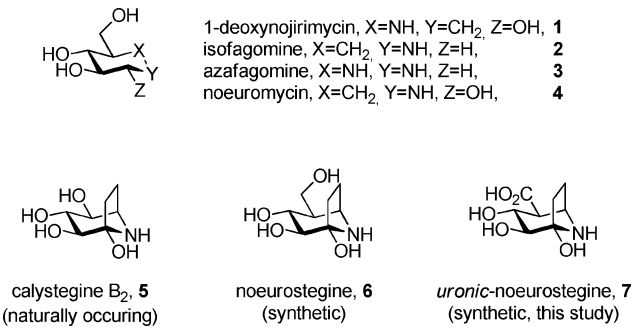


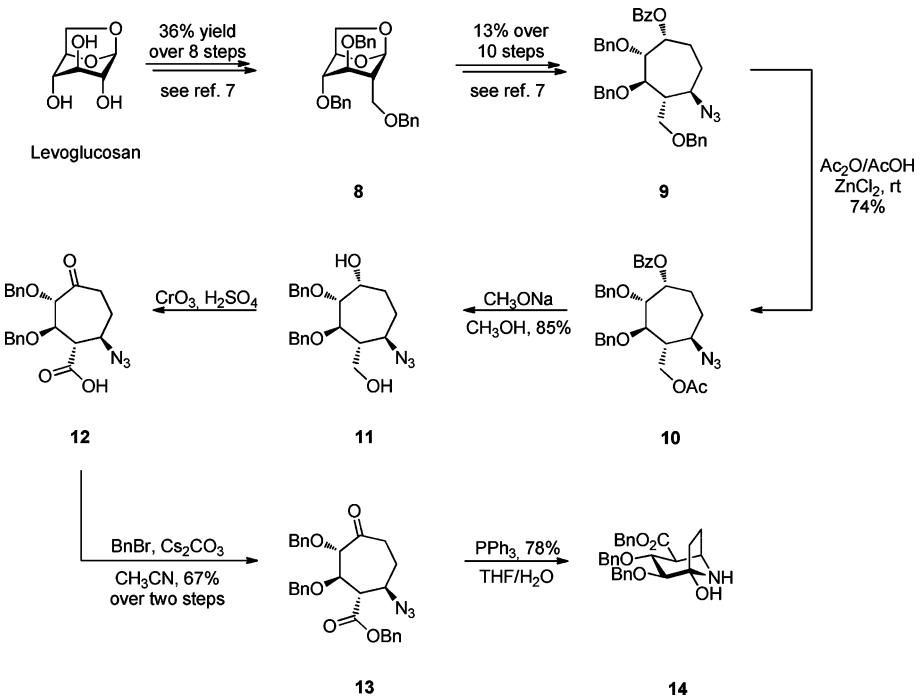
Fig. 1 Structures of various iminosugars/azasugars including the focus of this study, compound **7**.

A potentially valuable strategy for alleviating harmful side effects for the common colon cancer chemotherapeutic CPT-11 was demonstrated by Redinbo and co-workers in the journal *Science* in 2010.[9] Here, by studying *E. coli* β-glucuronidase the authors found potent non-carbohydrate-like uncompetitive enzyme inhibitors to be capable of diminishing the concentration of toxic CPT-11 metabolites in the gastrointestinal tract. Based on this newly established and important potential use of glucuronidase inhibitors, we decided to prepare a glucuronic acid analogue of noeurostegine and test its inhibition profile.

[a]*Department of Chemistry, Aarhus University, Langelandsgade 140, 8000, Aarhus C, Denmark. E-mail: hhj@chem.au.dk; Tel: +458942 3963*
[b]*Center for Insoluble Protein Structures (inSPIN) and Interdisciplinary Nanoscience Center (iNANO), Denmark*
[c]*Department of Hospital Pharmacy, University of Toyama, 930-01940, Japan*

† This paper is dedicated to Professor Mikael Bols on the occasion of his 50th birthday

‡ Electronic supplementary information (ESI) available: Molecular docking calculations, glycosidase inhibition, Michaelis–Menten plots, compound numbering and NMR spectra. See DOI: 10.1039/c1ob06038d



Scheme 1 Synthesis of protected *uronic*-noeurostegine (**14**) from levoglucusan.

Results and discussion

Similar to the synthesis of noeurostegine (**6**)[7] we started with levoglucosan as previously described. Using this known sequence of reactions, we are at this time able to achieve 36% of **8** over eight steps compared to the previous 28% (Scheme 1). The anhydrosugar **8** subsequently underwent a series of reactions also described earlier to obtain azidocycloheptane **9** in 13% yield over 10 steps.[7] Next, the primary benzyl ether was selectively acetolysed into the corresponding acetate by treatment with $ZnCl_2$ in acetic anhydride/acetic acid in 74% yield to give **10**. A di-deacylation to remove both the acetyl and benzoyl groups was conducted in methanol using sodium methoxide afforded **11** (85%), which was later oxidised by the Jones reagent to the keto carboxylic acid **12**. To avoid working with a zwitter ionic compound until the last step we decided to protect the carboxylic acid as a benzyl ester. This was accomplished by reaction of **12** with benzyl bromide in dry acetonitrile in the presence of cesium carbonate in 67% yield from diol **11**.[10] The azide function of **13** was next reduced by the Staudinger reaction in THF/water to spontaneously give bicyclo[3.2.1]-compound **14** as indicated by the disappearance of δ_C 208 ppm and appearance of δ_C 92.6 ppm by ^{13}C NMR.

To obtain the target compound (**7**), only protecting group removal remained. We have previously in our synthesis of noeurostegine (**6**) reported the uneventful removal of benzyl protecting groups by hydrogenolysis over $Pd(OH)_2/C$,[7] but in this project we experienced significant difficulties in this final step. Heating or increasing the H_2-pressure did not seem to have a noteworthy effect on starting material consumption. We assume this was due to a minute impurity that has poisoned and thereby to some extent inactivated the catalyst towards debenzylation. First the reaction was carried out in ethanol/water and went on for 10 days with intermediary filtration of the reaction mixture through Celite and addition of new catalyst. The product from this reaction turned out to be the *N*-ethyl congener of the desired product. We speculate that this product could originate from palladium catalysed dehydrogenation of ethanol to produce acetaldehyde, which later underwent reductive amination to give *N*-ethyl *uronic*-noeurostegine (**15**).[11]

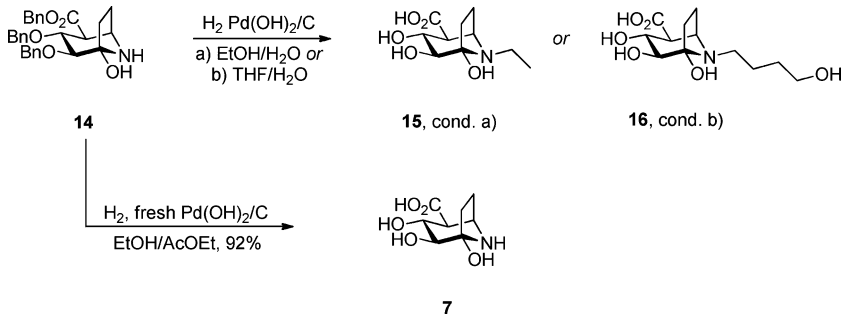
To avoid this unwanted side reaction during hydrogenolysis we decided to change the solvent to a THF/water mixture. The reaction also proceeded sluggishly in this medium over 5 days but resulted in a product different from *N*-ethyl *uronic*-noeurostegine (**15**) by TLC analysis. This however turned out to be the *N*-4-hydroxybutyl alkylated nortropane **16**.[11] In this case we speculate that a palladium catalysed dehydrogenation of THF in the presence of water ultimately will lead to formation of 4-hydroxybutanal, which then undergoes reductive amination with the amine to give *N*-4-hydroxybutyl *uronic*-noeurostegine (**16**) (Scheme 2).

Until this point an old batch of Pearlman's catalyst ($Pd(OH)_2/C$) had been used, which could be of weakened activity. After using a freshly purchased batch of Pearlman's catalyst for the debenzylation of **14** in ethanol/ethyl acetate/water the desired compound **7** was formed.

The stability of *uronic*-noeurostegine was evaluated by leaving it in either a neutral aqueous solution at ambient temperature or in acetate buffer (100 mM, pH 4.6) at 37 °C for several days. No change after this time was observed by NMR spectroscopy demonstrating again that these nortropane hemiaminal compounds indeed are stable.

Inhibition studies

In line with the recent study by Redinbo and co-workers where it was shown that *E. coli* and bovine liver β-glucuronidases are



Scheme 2 Hydrogenolysis of **14** using aged and fresh catalyst.

markedly different and that selective inhibition of the former could have great medicinal potential in cancer treatment, we decided to evaluate the synthesised nortropanes **7,15,16** against these enzymes.

We found that all three inhibitors showed similar and low micromolar competitive inhibition (K_i) against the mammalian enzyme from bovine liver and that the parent *uronic*-noeurostegine (**7**) was slightly more potent (K_i 2.3 μM) than the *N*-alkylated congeners (K_i 9.5 μM and 3.6 μM) (Table 1). These inhibitory constants are similar to what has been established for the *uronic*-azafagomine (**19**, K_i 1 μM, Fig. 2)[3c] and *uronic*-1-deoxynojirimycin (**18**, K_i 6.5 μM, Table 1),[12] but significantly less potent than *uronic*-isofagomine (**17**, K_i 79 nM).[2b] This is despite the fact that **17** does not possess a hydroxyl group in what would be the 2-position of the substrate, which is known to contribute greatly to binding.[13] The high potency of **17** over **18** is most likely due to the fact that β-glycosidases generally prefer a basic *N*-atom in what would be C-1 of the glycoside substrate.[5a] The noeurostegines have, like isofagomines, azafagonines and noeuromycins, an *N*-atom in the C-1 position, but require that the enzyme is capable of accommodating the ethylene bridge, which seems to be possible in this case.

The bacterial β-glucuronidase from *E. coli* was inhibited competitively and very potently by *uronic*-noeurostegine (**7**) with K_i of 60 nM whereas *N*-ethyl and *N*-4-hydroxybutyl *uronic*-noeurostegines (**15** and **16**) demonstrated a lower degree of inhibition with K_i-values of 1000 nM and 740 nM, respectively (Table 1). This demonstrates that the ethylene bridge of the nortropane skeleton can be accommodated by the enzymes (vide infra). *Uronic*-1-deoxynojirimycin (**18**) is known as a weak inhibitor of this bacterial enzyme[12] and thereby has an inverse inhibition profile compared to *uronic*-noeurostegine (**7**).

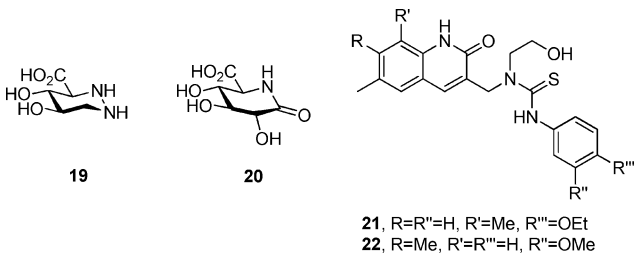


Fig. 2 Structure of *uronic*-azafagomine (**19**), glucaro-δ-lactam (**20**)[14] and uncompetitive inhibitors **21** and **22**.

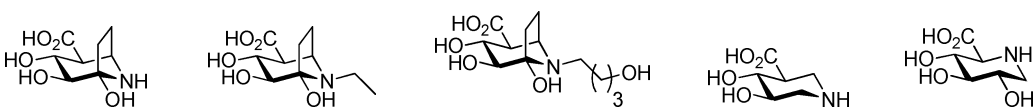
Inhibition by the best compound (**21**, Fig. 2) found by Redinbo and co-workers showed a K_i of 164 nM (uncompetitive) against *E. coli* β-glucuronidase and no inhibition of the bovine liver enzyme.[9]

To obtain a more complete picture of the inhibition profile of *uronic*-noeurostegines **7,15,16** were tested against a series of other glycosidases. Parent noeurostegine (**6**), was included in this study.

No significant inhibition of any of the tested glycosidases was found by the *uronic*-noeurostegines (**7,15,16**) at 1 mM concentration illustrating the selectivity displayed by these compounds (Table 2). In comparison, *uronic*-azafagomine (**19**) inhibits yeast α-glucosidase with K_i 160 μM and almond β-glucosidase with K_i 7 μM.[3c] Noeurostegine (**6**) has previously been found not to inhibit α-glucosidase from yeast, which we also found to be the case for the rice and rat intestinal enzymes. Mild inhibition of *Asp. niger* α-glucosidase was found with an IC_{50} of 240 μM.

β-Glucosidase from bovine liver (IC_{50} 96 μM) and β-glucosidase from human lysosome (IC_{50} 12 μM), was inhibited by noeurostegine, while α-galctosidase from human lysosome was inhibited at a higher concentration (IC_{50} 330 μM). Despite the glucose configuration of noeurostegine we previously found it to be an inhibitor of β-galactosidase from both *Asp. oryzae* and *E. coli*.

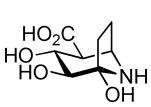
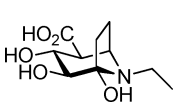
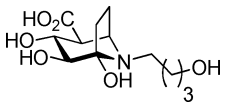
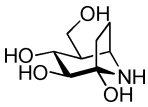
Table 1 K_i values in μM (—, not tested)



Compound	**7**	**15**	**16**	**17**	**18**
β-glucuronidase (bovine liver)[a]	2.3	9.5	3.6	0.079[b]	6.5[c]
β-glucuronidase (*E. coli*)[d]	0.060	1.0	0.74	—	400[c]

[a] pH 4.6, 37 °C. [b] Value from ref. 2b. [c] Value from ref. 12 [d] pH 6.8, 37 °C.

Table 2 IC_{50} values in μM. NI: IC_{50} > 1 mM; (—, not tested)

				
Compound	**7**	**15**	**16**	**6**
α-glucosidase (yeast)	NI	NI	NI	NI[a]
α-glucosidase (rice)	NI	NI	NI	NI
α-glucosidase (rat intestinal maltase)	—	—	—	NI
α-glucosidase (*Asp. niger*)	—	—	—	240
β-glucosidase (bovine liver)	NI	NI	NI	96
β-glucosidase (almonds)	NI	NI	NI	K_i 0.050[a]
β-glucosidase (human lysosome).	—	—	—	12
α-galactosidase (coffee beans)	NI	NI	NI	K_i 2.5[a]
α-galactosidase (human lysosome)	—	—	—	330
β-galactosidase (bovine liver)	NI	NI	NI	47
β-galactosidase (rat intestinal lactase)	—	—	—	0.25
α-mannosidse (jack bean)	NI	NI	NI	NI
β-mannosidase (snail)	NI	NI	NI	670
α-L-rhamnosidase (*P. decumbens*)	NI	NI	NI	470
α-L-fucosidase (bovine kidney)	NI	NI	NI	—
α-L-fucosidase (bovine epididymis)	—	—	—	NI
trehalase (porcine kidney)	NI	NI	NI	—
trehalase (rat intestine)	—	—	—	0.44
amyloglucosidase (*Asp. niger*)	NI	NI	NI	980

[a] Ref. 7.

Here we additionally found **6** to be an inhibitor of the bovine liver (IC_{50} 47 μM) and rat intestinal enzymes, with the latter being in the sub-micromolar region (IC_{50} 250 nM).

No inhibition of jack bean α-mannosidase and bovine α-fucosidase, whereas moderate inhibition of snail β-mannosidase (IC_{50} 670 μM), *P. decumbens* α-rhamnosidase (IC_{50} 470 μM) and *Asp. niger* amyloglucosidase (975 μM) was found.

Trehalase from rat intestine was inhibited potently with IC_{50} of 0.44 μM.

Molecular docking calculations

Redinbo and co-workers have succeeded in crystallising the *E. coli* β-glucuronidase in the presence of both the competitive inhibitor glucaro-δ-lactam (**20**) and the uncompetitive inhibitor **22** (Fig. 2). We decided to explore how *uronic*-noeuromycin (**7**) would bind to the bacterial enzyme by molecular docking calculations. We performed a re-docking of the co-crystallised glucaro-δ-lactam (**20**) as a reference, with the binding mode from the calculations overlapping perfectly (RMSD = 0.20 Å for all ligand atoms) with the one observed in the crystal structure. Additionally we were able to dock the novel competitive inhibitor **7** into the binding pocket in a position overlapping with the competitive inhibitor **20**. The NH group of **7** is overlapping with the amide carbonyl of glucaro-δ-lactam resulting in the carboxylic acid being located in the same pocket of the binding site.

Uronic-noeurostegine (**7**) was hence found to occupy the central substrate binding site and have interactions with the two active site carboxylates (Glu413 and Glu504) in addition to several other polar interactions, see Fig. 3. Furthermore, the N-atom of *uronic*-noeurostegine (**7**) occupies the position of the C-1 of the substrate and thereby it binds in a 'noeuromycin binding mode' rather than

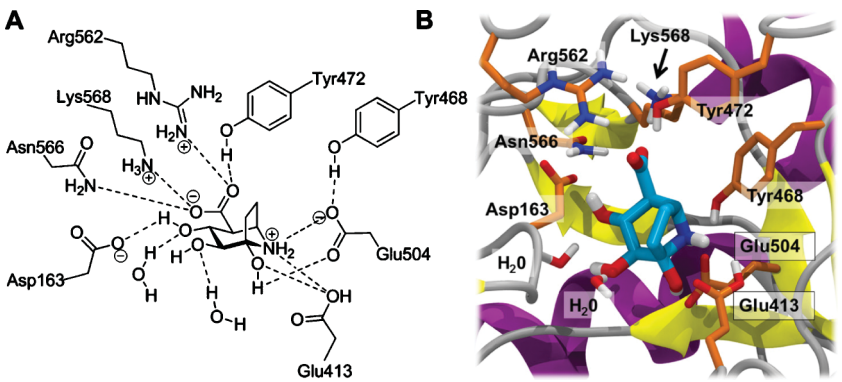


Fig. 3 Docking of **7** in *E. coli* β-glucuronidase (PDB : 3K4D) and the observed interactions between water and protein residues. The catalytic glutamates form interactions with the inhibitor **7** in addition to several other polar interactions with charges and hydrophilic residues within the binding pocket. (A) Schematic representation of the binding site interactions and (B) interactions within the central binding pocket. *Uronic*-noeurostegine (**7**) in cyan and protein side chains in orange. The protein is coloured by secondary structure with α-helices in purple and β-strands in yellow.

a '1-deoxynojirimycin binding mode' in which the N-atom takes the place of O-5 of the substrate. This binding mode has also been established for calystegine B_2 (**5**) in *T. maritima* β-glucosidase.[15]

The location of the uncompetitive inhibitor **22** was found by Redinbo and co-workers to slightly overlap with the binding site for *uronic*-noeurostegine determined by our docking calculations.

Conclusion

We have prepared a new nortropane type β-glucuronidase inhibitor in 24 steps from levoglucosan and christened this *uronic*-noeurostegine due to its resemblance to calystegine B_2 and noeuromycin. To the best of our knowledge, this is the first stable hemiaminal amino acid. The final deprotection using an older batch of Pearlman's catalyst was found to be sluggish and yielded the *N*-ethyl and *N*-4-hydroxybutyl congeners by reaction with ethanol and tetrahydrofuran, respectively.

The compounds synthesised in this study were all established to be potent inhibitors of both bovine liver and *E. coli* β-glucuronidase with the parent compound *uronic*-noeurostegine exerting the most powerful inhibition with a K_i of 60 nM against the latter. Inhibition against the bacterial enzyme was furthermore found to be more pronounced than against the enzyme of mammalian origin with a selectivity factor of 38. Given the recent promising results in colon cancer chemotherapy *uronic*-noeurostegine or other compounds of the iminosugar/azasugar family could be a useful medicinal candidate.

Noeurostegine synthesised in a previous study was here also tested against a wide panel of glycosidases.[7] In addition to our previous findings that this compound is a potent inhibitor of β-glucosidases from *T. maritima* and almonds we also found strong inhibition of rat intestinal lactase and trehalase.

General experimental information

Organic synthesis

All reagents except otherwise stated were used as purchased without further purification. Enzymes were purchased from Sigma. Dried glassware from the oven (*ca.* 120 °C) was used for reactions carried out under nitrogen or argon atmosphere. Solvents were dried using MB-SPS Solvent Purification System. Flash chromatography was performed with Merck silica 60 (230–400 mesh) as stationary phase. TLC was performed on silica-coated aluminium plates (Merck 60 F_{254}). TLC plates were first observed in UV-light and then visualized with ceric sulfate/ammonium molybdate in 10% H_2SO_4 stain. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded at a Varian Mercury 400 spectrometer. $CDCl_3$ (δ 7.26 ppm ($CHCl_3$) for proton and δ 77.16 ppm for carbon resonances) and D_2O (δ 4.79 ppm for proton) were used as internal references. Spectra were assigned based on *g*COSY, *g*HMQC and DEPT-135 experiments. MS spectra were recorded at a Micromass LC-TOF instrument by using electrospray ionization (ESI). High resolution spectra were recorded with one of the following compounds as internal standard: (Boc-L-alanine: $C_8H_{15}NO_4Na$: 212.0899; BzGlyPheOMe: $C_{19}H_{20}N_2O_4Na$: 363.1321; BocSer(OBn)SerLeuOMe: $C_{25}H_{39}N_3O_8Na$: 532.2635; erythromycin: $C_{37}H_{67}NO_{13}Na$: 756.4510) Masses of standards and analytes are calculated and reported in Daltons for uncharged species.

Melting points were measured on a Büchi B-540 instrument and are uncorrected. Optical rotation was measured on a PE-241 polarimeter and reported in units of deg cm^2 g^{-1}. Concentrations are reported in g/100 mL.

(1*R*,2*R*,3*R*,4*R*,5*R*)-4-(Acetoxymethyl)-5-azido-1-*O*-benzoyl-2,3-di-*O*-benzyl-cycloheptane (10). (1*R*,2*R*,3*R*,4*R*,5*R*)-5-Azido-1-*O*-benzoyl-2,3-di-*O*-benzyl-4-*C*-benzyloxymethyl-cycloheptane (**9**)7 (0.43 g, 0.73 mmol) was dissolved in acetic anhydride (10 mL) and acetic acid (5 mL). $ZnCl_2$ (1.57 g, 11.5 mmol, 15 eq) was added and the reaction mixture was stirred at rt for 3 h. The reaction mixture was poured onto a saturated aqueous solution of $NaHCO_3$ (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL) before the combined organic layers were washed with H_2O (2 × 100 mL), dried over $MgSO_4$, filtered and concentrated. The resulting residue was purified by flash column chromatography (pentane/EtOAc 15 : 1 → 10 : 1) to give the desired product **10** (0.29 g, 74%) as colourless crystals. Mp 120.5–122.5 °C. $[\alpha]_D^{20}$ 40.6 (*c* 1.0, $CHCl_3$). R_f (pentane/EtOAc 10 : 1) 0.23. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 7.93 (m, 2H, *o*-Bz), 7.50 (m, 1H, Ar*H*), 7.35 (m, 2H, Ar*H*), 7.23–7.17 (m, 8H, Ar*H*), 7.11–7.09 (m, 2H, Ar*H*), 5.37 (m, 1H, H1), 4.69–4.63 (m, 3H,CH_2Ph), 4.40–4.36 (m, 3H, OCH_2Ph, H8), 4.32 (dd, 1H, *J* 11.2 Hz, *J* 2.8 Hz, H8′), 3.96 (t, 1 H, *J* 6.4 Hz, H2), 3.66 (m, 1H, H5), 3.56 (dd, 1H, *J* 9.2 Hz, *J* 6.4 Hz, H3), 2.09 (tt, 1H, *J* 9.2 Hz, *J* 2.8 Hz, H4), 2.00 (m, 4 H, H6,H6′,H7,H7′), 1.98 (s, 3H, OCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 170.9 (*C*O), 165.9 (*C*O), 137.9, 133.2, 130.2, 129.7–127.8 (Ar*C*), 82.5 (C2), 78.1 (C3), 74.6, 74.3 (*C*H_2Ph), 73.9 (C1), 63.6 (C8), 60.6 (C5), 47.1 (C4), 28.4, 24.6 (C6, C7), 21.1 (OCO*C*H_3). HRMS(ES+): calcd. for $C_{31}H_{33}N_3O_6Na$: 566.2267, found 566.2271.

(1*R*,2*R*,3*R*,4*R*,5*R*)-5-Azido-2,3-di-*O*-benzyl-4-*C*-hydroxymethyl-cycloheptanol (11). $NaOCH_3$ (0.079 g, 1.47 mmol, 2 eq) was added to a solution of **10** (0.39 g, 0.72 mmol) in dry MeOH (10 mL) under an atmosphere of nitrogen. The reaction mixture was stirred overnight at rt before diluted with EtOAc (50 mL) and the organic phase was washed with H_2O (3 × 50 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane/EtOAc 2 : 1) to give the desired diol **11** (0.24 g, 85%). $[\alpha]_D^{20}$ −2.3 (*c* 1.0, $CHCl_3$). R_f (pentane/EtOAc 3 : 1) 0.24. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 7.38–7.29 (m, 10H, Ar*H*), 4.82 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.76 (d, 1 H, *J* 11.2 Hz, CH_2Ph), 4.63 (d, 2H, *J* 11.2 Hz, CH_2Ph), 3.83–3.75 (m, 2H, H1, H8), 3.72–3.65 (m, 3H, H2, H3, H8′), 3.59 (m, 1H, H5), 3.19 (d, 1H, *J* 4.4 Hz, O*H*1), 2.33 (t, 1H, *J* 6.0 Hz, O*H*8), 2.09 (m, 1H, H6), 1.95 (m, 1H, H4), 1.89–1.75 (m, 3H, H6′,H7,H7′). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 137.8, 137.4, 128.9–128.0 (Ar*C*), 85.0, 79.8 (C2, C3), 74.8, 74.5 (*C*H_2Ph), 72.2 (C1), 63.1 (C8), 61.1 (C5), 49.9 (C4), 27.7, 26.1 (C6, C7), 21.1 (OCO*C*H_3). HRMS(ES+): calcd. for $C_{22}H_{27}N_3O_4Na$: 420.1899, found 420.1898.

(1*S*,2*R*,3*S*,7*R*) Benzyl 7-azido-2,3-di-*O*-benzyl-4-oxocycloheptanecarboxylate (13). The diol **11** (0.21 g, 0.52 mmol) was dissolved in acetone (8 mL) and cooled to 0 °C. Jones reagent (1.2 mL; Jones reagent: 2.67 g of CrO_3 in concentrated sulfuric acid (2.3 mL) and then diluted with H_2O to 10 mL) was added in

three portions over 30 min. The reaction mixture was stirred at 0 °C for 2½ h. *i*-PrOH (25 mL) and H_2O (60 mL) were then added before the acetone was removed under reduced pressure and the aqueous phase extracted with EtOAc (5 × 60 mL) and the combined organic layers dried ($MgSO_4$), filtered and concentrated. The resulting crude product (**12**) (0.20 g) was dissolved in dry CH_3CN (8 mL) and Cs_2CO_3 (249 mg, 0.76 mmol, 1.5 eq) and BnBr (0.20 mL, 1.50 mmol, 3 eq) were added and the reaction mixture heated to 60 °C for 15 min. Then the reaction mixture was cooled to rt and diluted with EtOAc (50 mL). The organic phase was washed with H_2O (3 × 50 mL), dried over $MgSO_4$, filtered and concentrated. The resulting oil underwent flash column chromatography in pentane/EtOAc 20 : 1 → 10 : 1 which gave the desired benzyl ester **13** (174 mg, 67%) over two steps. $[\alpha]_D^{20}$ 13.5 (*c* 1.0, $CHCl_3$). R_f (pentane/EtOAc 10 : 1) 0.25. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 7.32–7.18 (m, 15H, Ar*H*), 5.06 (s, 2H, CH_2Ph), 4.64 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.51 (d, 1H, *J* 11.6 Hz, CH_2Ph), 4.47 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.37 (d, 1H, *J* 11.6 Hz, CH_2Ph), 4.21–4.17 (m, 2H, H2, H7), 4.07 (d, 1 H, *J* 6.8 Hz, H3), 2.89 (dd, 1 H, *J* 6.8 Hz, *J* 9.2 Hz, H1), 2.69 (m, 1H, H5), 2.51 (ddd, 1 H, *J* 3.6 Hz, *J* 7.2 Hz, *J* 15.2 Hz, H5′), 2.28 (m, 1H, H6), 2.05 (m, 1H, H6′). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 208.0 (*C*O), 170.8 (*C*O_2Bn), 137.3, 136.6, 135.4, 128.6–127.9 (Ar*C*), 86.8 (C3), 78.2 (C2), 73.5, 72.8 (*C*H_2Ph), 67.2 (COO*C*H_2Ph), 61.2 (C7), 54.0 (C1), 37.1 (C5), 27.7 (C6). HRMS(ES+): calcd. for $C_{29}H_{29}N_3O_5Na$: 522.2005, found 522.2000.

(1*R*,2*S*,3*R*,4*S*,5*R*) Benzyl 3,4-Di-*O*-benzyl-5-hydroxy-8-azabicyclo[3.2.1]octane-2-carboxylate (14). PPh_3 (0.21 g, 0.77 mmol, 2.5 eq) was added to a stirred solution of ketone **13** (0.16 g, 0.31 mmol) in THF (5 mL) and H_2O (0.5 mL). The reaction mixture was stirred at 40 °C for 4 h before it was concentrated and the crude product purified by flash column chromatography (CH_2Cl_2/Et_2O 4 : 1→ 2 : 1 → 1 : 1 → 0 : 1) to give the desired product **14** (0.11 g, 78%) as a colourless oil. $[\alpha]_D^{20}$ −5.0 (*c* 1.0, $CHCl_3$). R_f (EtOAc) 0.53. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 7.40–7.25 (m, 13H, Ar*H*), 7.17–7.15 (m, 2H, ArH), 5.17 (d, 1H, *J* 12.0 HZ, CH_2Ph), 5.10 (d, 1 H, *J* 12.0 Hz, CH_2Ph), 5.02 (d, 1H, *J* 11.2 Hz, CH_2Ph), 4.83 (d, 1H, *J* 10.8 Hz, CH_2Ph), 4.79 (d, 1 H, *J* 11.2 Hz, CH_2Ph), 4.65 (d, 1H, *J* 10.8 Hz, CH_2Ph), 3.99 (dd, 1H, *J* 8.0 Hz, *J* 10.0 Hz, H3), 3.57 (dd, 1H, *J* 6.8 Hz, *J* 3.2 Hz, H1), 2.74 (dd, 1H, *J* 10.0 Hz, *J* 3.2 Hz, H2), 2.30 (m, 1H, H6), 1.96 (m, 1H, H7), 1.76 (m, 1H, H7′), 1.52 (m, 1H, H6′). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 171.1 (*C*O_2Bn), 139.0, 138.7, 135.7, 128.7–127.6 (Ar*C*), 92.6 (C5), 87.5 (C4), 79.1 (C3), 75.5, 74.9, 66.8 (*C*H_2Ph), 55.6 (C2), 54.1 (C1), 30.9 (C6), 24.3 (C7). HRMS(ES+): calcd. for $C_{29}H_{31}NO_5Na$: 496.2100, found 496.2095.

(1*R*,2*S*,3*R*,4*S*,5*R*)-3,4,5-tri-hydroxy-8-azabicyclo[3.2.1]octane-2-carboxylic acid (7). The tribenzylated compound (**14**) (65 mg, 0.14 mmol) was dissolved in EtOH (4 mL) and EtOAc (2 mL) and flushed with N_2. Pearlman's catalyst (30 mg) was added and the reaction mixture was stirred at rt under an atmosphere of H_2 (balloon) for 8 h before the reaction mixture was filtered through Celite and concentrated. The resulting residue (ester deprotected) was re-dissolved in EtOH (4 mL) and flushed with N_2. Pearlman's catalyst (42 mg) was added and the reaction mixture stirred at rt under an atmosphere of H_2 (balloon) overnight. The mixture was then filtered through Celite, concentrated and the residue was dissolved in EtOH (4 mL) and H_2O (1 mL) and flushed with N_2. Pearlman's catalyst (36 mg) was again added and the reaction mixture stirred at rt for another 8 h under an atmosphere of H_2 (balloon) before being filtered through Celite, concentrated to give the desired product **7** (26 mg, 92%) as a clear oil. $[\alpha]_D^{20}$ 14 (*c* 1.0, H_2O). R_f (EtOAc/isopropanol/water 1 : 2 : 1) 0.29. ^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 3.85 (t, 1H, *J* 10.4 Hz, H3), 3.68 (m, 1H, H5), 3.54 (d, 1H, *J* 8.4 Hz, H2), 2.50 (m, 1H, H4), 2.07 (m, 2H, H6, H7), 1.73 (m, 1H, H6 or H7), 1.60 (m, 1H, H7 or H6). ^{13}C NMR (100 MHz, D_2O): δ_C (ppm) 176.7 (*C*OOH), 92.1 (C1), 76.8, 70.8 (C2, C3), 54.7, 54.6 (C4, C5), 27.5, 23.3 (C6, C7). HRMS(ES+): calcd. for $C_8H_{14}NO_5$: 204.0872, found 204.0870.

(1*R*,2*S*,3*R*,4*S*,5*R*)-8-Ethyl-3,4,5-tri-hydroxy-8-azabicyclo [3.2.1]octane-2-carboxylic acid (15). Treatment of **14** (47 mg, 0.1 mmol) in a similar manner as described for **7** using an old batch of $Pd(OH)_2$ resulted in formation of the unexpected compound **15** after stirring for 10 days. Repeatedly filtration through Celite, concentration under reduced pressure and treatment of the resulting residue with another portion of $Pd(OH)_2$ in EtOH/EtOAc was conducted. The crude product was purified by flash column chromatography (EtOAc/isopropanol/water 1 : 1 : 1) to give compound **15** (11 mg, 49%). R_f (EtOAc/isopropanol/water 1 : 1 : 1) 0.41. ^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 3.96 (m, 1H, H5), 3.90 (dd, 1H, *J* 10.4 Hz, *J* 8.8 Hz, H3), 3.75 (d, 1 H, *J* 8.8 Hz, H2), 3.27 (m, 1H, H9), 2.89 (m, 1H, H9′), 2.66 (dd, 1H, *J* 10.4 Hz, *J* 3.2 Hz, H4), 2.28–2.10 (m, 2H, H6, H7), 1.89–1.75 (m, 2H, H6′,H7′), 1.27 (t, 3H, *J* 7.2 Hz, H10). ^{13}C NMR (100 MHz, D_2O): δ_C (ppm) 176.4 (*C*OOH), 96.9 (C1), 74.9, 70.6, (C2, C3), 58.0, 51.5 (C4, C5), 39.4 (C9), 26.1, 21.1 (C6, C7), 10.3 (C10). HRMS(ES+): calcd. for $C_{10}H_{17}NO_5Na$: 254.1004, found 254.1003.

(1*R*,2*S*,3*R*,4*S*,5*R*)-3,4,5-tri-hydroxy-8-(4-hydroxybutyl)-8-azabicyclo[3.2.1]octane-2-carboxylic acid (16). A solution of **14** (40 mg, 0.08 mmol) in 50% aqueous THF was treated with $Pd(OH)_2$ (old batch) under an atmosphere of hydrogen. The reaction mixture was stirred for 5 days where repeatedly filtration through Celite, concentration under reduced pressure and treatment of the resulting residue with another portion of $Pd(OH)_2$ in THF/water was conducted. The product isolated after flash column chromatography (EtOAc/isopropanol/water 1 : 2 : 1) was the unexpected compound **16** (14 mg, 58%). R_f (EtOAc/isopropanol/water 1 : 1 : 1) 0.44. ^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 3.95 (m, 2H, H3, H5), 3.80 (d, 1H, *J* 8.8 Hz, H2), 3.67 (t, 2H, *J* 6.4 Hz, H12), 3.17 (m, 1H, H9), 2.86 (m, 1H, H9′), 2.75 (dd, 1H, *J* 12.0 Hz, *J* 4.0 Hz, H4), 2.30–2.15 (m, 2H, H6, H7), 1.90–1.63 (m, 6H, H6′,H7′, H10, H10′, H11, H11′). ^{13}C NMR (100 MHz, D_2O): δ_C (ppm) 176.6 (*C*OOH), 96.0 (C1), 74.5, 70.5, (C2, C3), 61.1 (C12), 58.3, 51.1 (C4, C5), 43.8 (C9), 29.0, 26.2, 22.3, 21.1 (C6,C7,C10,C11). HRMS(ES+): calcd. for $C_{12}H_{22}NO_6$: 276.1447, found 276.1449.

Inhibition studies

Inhibition constants (K_i's) were determined by measuring initial rates (<10% of substrate conversion) using *p*-nitrophenyl β-D-glucuronide at seven concentrations ranging from ¼– 4 times K_M monitoring at 400 nm with $A < 1$ using either a Varian Cary 100 Bio UV-vis Spectrophotometer or a PerkinElmer 2300 EnSpire Multilabel Plate Reader. Reduced volume Polystyren cuvettes were used for spectrophotometer measurements while PolySorb (flat bottom) Immuno 96 MicroWell Solid Plates from

Nunc was used for plate reader measurements. Measurements were conducted in 50 mM phosphate buffer (pH 6.8) at 37 °C for 2 min for *E. coli* β-glucuronidase using a continuous assay. Measurements of inhibition of bovine liver β-glucuronidase were conducted in 50 mM sodium acetate buffer (pH 4.6) at 37 °C for 2 min using a stopped assay. In this context it should be mentioned that the enzyme wasn't active at the same pH in citrate buffer. Temperature equilibration was conducted prior to enzyme addition and monitoring of reaction rates. K'_M and K_M are Michaelis–Menten constants with or without inhibitor present. K'_M and K_M were obtained from Hanes plot, which was also used to ensure that inhibition was competitive. K_i values were calculated as $K_i = [I]/((K'_M/K_M) - 1)$ having $[I] \approx K_i$. For Hanes plots, see the ESI.‡

Acknowledgements

We thank Mr Mikkel T. Jensen for technical support. We are furthermore grateful for financial support from the Faculty of Natural Sciences, OChem Graduate School, Aarhus University, The Danish National Science Research Foundation, The Lundbeck Foundation, and DCSC.

References

1 *Iminosugars as Glycosidase Inhibitors – Nojirimycin and Beyond*, A. E. Stütz, ed., Wiley-VCH, 1999.
2 (*a*) T. M. Jespersen, W. Dong, M. R. Sierks, T. Skrydstrup, I. Lundt and M. Bols, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1778–1779; (*b*) Y. Ichikawa, Y. Igarashi, M. Ichikawa and Y. Suhara, *J. Am. Chem. Soc.*, 1998, **120**, 3007–3018.
3 (*a*) M. Bols, R. G. Hazell and I. B. Thomsen, *Chem.–Eur. J.*, 1997, **3**, 940–947; (*b*) H. H. Jensen and M. Bols, *J. Chem. Soc., Perkin Trans. 1*, 2001, 905–909; (*c*) H. H. Jensen, A. Jensen, R. G. Hazell and M. Bols, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1190–1198.
4 H. Liu, X. Liang, H. Søhoel, A. Bülow and M. Bols, *J. Am. Chem. Soc.*, 2001, **123**, 5116–5117.
5 (*a*) V. H. Lillelund, H. H. Jensen, X. Liang and M. Bols, *Chem. Rev.*, 2002, **102**, 515–553; (*b*) *Iminosugars: From Synthesis to Therapeutic Applications*, ed. P. Compain and O. R. Martin, Wiley-VCH, 2007.
6 (*a*) A. Goldmann, M.-L. Milat, P.-H. Ducrot, J.-Y. Lallemand, M. Maille, A. Lepingle, I. Charpin and D. Tepfer, *Phytochemistry*, 1990, **29**, 2125–2127; (*b*) N. Asano, A. Kato, M. Miyauchi, H. Kizu, Y. Tomimori, K. Matsui, R. J. Nash and R. J. Molyneux, *Eur. J. Biochem.*, 1997, **248**, 296–303; (*c*) B. Dräger, *Nat. Prod. Rep.*, 2004, **21**, 211–223.
7 T. S. Rasmussen and H. H. Jensen, *Org. Biomol. Chem.*, 2010, **8**, 433–441.
8 T. S. Rasmussen, S. Allman, G. Twigg, T. D. Butters and H. H. Jensen, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1519–1522.
9 B. D. Wallace, H. Wang, K. T. Lane, J. E. Scott, J. Orans, J. S. Koo, M. Venkatesh, C. Jobin, L.-A. Yeh, S. Mani and M. R. Redinbo, *Science*, 2010, **330**, 831–835.
10 J. C. Lee, Y. S. Oh, S. H. Cho and J. D. Lee, *Org. Prep. Proced. Int.*, 1996, **28**, 480–483.
11 P. D. Bailey, M. A. Beard, H. P. T. Dang, T. R. Phillips, R. A. Price and J. H. Whittaker, *Tetrahedron Lett.*, 2008, **49**, 2150–2153.
12 Y. Yoshimura, C. Ohara, T. Imahori, Y. Saito, A. Kato, S. Miyauchi, I. Adachi and H. Takahata, *Bioorg. Med. Chem.*, 2008, **16**, 8273–8286.
13 D. L. Zechel and S. G. Withers, *Acc. Chem. Res.*, 2000, **33**, 11–18.
14 T. Niwa, T. Tsuruoka, S. Inouye, Y. Naito, T. Koeda and T. Niida, *J. Biochem.*, 1972, **72**, 207–211.
15 T. M. Gloster, R. Madsen and G. J. Davies, *ChemBioChem*, 2006, **7**, 738–742.