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Expanding the library of divalent fucosidase inhibitors with polyamino and triazole-benzyl bridged bispyrrolidines

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A small library of divalent fucosidase inhibitors containing pyrrolidine motifs and separated by polyamino and triazole-benzylated spacers, were prepared and evaluated as α -fucosidase inhibitors. Although weak multivalent effect was observed in polyamino derived dimers, useful structural information can be deduced about the length of the bridge, the number of nitrogen atoms present and the moieties close to the pyrrolidine. Within these investigations one of the best α -fucosidase inhibitors containing a pyrrolidine framework was obtained (**18**, $K_i = 3.7$ nM)

Introduction

Fucose containing glycans are involved in a variety of biological processes¹ such as infectious and inflammatory diseases,² blood coagulation cascade,³ antigenic determination⁴ and tumor progression.⁵ α -Fucosidases (FUCA) are exoglycosidases which participate in the processing of α -fucosyl residues by catalyzing either their cleavage or their glycosylation.⁶

Two retaining α -fucosidases assigned to the GH29 CAZY family are expressed in human. Of these, FUCA1 is found in lysosomes whereas FUCA2 is specifically localized in plasma.⁷ The accumulation of fucose-containing glycoconjugates because of deficiency of FUCA1 causes fucosidosis,⁸ a rare autosomal recessive lysosomal storage disorder. The level of FUCA1 activity is also a relevant biomarker for the diagnosis of hepatocellular cancer.⁹ Furthermore, FUCA1 is present in seminal plasma and sperm surface and may have important roles in reproduction.¹⁰ Recently, it has been shown that human FUCA2 is crucial for adhesion, growth and pathogenicity of the bacteria *Helicobacter pylori* which is related to the development of gastric cancer.^{7,11} In many cases, the biological role of FUCA has been assessed with the use of potent inhibitors. These invaluable tools have been successfully applied in the study or the control of infectivity,¹² of cancer,¹³ or of fertilization processes and sterility.¹⁴ Moreover, glycosidase inhibitors have been proposed as molecular chaperones¹⁵ that might stabilize misfolded proteins thus combating rare diseases. This approach has been successfully applied to lysosomal storage diseases such as Gaucher and Fabry diseases.¹⁶ Likewise, α -

fucosidase inhibitors might be useful to stabilize misfolded proteins thus combating fucosidosis. For these reasons, in the last years, many efforts have been devoted to the synthesis of α -fucosidase inhibitors, mainly of the family of iminosugars, reaching activities in the micro-, nano- and even pico-molar range.^{17,18}

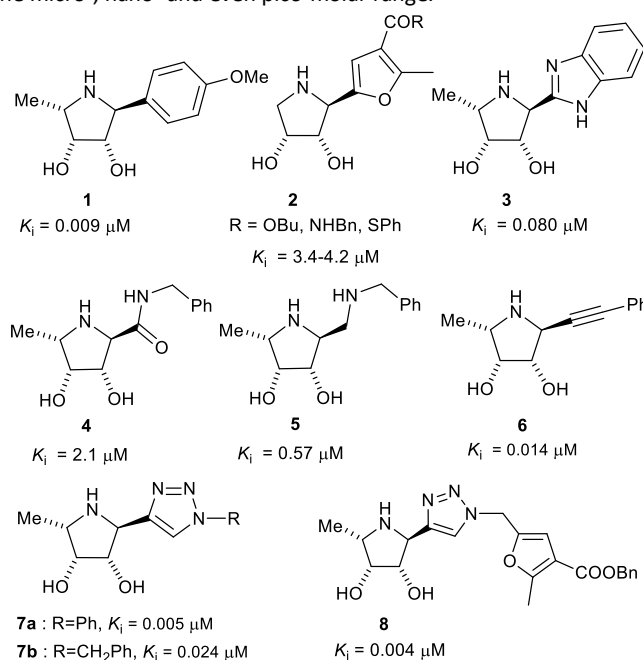


Figure 1. Structures of known fucosidase inhibitors^{13,20a-e}

Recent structure-activity studies have shown that the incorporation of aromatic/aliphatic moieties on iminosugars (compounds **1–8**, Figure 1) improves their inhibition properties as fucosidase inhibitors through additional secondary allosteric interactions.^{19,20} Moreover, several structural studies on bacterial α -fucosidases have been reported focusing on CAZY family GH29 isoform.^{13,21,22} Interestingly, structures were determined with five-membered iminocyclitols like **1**¹³, **3**^{22f} or **7a**^{22e} as ligands, which

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† Footnotes relating to the title and/or authors should appear here. Electronic Supplementary Information (ESI) available: [IC₅₀ curves, ¹H and ¹³C-NMR spectra of final compounds]. See DOI: 10.1039/x0xx00000x

revealed a transition-state mimicking (³E) conformation of the pyrrolidine ring and the adventitious interactions of the hydrophobic aglycon.

Our interest focuses in finding new ways to extend these successful results obtained with "monovalent" sugar mimics to multivalent systems. It is known that carbohydrate-templated multivalency not only increases the potency of the carbohydrate/receptor interactions but also may improve the solubility of the complex in aqueous media.²³ The affinity enhancement resulting from such multiple interactions is known as multivalent or "cluster" effect,²⁴ which has been extensively studied in the case of lectins.²⁵ Recent reports have extended the application of multivalent architectures to the field of enzyme inhibition.²⁶ In general, it is believed that multivalency may improve the binding affinity in enzymatic systems by (i) simultaneous binding or chelation of multiple (sub)ligands to affinal binding sites located on target proteins and/or (ii) the so called "statistical rebinding" or "proximity effect" which reflects the greater tendency for ligand rebinding when two relevant ligands are close to one another. The statistical interaction may operate with short spacers that prevent the simultaneous interaction of the ligand with different active sites of the protein, or when the protein has a unique active site.²⁷ Multivalency has been reported mostly with glycosidase inhibitors of α -mannosidases,²⁸ amyloglucosidases, α - and β -glucosidases and β -galactosidases.²⁹

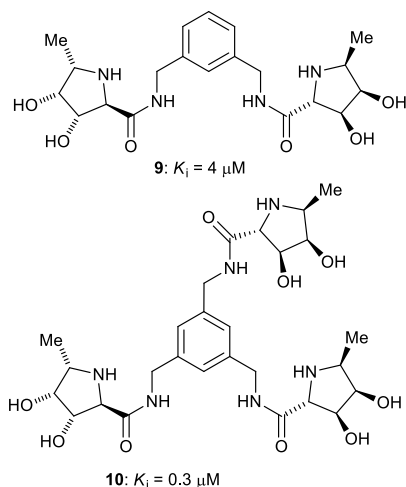


Figure 2. Structures of first generation multimeric fucosidase inhibitors.

In the case of α -fucosidases, we have recently reported the synthesis of divalent and trivalent inhibitors (compounds **9** and **10**, Figure 2).³⁰ In order to get more insights about the type of multivalency operating in α -fucosidase inhibition, in this paper we report the synthesis and biological evaluation of monovalent pyrrolidines **11–13** and their divalent counterparts **14–18** bearing chemically diverse spacers between the two *fuco*-configured pyrrolidine moieties (Figure 3). The spacers contain polyalkylamino chains of different lengths or 1,3-bis(triazole)methylbenzene moieties. These investigations have allowed the discovery of one of the most potent and specific α -fucosidase inhibitors (Compound **18**, $K_i = 3.7 \text{ nM}$) containing a pyrrolidine framework, reported to date.

Results and discussion

The synthesis of iminosugars **11,12,14,15** started from known allylpyrrolidines **19** and **22**.³¹ Using the (2*R*)-configured **19**, dihydroxylation (with AD-mix- α) followed by oxidative cleavage (NaIO_4 in EtOH-water as the solvent) afforded the expected aldehyde **20** (Scheme 1), which proved stable at room temperature.

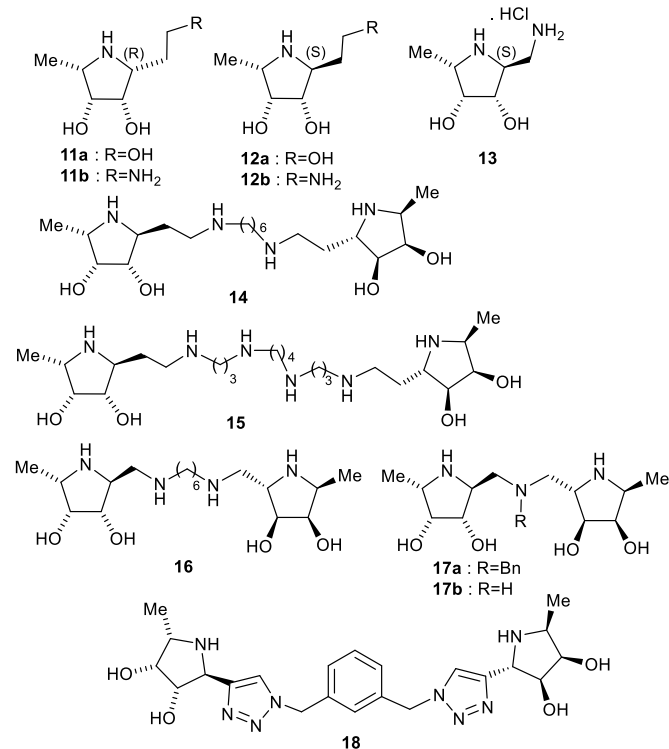
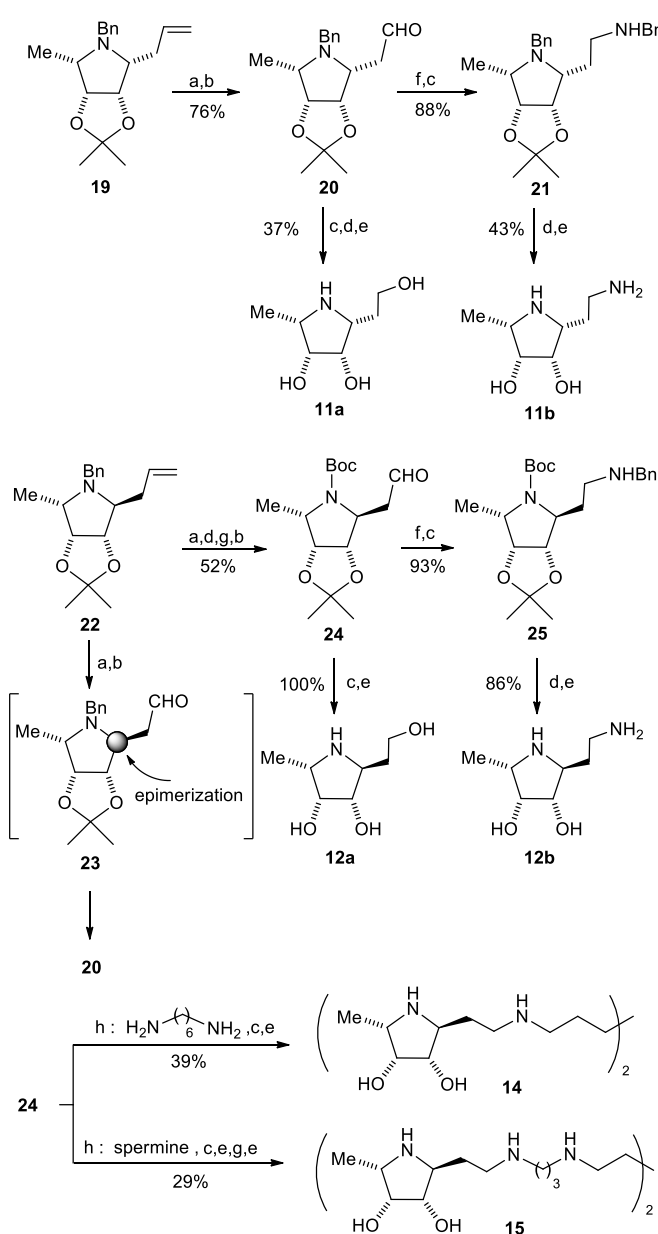


Figure 3. Structures of targeted divalent inhibitors and of the corresponding monomers.

Reduction of **20** with sodium borohydride followed by deprotection (H_2 -Pd/C then 1 M HCl) afforded pyrrolidinyl-ethanol **11a**.³² Reductive amination of **20** with benzylamine gave **21** which was deprotected to yield the (2*R*)-configured pyrrolidinyl-ethylamine **11b**. The synthesis of the (2*S*)-stereoisomers could not follow a similar route since aldehyde **23**, obtained from the (2*S*)-allylpyrrolidine **22**, epimerized spontaneously to **20**, impeding the use of this intermediate for synthetic purpose. Epimerization was mainly due to the transient opening of the pyrrolidine ring to form the corresponding conjugated aldehyde, a process which operates via protonation of the internal amine. This unusual transformation reported for the first time by us,³¹ was exploited recently to build an epimerization strategy to access stereoisomeric pyrrolidines.³³ Protective-group transformation from *N*-Bn to *N*-Boc prevents such a transformation and aldehyde **24** could be obtained in pure and stable state. Reduction and reductive amination were conducted under the same conditions than above to afford pyrrolidinyl-ethanol **12a**³⁴ and diamine **12b**, the epimers of **11a,b**, which were all assayed on α -fucosidase to assess the inhibition potencies of these monomeric entities (see below).

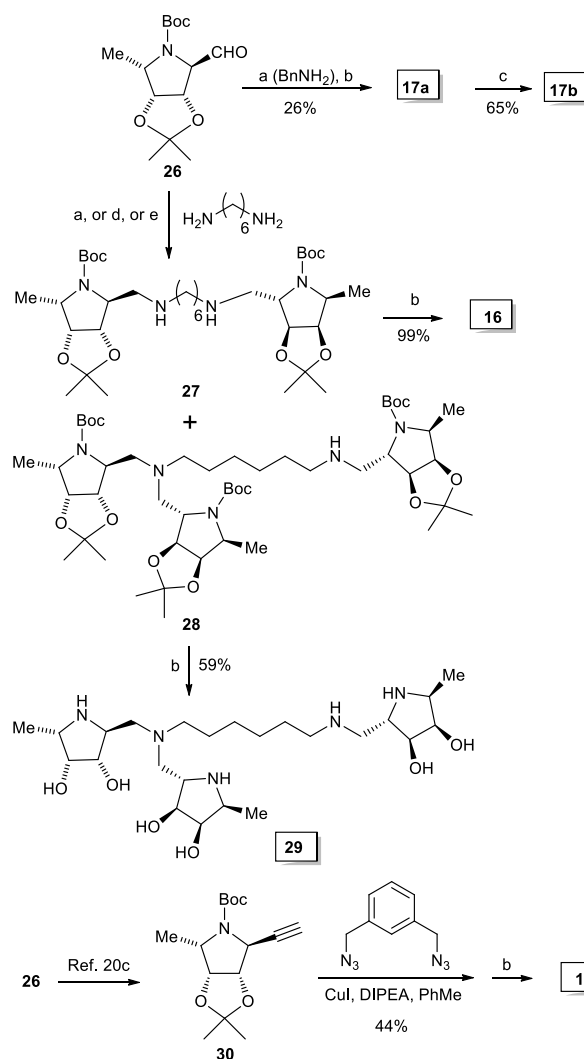


Scheme 1. Reagents and conditions. (a) AD-Mix- α , acetone-H₂O; (b) NaIO₄, EtOH-H₂O; (c) NaBH₄, MeOH; (d) H₂ or HCO₂NH₄, Pd-C (10%), MeOH; (e) HCl_{aq}-MeOH, then Amberlyst A-26 (OH⁻); (f) Bn-NH₂, MgSO₄, CH₂Cl₂; (g) Boc₂O, Et₃N, CH₂Cl₂; (h) polyamine (0.45 equiv.), MeOH-CH₂Cl₂, MgSO₄.

The dimers **14** and **15** were prepared starting from aldehyde **24**³¹ by an analogous synthetic route (Scheme 1). To this purpose, a two-fold excess of **24** was reacted with either hexamethylenediamine or spermine in a mixture of dichloromethane/methanol in the presence of MgSO₄ to produce the corresponding di-imine. After 5h the reaction was complete and reduction to the protected di-amine was effected with NaBH₄. Final deprotection was performed by stirring at room temperature overnight in a 1:1 mixture of MeOH and 1M aqueous HCl. Whereas divalent pyrrolidine **14**, featuring the hexamethylenediamino linker, was obtained in a pure state following this sequence, the congener **15**

with a spermine bridge contained some impurities. A further sequence of Boc protection/purification/deprotection (MeOH:HCl_{aq}) was required to obtain pure **15**. The symmetry in the structures of dimers **14**, **15** was clearly reflected by the NMR spectra, showing only one set of protons or carbons for both pyrrolidines as well as symmetry in the bridging moiety.

In parallel, the divalent **16,17**, analogues of known pyrrolidine **13**³⁵ were obtained from aldehyde **26**,^{20c} by following comparable strategies (Scheme 2).



Scheme 2. Reagents and conditions. (a) Amine, NaBH(OAc)₃, 1,2-DCE (**27**: 25%, **28**: 27%); (b) HCl-THF; (c) H₂, Pd-C (10%), MeOH; (d) amine, MgSO₄, CH₂Cl₂-MeOH, then NaBH₄; (e) amine, CF₃CH₂OH, then NaBH₄ (**27**: 49%, **28**: 5%).

Thus, the reaction of **26** (2.3-fold excess) with benzylamine and sodium triacetoxyborohydride followed by acidic deprotection afforded compound **17a**. Dimer **17b** was obtained after subsequent debenzilation. When the reductive amination reaction of **26** (2-fold excess) was performed with hexamethylenediamine, the expected divalent derivative **27** was obtained in only 25% yield, together with

the trivalent compound **28** (27% yield). Similar results were obtained in the reaction of **26** and hexamethylenediamine when following the conditions stated above for aldehyde **24** (dichloromethane/methanol as the solvent, MgSO_4 as a dehydrating agent and NaBH_4 as the reductant). Gratifyingly, reaction of **26** with the diamine and NaBH_4 in 2,2,2-trifluoroethanol afforded **27** in 49% yield, which after acidic deprotection gave divalent derivative **16**. To enlarge the scope of multimeric pyrrolidines, trimer **28** was also deprotected (HCl-THF) to afford **29** which was tested likewise towards fucosidase.

Inspired by the good inhibitory activities shown by our previously described (pyrrolidin-2-yl)triazoles **20c** we decided to synthesize divalent compound **18**. For this purpose, alkyne **30** was prepared, which after Cu(I)-catalyzed alkyne-azide cycloaddition with 1,3-bis(azidomethyl)benzene followed by acidic deprotection afforded divalent **18** in 44% yield.

Fucosidase inhibitory properties of all compounds were then examined. In a first set of experiments, the monomers **11a,b**, **12a,b**, were tested in order to establish the inhibition potencies of the monovalent references, but also to validate the choice of the (2S) configuration as the best one. Pyrrolidine **13**,^{20a, 35} the monomeric analogue of **16**, **17** and **29** had already been prepared and screened ($K_i = 1.9 \mu\text{M}$).³⁵ Nevertheless, for comparative purpose, we have determined the IC_{50} under the same conditions as the other compounds. Compound **7b**,^{20c} the monomeric partner of **18**, had been evaluated previously by our group. The results are outlined in Table 1.

Table 1. Bovine kidney fucosidase inhibition results

Compound	Valency (n)	IC_{50} (μM) (K_i)	Relative potency ^c (rp)	rp/n ^d
11a	1	2.4		
11b	1	29		
12a	1	0.373		
		($K_i = 0.090 \mu\text{M}$) ^b		
12b	1	13	-	-
14	2	1.2	10.8	5.4
15	2	6.3	2.1	1.0
13^f	1	3.2	-	-
16	2	4.7	0.68	0.34
17a	2	71	0.045	0.023
17b	2	2.2	1.5	0.75
29	3	4.4	0.73	0.24
7b	1	0.3^e	-	-
18	2	0.074	4.1	2.1
		($K_i = 0.0037 \mu\text{M}$)		

^a The IC_{50} of reference monomers are in bold.

^b In our hands compound **12a** was considerably less active than originally described.³⁴ K_i calculated through Cheng-Prusoff equation.

^c Relative potency = IC_{50} monovalent / IC_{50} multivalent

^d rp/n: Potency per iminosugar = relative potency / number of pyrrolidine moieties

^e Ref 20c

^f Hydrochloride salt

As could be predicted, compounds **12** are better inhibitors than pyrrolidines **11**, thus assuring the choice of the

(2S) configuration at the pyrrolidine moiety which mimics the α -fuco configuration. Alcohols **11a** and **12a** also showed better inhibitory activity than the corresponding amines **11b** and **12b**. In order to check if some multivalent effect could be observed with the dimeric structures, monomeric pyrrolidines **12b** and **13** were chosen as reference compounds and dimers **14**, **15**, **16** and **17** were assessed. The results show an increase in the activity for compounds **14** and **15** with regards to the parent compound **12b**. Regarding a possible multivalent effect, only **14** proved more active than the monomer, valency corrected (relative potency per iminosugar: 5.4). A longer spacer between the two iminosugars as in **15**, does not offer any advantage, since the potency per iminosugar for **15** is identical to the monomer.

On the contrary, compounds **16**, **17a,b** and **29** showed less potency per iminosugar than the corresponding monomer **13**. Significantly, the benzyl substitution at the central nitrogen atom (compound **17a**) reduced at large extent the inhibitory activity, probably because of a steric hindrance effect in reaching the active site, since **17b** is 32-fold more active. Dimer **16** and trimer **29** have identical potencies showing that an increase in the number of sugar motifs in polyamino-bridged pyrrolidines does not improve multivalent properties. For the triazole-benzyl bridged bispyrrolidine **18** ($\text{IC}_{50} = 0.074 \mu\text{M}$, $K_i = 3.7 \text{ nM}$), a weak multivalent effect was observed on comparing it with the reference compound **7b**.

K_i of known **12a**³⁴ was calculated through Cheng-Prusoff equation, and K_i for **18** was determined from the Lineweaver-Burk plots (see ESI[†] for details) indicating a competitive inhibition. For the best inhibitor **18**, and for compound **14** that showed the highest rp/n (best multivalent effect), selectivity was also evaluated by measuring the % of inhibition against other commercially available glycosidases: α -galactosidase from coffee beans, β -galactosidase from *Aspergillus oryzae* and from *Escherichia Coli*, α -glucosidase from rice, α -glucosidase from *Saccharomyces cerevisiae*, amyloglucosidase from *Aspergillus niger*, β -glucosidase from almonds, α -mannosidase from Jack beans, β -mannosidase from *Helix pomatia* and β -N-acetylglucosaminidase from Jack beans. Dimers **18** and **14** are specific inhibitors of α -fucosidase (100% inhibition), showing no inhibition against the other enzymes. Only **18** showed a weak inhibition (32%) towards amyloglucosidase from *Aspergillus niger*.

Conclusions

We have performed the synthesis of several dimers containing polyamino and triazole benzylated bridges that were evaluated as α -fucosidase inhibitors and compared with their respective monovalent pyrrolidines. Useful structural information can be deduced on the length and the rigidity of the bridge, the number of nitrogen atoms present and the moieties close to the pyrrolidine for the design of future fucosidase inhibitors. Although much more research is definitively needed to understand multivalency and its utility in the area of glycosidases in general, and particularly in α -fucosidase

inhibition, the results provided in this paper will help for the development of better multivalent inhibitors. In this way, new multimeric iminosugars **14-18,29** are valuable models that could be assayed on other unrelated glycosidases or glycosyltransferases to detect unexpected multivalent effect. Remarkably is the discovery of one of the most potent α -fucosidase inhibitors, compound **18**, at the low nM range containing a pyrrolidine motif.

Experimental

General considerations: The reagents and solvents were commercially available and used as received. Silica gel F-254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic solution of phosphomolybdic acid, *p*-anisaldehyde, ninhydrin or an aqueous solution of KMnO_4 (2%) / Na_2CO_3 (4%), followed by heating. Flash column chromatography was performed over silica gel M 9385 (40-63 μm and 63-200 μm) Kieselgel 60. NMR spectra were recorded on Bruker AC 250 (250 MHz for ^1H , 62.5 MHz for ^{13}C) or 600 (600 MHz for ^1H , 150 MHz for ^{13}C) and Bruker AV300 (300 MHz for ^1H , 75.4 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in parts per million (ppm) and were calibrated to the residual solvent peak. Coupling constants are in Hz and splitting pattern abbreviations are: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded with a Jasco FTIR-410 and are expressed in cm^{-1} . Optical rotations were determined at the indicated temperature with a Perkin-Elmer Model 241 or with a Jasco P-2000 polarimeter in the specified solvents. High Resolution Mass Spectra (HRMS) were performed on Q-TOF Micro micromass positive ESI (CV = 30 V) and QExactive spectrometres.

Synthesis of alcohol 11a

To a solution of aldehyde **20** (39 mg, 0.135 mmol), prepared from allylpyrrolidine **19** following the described procedure,³¹ in MeOH (1 mL) was added NaBH_4 (7 mg, 1.3 equiv.) at 0 °C. The solution was stirred and left to warm to rt overnight. Saturated NH_4Cl and EtOAc were successively added, followed by extraction. The organic layer was dried (MgSO_4) and concentrated. The residue (39 mg) was subjected to hydrogenolysis overnight with 10% Pd-C (24 mg) under an atmosphere of hydrogen. Filtration over celite and purification by silica gel chromatography ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$: 6/4/0.5) yielded the expected debenzylated product, which was further deprotected by stirring overnight in a solution of MeOH (1 mL) and 1 M HCl (1 mL). After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and evaporated. Purification by silica gel column chromatography (DCM/MeOH : 8/2 \rightarrow $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$: 6/4/1) gave pyrrolidine **11a** (8 mg, 37% from **20**, colorless oil).

11a: $[\alpha]_{\text{D}}^{20} = +5.33$ (c 1.1, CHCl_3); $^1\text{H-NMR}$ (D_2O , 250 MHz): δ 1.34 (d, 3H, $J = 6.9$ Hz, CH_3), 1.81-2.17 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-OH}$), 3.60-3.82 (m, 4H, H-2, H-5 and $-\text{CH}_2\text{-OH}$), 4.31-4.44 (m, 2H, H-3 and H-4); $^{13}\text{C-NMR}$ (D_2O , 63 MHz): δ 12.3 (CH_3), 29.2 ($\text{CH}_2\text{-CH}_2\text{-OH}$), 56.6, 58.5 (C-

2 and C-5), 58.7 ($-\text{CH}_2\text{-OH}$), 71.1, 71.2 (C-3 and C-4); HRMS-ESI⁺ (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_7\text{H}_{16}\text{NO}_3$: 162.1130, found 162.1127.

Synthesis of amine 11b

To a solution of aldehyde **20**³¹ (129 mg, 0.45 mmol), in MeOH (1 mL) was added MgSO_4 (535 mg, 10 equiv) and benzylamine (53 μL , 0.491 mmol). The solution was stirred at rt for 5 h. After filtration and concentration, the resulting material was dissolved in MeOH (2 mL) and NaBH_4 (22 mg, 0.58 mmol, 1.3 equiv.) was added at 0 °C. This solution was stirred and left to warm to rt overnight. Saturated NH_4Cl and EtOAc were successively added, followed by extraction. The organic layer was dried (MgSO_4) and concentrated. Purification by silica gel column chromatography ($\text{Et}_2\text{O}/\text{MeOH}$: 9/1) yielded *N*-benzylidiamine **21** (149 mg, 88%, colorless oil). The compound (100 mg, 0.263 mmol) was debenzylated with HCO_2NH_4 (116 mg, 1.84 mmol) and Pd/C 10% (100 mg) in MeOH (1.5 mL) at 60 °C for 1.5 h. Filtration over celite and purification by silica gel chromatography ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$: 6/4/0.5) yielded the expected ethylaminopyrrolidine (34 mg), which was further deprotected by stirring overnight in a solution of MeOH (1 mL) and 1 M HCl (1 mL). After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and evaporated. Purification by silica gel column chromatography (DCM/MeOH : 8/2 \rightarrow $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$: 6/4/1) yielded diamine **11b** (18 mg, 43% from **21**, colorless oil). **11b:** $[\alpha]_{\text{D}}^{20} = -2.7$ (c 0.37, H_2O); $^1\text{H-NMR}$ (D_2O , 600 MHz): δ 1.23 (d, 3H, $J = 6.8$ Hz, CH_3), 1.90 (td, 1H, $J = 15.6, 7.8$ Hz, $\text{CH}_2\text{-CH}_2\text{-NH}_2$), 2.08 (td, 1H, $J = 14.3, 7.8$ Hz, $\text{CH}_2\text{-CH}_2\text{-NH}_2$), 3.07 (t, 2H, $J = 7.8$ Hz, H-1'a), 3.32 (dd, 1H, $J = 13.6, 6.5$ Hz, H-2), 3.34-3.40 (m, 1H, H-5), 4.22 (t, $J = 5.2$ Hz, 1H, H-4), 4.32 (t, $J = 5.2$ Hz, 1H, H-3). $^{13}\text{C-NMR}$ (D_2O , 63 MHz): δ 11.3 (CH_3), 25.2 ($\text{CH}_2\text{-CH}_2\text{-NH}_2$), 35.8 ($-\text{CH}_2\text{-NH}_2$), 54.2 (C-5), 56.0 (C-2), 70.2 (C-3), 70.7 (C-4); HRMS-ESI⁺ (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_7\text{H}_{17}\text{N}_2\text{O}_2$: 161.1290, found 161.1289.

Synthesis of alcohol 12a

To a solution of aldehyde **24** (9 mg, 0.03 mmol), prepared from allylpyrrolidine **22** following the described procedure,³¹ in MeOH (1 mL) was added NaBH_4 (1.5 mg, 1.3 equiv.) at 0 °C. The solution was stirred and left to warm to rt overnight. Saturated NH_4Cl and EtOAc were successively added, followed by extraction. The organic layer was dried (MgSO_4) and concentrated. The residue was further deprotected by stirring overnight in a solution of MeOH (1 mL) and 1 M HCl (1 mL). After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-). Evaporation of the solvents gave pyrrolidine **12a** (5 mg, 100% from **24**) the NMR data of which correlated with those reported by Defoin.³⁴

Synthesis of amine 12b

To a solution of aldehyde **24**³¹ (30 mg, 0.100 mmol), in MeOH (1 mL) was added MgSO_4 (120 mg, 10 equiv) and benzylamine (13 μL , 0.120 mmol). The solution was stirred at rt for 5 h. After filtration and concentration, the resulting material was dissolved in MeOH (2 mL) and NaBH_4 (5 mg, 1.3 equiv.) was added at 0 °C. This solution was stirred and left to warm to rt overnight. Saturated NH_4Cl and EtOAc were successively

added, followed by extraction. The organic layer was dried (MgSO_4) and concentrated. Purification by silica gel column chromatography (EtOAc/MeOH: 19/1 \rightarrow 9/1) yielded *N*-benzylamine **25** as a colorless oil (43 mg, 93%). The compound (47 mg, 0.120 mmol) was debenzylated with HCO_2NH_4 (53 mg, 0.84 mmol) and Pd/C 10% (47 mg) in MeOH (1 mL) at 60 °C for 1.5 h. Filtration over celite and purification by silica gel chromatography (EtOAc/MeOH: 8/2) yielded the expected *N*-Boc-ethylaminopyrrolidine (31 mg, 86%), which was further deprotected by stirring a sample (19 mg, 0.063 mmol) overnight in a solution of MeOH (1 mL) and 1 M HCl (1 mL). After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and evaporated. Purification by silica gel column chromatography (DCM/MeOH: 8/2 \rightarrow $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$: 6/4/1) yielded diamine **12b** as a yellow oil (10 mg, 100%).

12b: $[\alpha]_{\text{D}}^{20} = -41.0$ (c 0.36, MeOH); $^1\text{H-NMR}$ (CD_3OD , 500 MHz): δ 1.34 (d, 3H, $J = 6.7$ Hz, CH_3), 2.03–2.16 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-NH}_2$), 3.10 (t, 2H, $J = 7.4$ Hz, $-\text{CH}_2\text{-NH}_2$), 3.40 (q, 1H, $J = 8.0$ Hz, H-2), 3.55–3.67 (m, 1H, H-5), 3.96 (t, 1H, $J = 3.5$ Hz, H-4), 4.03 (dd, 1H, $J = 8.0$, 3.5 Hz, H-3). $^{13}\text{C-NMR}$ (CD_3OD , 126 MHz): δ 12.9 (CH_3), 31.0 ($\text{CH}_2\text{-CH}_2\text{-NH}_2$), 38.4 ($-\text{CH}_2\text{-NH}_2$), 57.8 (C-5), 59.9 (C-2), 73.6 (C-4), 78.4 (C-3); HRMS-ESI $^+$ (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_7\text{H}_{17}\text{N}_2\text{O}_2$: 161.1290, found 161.1283.

Synthesis of dimer 14

To hexamethylenediamine (8 mg, 0.07 mmol, 1 equiv.) in a solution of DCM/MeOH: 2/1 (3 mL), MgSO_4 (77 mg, 0.7 mmol, 10 equiv.) and aldehyde **24** (44 mg, 0.147 mmol, 2.2 equiv) were successively added. The solution was stirred at room temperature for 5 h. Then the mixture was filtered, concentrated and the resulting material was dissolved in MeOH (1 mL). After addition of NaBH_4 (6.5 mg, 0.174 mmol, 2.6 equiv.) at 0 °C, the solution was allowed to warm to room temperature and stirred overnight. Saturated NH_4Cl and EtOAc were added at 0 °C followed by extraction with EtOAc. The combined organic layers were dried over MgSO_4 and concentrated. The residue was purified by silica gel chromatography (EtOAc \rightarrow $\text{CHCl}_3/\text{MeOH}/1\text{M } \text{NH}_4\text{OH}$: 6/4/1) yielding the corresponding protected dimer (26 mg) as colorless oil. The dimer (26 mg, 0.038 mmol) in MeOH (1 mL) was then treated with 1 M HCl (1 mL) and stirred at room temperature overnight. After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and lyophilized to yield dimer **14** (18 mg, 39%) as a white solid.

14: $[\alpha]_{\text{D}}^{20} = -34.3$ (c 0.40, $\text{H}_2\text{O}/\text{MeOH}$, 3/1); $^1\text{H-NMR}$ (CD_3OD , 500 MHz): δ 1.33 (d, 6H, $J = 6.6$ Hz, CH_3), 1.42–1.49 (m, 4H, 11-H), 1.67–1.77 (m, 4H, 10-H), 2.08–2.22 (m, 4H, pyr- CH_2 -), 2.99 (t, 4H, $J = 7.7$ Hz, $-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2$ -), 3.05–3.19 (m, 4H, pyr- $\text{CH}_2\text{-CH}_2$ -), 3.42 (q, 2H, $J = 8.5$ Hz, H-2), 3.65 (qd, 2H, $J = 6.6$, 3.1 Hz, H-5), 3.94 (t, 2H, $J = 3.1$ Hz, H-4), 4.03 (dd, 2H, $J = 8.5$, 3.1 Hz, H-3); $^{13}\text{C-NMR}$ (CD_3OD , 126 MHz): 12.7 (CH_3), 26.9 ($-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2$ -), 27.2 ($-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2$ -), 29.2 (pyr- CH_2 -), 46.2 (pyr- $\text{CH}_2\text{-CH}_2$ -), 48.9 ($-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2$ -), 58.0 (C-5), 59.9 (C-2), 73.4 (C-4), 78.1 (C-3). HRMS-ESI $^+$ (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{43}\text{N}_4\text{O}_4$: 403.3282, found 403.3296.

Synthesis of dimer 15

To spermine (14 mg, 0.07 mmol, 1 equiv.) in a solution of DCM/MeOH: 2/1 (3 mL), MgSO_4 (77 mg, 0.669 mmol, 10 equiv.) and aldehyde **24** (44 mg, 0.147 mmol, 2.2 equiv) were successively added. The solution was stirred at room temperature for 5 h. The mixture was filtered, concentrated and the resulting material was dissolved in MeOH (1 mL). After addition of NaBH_4 (6.5 mg, 0.174 mmol, 2.6 equiv.) at 0 °C, the solution was allowed to warm to room temperature and stirred overnight. Saturated NH_4Cl and EtOAc were added at 0 °C followed by extraction with EtOAc. The combined organic layers were dried over MgSO_4 and concentrated. The residue was purified by silica gel chromatography (EtOAc \rightarrow $\text{CHCl}_3/\text{MeOH}/1\text{M } \text{NH}_4\text{OH}$: 6/4/1) yielding the corresponding protected dimer (44 mg) as colorless oil. The dimer (44 mg, 0.057 mmol) in MeOH (1 mL) was then treated with 1 M HCl (1 mL) and stirred at room temperature overnight. After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and lyophilized.

The crude material was dissolved in DCM (2 mL) and cooled down to 0 °C. After addition of Et_3N (41 μL , 0.295 mmol, 5 equiv.) and Boc_2O (116 mg, 0.531 mmol, 9 equiv.) the solution was allowed to warm slowly to room temperature and was stirred for 24 h. Distilled water was then added and the aqueous layer was extracted with DCM (3 times). The combined organic layers were dried (MgSO_4), filtered and evaporated under vacuum. The crude residue was purified by silica gel column chromatography (EtOAc/PE: 7/3 \rightarrow EtOAc) yielding protected pyrrolidine (16 mg).

The compound was dissolved in a solution of 1 M HCl/MeOH: 1/1 (2 mL) and stirred at room temperature overnight. After completion of the reaction the solution was neutralized with Amberlyst® A-26 (OH^-) and lyophilized to yield dimer **15** (10 mg, 29%) as a white powder.

15: $[\alpha]_{\text{D}}^{20} = -3.7$ (c 0.48, $\text{H}_2\text{O}/\text{MeOH}$, 1/1); $^1\text{H-NMR}$ (CD_3OD , 500 MHz): δ 1.24 (d, 6H, $J = 6.8$ Hz, CH_3), 1.66–1.78 (m, 4H, $-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-}$), 1.79–1.89 (m, 2H, pyr- CH_2 -), 1.89–2.06 (m, 4H, pyr- CH_2 - and $-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-}$), 2.84–3.05 (m, 16H), 3.23 (td, 2H, $J = 8.0$, 5.3 Hz, H-2), 3.36–3.43 (m, 2H, H-5), 4.00–4.10 (m, 4H, H-3 and H-4); $^{13}\text{C-NMR}$ (CD_3OD , 126 MHz): δ 13.1 (CH_3), 24.4 ($-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-}$), 25.5 ($-\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH-}$), 31.5 (pyr- CH_2 -), 45.6, 45.7, 45.9, 47.5, 54.6 (C-5), 58.5 (C-2), 73.6 (C-4), 77.7 (C-3); HRMS-ESI $^+$ (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{53}\text{N}_6\text{O}_4$: 489.4128, found 489.4126.

Synthesis of dimer 16

To a stirred solution of hexamethylenediamine (21 mg, 0.175 mmol) in 2,2,2-trifluoroethanol (TFE) (0.5 mL), a solution of aldehyde **26**^{20c} (100 mg, 0.35 mmol) in TFE (0.5 mL) was added. The mixture was stirred at 30 °C for 30 min and then NaBH_4 (32 mg, 0.842 mmol) was added. After 30 min, the mixture was filtered through celite and the residue washed with TFE and concentrated. The resulting residue was purified by chromatography column on silica gel (DCM/MeOH/ NH_4OH : 30/1/0.25) affording **27** (56 mg, 49%) and **28** (8 mg, 5%) as white solids. A solution of **27** (56 mg, 0.086 mmol) in TFA: H_2O 4:1 (2 mL) was stirred at rt for 2 h. Then, concentration under vacuum afforded a residue that was purified by chromatography column on silica gel (DCM/MeOH/ NH_4OH : 1/1/0.25) to yield **16** (32 mg, 99%) as a white solid.

16: $[\alpha]_{\text{D}}^{25} = -56.3$ (c 0.5, H_2O); IR ν_{max} : 3272 (OH), 2932, 1445, 1047 cm^{-1} . $^1\text{H-NMR}$ (D_2O , 300 MHz): δ 1.21 (d, 6H, $J_{\text{Me},5} = 6.7$ Hz, CH_3),

1.38–1.47 (m, 4H, CH₂), 1.65–1.78 (m, 4H, CH₂), 3.06 (apt, 4H, NH-CH₂-CH₂), 3.16 (dd, 2H, $J_{1'a,1'b} = 13.1$ Hz, $J_{1'a,2} = 9.0$ Hz, H-1'a), 3.28 (dd, 2H, $J_{1'b,1'a} = 13.1$ Hz, $J_{1'b,2} = 4.4$ Hz, H-1'b), 3.38 (qd, 2H, $J_{5,Me} = 6.7$ Hz, $J_{5,4} = 2.8$ Hz, H-5), 3.49 (dt, 2H, $J_{2,1'a} = J_{3,2} = 8.7$ Hz, $J_{2,1'b} = 4.3$ Hz, H-2), 3.99 (apt, 2H, H-4), 4.11 (dd, 2H, $J_{3,2} = 8.5$ Hz, $J_{3,4} = 3.9$ Hz, H-3); ¹³C-NMR (D₂O, 75.4 MHz): δ 12.5 (CH₃), 25.1, 25.3 (CH₂), 48.1 (NH-CH₂-CH₂), 50.0 (C-1'), 55.6 (C-5), 57.5 (C-2), 73.1 (C-4), 75.9 (C-3); HRMS-ESI (m/z): [M+H]⁺ calcd for C₁₈H₃₉N₄O₄: 375.2966, found 375.2961.

Synthesis of dimer 17a

To a stirred solution of **26**^{20c} (100 mg, 0.35 mmol) in 1,2-dichloroethane (2 mL), benzylamine (17 μ L, 0.155 mmol) and NaBH(OAc)₃ (136 mg, 0.643 mmol) were added under Ar. After stirring at rt for 5 h, sat. aq. sol. of NaHCO₃ (1 mL) was added and the mixture extracted with CH₂Cl₂ (4 x 2 mL). The organic phase was then dried over Na₂SO₄, filtered and concentrated under reduced pressure. The resulting residue was purified by chromatography column on silica gel (EtOAc/cyclohexane: 1/7) affording protected **17a** (42 mg, 0.065 mmol, 42%) as an oil. 4 M HCl (2 mL) was added to a solution of protected **17a** (102 mg, 0.158 mmol) in THF (1 mL) and the mixture was stirred at rt for 3 h. After evaporation, the residue was purified through a Dowex 50WX8 (100–200 mesh) column and eluted, successively with MeOH, H₂O and NH₄OH. The fractions containing the unprotected product were concentrated and purified again by flash chromatography on silica gel (CH₂Cl₂/MeOH/NH₄OH: 1/1/0.15) to afford **17a** (35 mg, 0.096 mmol, 61%) as a solid.

17a: [α]_D²⁵ = -3.8 (c 0.5, MeOH); IR ν_{max} : 3291, 2920, 1060, 746 cm⁻¹; ¹H-NMR (CD₃OD, 300 MHz): δ 1.19 (d, 6H, $J_{Me,5} = 6.7$ Hz, CH₃), 2.68 (d, 4H, $J_{1',2} = 6.8$ Hz, H-1'), 3.18 (qd, 2H, $J_{5,Me} = 6.7$ Hz, $J_{5,4} = 2.5$ Hz, H-5), 3.33–3.36 (m, 2H, H-2), 3.68 (d, 1H, $J = 13.6$ Hz, CH₂-Ph), 3.75 (d, 1H, $J = 13.6$ Hz, CH₂-Ph), 3.80–3.84 (m, 4H, H-4, H-3), 7.24–7.41 (m, 5H, H(Ph)); ¹³C-NMR (CD₃OD, 75.4 MHz): δ 14.3 (CH₃), 56.0 (C-5), 58.2 (C-1'), 60.5 (CH₂-Ph), 61.0 (C-2), 74.9, 77.9 (C-3, C-4), 128.3, 129.4, 130.5 (C(Ph)), 139.9 (C_q(Ph)); HRMS-ESI (m/z): [M+H]⁺ calcd for C₁₉H₃₂O₄N₃: 366.2387, found 366.2382.

Synthesis of dimer 17b

A solution of **17a** (25 mg, 0.068 mmol) in MeOH (1 mL) was hydrogenated with Pd/C (10%) as catalyst. After 4 h the catalyst was removed by filtration through celite and the solution concentrated. The resulting crude product was purified by column chromatography (CH₂Cl₂/MeOH/NH₄OH: 1/1/0.25) affording **17b** (12 mg, 0.044 mmol, 65%) as a solid.

17b: [α]_D²⁵ = -3.7 (c 0.5, MeOH); IR ν_{max} : 3280, 2927, 1042 cm⁻¹; ¹H-NMR (CD₃OD, 300 MHz): δ 1.37 (d, 6H, $J_{Me,5} = 6.7$ Hz, CH₃), 2.93–3.04 (m, 4H, H-1'), 3.45 (ddd, 2H, $J_{2,3} = 8.5$ Hz, $J_{2,1'a} = 6.9$ Hz, $J_{2,1'b} = 4.9$ Hz, H-2), 3.62 (qd, 2H, $J_{5,Me} = 6.7$ Hz, $J_{5,4} = 3.0$ Hz, H-5), 3.93–3.96 (m, 2H, H-4), 4.04 (dd, 2H, $J_{3,2} = 8.6$ Hz, $J_{3,4} = 3.8$ Hz, H-3); ¹³C-NMR (CD₃OD, 75.4 MHz): δ 12.9 (CH₃), 50.1 (C-1'), 57.7 (C-5), 62.3 (C-2), 74.1 (C-4), 75.7 (C-3); HRMS-ESI (m/z): [M+H]⁺ calcd for C₁₂H₂₆O₄N₃: 276.1918, found 276.1913.

Synthesis of dimer 18

DIPEA (124 μ L, 0.707 mmol) and CuI (12 mg, 0.06 mmol) were added to a solution of 1,3-bis(azidomethyl)benzene³⁶ (35 mg, 0.186

mmol) and alkynyl pyrrolidine **30**^{20c} (115 mg, 0.409 mmol) in toluene (2.0 mL). The mixture was stirred at rt overnight and then, a sat. aq. sol. of NaHCO₃ (5 mL) was added and the mixture was extracted with CH₂Cl₂ (4 x 5 mL). The organic phases were dried, filtered and concentrated under reduced pressure. Deprotection of the crude product was carried out in 4 M HCl-THF 1:1 (3 mL) at rt for 3 h. The solvent was then evaporated and the residue was purified by chromatography column on silica gel (CH₂Cl₂/MeOH/NH₄OH: 3/1/0.5) to yield compound **18** (38 mg, 0.081 mmol, 44 %) as a solid.

18: [α]_D²⁵ = -5.2 (c 0.55, H₂O); IR ν_{max} : 3273, 2929, 1125, 1053, 739 cm⁻¹; ¹H-NMR (CD₃OD, 300 MHz): δ 1.19 (d, 6H, $J_{Me,5} = 6.7$ Hz, CH₃), 3.43 (qd, 2H, $J_{5,Me} = 6.6$ Hz, $J_{5,4} = 3.4$ Hz, H-5), 3.95 (apt, 2H, H-4), 4.25 (d, 2H, $J_{2,3} = 8.0$ Hz, H-2), 4.34 (dd, 2H, $J_{3,2} = 8.0$ Hz, $J_{3,4} = 4.1$ Hz, H-3), 5.58 (s, 4H, CH₂), 7.28–7.42 (m, 4H, H(Ph)), 7.89 (s, 2H, H(Triazole)); ¹³C-NMR (CD₃OD, 75.4 MHz): δ 15.0 (CH₃), 54.5 (CH₂), 56.6 (C-5), 58.6 (C-2), 75.2 (C-4), 80.2 (C-3), 123.9 (CH(Triazole)), 128.8, 129.3, 130.7 (C(Ph)), 137.7 (C_q(Ph)), 150.5 (C(Triazole)); HRMS-ESI (m/z): [M+Na]⁺ calcd for C₂₂H₃₀O₄N₈Na: 493.2282, found 493.2272.

Synthesis of trimer 29

Deprotection of **28** (87 mg, 0.094 mmol) following the conditions described for the preparation of **16**, afforded **29** (28 mg, 59%) as a white solid.

29: [α]_D²⁵ = -69.0 (c 0.62, H₂O); IR ν_{max} : 3217 (OH), 2935, 1448, 1039 cm⁻¹; ¹H-NMR (D₂O, 300 MHz): δ 1.22 (d, 3H, $J = 6.7$ Hz, CH₃'), 1.31–1.61 (m, 6H, 3CH₂'), 1.38 (d, 6H, $J = 6.8$ Hz, 2CH₃'), 1.64–1.77 (m, 2H, CH₂'), 2.66 (t, 2H, $J = 7.7$ Hz, N-CH₂-CH₂'), 2.90 (dd, 2H, $J = 13.9$ Hz, $J = 10.0$ Hz, pyrr-CH₂'), 2.97 (dd, 2H, $J = 14.4$ Hz, $J = 4.9$ Hz, pyrr-CH₂'), 3.06 (t, 2H, $J = 7.8$ Hz, N-CH₂-CH₂'), 3.20 (dd, 1H, $J = 13.1$ Hz, $J = 9.1$ Hz, pyrr'-CH₂'), 3.31 (dd, 1H, $J = 13.3$ Hz, $J = 4.4$ Hz, pyrr'-CH₂'), 3.37–3.47 (m, 1H, H-5'), 3.52 (td, 1H, $J = 8.5$ Hz, $J = 4.3$ Hz, H-2'), 3.61–3.72 (m, 2H, H-2), 3.74–3.85 (m, 2H, H-5), 4.00 (apt, 1H, H-4'), 4.09–4.17 (m, 5H, H-3, H-4, H-3'). ¹³C-NMR (D₂O, 75.4 MHz): δ 11.2 (CH₃'), 12.3 (CH₃'), 22.8, 25.5, 25.9 (CH₂'), 48.2 (N-CH₂-CH₂'), 49.7 (pyrr'-CH₂'), 52.5 (N-CH₂-CH₂'), 53.2 (pyrr-CH₂'), 55.8 (C-5'), 56.7 (C-5), 57.4 (C-2'), 57.8 (C-2), 71.9 (C-3 or C-4), 72.9 (C-4'), 74.2 (C-3 or C-4), 75.7 (C-3'). HRMS-ESI (m/z): [M+H]⁺ calcd for C₂₄H₅₀N₅O₆: 504.3756, found 504.3745.

Glycosidase inhibition assays

The experiments were performed essentially as previously described.³⁷ The initial screening to calculate the % inhibition was carried out in a multiwell plate. For those compounds that show inhibition higher than 80%, the IC₅₀ (concentration of inhibitor required for 50% inhibition of the enzyme activity) is determined. IC₅₀ values were calculated from plots of percentage of inhibition versus inhibitor concentration. For the best inhibition values (compounds **12a** and **18**), K_i towards α -fucosidase (from bovine kidney) were determined. K_i of known **12a**³⁴ was calculated through Cheng-Prusoff equation, and K_i for **18** was determined from the Lineweaver–Burk plots (see ESI[†] for details). All the experiments were performed by duplicate. Before each assay the enzyme is calibrated by adding the corresponding substrate and measuring its absorbance. Briefly, the general protocol is: 0.01 – 0.5 units/mL of enzyme and inhibitor (at 1mM concentration) were pre-

incubated for 5 min at r.t., and the reaction started by addition of the substrate, buffered to the optimal pH of the enzyme. After 20 min of incubation at 37 °C, the reaction was stopped by addition of sodium borate buffer pH 9.8. The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 405 nm. The absorbance is related with the percentage of inhibition. Then IC₅₀ of the inhibitors were calculated. Double-reciprocal Lineweaver-Burk plots were used to determine the inhibition characteristics

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