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FULL PAPER

Physiochemical tuning of potent *E. coli* antiadhesives by microencapsulation and methylene homologation.

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Abstract: Thiazolylaminomannosides (TazMan) are FimH antagonists with anti-adhesive potential against adherent-invasive *Escherichia coli* (AIEC) promoting gut inflammation in patients with Crohn's disease (CD). The lead TazMan is highly potent *in vitro* but shows limited *in vivo* efficiency probably due to low pH stability and water solubility. We recently developed a second generation of stable TazMan but the anti-adhesive effect was decreased compared to the first. Here, we report the co-crystal structure of the lead TazMan in FimH, revealing that the anomeric NH and the second thiazole moiety provide a positive H-bonding interaction with a trapped water molecule, and π-stacking with Tyrosine 48 of FimH, respectively. Consequently, we have developed NeoTazMan homologated with a methylene group for low pH and mannosidase stability with a conserved NH group and bearing various heterocyclic aglycons. Microencapsulation of the lead NeoTazMan in a γ-cyclodextrin dramatically improved the water solubility without disrupting the FimH affinity or the anti-adhesive effect against AIEC isolated from patients with CD.

Introduction

The anti-adhesive strategy is an appealing alternative approach to antibiotic treatments that consist in preventing or disrupting bacterial adherence to the host cells.[1–4] One of the most studied target is the FimH adhesin, a mannose-binding lectin situated at the tip of rod-like organelles (pil) expressed by most *E. coli.*[5,6] The concept was proposed more than 30 years ago, when aryl-mannosides were shown to be FimH antagonists, with anti-adhesive effects observed in cell-based assays.[7] Alkyl-mannosides were identified as a second class of potent inhibitors, with heptylmannosides (HM) being the most potent of the series, and showing an *in vivo* anti-adhesive effect when coadministred with uropathogenic *E. coli* strains to mouse bladder.[8] These pioneering works drived the development of alternative treatments for urinary tract infections (UTIs) at the academic and industrial level[9]. Potent *in vitro*[10] and *in vivo*[11–14] anti-adhesive effects were observed, clearly suggesting the high potential of the approach for the treatment of *E.coli*-induced UTIs. Complementarily, multivalent glycoconjugates bearing multiples copies of specific mannosides were also extensively investigated by us[15–17] and others[18–21]. This particular class of compounds was shown to induce the formation of bacterial aggregates and to be more effective *in vitro* and *in vivo* compared to their monovalent analogues.[16,18,22]

Recently, we investigated the potential of FimH antagonists for treating *E. coli*-induced inflammation in Crohn's disease (CD).[23–25] CD is characterized by a dysfunction of the immune system in response to an altered microbiota. A pathogenic group of bacteria called adherent-invasive *E. coli* (AIEC) has been shown to induce inflammatory cytokine expression after adhesion to a mannosylated receptor (CEACAM6), overexpressed in the ileum of CD patients compared to healthy controls. The synthetic mannosides were shown to decrease AIEC colonization in the feces, gut and ileum of the CEABAC 10 mouse model mimicking CD after oral administration at a dose of 10 mg/kg.[24,25] Importantly this was correlated with a decrease in the inflammatory syndrome. These results strongly suggested an alternative approach for CD patients to the current treatment based on the administration of immunosuppressive agents (such as anti TNF-α).

The thiazolylaminomannosides (TazMans) family (*i.e.* compounds **1** and **2**, Figure1), recently developed in the group,[23] are highly potent *E. coli* anti-adhesives in eukaryotic cells, preventing the adhesion of a broad range of *E. coli* strains isolated from patients with UTIs, CD or osteoarticular infections.[26] However, this first generation suffered from an anomerization of the heteroarylamino groups at low pH from the active α to the inactive β-form. This may be problematic in a potential oral administration, in which the compound passes through the stomach (pH = 2). We recently designed a second generation of TazMan,[27] in which the anomeric NH was replaced by O-CH_2, S-

CH_2, CH_2-CH_2, CH_2-S or O-CH_2CH_2 groups. These compounds proved to be stable in acidic media but *in vitro* tests showed a decreased in anti-adhesive potency compared to **1**, probably due in part to the absence of a stabilizing hydrogen bonding between anomeric NH and a water molecule trapped in the FimH binding sites as seen in the **1**-FimH co-crystal structure (Figure 1B). Such a stabilizing interaction has recently been observed by Janetka and co-workers with biphenyl-C-mannosides bearing anomeric hydroxyalkyl groups.[28] The compounds included in our recent study also lacked the second thiazole and pyrazine ring of **2**, the most potent FimH antagonist of the serie, that showed outstanding *in vitro* anti-adhesive effects[23,26]. In this work, we developed a new series of TazMans based on a co-crystal structure of **2**-FimH (Figure 1C). The anomeric amino linkage was replaced by a methylamino group to improve the chemical stability at low pH and prevent hydrolysis by glycosidases. The pyrazine pharmacophore of **2** was replaced by a small library of heterocyclic aglycons to modulate both the FimH affinity and water solubility of the compounds. The FimH affinity of the small library was evaluated as well as their potency in inhibiting AIEC adhesion to intestinal cells.

Results and Discussion

Prior to starting our investigation, we wanted to gain more insight into the generally high anti-adhesive potency of the TazMan series and particularly of the best compound **2** which is around 100-fold more potent than the reference compound heptyl-mannoside (HM), and 50 to 100-fold more potent than **1**.[26] Co-crystals were obtained using the vapor diffusion method similar to a previously published protocol.[8] The obtained complex crystallized in tetragonal space group $P4_32_12$, with two molecules in the asymmetric unit. Data collection and refinement statistics are presented as supplemental information (Table S1). The orientation of the ligand is similar to that observed in compound **1**. Nevertheless, the additional thiazol moiety born by **2** interacts more evidently through π-π stacking with phenol ring of Tyr48, keeping it into it the half-open tyrosine gate conformation. The *N*-glycosidic linker atom is hydrated like in the crystal structure of FimH in complex with compound **1**,[23] and forms a weak hydrogen bond (3.2 – 3.4 Å depending on the molecule in the asymmetric unit).

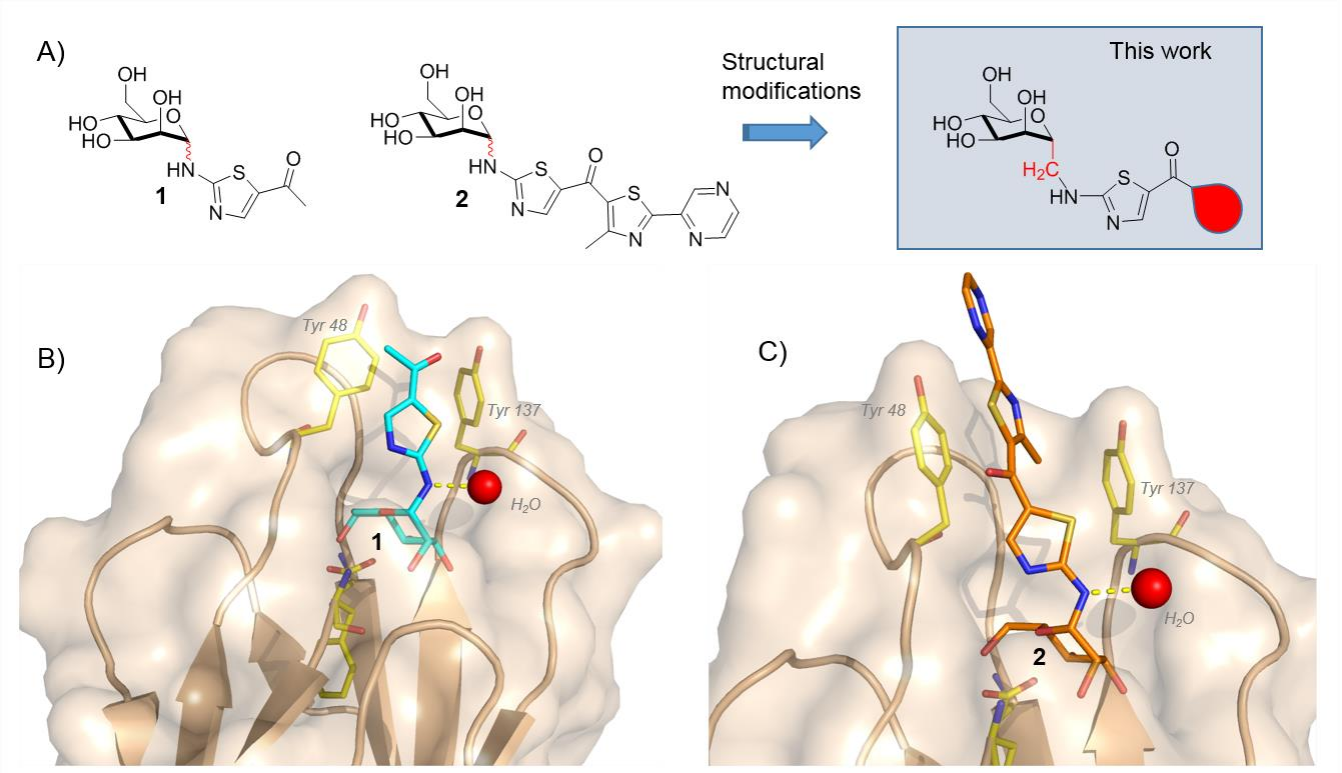


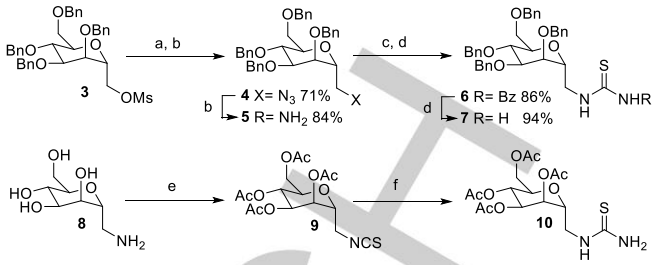
Figure 1. A) Chemical structure of previously described TazMans **1** and **2**.[23] **2** is around 50-100 fold more potent as an *E. coli* anti-adhesive compared to **1** but the aglycone group partially anomerizes at low pH from the α to the inactive β form. To prevent anomerization and improve chemical and enzymatic stability, we developed a new TazMan family homologated with a methylene group and bearing diverse pharmacophores. B) The structure of **1** co-crystalized with FimH(PDB entry 3zl2, 1.25 Å resolution)[23] shows anomericNH bonding with a water molecule. C) A similar interaction is observed in the co-cristal structure of **2**-FimH (PDB entry 5MTS, 2.6 Å resolution).

The chemical synthesis of the homologated TazMans required protected α-mannosides with the methylthiourea group in the anomeric position to be obtained for the addition-cyclization step leading to a thiazole ring. Armed and disarmed compounds **7** and **10** bearing benzyles and acetates on the mannose hydroxyl groups were both synthesized (Scheme1), as reactivity during the critical thiazole formation was differently affected depending on the substrates.

The synthesis of benzyl-protected **7** started from α-C-mannoside **3** obtained in seven steps from mannose as we

previously reported.[27] The mesylate group of **3** was substituted by an azido-group with sodium azide and *tert*-butylammonium iodide and then the crude compound **4** obtained was further reduced by a Staudinger procedure to form amine **5**[29] in 60% yield over two steps. **5** was directly converted into the benzoyl-protected thiourea **6** with 86% yield using potassium isothiocyanate and benzoyl chloride in acetone. After flash chromatography purification, the benzoyl group was easily deprotected with sodium hydroxide to form **7**.

The synthesis of acetyl protected thiourea **10** started from aminomethylmannoside **8** obtained in three steps and as a pure alpha-form using a previously described procedure.[30] The amino group of **8** was first converted to an isothiocyanate in a mixture of calcium carbonate and carbone disulfide and the product was acetylated to form **9**.[29] Finally, treatment of isothiocyanate **8** with HMDS in DMF yielded thiourea **10**.



Scheme1. Synthesis of thiourea **7** and **10**. *Reagents and conditions*: a) NaN_3 DMF, 71%; b) PPh_3, THF/H_2O, 84%, c) KNCS, BzCl, acetone, 86%; d) NaOH, MeOH, 94%; e) CS_2, $CaCO_3$ then Ac_2O, Pyr 38% two steps; f) HMDS DMF, 79%.

After addition of DMF-DMA, the corresponding benzyl or acetyl protected *C*-mannosides were engaged in the critical addition-cyclization step with diverse chloroketones to form protected compounds **11**-**22**. The benzyl and acetate groups of the corresponding cycloadducts were removed with trichloroborane or sodium methanolate, respectively, leading to unprotected *C*-mannoside **23**-**34** with yields ranging from 64 to 96% (Figure 2).

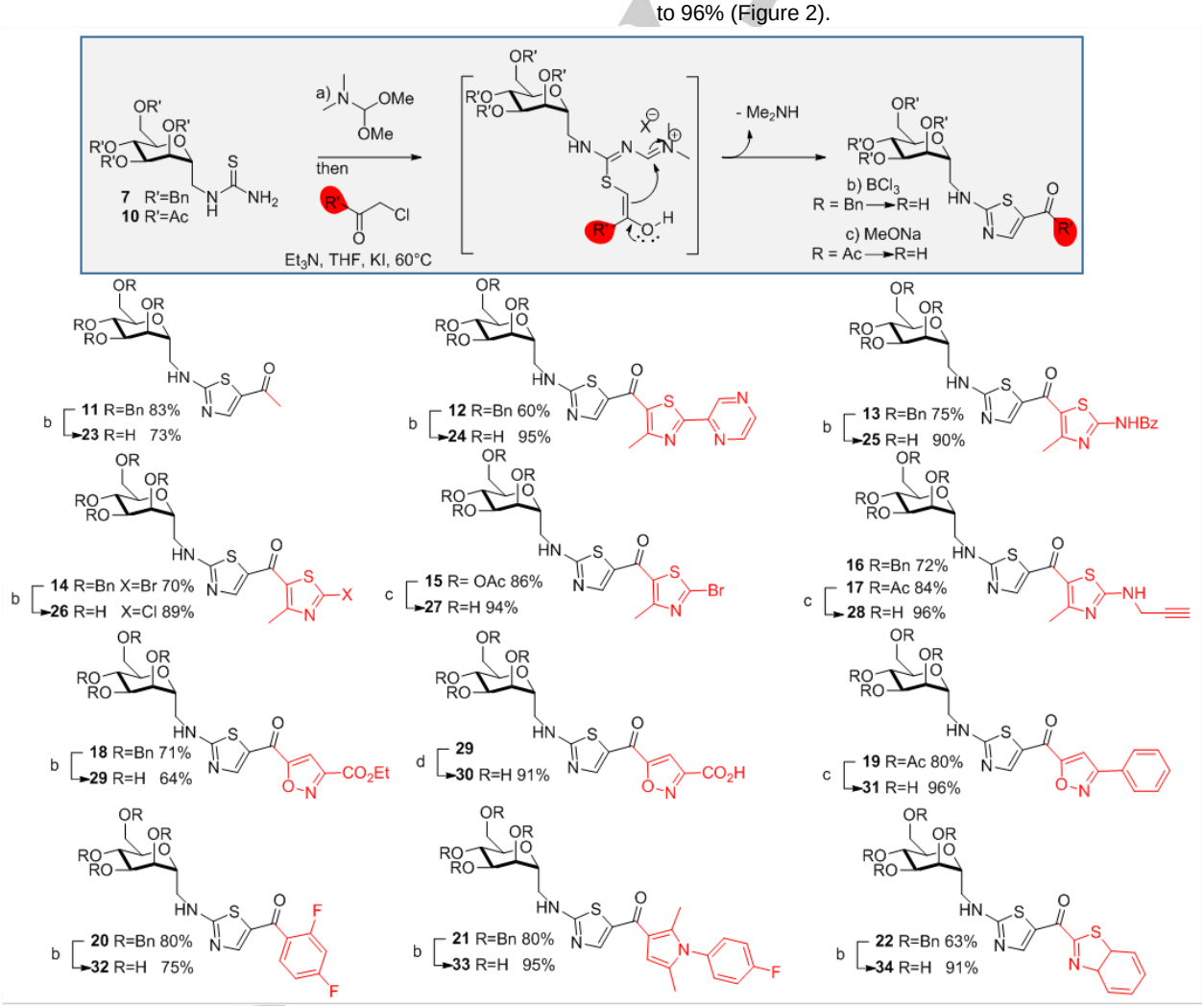
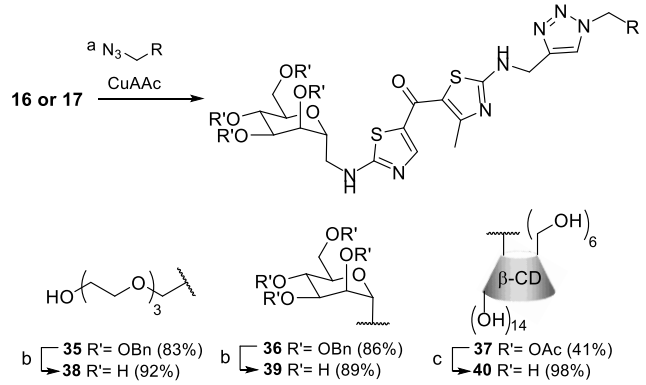


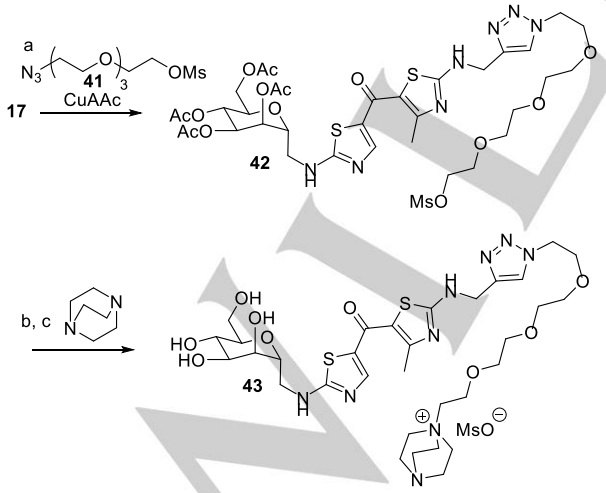
Figure 2. Structure of cycloadducts **11**-**33**. *Reagents and conditions* a) DMF-DMA, chlorocetone, Et3N, KI, THF; b) BCl_3, DCM for Bn; c) MeOH, MeONa for acetates; d) LiOH, $MeOH/H_2O$.

The presence of the conjugated aglycons was shown to significantly lower the water solubility of the compounds. In order to obtain fully water-soluble *C*-mannosides, we designed a subset of compounds bearing hydrophilic moieties at position 2 of the second thiazole starting from alkynes **16** or **17** (Scheme 2). The new pharmacophores should not disrupt FimH binding as they point out of the protein-carbohydrate binding domain. **16** or **17** were reacted in a CuAAC protocol with highly hydrophilic groups which were an azido-functionalized triethyleneglycol, a *C*-mannoside or a beta-cyclodextrin.



Scheme2. Synthesis of hydrophilic analogues **38**-**40**. *Reagents and conditions*: a) sodium ascorbate, $CuSO_4$, dioxane/H_2O; b) BCl_3, DCM for Bn; c) MeOH, MeONa for acetates.

In addition, we synthesized the highly polar compound **43** bearing a permanent positive charge on the nitrogen atom of DABCO (Scheme 3). **43** was obtained in three steps starting from the hetero-functionalized tetraethyleneglycol **41** and alkyne **17**. After insertion of the linker by CuAAc, protective acetates were removed from the sugar and the mesylate was substituted with DABCO.



Scheme 3. Synthesis of hydrophilic compound **43**. *Reagents and conditions*: a) sodium ascorbate, $CuSO_4$, dioxane/H_2O, 98%; b) NaOMe, MeOH; c) AcOEt, DABCO, MeOH, 90% two steps.

The whole set of compounds was evaluated side-by-side in a cell-based assay to measure their potency in preventing the adhesion of the pathogenic AIEC LF82 strain (isolated from patients with CD) to T84 intestinal epithelial cells. T84 express the mannosylated CEACAM6 protein, a GPI-anchored protein abnormally expressed at the ileal mucosa of CD, and allowing FimH-mediated AIEC attachment to the cells. The residual percentage of bacterial adhesion obtained in the presence of a 10 µM concentration of the compounds, compared to the non-treated wells is presented in Figure 3. HM was included in the assay as an internal reference. HM has previously been shown to display a low nanomolar affinity for FimH (5 nM by SPR) and a strong *in vitro* anti-adhesive effect against AIEC LF82, but it failed to reduce AIEC levels *in vivo* using a transgenic mouse model mimicking CD.[25]

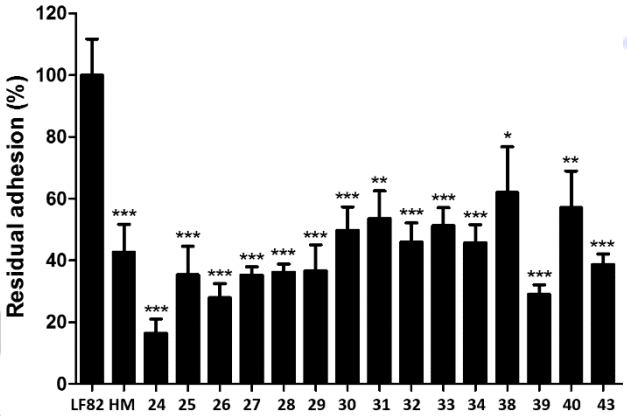


Figure 3. Inhibitory effect of the compounds on the ability of the LF82 strain to adhere to intestinal epithelial cells T84. Cells were infected at a multiplicity of infection of 10 bacteria per cell, for a 3-hour period. Compounds were incubated with AIEC bacteria for 1h before infection of cells at a concentration of 10µM. Results are expressed in percentage of bacteria associated with the cells (n=6 experiments, means ± SEM; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0,001$). LF82 infection in the absence of treatment was normalized to 100%.

Although a larger number of molecules would be required in the library to investigate structure-activity relationships extensively, interesting information can be extracted from the results. Most of the compounds were shown to prevent significantly the bacterial adhesion to the cells, with seven out of the fifteen compounds tested being more effective than the HM reference compound. The only exception was compound **23**, lacking additional substituents after the methylcarbonyl group. To observe a significant reduction in the bacterial attachment the concentration of **23** had to be increased to 50 µM, which was 5 times higher than the concentration used to assay the whole set of molecules (data not shown). Thus, the addition of a second heterocycle (substituted thiazole, pyrrole or isoxazole) after the carbonyl moiety was highly beneficial for improving the anti-adhesive effect in all cases studied here. This can be rationalized by the crystal structure of **2**-FimH showing a stacking interaction between Tyr 48 and the second thiazole ring. Although Tyr48 can adopt different conformation, the X-ray structure of **1**-FimH shows a parallel but staggered orientation of the thiazole ring of **1** relative to the phenyl group of Tyr 48. Modifications beyond the second heterocycle also impact the anti-adhesive effect but to a lesser extent as the inhibition values ranged from 16% to 62% at a fixed concentration of 10 µM for all compounds (Figure 3). As seen in the **2**-FimH co-crystal, the additional pyrazine group is

pointing out of the binding domain were possible steric clashes and positive interactions with the protein are more limited. Substitution of this group by hydrophilic substituents enabled us to design the water-soluble compounds **38**-**40** and **43** with conserved anti-adhesive potencies. It should be noted that compound **39** can potentially bind two FIMH due to the presence of two mannose moieties.

Compound **24**, the homologated analogue of **2**, was significantly more potent, with a residual adhesion level of only 16%. This value also exceeded the anti-adhesive effects that we observed with the same pre-incubation protocol and at the same concentration with our previously published second generation of enzymatically stable TazMans (values ranging from 44 to 78%),[27] and heptyl-mannoside derivatives (37-95%).[24]

The low residual adhesion value of 17% for **24** at 10 µM is promising considering that compounds with higher levels of *in vitro* anti-adhesive effect (ranging from 40-75%) showed AIEC decolonization and reduced inflammatory syndromes in a CD mouse model after oral administration (10 mg/kg).[25]

We previously identified compounds **1** and **2** as very potent FimH antagonists *in vitro*.[23] However these two compounds were not suited for *in vivo* application due to their anomerization to the inactive β-form at the low pH encountered in the stomach and their potential instability towards mannosidases hydrolysis. The homologation by a methylene group to form analogs **23** and **24**, respectively now fully prevents these phenomena. To quantify better the potential loss of affinity provided by such homologation, the binding affinity for FimH of **1**, **2**, **23** and **24** was compared side by side in an enzyme-linked lectinosorbent assay (ELLSA). In this assay, the highly mannosylated RNAseB protein was coated on the surface of immunological wells and FimH was added in the presence or absence of inhibitors. The surface-bound FimH was detected spectrophotometrically with anti-FimH and secondary-labeled antibodies. Dose-response curves obtained from testing compounds at eight different concentrations enabled the determination of the minimal inhibitory concentration to achieve 50% inhibition (IC_{50}). All four compounds showed an IC_{50} below 500 nM, and down to 70 nM for the best compound **2**. The homologated **23** and **24** were 2.4-fold (494 vs. 205 nM) and 2.8-fold (194 vs. 70 nM) less potent than **1** and **2**, respectively (Figure 4). The decreased FimH affinity is therefore significant after homologation but remains acceptable considering the very high *in vitro* potency of **1** and **2**. Interestingly, the pyrazinylthiazolyl moiety improved FimH binding to a similar level of 2.5 and 2.9 fold when switching from **1** to **2** and **23** to **24**, respectively.

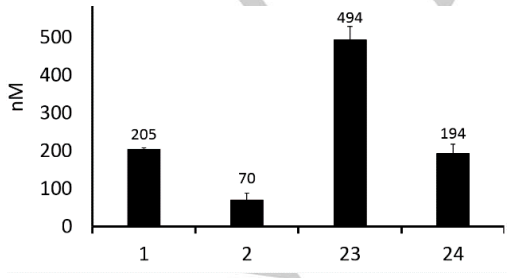


Figure 4. Binding affinity of TazMans **1**, **2**, **23**, and **24** for FimH determined by ELLSA. Average of three measurements. IC_{50} values expressed in nanomolar.

Addition of the pyrzinylthiazolyl moiety also significantly altered the water solubility of compounds and stock solutions of **2** and **24** had to be prepared in DMSO prior to running the *in vitro* assays. Although we showed that DMSO did not impact the result in cell-based assays, this vector is not optimal for *in vivo* evaluation. To overcome this disadvantage, we planned to encapsulate the compounds by host molecules possessing a hydrophobic cavity. Cyclodextrins are particularly suited for microencapsulation and are extensively used in formulation to improve the therapeutic index of hydrophobic molecules. **2** and **24** were mixed with α-, β- and γ-cyclodextrins (CD) possessing hydrophobic cavities of 4.5, 7 and 8Å, respectively. After addition of ten volumes of water to the compounds-CD mixtures dissolved in DMSO, the samples were lyophilized. **2** and **24** were shown to form 1-1 stoechiometric inclusion complexes in γCD but not in α or β. The solubility of the compounds was dramatically improved allowing around 10 $mg.mL^{-1}$ of the two complexes **2**@γCD and **24**@γCD to be dissolved in water.

The affinity of the two complexes was then evaluated in our ELLSA and the IC_{50} values were 109 and 135 nM for **2**@γCD and **24**@γCD, respectively. Thus, the complexes display similar level of affinity for their targets compared to the free molecules, meaning that **2** and **24** can easily "escape" from the CD ring to interact with FimH. γCD was also included in the assay, but no inhibition was observed at the higher dose tested (1 mM).

2@γCD and **24**@γCD (dissolved in water) were then tested using the preincubation protocol to measure their AIEC anti-adhesive efficiency. The inclusion complexes were tested at five different concentrations of 100, 10, 1, 0.1 and 0.01 µM in order to provide an estimation of the IC_{50}, defined as the concentration of compounds required to decrease the AIEC adhesion level by 50%. Compound **2** (dissolved in DMSO) was also included in the assay as a reference to account for a potential loss of affinity with **2**@γCD. The results presented in Figure 5 clearly show that no loss of anti-adhesive effect was observed with **2**@γCD compared to **2,** consistent with the results from the ELLSA assay. This was confirmed by the calculation of the IC_{50} which were virtually identical and equal to 0.7 µM for **2** and **2**@γCD.

Thus, microencapsulation does not impair FimH binding and is an interesting strategy to enhance the water-solubility of anti-adhesive compounds without altering their anti-adhesive effect The determination of the IC_{50} for **24**@γCD was more approximate because the residual adhesion at concentrations of 0.1 and 0.01 exceeded 100%, a phenomenon that we previously observed with heptavalent HM covalently linked to a cyclodextrin core.[25] Curve fitting gave an IC_{50} of 2 µM for **24**@γCD which is around three time higher than for **2**@γCD. Thus, the results obtained in the cell-based assay are consistent with the loss of FimH affinity by compound **24** compared to **2**. This decreased efficiency is limited considering that **2** is very potent in preventing AIEC attachment to intestinal cells at around 10000-fold and 100-fold lower concentrations than mannose and the potent FimH antagonist HM, respectively.[23]

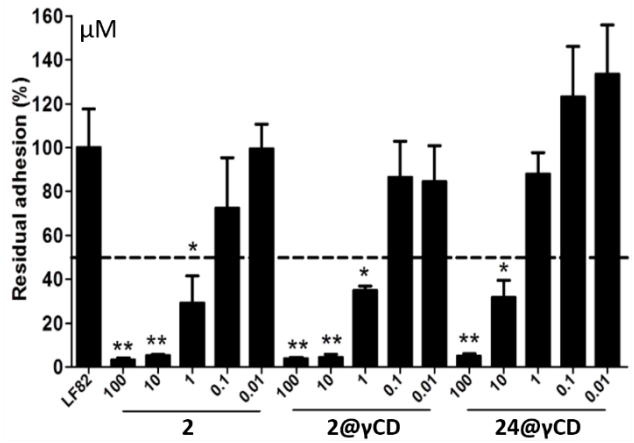


Figure 5. Inhibitory effect, measured at five different concentrations of **2**, **2**@γCD and **24**@γCD on the ability of the LF82 strain to adhere to intestinal epithelial cells.

Conclusions

The co-crystal structure of the potent TazMan **2** in the FimH binding site and the ligands and protein amino-acid (Tyr48 and Tyr137) conformations were compared with the previously published structure of **1**-FimH. An anomeric NH bonding interaction with a water molecule was conserved in both structures. The Tyr 48 orientation has significantly shifted to form a stabilizing stacking interaction with the second thiazole of **2**, probably explaining its higher FimH affinity compared to **1**. Based on these structures, and to provide chemically and enzymatically stable FimH antagonists for potential *in vivo* applications, we designed fifteen homologated *C*-mannosides with an NH group, and functionalized thiazole, pyrrole or isoxazole as a second heterocyclic moiety for a π-stacking interaction withTyr 48. The most potent compound of the serie was **24**, the analogue of **2** homologated by a methylene group. This new compound should be fully stable towards enzymatic and acidic hydrolysis and showed a limited affinity loss for FimH compared to **2**. **24** has the higher AIEC anti-adhesive effect measured so far *in vitro* for a stable TazMan. The compound was formulated as a water soluble γCD complex that escape from the hydrophobic cavity to interact with FimH. **24**@γCD is a promising formulation in an *E. coli* anti-adhesive therapy considering that heptylmannoside derivatives with much lower *in vitro* potency have proved effective in reducing bacterial levels and inflammatory syndromes in a transgenic mouse model of Crohn's disease.

Experimental Section

General experimental details. NMR spectra were recorded at room temperature with a Bruker Avance 300 Ultra Shield or eBruker Avance III 400 spectrometer and chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak peak ($CHCl_3$: ^1H: δ=7.26, ^{13}C: δ=77.2; DMSO-d6: ^1H: δ=2.54, ^{13}C: δ=40.4). Peak multiplicity is reported as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Peak multiplicity and chemical shifts are reported for α compounds in case of anomeric mixtures in equilibrium. High resolution mass spectra HRMS where obtained by Electrospray Ionisation (ESI) on a Micromass-Waters Q-TOF Ultima Global or with a Bruker Autoflex III SmartBeam spectrometer (MALDI). Low-resolution mass spectra (MS) were recorded with a Thermo electron DSQ spectrometer. All reagents were purchased from Acros Organics or Aldrich and were used without further purification. Column chromatography was conducted on silica gel Kieselgel SI60 (40-63 µm) from Merck. Reactions requiring anhydrous conditions were performed under argon. Dichloromethane was distilled from calcium hydride under nitrogen prior to use. Microwave experiments were conducted in sealed vials in commercial microwave reactors especially designed for synthetic chemistry. (MultiSYNTH, Milestone). The instrument features a special shaking system that ensures high homogeneity of the reaction mixtures. Optical rotations were measured on a 343 PERKIN ELMER at 20°C in a 1cm cell in the stated solvent; $[\alpha]_D$ values are given in 10^{-1} deg.cm^2 g^{-1} (concentration c given as g/100 mL).

GP1 = First general procedure for the addition-cyclization step The thiourea **7** or **10** (1eq) was dissolved in acetonitrile or THF (20 mL/mmol), DMF-DMA (1.3 eq) was added and the mixture was warmed at 60°C for 40 min. After completion, as indicated by TLC, α-halogenoketone (1.2 eq) was added with a catalytic amount of potassium iodide (0.05 eq). After 15 min of stirring at room temperature, triethylamine (2 eq) was added and the mixture was heated at 60°C until reaction completion, as indicated by TLC. The mixture was washed with brine, extracted by AcOEt, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel.

GP2 = General procedure for the benzyl deprotection step The protected carbohydrate (1eq) was dissolved in DCM (2mL/mmol) under inert atmosphere and the solution was stirred at -10°C. BCl_3 1M in DCM (3eq per function) was added dropwise and the mixture was stirred at rt for 20h. Methanol was added slowly and the mixture was concentrated under vacuum. This operation was repeated 4 times. Then the resulting mixture was purified by flash chromatography on silica gel.

GP3 = General procedure for the acetyl deprotection step The protected carbohydrate (1 equiv.) was dissolved in dry MeOH (30 mL) and sodium methoxide (1 M solution in MeOH, 10% mol per AcO) was added. The mixture was stirred for 4 h, neutralized with Amberlite IR120 (H), filtered and the solvents evaporated to dryness. The substrate was dissolved in water and subjected to lyophilization.

2,3,4,6-tetra-O-benzyl-1-azidomethyl-α-D-mannopyranose (4). Compound **3**[27] (900 mg, 1.42 mmol) was dissolved in DMF (15 mL), NaN_3 (462 mg, 6 eq) and TBAI (1eq, 523 mg) were added. The mixture was heated to 110°C for 24h then extracted by Et_2O. The organic layer was washed with brine 5 times, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified on silica gel (PE/AcOEt 9:1) to afford 587mg (71% yield) of **4** as a colourless oil. $[\alpha]_D^{20}$ = +28 (c = 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ = 7.18-7.36 (20H, m, HBn), 4.41-4.57 (8H, m, CH_2Bn), 4.10 (1H, ddd, $^3J_{5\text{-}4}$=2.6 Hz, $^3J_{5\text{-}6}$=6.0 Hz, $^3J_{5\text{-}6}$=6.4 Hz, H-5), 4.06 (1H, ddd, ^3J=4.5 Hz, ^3J=5.5 Hz, ^3J=8.5 Hz, H-1), 3.82 (1H, dd, $^3J_{6\text{-}5}$=6.6 Hz, $^2J_{6\text{-}6'}$=10.1 Hz, H-6), 3.76-3.81 (2H, m, H-3, H-4), 3.76 (1H, dd, ^3J=2.8 Hz, ^3J=8.5Hz, H-2),3.71 (1H, dd, $^3J_{5\text{-}6'}$= 6.0 Hz, $^2J_{6\text{-}6'}$=10.1Hz, H-6'), 3.40-3.48 (2H, m, H7); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.4 (CBn_{IV}), 138.1 (CBn_{IV}), 138.0 (CBn_{IV}), 137.9 (CBn_{IV}), 127.7-128.6 (CBn), 74.6 (C-5), 74.2 (C-4), 73.8 (C-3), 73.7 (C-2), 73.3 (CH_2Bn), 72.6 (CH_2Bn), 72.3 (CH_2Bn), 71.6 (CH_2Bn), 69.9 (C-1), 68.2 (C-6), 51.7 (C-7); HRMS (ES+) *m/z* calcd for $C_{35}H_{38}N_3O_5$: 580.2811, found 580.2787.

2,3,4,6-tetra-O-benzyl-1-aminomethyl-α-D-mannopyranose (5). 4 (587 mg, 1.01 mmol) was dissolved in THF (15mL) and a few drops of water were added. Triphenylphosphine (345 mg, 1.3 eq) was added and the mixture was heated to reflux for 2h. The mixture was concentrated in vacuo, rinsed by PE, concentrated and dissolved in

Et_2O. The triphenylphosphine oxide precipitated and was filtered off. The mixture was dried over $MgSO_4$, filtered,and concentrated in vacuum. The residue was purified on silica gel ($CHCl_3$/MeOH 1:0 to 97:3) to afford the 469 mg (84% yield) of **5** as a colourless oil. $[\alpha]_D^{20}$ = +20 (c = 0.5, $CHCl_3$); NMR previously described in litt;[29] HRMS (MALDI) *m/z* calcd for $C_{35}H_{40}NO_5$: 554.2901, found 554.2873.

***N*-benzoyl-*N'*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl) methylthiourea (6).** Potassium isothiocyanate **(**3 eq, 411 mg), was dissolved in acetone (10 mL). Benzoyl chloride (2 eq, 400 mL) was added and the white suspension was stirred for 20 min, then **5** (780 mg, 1.41 mmol) diluted in DCM (5 mL) was added to the mixture. After 10 min, the reaction was complete. The mixture was washed by brine, extracted by DCM, dried over $MgSO_4$ and concentrated under vacuum. The resulting oil was purified by flash chromatography on silica gel (PE/AcOEt 8:2 to 7:3) to afford 870 mg (86% yield) of **6** as a colourless oil. Broad NMR signals were obtained due to the presence of rotamers; HRMS (MALDI) *m/z* calcd for $C_{43}H_{44}N_2O_6SNa$: 739.2812, found 739.2783.

***N*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylthiourea (7).** **6** (870 mg, 1.21 mmol) was diluted in MeOH (6mL), sodium hydroxide pellets were added. After 10 min, the reaction was complete. The mixture was filtered, then neutralised by 2M HCl, extracted by DCM, washed by brine, dried over $MgSO_4$ and concentrated under vacuum. The resulting oil was purified by flash chromatography on silica gel (PE/AcOEt 1:1) to afford 698 mg (94% yield) of **7** as colourless oil. Severals conformers were observed by NMR and broad signals were obtained; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.21-7.53 (20H, m, 4 x BnO), 4.42-4.55 (8H, m, 4 x BnO), 4.07 (1H, m, H-1), 3.86-3.93 (3H, m, H5, H-6, H-7), 3.72-3.79 (3H, m, H-3, H-4, H-7'), 3.58-3.63 (2H,m, H-2, H-6'); ^{13}C NMR (127 MHz, CD_3OD): δ = 45.8 (CH_2, C-7), 68.1 (CH_2, C-6, several rotamers), 69.7-79.2 (5CH, 4CH_2, several rotamers), 128.3-133.7 (20CH, 4 x BnO, several rotamers), 139.4-139.5 (4C, 4 x BnO), 183.7 (C, thiourea); HRMS (MALDI) *m/z* calcd for $C_{36}H_{41}N_2O_5S$: 613.2731, found 613.2706.

Compound (9). To a solution of amine **8** (1.88 g, 9.741 mmol) in 6:4 H_2O/acetone (50 mL) was added $CaCO_3$ (2.92 g, 29.223 mmol) and $CSCl_2$. After 4 h, the mixture was filtered under Celite pad and concentrated. The isothiocyanate crude was solved in Py (20 mL) and Ac_2O (20 mL) and DMAP (20 mg) were added. After 8 h, the reaction mixture was concentrated and the obtained crude was purified by silica gel column chromatography (PE/EtOAc 7/3 as eluent) to give the protected isothiocyanate **10** (1.53 g, 3.796 mmol, 39%), whose spectroscopic dates are in concordance with the bibliographic ones.[29]

***N*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)methylthiourea (10).** To a solution of **9** (50 mg, 0.124 mmol) in DMF (0.6 mL), at 0° C and under N_2, was added HMDS (258 µL, 1.240 mmol). After 8 h, the mixture was concentrated and the crude was purified by silica gel column chromatography (EtOAc as eluent) to give the thiourea **10** (41 mg, 0.098 mmol, 79%) as a colorless oil. $[\alpha]_D^{20}$ = +12 (c = 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.94 (3H, s, AcO), 1.97 (3H, s, AcO), 1.99 (6H, s, 2 x AcO), 3.74 (2H, m, H-7), 3.89-4.15 (2H, m, H-1, H-5, H-6a), 4.36 (1H, m, H-6b), 4.97-5.11 (2H, m, H-4, H-2), 5.15 (1H, dd, J3,4 = 7.5 Hz, J3,2 = 3.3 Hz, H-3), 6.42 (2H, bs, NH2), 7.37 (1H, bs, NH); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 20.4, 20.47, 20.49, 20.55 (4CH_3, 4 x AcO), 43.5 (CH_2, C-7), 61.4 (CH_2, C-6, several rotamers), 66.8 (CH, C-4, several rotamers), 67.6 (CH, C-3, several rotamers), 68.1 (CH, C-3, several rotamers), 68.4 (CH, C-1), 70.9-72.1 (2CH, C-1, C-5, several rotamers), 169.4 (2C, 2 x AcO), 169.9 (C, AcO), 170.7 (C, AcO), 183.7 (C, thiourea); HRMS (ESI) m/z calcd for C16H25N2O9S [M + H]+ 421.1273, found 421.1275.

5-acetyl-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methyl aminothiazole (11). Prepared following GP 1, starting from **7** (166 mg, 0.271mmol) and chloroacetone. After purification over silica gel (PE/AcOEt 3:6) and a second purification ($CHCl_3$/AcOEt 7:3), 152 mg (83% yield) of **11** was obtained as a slightly yellow oil. $[\alpha]_D^{20}$ = +14 (c = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.76 (1H, s, H-9), 7.16-7.37 (20H, m, H-Bn), 6.05 (1H, bt, NH), 4.36-4.55 (8H, m, H-CH_2Bn), 4.03-4.10 (2H, m, H-1, H-5) , 3.79-3.86 (2H, m, H-4, H-6), 3.67-3.72 (2H, m, H-2, H-3), 3.61 (1H, dd, $^2J_{6\text{-}6'}$ =10.3Hz, $^3J_{6'\text{-}5}$ = 5.6 Hz, H-6'), 3.43-3.61 (2H, m, H-7), 2.43 (3H, s, H-12); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.2 (C-11), 175.1 (C-8), 148.1 (C-9), 138.2 (C-Bn_{IV}), 137.9 (2C-Bn_{IV}), 137.6 (C-Bn_{IV}), 129.4 (C-10), 127.8-128.8 (C-Bn), 74.9 (C-5), 74.4 (C-2), 74.3 (C-3), 74.3 (C-4), 73.4 (C-CH_2Bn), 72.9 (C-CH_2Bn), 72.4 (C-CH_2Bn), 71.6 (C-CH_2Bn), 68.2 (C-1), 67.99 (C-6), 46.5 (C-7), 26.1 (C-12); HRMS (MALDI) *m/z* calcd for $C_{40}H_{43}N_2O_6S$: 679.2836, found 679.2836.

5-(4-methyl-2-(pyrazin-2-yl)thiazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (12). Prepared following GP 1, starting from **7** (300 mg, 0.49mmol) and 2-bromo-1-(4-methyl-2-(pyrazine-2-yl)thiazol-5-yl)ethanone. After purification over silica gel (PE/AcOEt 4:6) 249 mg (60% yield) of **12** was obtained as a yellow oil. $[\alpha]_D^{20}$ = +2 (c = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.46 (1H, d, $^3J_{19\text{-}18}$ *=1.5Hz,* H-19), 8.64 (1H, d, $^3J_{17\text{-}18}$ *=2.5Hz* H-17), 8.58 (1H, dd, $^3J_{17\text{-}18}$ *=2.5Hz,* , $^3J_{19\text{-}18}$ *=1.5Hz,* H-18), 7.96 (1H, s, H-9), 7.17-7.37 (20H, m, H-Bn), 6.24 (1H, bt, NH), 4.36-4.56 (8H, m, H-CH_2Bn), 4.05-4.13 (2H, m, H-1, H-5) , 3.81-3.87 (2H, m,H-4, H-6), 3.68-3.73 (2H, m, H-2, H-3), 3.61 (1H, dd, $^2J_{6\text{-}6'}$ *=10.2Hz,* $^3J_{6'\text{-}5}$ *= 5.6 Hz*, H-6'),3.47-3.63 (2H, m, H-7), 2.75 (3H, s, H-14); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.3 (C-11), 175.4 (C-8), 166.2 (C-15), 159.4 (C-12), 149.9 (C-9), 146.5 (C-16), 145.9 (C-17), 144.2 (C18), 142.0 (C-19), 138.1 (C-Bn_{IV}) ,137.9 (C-Bn_{IV}), 137.6 (C-Bn_{IV}), 130.3 (C-13), 130.1 (C-10), 127.8-128.8 (C-Bn), 75.0 (C-5), 74.3 (C-4), 73.6 (C-3), 73.4 (C-2), 73.4 (C-CH_2Bn), 73.0 (C-CH_2Bn), 72.3 (C-CH_2Bn), 71.6 (C-CH_2Bn), 68.0 (C-1), 68.0 (C-6), 46.7 (C-7), 20.0 (C-14); HRMS (MALDI) *m/z* calcd for $C_{47}H_{46}N_5O_6S_2$: 840.2884, found 840.2857.

5-(2-benzoylamino-4-methylthiazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (13). Prepared following GP 1, starting from **7** (50 mg, 0.082 mmol) and *N*-(5-(2-chloroacetyl)-4-methylthiazol-2-yl)benzamide (36 mg, 0.123 mmol) as starting materials, the derivative **13** (54 mg, 0.061 mmol, 75%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 1:1 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +48 (c = 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.47 (3H, s, methylthiazol), 3.52 (1H, dd, $J_{7a,7b}$ = 12.9 Hz, $J_{7a,1}$ = 6.6 Hz, H-7a), 3.62-3.68 (2H, m, H-7b, H-6a), 3.71-3.76 (2H, m, H-2, H-3), 3.82-3.89 (2H, m, H-4, H-6b), 4.09-4.17 (2H, m, H-1, H-5), 4.41-4.57 (8H, m, 4 x BnO), 6.94 (1H, bs, NH), 7.21-7.38 (20H, m, 4 x BnO), 7.54 (2H, bt, J = 7.5 Hz, benzamide), 7.63 (1H, bt, J = 7.5 Hz, benzamide), 7.94 (1H, s, H9), 7.98 (1H, bd, J = 7.5 Hz, benzamide); ^{13}C NMR (127 MHz, $CHCl_3$): δ = 17.5 (CH_3, methylthiazol), 46.8 (CH_2, C-7), 67.9 (CH_2, C-6), 68.3 (CH, C-1), 71.3, 72.1, 72.6, 73.2 (4CH_2, 4 x BnO), 73.4 (CH, C-4), 73.6, 74.1 (CH, C-2, C-3), 74.6 (CH, C-5), 121 (C), 127.6-128.5 (21CH, 4 x BnO, benzamide), 129.0 (CH, benzamide), 129.9 (C), 131.7 (C), 133.2 (CH, benzamide), 137.4 (C, BnO), 137.7 (2C, 2 x BnO), 137.9 (C, BnO), 148.4 (CH, C-9), 154.2 (C), 159.6 (C), 165.4 (C), 175.1 (C), 175.2 (C, C-10); HRMS (ESI) m/z calcd for C50H48N4O7S2 [M + H]+ 881.3027, found 881.3043.

5-(2-bromo-4-methylthiazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (14). Prepared following GP 1, starting from **7** (50 mg, 0.082 mmol) and 1-(2-bromo-4-methylthiazol-5-yl)-2-chloroethanone (31 mg, 0.123 mmol) as starting materials, the derivative **14** (48 mg, 0.057 mmol, 70%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 70:30 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +18 (c = 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.64 (3H, s, methylthiazol), 3.49 (1H, dd, $J_{7a,7b}$ = 12.7 Hz, $J_{7a,1}$ = 6.2 Hz, H-7a), 3.57-3.65 (2H, m, H-7b, H-6a), 3.67-3.71 (2H, m, H-2, H-3), 3.81-3.87 (2H, m, H-4, H-6b), 4.04-4.13 (2H, m, H-1, H-5), 4.34-4.56 (8H, m, 4 x BnO), 6.26 (1H,

bs, NH), 7.17-7.36 (20H, m, 4 x BnO), 7.82 (1H, s, H9); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 17.7 (CH_3, methylthiazol), 46.8 (CH_2, C-7), 67.7 (CH_2, C-6), 68.0 (CH, C-1), 71.3, 72.1, 72.6, 73.2 ($4CH_2$, 4 x BnO), 73.0 (CH, C-4), 73.4, 74.0 (CH, C-2, C-3), 74.7 (CH, C-5), 127.6-128.5 (20CH, 4 x BnO), 128.9 (C), 131.2 (C), 137.3 (C, BnO), 137.6 (2C, 2 x BnO), 137.8 (C, BnO), 148.7 (CH, C-9), 157.4 (C), 175.3 (C), 175.5 (C); HRMS (ESI) m/z calcd for $C_{43}H_{43}BrN_3O_6S_2$ [M + H]+ 840.1774, found 840.1771.

5-(2-bromo-4-methylthiazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)methylaminothiazole (15). Prepared following GP 1, using the thiourea **10** (30 mg, 0.0714 mmol) and 1-(2-bromo-4-methylthiazol-5-yl)-2-chloroethanone (25 mg, 0.0928 mmol) as starting materials, **15** (39 mg, 0.0615 mmol, 86%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 30:70 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +63 (c = 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.07 (3H, s, AcO), 2.09 (3H, s, AcO), 2.10 (3H, s, AcO), 2.12 (3H, s, AcO), 2.62 (3H, methylthiazol), 3.60 (2H, m, H-7), 3.97 (1H, dd, $J_{6a,6b}$ = 12.1 Hz, $J_{6a,5}$ = 3.5 Hz, H-6a), 4.05 (1H, m, H-5), 4.27 (1H, m, H-1), 4.77 (1H, dd, $J_{6b,6a}$ = 12.1 Hz, $J_{6a,5}$ = 8.3 Hz, H-6b), 4.98 (1H, dd, $J_{4,3}$ = 5.5 Hz, $J_{4,5}$ = 3.6 Hz, H-4), 5.11 (1H, dd, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.32 (1H, dd, $J_{3,4}$ = 5.3 Hz, $J_{3,2}$ = 3.3 Hz, H-3), 6.61 (1H, bs, NH), 7.80 (1H, s, thiazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.4 (CH_3, methylthiazol), 20.69, 20.75, 20.77, 20.81 (4CH3, 4 x AcO), 34.7 (CH2, C-7), 60.7 (CH2, C-6), 67.0, 67.4, 67.9, 68.9 (4CH), 73.4 (CH, C-1), 116.6 (C), 130.5 (C), 146.0 (CH, thiazol), 158.4(C), 169.26, 169.49, 169.64, 169.75 (4C, 4 x AcO), 171.0, 173.9, 176.0 (3C); HRMS (ESI) m/z calcd for $C_{23}H_{27}BrN_3O_{10}S_2$ [M + H]+ 648.0321, found 648.0329.

5-(2-(prop-2-yn-1-yl)amino-4-methylthiazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (16). According to the general procedure GP 1, using the thiourea **7** (130 mg, 0.212 mmol) and 2-chloro-1-(2-(prop-2-yn-1-ylamino)thiazol-5-yl)ethanone chloroacetone alkyne (63 mg, 0.276 mmol) as starting materials, the derivative **16** (125 mg, 0.153 mmol, 72%) was obtained after purification by silica gel column chromatography (EtOAc as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +35 (c = 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.35 (1H, t, J = 2.5 Hz, propargyamine), 2.57 (3H, s, methylthiazol), 3.49 (1H, dd, $J_{7a,7b}$ = 13.4 Hz, $J_{7a,1}$ = 6.6 Hz, H-7a), 3.57-3.63 (2H, m, H-7b, H-6a), 3.67-3.72 (2H, m, H-2, H-3), 3.81-3.87 (2H, m, H-4, H-6b), 4.05-4.11 (2H, m, H-1, H-5), 4.14 (2H, d, J = 2.5 Hz, propargyamine), 4.37-4.55 (8H, m, 4 x BnO), 6.36 (1H, bs, NH), 7.18-7.36 (20H, m, 4 x BnO), 7.87 (1H, s, thiazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.3 (CH_3, methylthiazol), 34.8 (CH_2, propargyamine), 46.5 (CH_2, C-7), 67.8 (CH_2, C-6), 68.9 (CH, C-1), 71.4, 72.2, 72.7, 73.2 (4CH2, 4 x BnO), 73.2, 73.4, 74.1, 74.7 (4CH), 77.2 (CH, propargyamine), 77.9 (C, propargyamine), 117.2 (C), 127.7-128.5 (20CH, 4 x BnO), 130.1 (C), 132.0 (C), 137.4, 137.70, 137.73, 137.9 (4C, 4 x BnO), 146.3 (CH, thiazol), 169.0 (C), 174.1 (C), 176.0 (C); HRMS (ESI) m/z calcd for $C_{46}H_{47}N_4O_6S_2$ [M + H]+ 815.2941, found 815.2932.

5-(2-(prop-2-yn-1-yl)amino-4-methylthiazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)methylaminothiazole (17). Prepared following GP1, using the thiourea **10** (200 mg, 0.476 mmol) and 2-chloro-1-(2-(prop-2-yn-1-ylamino)thiazol-5-yl)ethanone chlorocetone alkyne (142 mg, 0.619 mmol) as starting materials, **17** (249 mg, 0.399 mmol, 84%) was obtained after purification by silica gel column chromatography (EtOAc as eluents) as a yellowish amorphous solid. $[\alpha]_D^{20}$ = +61 (c = 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.06 (3H, s, AcO), 2.03 (3H, s, AcO), 2.099 (3H, s, AcO), 2.104 (3H, s, AcO), 2.34 (1H, t, J = 2.5 Hz, propargylamine), 2.56 (3H, s, thiazol), 3.57 (2H, m, H-7), 4.02 (1H, dd, $J_{6a,6b}$ = 12.0 Hz, J6a,5 = 3.7 Hz, H-6a), 4.07-4.14 (3H, m, propargylamine, H-5), 4.25 (1H, m, H-1), 4.69 (1H, dd, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 8.2 Hz, H-6a), 4.99 (1H, dd, $J_{2,3}$ = 5.5 Hz, $J_{2,1}$ = 3.8 Hz, H-2), 5.12 (1H, dd, $J_{4,5}$ = 7.6 Hz, $J_{4,3}$ = 3.4 Hz, H-4), 5.32 (1H, dd, $J_{3,2}$ = 5.5 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 6.92 (1H, bs, NH), 7.23 (1H, bs, NH), 7.85 (1H, m, thiazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.4 (CH_3, methylthiazol), 20.69, 20.75, 20.77, 20.81 ($4CH_3$, 4 x AcO), 34.7 (CH_2, C-7), 45.4 (CH_2, propargylamine), 60.7 (CH_2, C-6), 67.0, 67.4, 67.9, 68.9 (4CH), 72.9 (CH, propargylamine), 73.4 (CH, C-1), 78.0 (C, propargylamine), 116.6 (C), 130.5 (C), 146.0 (CH, thiazol), 158.4(C), 169.26, 169.49, 169.64, 169.75 (4C, 4 x AcO), 171.0, 173.9, 176.0 (3C); HRMS (ESI) m/z calcd for $C_{26}H_{31}N_4O_{10}S_2$ [M + H]+ 623.1484, found 623.1476.

5-(3-ethoxycarbonylisoxazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (18). Prepared following GP 1, starting from **7** (50 mg, 0.082 mmol) and ethyl 5-(2-bromoacetyl)isoxazole-3-carboxylate as starting material, the derivative **18** (47 mg, 0.058 mmol, 71%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 70:30 → 50:50 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +63 (c = 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.36 (3H, t, J = 7.13 Hz, COOEt), 3.45 (1H, dd, $J_{7a,7b}$ = 13.4 Hz, $J_{7a,1}$ = 6.3 Hz, H-7a), 3.51-3.59 (2H, m, H-7b, H-6a), 3.61-3.65 (2H, m, H-2, H-3), 3.75-3.81 (2H, m, H-4, H-6b), 3.99-4.09 (2H, m, H-1, H-5), 4.27-4.49 (10H, m, COOEt, 4 x BnO), 7.00 (1H, bs, NH), 7.11-7.29 (21H, m, isoxazole, 4 x BnO), 8.41 (1H, s, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0 (CH_3, COOEt), 46.9 (CH_2, C-7), 62.4 (CH2, COOEt), 67.6 (CH_2, C-6), 67.8 (CH, C-1), 71.2, 72.0, 72.6, 72.9 (4 x CH2, 4 x BnO), 73.2 (CH, C-4), 73.3, 73.9 (CH, C-2, C-3), 74.7 (CH, C-5), 108.2 (CH, isoxazole), 126.5 (C), 127.6-128.5 (20CH, 4 x BnO), 137.3, 137.63, 137.65, 137.9 (4C, 4 x BnO), 152.6 (CH, C-9), 159.1, 168.4, 169.1 (3C), 176.2 (C, COOEt); HRMS (ESI) m/z calcd for $C_{45}H_{45}N_3O_9SNa$ [M + Na]+ 826.2770, found 826.2774.

5-(3-phenylisoxazol-5-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)methylaminothiazole (19). Prepared following GP 1, using the thiourea **10** (30 mg, 0.0714 mmol) and 2-bromo-1-(3-phenylisoxazol-5-yl)ethanone (25 mg, 0.0928 mmol) as starting materials, the derivative **19** (35 mg, 0.0568 mmol, 80%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 30:70 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +58 (c = 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.08 (6H, s, 2 x AcO), 2.1 (6H, s, 2 x AcO), 3.63 (2H, m, H-7), 3.99 (1H, dd, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 3.8 Hz, H-6a), 4.11 (1H, ddd, $J_{5,6}$ = 8.4 Hz, $J_{5,6}$ = 3.5 Hz, $J_{5,4}$ = 3.5 Hz, H-5), 4.31 (1H, m, H-1), 4.79 (1H, dd, $J_{6b,6a}$ = 12.0 Hz, $J_{6a,5}$ = 8.5 Hz, H-6b), 5.00 (1H, dd, J4,3 = 5.3 Hz, J4,5 = 3.5 Hz, H-4), 5.13 (1H, dd, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.34 (1H, dd, $J_{3,4}$ = 5.3 Hz, $J_{3,2}$ = 3.3 Hz, H-3), 7.30 (1H, s, phenylisoxazol), 7.48 (3H, m, phenylisoxazol), 7.85 (2H, m, phenylisoxazol), 8.54 (1H, s, thiazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 20.69, 20.72, 20.79, 20.80 ($4CH_3$, 4 x AcO), 45.7 (CH_2, C-7), 60.5 (CH_2, C-6), 66.9 (CH, C-2), 67.2 (CH, C-3), 67.9 (CH, C-4), 68.4 (CH, C-1), 73.8 (CH, C-5), 106.3 (CH, phenylisoxazol), 126.8-129.1 (5 x CH, phenylisoxazol), 127.9 (C, phenylisoxazol), 130.5 (C, phenylisoxazol), 151.9 (C, phenylisoxazol), 162.6 (C), 162.7 (CH, thiazol), 167.4 (C), 169.2-170.27 (4C, 4 x AcO); HRMS (ESI) m/z calcd for $C_{28}H_{30}N_3O_{11}S$ [M + H]+ 616.1595, found 616.1596.

5-(2,4-difluorobenzoyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (20). Prepared following GP1, using the thiourea **7** (50 mg, 0.082 mmol) and 2'-chloro-2,4-difluoroacetophenone (20 mg, 0.106 mmol) as starting materials, the derivative **20** (39 mg, 0.050 mmol, 61%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 80:20 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +13 (c = 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.45 (1H, dd, $J_{7a,7b}$ = 12.8 Hz, $J_{7a,1}$ = 6.1 Hz, H-7a), 3.54-3.60 (2H, m, H-7b, H-6a), 3.64-3.68 (2H, m, H-2, H-3), 3.75-3.81 (2H, m, H-4, H-6b), 3.99-4.07 (2H, m, H-1, H-5), 4.30-4.49 (8H, m, 4 x BnO), 6.44-6.56 (2H, m, difluoroacetophenone), 7.13-7.33 (21H, m, 4 x BnO, difluoroacetophenone), 7.53 (1H, s, H-9); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 46.5 (CH_2, C-7), 67.7 (CH_2, C-6), 68.1 (CH, C-1), 71.3, 72.1, 72.6, 73.2 (4 x CH_2, 4 x BnO), 73.2 (CH, C-4), 73.4, 74.0 (CH, C-2, C-3), 74.7 (CH, C-5), 102.9 (CH, d, JC,F = 24.7 Hz, difluoroacetophenone), 104.7 (CH, d, JC,F = 22.2 Hz,

difluoroacetophenone), 124.0 (C, d, JC,F = 2.4 Hz, difluoroacetophenone), 127.6-128.5 (20CH, 4 x BnO), 128.8 (C), 132.2 (CH, d, JC,F = 10.8 Hz, difluoroacetophenone), 137.4, 137.69, 137.72, 137.9 (4C, 4 x BnO), 150.2 (CH, C-9), 152.7 (C, d, JC,F = 10.3 Hz, difluoroacetophenone), 164.7 (C, d, JC,F = 247.9 Hz, difluoroacetophenone), 175.1 (C), 187.5 (C, C-10); HRMS (ESI) m/z calcd for $C_{45}H_{42}F_2N_2O_6SNa$ [M + Na]+ 776,2732, found 776,2729.

5-((4-fluorophenyl)-2,5-dimethyl-1*H*-pyrrol-3-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (21). Prepared following GP 1, starting from **7** (50 mg, 0.082 mmol) and 2-chloro-1-[1-(4-fluorophenyl)-2,5-dimethyl-1*H*-pyrrol-3-yl]ethanone (33 mg, 0.123 mmol) as starting materials, the derivative **21** (38 mg, 0.045 mmol, 55%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 80:20 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +31 (c = 1.3, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.95 (3H, s, methylpyrrol), 2.22 (3H, s, methylpyrrol), 3.46 (1H, dd, $J_{7a,7b}$ = 13.1 Hz, J7a,1 = 6.7 Hz, H-7a), 3.52-3.61 (2H, m, H-7b, H-6a), 3.66-3.69 (2H, m, H-2, H-3), 3.75-3.81 (2H, m, H-4, H-6b), 3.99-4.09 (2H, m, H-1, H-5), 4.35-4.49 (8H, m, 4 x BnO), 6.32 (1H, bs, methylpyrrol), 7.12-7.31 (24H, m, 4 x BnO, fluorophenyl), 7.81 (1H, s, H-9); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 12.6 (2 x CH_3, methylpyrrol), 46.2 (CH_2, C-7), 67.9 (CH_2, C-6), 68.5 (CH, C-1), 71.5, 72.3, 72.6, 73.2 (4 x CH_2, 4 x BnO), 73.5 (CH, C-4), 73.8, 74.2 (CH, C-2, C-3), 74.6 (CH, C-5), 101.8 (CH, methylpyrrol), 116.3 (2 x CH, d, JC,F = 23.0 Hz, fluorophenyl), 119.2 (C, fluorophenyl), 127.6-128.4 (20CH, 4 x BnO), 128.8 (C), 129.8 (2 x CH, d, JC,F = 8.9 Hz, fluorophenyl), 130.8 (C), 133.5 (C, d, JC,F = 3.3 Hz, fluorophenyl), 135.8 (C), 137.5, 137.79, 137.81, 138.0 (4C, 4 x BnO), 146.3 (CH, C-9), 162.4 (C, d, JC,F = 10.3 Hz, fluorophenyl), 173.6 (C), 181.8 (C, C-10); HRMS (ESI) m/z calcd for $C_{51}H_{51}FN_3O_6S$ [M + H]+ 852.3506, found 852.3483.

5-(benzo[d]thiazol-2-ylcarbonyl)-2-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)methylaminothiazole (22). Prepared following GP 1, starting from **7** (75 mg, 0.122 mmol) and 1-(benzo[d]thiazol-2-yl)-2-bromoethanone (34 mg, 0.134 mmol) as starting materials, the derivative **22** (61 mg, 0.076 mmol, 63%) was obtained after purification by silica gel column chromatography (petroleum ether/EtOAc, 80:20 as eluents) as a yellowish oil. $[\alpha]_D^{20}$ = +18 (c = 0.9, $CDCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.60 (1H, dd, J7a,7b = 13.1 Hz, J7a,1 = 6.6 Hz, H-7a), 3.66-3.72 (2H, m, H-7b, H-6a), 3.76-3.79 (2H, m, H-2, H-3), 3.86-3.94 (2H, m, H-4, H-6b), 4.11-4.21 (2H, m, H-1, H-5), 4.41-4.56 (8H, m, 4 x BnO), 7.22-7.39 (24H, m, 4 x BnO), 7.54 (2H, m, benzothiazol), 7.99 (1H, m, benzothiazol), 8.22 (2H, m, benzothiazol), 9.04 (1H, s, H9); ^{13}C NMR (127 MHz, $CHCl_3$): δ = 46.8 (CH2, C-7), 67.7 (CH2, C-6), 68.1 (CH, C-1), 71.3, 72.1, 72.6, 73.2 (4CH2, 4 x BnO), 73.2 (CH, C-4), 73.3, 74.0 (CH, C-2, C-3), 74.6 (CH, C-5), 122.1, 125.2, 126.7, 127.0 (4CH, benzothiazol), 127.6-128.4 (20CH, 1C, 4 x BnO, benzothiazol), 136.6 (C), 137.4 (C, BnO), 137.7 (2 x C, 2 x BnO), 137.9 (C, BnO), 153.6 (CH, C-9), 153.7 (C), 167.2 (C), 174.8 (C), 176.5 (C, C-10); HRMS (ESI) m/z calcd for $C_{46}H_{44}N_3O_6S_2$ [M + H]+ 798.2686, found 798.2672.

5-acetyl-2-(α-D-mannopyranosyl)methylaminothiazole (23). Prepared following GP 2, starting from **11** (152mg, 0.224mmol). After purification over silica gel (DCM/MeOH 9:1) and lyophilisation, 52.15mg (73% yield) of **23** was obtained as a light white solid. $[\alpha]_D^{20}$ = +44 (c = 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ = 8.15 (1H, s, H-9), 4.27 (1H, m, H-1), 4.06 (1H, t, ^3J=3.2 Hz, H-2), 3.84-3.93 (4H, m, H-3, H-6, H7'), 3.79 (1H, t, ^{3} =8.3 Hz, H-4), 3.67-3.74 (2H, m, H-5, H-7') 4.03-4.10 (2H, m, H-1, H-5) , 3.79-3.86 (2H, m,H-4, H-6), 3.67-3.72 (2H, m, H-2, H-3), 3.61 (1H, dd, $^2J_{6\text{-}6'}$ =10.3Hz, $^3J_{6'\text{-}5}$= 5.6 Hz, H-6'), 3.43-3.61 (2H, m, H-7), 2.43 (3H, s, H-12); ^{13}C NMR (100 MHz, D_2O): δ = 193.5 (C-11), 174.1 (C-8), 144.9 (C-9), 126.8 (C-10), 75.3 (C-5), 74.9 (C-1), 70.7 (C-3), 68.7 (C-2), 67.4 (C-4), 60.8 (C-6), 43.9 (C-7), 25.2 (C-12); HRMS (MALDI) *m/z* calcd for $C_{12}H_{19}N_2O_6S$: 319.0958, found 319.0949.

5-(4-methyl-2-(pyrazin-2-yl)thiazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (24). Prepared following GP2 starting from **12** (239 mg, 0.284 mmol). After purification over silica gel (DCM/MeOH 8:2) and lyophilisation, 130 mg (95% yield) of **24** was obtained as a yellow powder. $[\alpha]_D^{20}$ = +29 (c = 0.5, DMSO); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.36 (1H, d, $^3J_{19\text{-}18}$ =1.4Hz, H-19), 8.94 (1H, bs, NH) 8.82 (1H, d, $^3J_{17\text{-}18}$ =2.5Hz, H-17), 8.77 (1H, dd, $^3J_{17\text{-}18}$ =2.5Hz, $^3J_{19\text{-}18}$ =1.4Hz, H-18), 8.01 (1H, s, H-9), 4.83 (1H, d, ^2J=5.0 Hz, OH), 4.79 (1H, d, ^2J=4.3 Hz, OH), 4.70 (1H, d, ^2J =5.4 Hz, OH), 4.41 (1H, dd, ^2J=5.1 Hz, ^2J =6.5 Hz, OH), 3.86 (1H, m, H-1), 3.57-3.71 (4H, m, H-3, H-4, H-6, H-7), 3.42-3.57 (4H, m, H-2, H-5 H-6', H-7'), 2.62 (3H, s, H-14); ^{13}C NMR (100 MHz, DMSO): δ = 183.0 (C-11), 176.5 (C-8), 166.0 (C-15), 157.6 (C-12), 151.6 (C-9), 147.1 (C-17), 145.7 (C-16), 145.2 (C-18), 141.2 (C-19), 130.8 (C-13), 127.9 (C-10), 78.2 (C-2), 72.2 (C-1), 71.3 (C-3), 68.9 (C-5), 67.81 (C-4), 60.9 (C-6, C-7), 17.9 (C-14); HRMS (ESI) *m/z* calcd for $C_{19}H_{21}N_5O_6S_2Na$: 502.0831, found 502.0841.

5-(2-benzoylamino-4-methylthiazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (25). Prepared following GP 2, using the derivative **13** (37 mg, 0.042 mmol) as starting materials, the derivative **25** (20 mg, 0.038 mmol, 91%) was obtained after purification by silica gel column chromatography (DCM/MeOH, 80:20 as eluents) as an amorphous white solid. $[\alpha]_D^{20}$ = +32 (c = 0.6, MeOH); ^{1}H NMR (400 MHz, DMSO) δ = 2.52 (3H, s, methylthiazol), 3.34-3.70 (H, m), 3.87 (1H, m, H-1), 7.55 (2H, bt, J = 7.5 Hz, benzamide), 7.66 (1H, bt, J = 7.5 Hz, benzamide), 7.93 (1H, s, thiazol), 8.11 (1H, bd, J = 7.5 Hz, benzamide), 8.94 (1H, bs, NH), 13.0 (1H, bs, NH); ^{13}C NMR (127 MHz, DMSO): δ = 17.6 (CH_3, methylthiazol), 49.9 (CH_2, C-7), 60.4 (CH_2, C-6), 67.5 (CH), 68.5 (CH), 70.8 (CH), 71.9 (CH, C-1), 77.7 (CH), 127.9 (C), 128.3, 128.7 (CH, benzamide), 131.6 (C), 133.0 (CH, benzamide), 137.3 (C), 149.1 (CH, thiazol), 153.1 (C), 159.1 (C), 162.4 (C), 176.7 (C); HRMS (ESI) m/z calcd for $C_{22}H_{25}N_4O_7S_2$ [M + H]+ 521.1166, found 521.1159.

5-(2-chloro-4-methylthiazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (26). Prepared following GP 3, using the derivative **14** (30 mg, 0.036 mmol) as starting materials, the derivative **26** (14 mg, 0.032 mmol, 89%) was obtained after purification by silica gel column chromatography (DCM/MeOH, 85:15 as eluent) as an amorphous white solid. $[\alpha]_D^{20}$ = +21 (c = 1.1, MeOH); ^{1}H NMR (400 MHz, MeOD) δ = 2.53 (3H, s, methylthiazol), 3.62-3.78 (6H, m), 3.82 (1H, dd, J2,3 = 4.9 Hz, J2,1 = 3.2 Hz, H-2), 3.89 (1H, dd, J6b,6a = 11.9 Hz, J6b,5 = 6.8 Hz, H-6b), 4.07 (1H, m, H-1), 7.89 (1H, s, thiazol); ^{13}C NMR (127 MHz, MeOD): δ = 17.7 (CH_3, methylthiazol), 45.4 (CH_2, C-7), 62.2 (CH_2, C-6), 69.4 (CH, C-2), 69.8 (CH), 72.4 (CH), 74.6 (CH, C-1), 78.2 (CH), 129.2 (C), 131.3 (C), 151.6 (CH, thiazol), 151.3(C),154.1 (C), 156.7 (C), 177.1 (C); HRMS (ESI) m/z calcd for $C_{15}H_{19}ClN_3O_6S_2$ [M + H]+ 436.0396, found 436.0398.

5-(2-bromo-4-methylthiazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (27). Prepared following GP3, using the derivative **15** (25 mg, 0.039 mmol) as starting material, the derivative **27** was obtained after lyophilization (17 mg, 0.036 mmol, 94%) as an amorphous white solid. $[\alpha]_D^{20}$ = +42 (c = 1.1, CD_3OD); ^{1}H NMR (400 MHz, CD_3OD) δ = 2.56 (3H, s, methylthiazol), 3.64-3.83 (6H, m), 3.81 (1H, dd, J2,3 = 5.2 Hz, J2,1 = 3.2 Hz, H-2), 3.90 (1H, dd, J6a,6b = 11.8 Hz, J6a,5 = 6.9 Hz, H-6a), 4.06 (1H, dt, J = 8.0 Hz, J = 4.8 Hz, H-1), 7.89 (1H, s, thiazol); ^{13}C NMR (127 MHz, MeOD): δ = 17.6 (CH3, methylthiazol), 45.6 (CH2, C-7), 62.2 (CH2, C-6), 69.3 (CH, C-2), 69.9 (CH), 72.4 (CH), 74.4 (CH, C-1), 78.4 (CH), 129.2 (C), 132.9 (C), 139.2 (C), 150.9 (CH, thiazol), 158.1 (C), 176.5 (C), 177.0 (C); HRMS (ESI) m/z calcd for $C_{15}H_{19}BrN_3O_6S_2$ [M + H]+ 479.9893, found 479.9880.

5-(2-(prop-2-yn-1-yl)amino-4-methylthiazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (28). Prepared following GP3, using **17** (29 mg, 0.046 mmol) as starting materials, **28** (20 mg,

0.044 mmol, 96%) was obtained after lyophilization. $[\alpha]_D^{20}$ = +37 (c = 1.3, CD_3OD); ^1H NMR (400 MHz, CD_3OD) δ = 2.49 (3H, s, methylthiazol), 2.69 (1H, t, J = 2.5 Hz, propargylamine), 3.59-3.79 (8H, m), 3.83 (1H, dd, $J_{2,3}$ = 4.7 Hz, $J_{2,1}$ = 3.4 Hz, H-2), 3.88 (1H, dd, $J_{6b,6a}$ = 11.8 Hz, $J_{6b,5}$ = 7.1 Hz, H-6b), 4.06 (1H, dt, J = 7.8 Hz, 4.9, H-1), 4.16 (1H, d, J = 2.5 Hz, propargylamine), 7.85 (1H, s, thiazol); ^{13}C NMR (127 MHz, CD_3OD): δ = 18.6 (CH3, methylthiazol), 34.5 (CH_2, propargylamine), 45.3 (CH_2, C-7), 62.3 (CH_2, C-6), 69.5(CH), 69.9 (CH), 72.5 (CH, C-1), 73.2 (CH), 74.7 (CH), 78.2 (CH, propargylamine), 79.9 (C, propargylamine), 117.2 (C), 130.3 (C), 147.9 (CH, thiazol), 160.1 (C), 171.3 (C), 175.5 (C), 178.1 (C); HRMS (ESI) m/z calcd for $C_{18}H_{22}N_4O_6S_2$ [M + H]+ 455.1063, found 455.1054.

5-(3-ethoxycarbonylisoxazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (29). Prepared following GP 3, starting from **18** (40 mg, 0.049 mmol) as starting materials, the derivative **29** (14 mg, 0.032 mmol, 64%) was obtained after purification by silica gel column chromatography (DCM/MeOH, 80:20 as eluents) as an amorphous white solid. $[\alpha]_D^{20}$ = +13 (c = 0.8, H_2O); ^1H NMR (400 MHz, D_2O) δ = 1.47 (3H, t, J = 7.2 Hz, COOEt), 3.68 (1H, dd, J7a,7b = 14.2 Hz, J7a,1 = 4.0 Hz, H-7a), 3.73 (1H, m, H-5), 3.81 (1H, t, J = 8.6 Hz, H-4), 3.83 (1H, dd, J6a,6b = 14.6 Hz, J6a,5 = 4.7 Hz, H-6a), 3.88-3.93 (3H, m, H-3, H-6b, H-7b), 4.08 (1H, t, J = 3.0 Hz, H-2), 4.26 (1H, ddd, $J_{1,7b}$ = 9.8 Hz, $J_{1,7a}$ = 4.1 Hz, $J_{1,2}$ = 2.9 Hz, H-1), 4.53 (2H, q, J = 7.2 Hz, COOEt), 7.43 (1H, s, isoxazole), 8.32 (1H, s, thiazol); ^{13}C NMR (127 MHz, D_2O): δ = 13.3 (CH_3, COOEt), 43.3 (CH2, C-7), 60.9 (CH_2, C-6), 63.8 (CH_2, COOEt), 67.3 (CH, C-4), 69.0 (CH, C-2), 70.8 (CH, C-3), 75.1 (CH, C-5), 75.5 (CH, C-1), 108.5 (CH, isoxazole), 125.1 (C), 153.6 (CH, thiazol), 159.8, 159.9, 167.1, 169.9 (4C); HRMS (ESI) m/z calcd for $C_{17}H_{21}N_3O_9SNa$ [M + Na]+ 466.0895, found 466.0895.

5-(3-carboxyisoxazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (30). To a solution of **29** (33 mg, 0.0745 mmol) in a mixture 3:1 of MeOH/H_2O (2 mL) was added LiOH (3.6 mg, 0.149 mmol). The mixture was stirred at 50° C for 8 h, neutralized with Amberlite IR120 (H), filtered and the solvents evaporated to dryness. The substrate was dissolved in water and subjected to lyophilization to give **30** (28 mg, 0.0675 mmol, 91%) as an amorphous white solid. $[\alpha]_D^{20}$ = +19 (c = 0.6, H_2O); ^1H NMR (400 MHz, MeOD) δ = 3.67 (1H, dd, J6a,6b = 11.6 Hz, J6a,5 = 2.5 Hz, H-6a), 3.71-3.87 (6H, m), 4.03(1H, dd, J6b,6a = 11.6 Hz, J6b,5 = 7.8 Hz, H-6b), 4.08 (1H, m, H-1), 7.52 (1H, s, isoxazole), 8.49 (1H, s, thiazol); ^{13}C NMR (127 MHz, MeOD): δ = 47.2 (CH_2, C-7), 61.4 (CH_2, C-6), 68.2 (CH), 70.4 (CH), 71.9 (CH), 72.1 (CH), 79.7 (CH), 110.2 (CH, isoxazole), 126.2 (C), 142.65 (CH, thiazol), 142.67, 159.0, 161.4, 168.0, 171.1 (5C); HRMS (ESI) m/z calcd for $C_{15}H_{18}N_3O_9S$ [M + H]+ 416.0754, found 416.0758.

5-(3-phenylisoxazol-5-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (31). Prepared following GP 3, using **19** (20 mg, 0.0325 mmol) as starting materials, the derivative **31** (14 mg, 0.0313 mmol, 96%) was obtained after lyophilization. $[\alpha]_D^{20}$ = +12 (c = 0.5, MeOH); ^1H NMR (400 MHz, DMSO-d6) δ = 3.45-3.57 (4H, m), 3.59-3.76 (4H, m), 3.89 (1H, m, H-1), 7.56 (3H, m, phenylisoxazol), 7.89 (1H, s, phenylisoxazol), 8.01 (2H, m, phenylisoxazol), 8.43 (1H, s, thiazol); ^{13}C NMR (127 MHz, , DMSO-d6): δ = 49.5 (CH_2, C-7), 60.3 (CH2, C-6), 67.2 (CH), 68.3 (CH), 68.4 (CH), 70.6 (CH), 71.6 (CH, C-1), 77.7 (CH), 106.1 (CH, phenylisoxazol), 125.1 (C, phenylisoxazol), 126.8 (3CH, phenylisoxazol), 129.1 (2CH, phenylisoxazol), 130.6 (C, phenylisoxazol), 152.3 (CH, thiazol), 162.4 (2 x C), 166.2 (C), 169.4 (C); HRMS (ESI) m/z calcd for $C_{20}H_{22}N_3O_7S$ [M + H]+ 448.1173, found 448.1173.

5-(2,4-difluorobenzoyl)-2-(α-D-mannopyranosyl)methylaminothiazole (32). Prepared following GP2, using the derivative **20** (40 mg, 0.0515 mmol) as starting materials, the derivative **32** (16 mg, 0.0385 mmol, 75%) was obtained after purification by silica gel column chromatography (AcOEt/MeOH, 70:30 as eluent) as an amorphous white solid. $[\alpha]_D^{20}$ = +42 (c = 1.4, CD_3OD); ^1H NMR (400 MHz, CD_3OD) δ = 3.62-3.77 (6H, m), 3.81 (1H, dd, J = 4.9 Hz, J = 3.1 Hz), 3.89 (1H, dd, J = 11.9 Hz, J = 7.1 Hz, H-6b), 4.06 (1H, m, H-1), 6.55-7.65 (3H, m, difluoroacetophenone), 7.54 (1H, s, thiazol); ^{13}C NMR (127 MHz, CD_3OD): δ = 45.4 (CH2, C-7), 62.3 (CH2, C-6), 69.5 (CH), 69.9 (CH), 72.5 (CH), 74.6 (CH, C-1), 78.3 (CH), 104.2 (CH, d, JC,F = 25.0 Hz, difluoroacetophenone), 105.9 (CH, d, JC,F = 22.5 Hz, difluoroacetophenone), 128.6 (C), 133.3 (C, d, JC,F = 11.1 Hz, difluoroacetophenone), 132.2 (CH, d, JC,F = 10.8 Hz, difluoroacetophenone), 152.1 (CH, thiazol), 153.3 (C, d, JC,F = 10.3 Hz, difluoroacetophenone), 166.2 (C, d, JC,F = 246.1 Hz, difluoroacetophenone), 175.1 (C), 189.8 (C); HRMS (ESI) m/z calcd for $C_{17}H_{19}F_2N_2O_6S$ [M + H]+ 417.0938, found 417.0926.

5-((4-fluorophenyl)-2,5-dimethyl-1*H*-pyrrol-3-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (33). Prepared following GP2, using the derivative **21** (31 mg, 0.0364 mmol) as starting materials, the derivative **33** (17 mg, 0.0345 mmol, 95%) was obtained after purification by silica gel column chromatography (DCM/MeOH, 80:20 as eluent) as an amorphous white solid. $[\alpha]_D^{20}$ = +19 (c = 0.3, MeOH); ^1H NMR (400 MHz, CD_3OD) δ = 2.01 (3H, s, methylpyrrol), 2.20 (3H, s, methylpyrrol), 3.61-3.79 (7H, m), 3.85 (1H, dd, J2,3 = 4.8 Hz, J2,1 = 3.3 Hz, H-2), 3.89 (1H, dd, $J_{6b,6a}$ = 11.9 Hz, $J_{6b,5}$ = 6.9 Hz, H-6b), 4.09 (1H, ddd, J = 10.2 Hz, J = 5.1 Hz, J = 5.1 Hz, H-1), 6.39 (1H, bs, pyrrol), 7.29-7.32 (4H, m, fluorophenyl), 7.82 (1H, s, H-9); ^{13}C NMR (127 MHz, CD_3OD): δ = 12.7 (CH_3, methylpyrrol), 12.9 (CH_3, methylpyrrol), 45.2 (CH_2, C-7), 62.4 (CH_2, C-6), 69.6, 69.8, 72.5 (3 x CH), 74.9 (CH, C-1), 78.1 (CH), 109.0 (CH, methylpyrrol), 117.5 (2 x CH, d, JC,F = 23.2 Hz, fluorophenyl), 120.3 (C, fluorophenyl), 130.6 (C), 131.3 (2 x CH, d, JC,F = 8.9 Hz, fluorophenyl), 134.9 (C, d, JC,F = 3.9 Hz, fluorophenyl), 137.1 (C), 148.3 (CH, thiazol), 163.9 (C, d, JC,F = 246.9 Hz, fluorophenyl), 175.1 (C), 184.1 (C); HRMS (ESI) m/z calcd for $C_{23}H_{27}FN_3O_6S$ [M + H]+ 492.1597, found 492.1599.

5-(benzo[d]thiazol-2-ylcarbonyl)-2-(α-D-mannopyranosyl)methylaminothiazole (34). Prepared following GP 2, starting from **22** (30 mg, 0.0376 mmol) as starting materials, the derivative **34** (15 mg, 0.0343 mmol, 91%) was obtained after purification by silica gel column chromatography (AcOEt/MeOH, 70:30 as eluent) as an amorphous white solid. $[\alpha]_D^{20}$ = +51 (c = 0.2, MeOH); ^1H NMR (400 MHz, DMSO-d6) δ = 3.47-3.70 (8H, m), 3.91 (1H,ddd, J = 8.7 Hz, J = 4.9 Hz, J = 4.9 Hz, H-1), 4.50 (1H, bs, OH), 4.76 (1H, bs, OH), 4.89 (2H, bs, OH), 7.64 (2H, m, benzothiazol), 8.25 (2H, bd, J = 7.9 Hz, benzothiazol), 8.89 (1H, s, thiazol), 9.34 (1H, bs, NH); ^{13}C NMR (127 MHz, DMSO-d6): δ = 49.8 (CH_2, C-7), 60.4 (CH_2, C-6), 67.4, 68.5, 70.8 (3CH), 71.8 (CH, C-1), 77.8 (CH, C-5), 122.7 (C), 122.9, 124.9, 127.4, 127.6 (4CH, benzothiazol), 135.8, 153.2 (2C, benzothiazol), 154.2 (CH, thiazol), 167.4 (C), 173.6 (C), 174.1 (C); HRMS (ESI) m/z calcd for $C_{18}H_{20}N_3O_6S_2$ [M + H]+ 438.0788, found 438.0790.

The following compounds were not named due to the complexity of the structures.

Compound (35). To a solution of mannosyl alkyne **16** (20 mg, 0.0245 mmol) and azidotetraethylenglycol (11 mg, 0.0491 mmol) in a mixture 3:1 of DMF-H_2O (0.8 mL) were added $CuSO_4$ (1 mg, 0.005 mmol) and sodium ascorbate (2 mg, 0.010 mmol) and the mixture was warmed to 70°C. After 8 h, the mixture was concentrated and the crude was purified by silica gel column chromatography (AcOEt /MeOH: 95/5 → 90/10 as eluents) to give **35** (21 mg, 0.0203 mmol, 83%) as a colorless oil. $[\alpha]_D^{20}$ = +51 (c = 0.7, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ = 2.56 (3H, s, methylthiazol), 3.45-3.85 (24H, m), 4.03-4.15 (2H, m, H-1, H-5), 4.30 (1H, m), 4.38-4.54 (8H, m, 4 x BnO), 4.64 (2H, s, propargylamine), 6.57 (1H, bs, NH), 7.18-7.37 (20H, m, 4 x BnO), 7.87 (1H, s, thiazol), 7.86 (1H, s, triazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.4 (CH_3, methylthiazol), 40.7 (CH_2, NH-CH_2-triazol), 46.3 (CH_2, C-7), 50.3 (CH_2,

CH_2-triazol), 61.3 (CH_2, TEG), 67.9 (CH2, C-6), 68.5 (CH, C-1), 69.2 (CH_2, TEG), 70.16-70.39 (4CH_2, 4 x BnO), 71.4, 72.4 (2CH_2, TEG), 72.5 (2CH_2, TEG), 73.2 (CH_2, TEG), 73.4 (CH, C-4), 73.8, 74.1 (2CH, C-2, C-3), 74.6 (CH, C-5), 116.0 (C), 123.6 (CH, triazol), 127.6-128.4 (20CH, 4 x BnO), 130.9 (C), 137.5 (C, BnO), 137.7 (2C, 2 x BnO), 137.9 (C, BnO), 143.4 (C), 146.2 (CH, C-9), 158.7 (C), 169.2 (C), 176.1 (C); HRMS (ESI) m/z calcd for $C_{54}H_{64}N_7O_{10}S_2$ [M + H]+ 1034.4161, found 1034.4151.

Compound (36). To a solution of mannosyl alkyne **16** (140 mg, 0.170 mmol) and 2,3,4,6-tetra-*O*-benzyl-1-azidomethyl-α-D-mannopyranose (129 mg, 0.223 mmol) in a mixture 3:1 of 1,4-dioxane-H_2O (3.7 mL) were added $CuSO_4$ (5.5 mg, 0.034 mmol) and sodium ascorbate (13 mg, 0.068 mmol) and the mixture was warmed up at 70° C. After 8 h, the mixture was concentrated and the crude was purified by silica gel column chromatography (DCM/AcOEt: 50/50 → 30/70 as eluents) to give **36** (204 mg, 0.146 mmol, 86%) as a colorless oil. $[\alpha]_D^{20}$ = +10 (c = 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.58 (3H, s, methylthiazol), 3.48-3.86 (12H, m), 4.04-4.26 (4H, m, H-1, H-1', H-5, H-5'), 4.37-4.55 (19H, m, 8 x BnO, CH2-triazol, H-7'a), 4.69 (1H, dd, J7a,7b = 14.4 Hz, J7a,1 = 2.5 Hz, H-7'b), 6.83 (1H, bs, NH), 7.03 (1H, bs, NH), 7.18-7.37 (40H, m, 8 x BnO), 7.86 (1H, s, thiazol), 7.88 (1H, s, triazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.6 (CH_3, methylthiazol), 40.3 (CH_2, CH_2-triazol), 46.4 (CH_2, C-7), 51.0 (CH_2, C-7'), 67.7, 67.9 (2CH_2, C-6, C-6'), 68.6 (2CH, C-1, C-1'), 71.2, 71.3, 71.9, 72.3, 72.5, 72.7, 73.0, 73.18 (8CH_2, 8 x BnO), 72.6, 73.13, 73.4, 73.9, 74.1, 74.2, 74.5, 74.7 (8CH), 116.2 (C), 123.8 (CH, triazol), 127.5-128.4 (40CH, 8 x BnO), 128.9 (C), 137.4-137.9 (8C, 8 x BnO), 143.1 (C), 148.1 (CH, thiazol), 159.0, 169.3, 174.2, 176.0 (4C); HRMS (ESI) m/z calcd for $C_{81}H_{84}N_7O_{11}S_2$ [M + H]+ 1394.5684, found 1394.5665.

Compound (37). To a solution of mannosyl alkyne **17** (20 mg, 0.032 mmol) and azido-β-cyclodextrine (58 mg, 0.029 mmol) in a mixture 3:1 of DMF-H_2O (1.3 mL) were added $CuSO_4$ (1 mg, 0.0064 mmol) and sodium ascorbate (2.5 mg, 0.0128 mmol) and the mixture was warmed to 70°C. After 8 h, the mixture was concentrated and the crude was purified by silica gel column chromatography (AcOEt → AcOEt /MeOH: 80/20 as eluents) to give **37** (34 mg, 0.0129 mmol, 41%) as a colorless oil. $[\alpha]_D^{20}$ = +81 (c = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.99 (3H, s, AcO), 2.01 (3H, s, AcO), 2.02 (3H, s, AcO), 2.03 (3H, s, AcO), 2.04 (3H, s, AcO), 2.05 (3H, s, AcO), 2.07-2.11 (48H, 16 x AcO), 2.13 (3H, s, AcO), 2.14 (3H, s, AcO), 2.55 (3H, s, methylthiazol), 3.51-5.38 (59H, m), 5.64 (1H, d, J = 4.0 Hz, H-1 CD), 6.42 (1H, bs, NH), 7.12 (1H, bs, NH), 7.69 (1H, s, triazol), 7.84 (1H, s, thiazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.5 (CH_3, methylthiazol), 20.7-20.8 (24CH_3, 24 x AcO), 40.6 (CH_2), 45.1 (CH_2, C-7), 49.9 (CH_2, C-6 CD), 60.7 (CH_2, C-6), 62.2-62.8 (6CH2, C-6 CD), 67.1-78.1 (33CH), 96.36 (CH, C-1 CD), 96.42 (CH, C-1 CD), 96.66 (2CH, 2 x C-1 CD), 96.8 (CH, C-1 CD), 96.9 (CH, C-1 CD), 97.1 (CH, C-1 CD), 116.2 (C), 124.9 (CH, triazol), 130.8 (C), 131.4 (C), 143.3 (C, triazol), 145.7 (CH, thiazol), 162.5 (C), 169.3-171.1 (24C, 24 x AcO), 173.5 (C), 176.0 (C); HRMS (ESI) m/z calcd for $C_{108}H_{141}N_7O_{64}S_2$ [M + 2H]2+ 1311.8710, found 1311.8712.

Compound (38). Prepared following GP 2, using the derivative **35** (20 mg, 0.0194 mmol) as starting materials, **38** (12 mg, 0.0178 mmol, 92%) was obtained after purification by silica gel column chromatography (AcOEt/MeOH, 70:30 as eluent) as an amorphous white solid. $[\alpha]_D^{20}$ = +39 (c = 0.6, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ = 2.53 (3H, s, methylthiazol), 3.51-4.00 (22H), 4.07 (1H, m, H-1), 4.61 (2H, m, H-7), 4.74 (2H, s, CH2-triazol), 7.96 (1H, s, thiazol), 8.14 (1H, s, triazol); ^{13}C NMR (127 MHz, CD_3OD): δ = 18.8 (CH3, methylthiazol), 40.7 (CH2, CH2-triazol), 45.3 (CH2, C-7), 51.5 (CH2), 62.2, 62.3 (2CH2), 69.5, 69.8 (2CH), 70.4 (CH2, C-6), 71.38, 71.41, 71.49, 71.54 (4CH2), 72.5 (CH), 73.6 (CH2), 74.6 (CH, C-1)), 78.2 (CH), 116.9 (C), 125.4 (CH, triazol), 130.5 (C), 145.3 (C, triazol), 147.7 (CH, thiazol), 160.5, 171.5, 175.4, 177.9 (4C); HRMS (ESI) m/z calcd for $C_{26}H_{39}N_7O_{10}S_2Na$ [M + Na]+ 696.2089, found 696.2092.

Compound (39). Prepared following GP2, using the derivative **36** (150 mg, 0.107 mmol) as starting materials, the derivative **39** (64 mg, 0.095 mmol, 89%) was obtained after purification by silica gel column chromatography (AcOEt/MeOH, 70:30 as eluent) as an amorphous white solid. $[\alpha]_D^{20}$ = +13 (c = 0.7, MeOH); ^{1}H NMR (400 MHz, D_2O) δ = 2.45 (3H, s, methylthiazol), 3.63-3.86 (12H, m), 3.99 (2H, q, J = 3.2 Hz), 4.19 (1H, m, H-1), 4.32 (1H, m, H-1'), 4.67 (1H, dd, J6a,6b = 15.0 Hz, J6a,5 = 3.6 Hz, H-6a), 4.74 (2H, s, CH2-triazol), 4.89 (1H, dd, J6b,6a = 15.0 Hz, J6b,5 = 3.6 Hz, H-6b), 7.95 (1H, s, triazol), 8.16 (1H, s, thiazol); ^{13}C NMR (127 MHz, D_2O): δ = 15.1 (CH_3, methylthiazol), 40.9 (CH_2, CH_2-triazol), 44.3 (CH_2, C-7'), 48.3 (CH_2, C-7), 60.6, 60.7 (2CH2, C-6, C-6'), 67.3, 67.4, 68.5, 68.6, 70.63, 70.66, 74.7, 75.2, 75.5, 76.0 (10CH), 115.1 (C), 125.4 (CH, triazol), 126.6, 141.5, 143.7 (3C), 149.4 (CH, thiazol), 169.3, 173.6, 176.3 (3C); HRMS (ESI) m/z calcd for $C_{25}H_{36}N_7O_{11}S_2$ [M + H]+ 674.1914, found 674.1921.

Compound (40). According to the general procedure GP2, using **37** (30 mg, 0.0114 mmol) as starting materials, the derivative **40** (18 mg, 0.0111 mmol, 98%) was obtained after lyophilization. $[\alpha]_D^{20}$ = +71 (c = 0.3, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.55 (3H, s, methylthiazol), 3.51-5.38 (59H, m), 5.64 (1H, d, J = 4.0 Hz, H-1 CD), 6.42 (1H, bs, NH), 7.12 (1H, bs, NH), 7.69 (1H, s, triazol), 7.84 (1H, s, thiazol); HRMS (ESI) m/z calcd for $C_{60}H_{91}N_7O_{40}S_2Na$ [M + Na]+ 1636.4635, found 1636.4590.

Compound (42). To a solution of mannosyl alkyne **17** (40 mg, 0.0642 mmol) and azidotetraethyleneglycolmethanesulfonate **41** (23 mg, 0.0770 mmol) in a mixture 3:1 of dioxane-H_2O (2.6 mL) were added CuSO4 (2 mg, 0.013 mmol) and sodium ascorbate (5 mg, 0.026 mmol) and the mixture was warmed to 70° C. After 3 h, the mixture was concentrated and the crude was purified by silica gel column chromatography (AcOEt → AcOEt /MeOH: 90/10 as eluent) to give the triazol **42** (58 mg, 0.0630 mmol, 98%) as a colorless oil. $[\alpha]_D^{20}$ = +51 (c = 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.05 (3H, s, AcO), 2.07 (3H, s, AcO), 2.09 (3H, s, AcO), 2.10 (3H, s, AcO), 2.56 (3H, s, methylthiazol), 3.04 (3H, s, MsO), 3.55-3.66 (12H, m, H-7a, H-7b, TEG) 3.74 (1H, m), 3.86 (2H, t, J = 4.9 Hz), 4.01-4.12 (2H, m, H-5, H-6a), 4.30 (1H, m, H-1), 4.35 (2H, m), 4.53 (2H, t, J = 4.9 Hz), 4.62 (1H, m, H-6b), 4.70 (2H, s, CH_2-triazol), 5.04 (1H, dd, J4,3 = 6.0 Hz, J4,5 = 4.5 Hz, H-4), 5.17 (1H, dd, J2,1 = 6.7 Hz, J2,3 = 3.2 Hz, H-2), 5.31 (1H, dd, J3,4 = 6.1 Hz, J3,2 = 3.3 Hz, H-3), 7.42 (1H, bs, NH), 7.81 (1H, s, thiazol), 7.89 (1H, s, triazol); ^{13}C NMR (127 MHz, $CDCl_3$): δ = 18.6 (CH_3, methylthiazol), 20.69 (CH3, AcO), 20.76 (2CH3, 2 x AcO), 20.81 (CH_3, AcO), 37.6 (CH_3, MsO), 39.9 (CH_2, CH_2-triazol), 45.6 (CH_2, C-7), 50.4 (CH_2), 60.9 (CH_2, C-6), 67.3 (CH, C-4), 67.6 (CH, C-3), 67.7 (CH, C-2), 68.9, 69.2, 69.3 (3CH_2), 69.5 (CH, C-1), 70.39, 70.42, 70.5, 70.6 (4CH2), 73.0 (CH, C-5), 115.7 (C), 123.4 (CH, triazol), 130.8 (C), 131.4 (C), 143.5 (C, triazol), 145.6 (CH, thiazol), 159.2 (C), 169.3, 169.6, 169.8, 170.9 (4C, 4 x AcO), 174.2 (C), 175.9 (C); HRMS (ESI) m/z calcd for $C_{35}H_{50}BrN_7O_{16}S_3$ [M + H]+ 920.2471, found 920.2460.

Compound (43). According to the general procedure GP3, using the methanesulfonate **42** (39 mg, 0.0424 mmol) as starting material, the deprotected derivative was obtained. The crude was dissolved in a mixture 3:1 of AcOEt-MeOH (3 mL) and DABCO (47 mg, 0.424 mmol) was added. After 4 h, the mixture was concentrated and washed with Et_2O (2 mL x 4). The derivative **43** was obtained after lyophilization (33 mg, 0.038 mmol, 90%) as a colorless oil. $[\alpha]_D^{20}$ = +55 (c = 0.8, MeOH); ^{1}H NMR (400 MHz, D_2O) δ = 2.31 (3H, s, methylthiazol), 2.77 (3H, s, MsO), 3.13 (6H, bt, J = 7.4 Hz, DABCO), 3.41 (6H, bt, J = 7.4 Hz, DABCO), 3.44-3.45 (16H), 3.89 (2H, t, J = 4.9 Hz), 3.95 (1H, t, J = 2.9 Hz, H-2), 4.12 (1H, m, H-1), 4.51 (2H, s, CH_2-triazol), 4.57 (2H, bt, J = 4.7 Hz), 7.60 (1H, s, thiazol), 8.03 (1H, s, triazol); ^{13}C NMR (127 MHz, D_2O): δ = 18.1 (CH3, methylthiazol), 38.4 (CH_3, MsO), 42.8 (CH_2, C-7), 44.1 (3CH_2, DABCO), 50.5 (CH2), 52.9 (3CH_2, DABCO), 60.8 (CH_2, C-6), 63.2, 63.6 (2CH_2), 67.2, 68.7, 68.9 (3CH), 69.4-70.7 (6CH_2), 75.0 (CH), 75.4 (CH, C-1), 114.9 (C), 124.7 (CH, triazol), 128.6 (C), 143.5 (C, triazol), 146.4 (CH, thiazol), 160.9, 170.9, 173.8, 176.1 (4C); HRMS

(ESI) m/z calcd for $C_{32}H_{50}N_9O_9S_2$ [M – MsO]+ 768.3155, found 768.3167.

Co-crystallization. Co-crystals were obtained by the vapor diffusion method and 1.0 M lithium sulfate, 0.1 M Tris-HCl at pH 8.5 and 0.01 M nickel (II) chloride as precipitant similar to a previously published protocol.[8] FimH was concentrated to 17.3 mg.ml^{-1} and 1 mM of compound **2** was added prior to a 1:1 mix with the precipitant into 1 µl hanging drops.

Enzyme-Linked Lectinosorbent Assay (ELLSA). Immunosorbent microplates (Nunc, Maxisorp) were coated with 100 µL of RNase B (5 mg/mL) in 100 mM carbonate/bicarbonate buffer, pH 9.6. Plates were incubated at 4°C overnight and then washed (300 µL/well) three times with 10 mM phosphate-buffered saline (PBS) containing 0.15% Tween-20 (PBST). All wells were blocked with 200µL 3% bovine serum albumin (BSA) in PBST and incubated for 2 h at 37°C. They were then washed three times with PBST. Mannosides were dissolved in PBST at the necessary concentrations and added to the microwells. FimH was diluted in PBST to 0.07µM, added to each plate well and incubated for 1h at room temperature. Wells were washed three times with PBST and incubated with 100 µL of rabbit-antiFimH antibodies IgG (aFimH) diluted 1:5000 in PBST for 1 h at room temperature. Then wells were washed three times with PBST, and incubated with 100 µL of goat anti-rabbit HRP-labeled secondary antibody (Enzo Life Sciences (2ndAb-HRP)) diluted 1:10000 in PBST for 1 h at room temperature. The wells were washed three times with PBST and 100 µL of 3,3′ ,5,5′ - tetramethylbenzidine (TMB) was added to each well and incubated in darkness for 5-15 min. The reaction was stopped with 100 µL/well of 1N sulfuric acid. Plate absorbance was analyzed at 450 nm using a microplate reader BioTekELx800.

Adhesion assays of AIEC LF82 on intestinal cells: *E. coli* strain LF82 isolated from an ileal biopsy of a CD patient was used as the AIEC reference strain. Bacteria were grown overnight at 37 °C in Luria−Bertani (LB) broth and a bacterial suspension was prepared at a concentration of 1.5×10^8 bacteria/mL in phosphate buffer saline (PBS) for adhesion assays. The human intestinal cell line T84, purchased from the American Type Culture Collection (ATCC, CCL-248), was maintained in an atmosphere containing 5% CO_2 at 37 °C in the culture medium recommended by ATCC. T84 cells were seeded in 48-well tissue culture plates at a density of 1.5×10^5 cells/well and incubated at 37 °C for 48 h.

Pre-incubation: Before infection, cells were washed with PBS and incubated for 1 h with TazMans at a final concentration of 10 µM (or 0.01 to 100 µM for the dose-effect experiment). Epithelial cells were then infected in the presence of inhibitory compounds with the AIEC reference strain LF82 for 3 h at a multiplicity of infection (MOI) of 10 bacteria per cell (1.5×10^6 bacteria/well). Monolayers were washed three times with PBS and lysed with 1% Triton X-100 (Sigma) in deionized water. Samples were diluted and plated onto LB agar plates to determine the number of colony-forming units (CFU).

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Keywords: Crohn's disease • FimH antagonist • C-Mannosides • Anti-adhesive therapy • Microencapsulation

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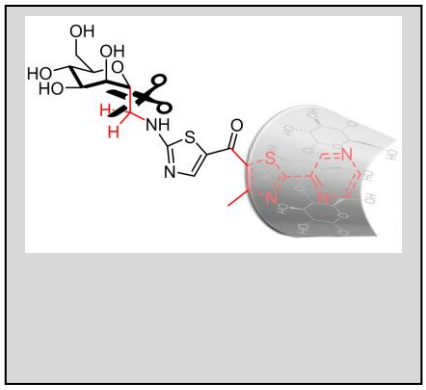

We developed a new generation of potent anti-adhesives of pathogenic *E. coli* strains promoting the gut inflammation in Crohn's disease. The neo-thiazolylmannosides were homologated by a methyl group and included in a γ-cyclodextrin to form a water soluble NeoTazMan@γCD complex with retained anti-adhesive effect against *E.coli*.