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Aromatic thioglycoside inhibitors against the virulence factor LecA from *Pseudomonas aeruginosa*†

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Three small families of hydrolytically stable thioaryl glycosides were prepared as inhibitors of the LecA (PA-IL) virulence factor corresponding to the carbohydrate binding lectin from the bacterial pathogen *Pseudomonas aeruginosa*. The monosaccharidic arylthio β-D-galactopyranosides served as a common template for the major series that was also substituted at the *O*-3 position. Arylthio disaccharides from lactose and from melibiose constituted the other two series members. In spite of the fact that the natural ligand for LecA is a glycolipid of the globotriaosylceramide having an α-D-galactopyranoside epitope, this study illustrated that the β-D-galactopyranoside configuration having a hydrophobic aglycon could override the requirement toward the anomeric configuration of the natural sugar. The enzyme linked lectin assay together with isothermal titration microcalorimetry established that naphthyl 1-thio-β-D-galactopyranoside (**11**) gave the best inhibition with an IC_{50} twenty-three times better than that of the reference methyl α-D-galactopyranoside. In addition it showed a K_D of 6.3 μM which was ten times better than that of the reference compound. The X-ray crystal structure of LecA with **11** was also obtained.

Introduction

Infection by pathogens is generally initiated by crucial steps of recognition and adhesion on host epithelial surfaces. Very frequently, the strategy used by micro-organisms involves binding to host glycoconjugates by sugar-binding proteins, lectins, which are specific for the target tissue. The dependence between pathogen receptors and host glycans leads to the concept of "glycoecology".[1] *Pseudomonas aeruginosa* is an opportunistic pathogen implicated in the development of lung infections in cystic fibrosis (CF) patients through these sugar–protein binding. These infections are triggered by the abnormal abundance of mucus in CF patients, thus offering an accumulation of anchoring sites for bacteria. *Pseudomonas aeruginosa*'s mechanism of action is managed by the adhesion of proteins called lectins LecA (PA-IL) and LecB (PA-IIL) specific to the galactose and fucose subunit, respectively.[2–4]

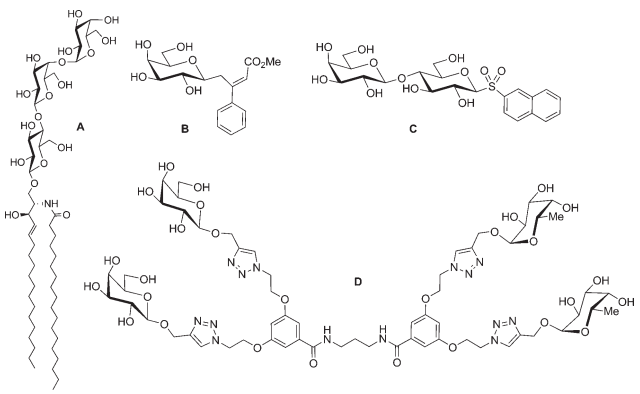

Fig. 1 Structure of selected *P. aeruginosa* LecA inhibitors. (A) Natural ligand: globotriaosylceramide (αGal(1–4)βGal(1–4)βGlc-Cer);[15] (B) synthetic C-galactopyranoside;[8] (C) naphthyl sulfone lactoside;[20] and (D) α-L-fucoside and β-D-galactopyranoside dendrimer hybrid[21] allowing the inhibition of both LecA and LecB.

LecA, the galactoside-binding lectin, has been demonstrated to be involved in adhesion to lung tissues and leads to alveolar destruction during the first steps of bacterial infection in a mice model,[5] and treatment with galactose or *N*-acetylgalactosamine (GalNAc) has a protective effect. Therefore, the design and synthesis of galactose-based inhibitors for LecA is an attractive strategy for new anti-infectious compounds. Several LecA inhibitors have been reported in the literature[6–14] and some of them are displayed in Fig. 1, but none of them

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have given rise to a well-established treatment against nosocomial infections.

The lectin crystal structure is tetrameric and one calcium ion is present in each binding site.[2] The natural ligand of LecA in the lung has been proposed to be glycolipids terminated with the αGal(1–4)Gal epitope (globotriaosylceramide).[15] Indeed, the lectin has a strong specificity for αGal terminating oligosaccharides and glycoconjugates, while lactose or other βGal oligosaccharides are not bound. However, β-D-galactopyranosides possessing an aromatic aglycon were reported to be efficient ligands for LecA.[16] The affinity of LecA for *p*-nitrophenyl β-D-galactopyranoside (PNP-β-Gal, K_d = 14 μM)[12] or a larger *O*-phenyl derivative (K_d = 6 μM)[12] is stronger than that for αMeGal or αGal disaccharides (50 to 100 μM).[15] The recent crystal structure of the LecA/PNP-β-Gal complex demonstrated the stabilizing interaction between the aromatic ring and a histidine residue.[10] It is therefore of interest to further investigate the role played by hydrophobic aglycones together with anomeric configurations in binding to LecA.

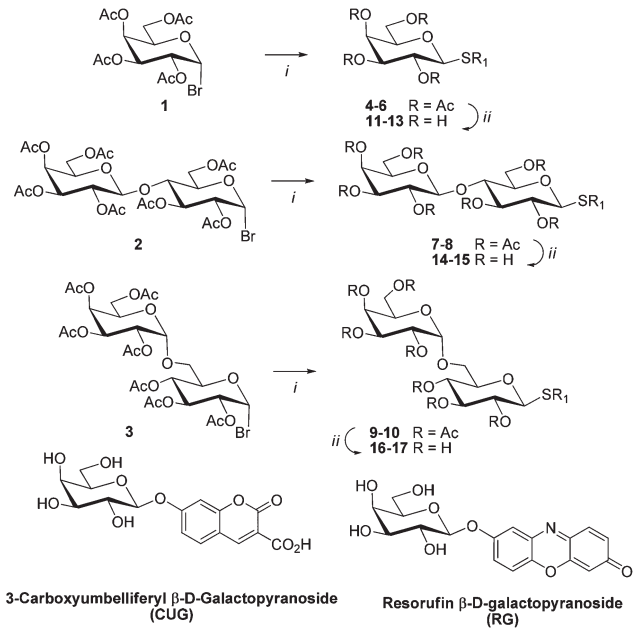
To this end, a series of galactopyranoside (β-D-Gal), lactoside (β-D-Gal(1–4)β-D-Glc), and melibioside (α-D-Gal(1–6)β-D-Glc) derivatives was prepared. These derivatives were synthesized keeping in mind their potential usage as therapeutic agents and were thus prepared as hydrolytically stable *S*-glycosides. Additionally, given the possible common binding modes between the *P. aeruginosa* bacterial lectin LecA and the family of galectins toward aryl β-D-galactopyranosides,[17–19] we included the syntheses of 3-*O*-substituted analogs, a modification that was beneficial for the human galectin series.[20]

Results and discussion

Chemistry

Previous studies by our group[18–20] and others[22] have uncovered that *S*-aryl galactopyranosides were potent inhibitors for human galectins. Moreover, recent biological evaluations have proven that the use of *S*-naphthyl group at the anomeric position led to positive adhesion of the inhibitors to the target lectin.[20] Synthesis of a family of inhibitors based on the *S*-naphthyl moiety could be easily achievable through the phase transfer catalysis (PTC) reaction developed in our research laboratories.[23,24] As an initial step towards the synthesis of potent LecA inhibitors, we focused our work on the syntheses of two families of inhibitors. The first family relied on modifications to the aglyconic part of the three sugars discussed above using the PTC reaction. The second family was focusing on the functionalization at the *O*-3 position of the glycosides using the Sonogashira and Glaser–Hay coupling reactions. The purpose of the second library family was to narrow the complexity of the oligosaccharide moiety by keeping only the key galactopyranoside in direct contact with the LecA receptors. These modifications were encouraged by the modeling analysis carried out on the naphthylsulfonyl lactoside **C** described in Fig. 1.

The stepwise synthesis of the targeted three thioglycoside families is described in Scheme 1. It started with a phase



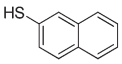
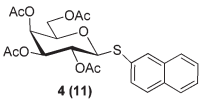
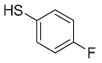
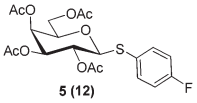
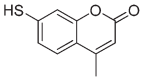
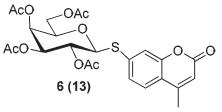
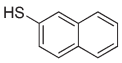
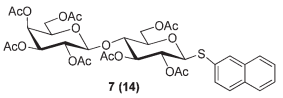
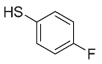
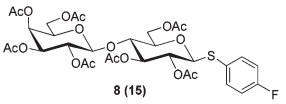
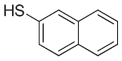
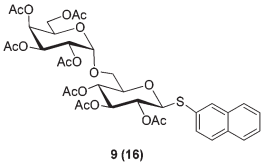
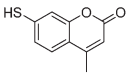
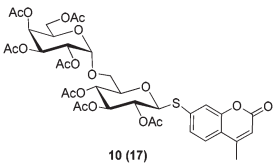
Scheme 1 Syntheses of three families of thioglycosides under PTC conditions. *Reagents and conditions*: (i) HSR_1 (see Table 1 for details), TBAHS, Na_2CO_3 1.0 M, EtOAc, 12 h, r.t. (ii) NaOMe 1.0 M, MeOH, o.n., r.t.

transfer catalyzed (PTC) reaction on the readily and commercially available glycosyl bromides **1–3.** The desired arylthio glycosides were all obtained in good to excellent yields (Table 1). The mild conditions and complete anomeric stereoselectivity of the PTC reaction gave access to pure β-anomers without having to use various protecting groups or harsher reaction conditions, such as Lewis acid catalyzed conditions that might damage the anomeric integrity of the desired thioglycosides.[25]

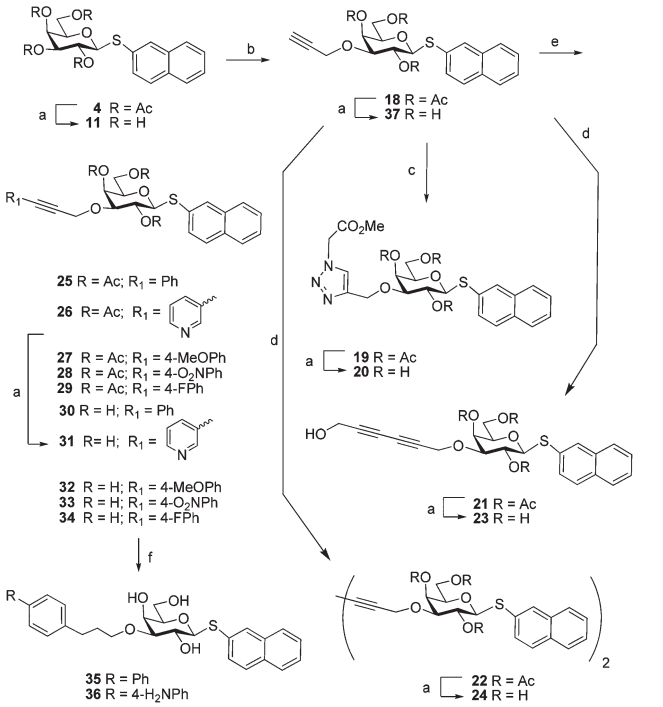
The anomeric configurations of all molecules have been proven by ^{1}H NMR and correlation experiments (COSY) as all $J_{H1,2}$ coupling constants were in the range of 8–10 Hz, thus establishing the 1,2-*trans* relationship at the anomeric centers. For each of the three family members **1–3** (galactoside (**1**), lactoside (**2**), and melibioside (**3**)), 2-naphthylthiol, 4-fluorobenzenethiol, and 7-mercapto-4-methylumbelliferone were used to afford thioglycosides **4–6**, **7–8**, and **9–10**, respectively (Scheme 1, Table 1). For comparison purposes, the commercially available fluorogenic substrates 3-carboxyumbelliferyl β-D-galactopyranoside (**CUG**) and resorufin β-D-galactopyranoside (**RG**) (Scheme 1) were added to the panel of thioglycosides.

Based on previous biological data[15] and on molecular modeling, the second family of analogs was derived from naphthyl 1-thio-β-D-galactopyranoside (**4**) (Scheme 2). The main purpose of this series was to further functionalize the *O*-3 position to increase potential binding interactions between the sugar inhibitor and the receptor by taking advantage of additional contacts from the lectin's binding site. This series of inhibitors was synthesized using three different routes starting from a regiochemically installed 3-*O*-propargyl ether on **4** to afford intermediate **18** using tin chemistry. The common intermediate **18** was prepared from compound **4** by classical Zemplén

Table 1 Thioglycosides as potential inhibitors of LecA synthesized by PTC reaction

Entry	R_1	Product[a]	Yield[b,c] (%)
1		 **4 (11)**	91 (88)
2		 **5 (12)**	88 (>95)
3		 **6 (13)**	94 (88)
4		 **7 (14)**	85 (86)
5		 **8 (15)**	51 (90)
6		 **9 (16)**	67 (92)
7		 **10 (17)**	56 (>95)

[a] Numbers in parentheses correspond to de-*O*-acetylated products. [b] PTC reaction yields. [c] Deprotection yields are shown in parentheses.



Scheme 2 Functionalization on the *O*-3 position of 2-naphthyl 1-thio-β-D-galactopyranoside. *Reagents and conditions*: (a) NaOMe 1.0 M, MeOH, 24 h, r.t.; (b) (i) Bu_2SnO, MeOH, reflux, 3 h, (ii) TBAI, propargyl bromide, dioxane, reflux 3 h, (iii) Ac_2O, pyridine, 24 h, r.t.; (c) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF–H_2O, r.t.; (d) propargyl alcohol, CuCl, TMEDA, DMF, O_2, 40 °C; (e) Ar–I, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 3 h, r.t.; (f) Pd/C, H_2, MeOH, 5 h, r.t.

de-*O*-acetylation (NaOMe, MeOH) to provide unprotected thiogalactoside **11** in 88% yield. Compound **11** was then treated with dibutyltin oxide in methanol under reflux to give the intermediate tin ketal (not isolated) which was then heated under reflux in the presence of propargyl bromide and tetrabutylammonium iodide in dioxane. The resulting crude product was peracetylated in pyridine and acetic anhydride to afford key intermediate **18** in 88% yield over three steps.

Then, "Click Chemistry"[26] was used in order to attach a triazole moiety at the *O*-3 position of the galactoside **18** to provide derivative **19**. For this, compound **18** was treated with methyl azidoacetate under standard conditions ($CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF–H_2O, r.t.) to give the expected adduct **19** in 68% yield which upon deprotection under Zemplén conditions (NaOMe, MeOH) gave compound **20**. The low yield (13%) for this simple deprotection may be accounted for by the presence of traces of water in the methanolic solution, hence providing the corresponding acid directly (*vide infra*).

Secondly, by sequentially using Glaser–Hay[27] alkyne oxidative homo- and cross-coupling of **18** with propargyl alcohol, heterodimer **21** followed by homodimer **22** were prepared. Toward this second series, propargylic ether **18** was treated with propargyl alcohol under Glaser–Hay coupling conditions (CuCl, TMEDA, DMF, O_2, 40 °C)[29] to provide **21** in 57% yield. The homodimer was similarly obtained under the above conditions (without propargylic alcohol) to give compound **22** in 77% yield.

The third series was prepared using palladium catalyzed Sonogashira[28] cross coupling with various aryl groups at the propargylic carbon after which intermediates **30–36** could be obtained in good yields after deprotection. To this end, compound **18** was subjected to the usual coupling conditions with diverse aryl iodides ($Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 3 h, r.t.) to afford compounds **25–29** in yields ranging from 54 to 77%. Of these, compounds **30** and **33** were subjected to hydrogenation under a hydrogen atmosphere to provide analogs **35** and **36** in order to give further flexibility to the side chain of the newly formed molecules (Scheme 2).

Lectin inhibition

The ability of the molecules described above to inhibit the binding of LecA lectin to a galactosylated surface was determined by an enzyme-linked lectin assay (ELLA) using its biotinylated form. Related IC_{50} values are listed in Table 2

Table 2 Inhibition of LecA binding to polymeric **α-Me-Gal** tested by ELLA. Methyl α-D-galactopyranoside was used as the reference to provide the relative inhibitory potency (RIP)

Inhibitor[a]	Mean IC_{50} (μM)	RIP
α-MeGal	70	1
11	3	23
12	20	3.5
13	5	14
14	1490	0.05
16	170	0.4
17	50	1.4
23	380	0.18
24	190	0.37
30	1490	0.05
31	1450	0.05
32	1670	0.04
33	810	0.09
34	1520	0.05
35	1510	0.05
37	1670	0.04

[a] Thioaryl aglycones alone and compounds **15**, **20**, and **36** gave no inhibition at the tested concentration.

together with inhibitory potency relative to αMeGal. When looking at disaccharides, lactose-based compound (**14**) was not a good inhibitor, while melibiose-containing molecules (**16** and **17**) with αGal(1–6)Glc linkage were slightly better than the monosaccharide. These data are in good agreement with a previous analysis of specificity toward LecA performed by inhibition of hemaglutination[30] or by a glycan array analysis.[15] LecA is indeed described as an αGal binding lectin with a preference for 1–6, 1–4, and 1–3 linkages. Our present results indicate that in most cases, LecA binding to these disaccharides was not affected by the presence of a large aromatic group at the anomeric position. Substitutions on position 3 of the galactose ring also showed a negative effect on the relative inhibitory potency. Only compound **24** with two galactose rings bridged by their *O*3 oxygen retained some activity, albeit lower than the monosaccharide standard. All β-D-galactopyranoside derivatives with an aromatic ring at the anomeric position were good inhibitors, the best one being compound **11** with an IC_{50} of 3 μM, a 23-fold enhancement over the reference αMeGal. Compound **13**, which contains a large thiocoumaryl group at C1, had a similar inhibitory potency (5 μM, 14-fold enhancement). These results confirm the previous observation that galactose derivatives with aromatic groups in the β-configuration represent very efficient ligands toward LecA.[12,16]

Isothermal titration calorimetry

In order to evaluate the affinity of the best compounds in solution, titration microcalorimetry experiments (ITC) were run on the highest affinity monosaccharide derivatives **11** and **13** and disaccharide derivative **17**. A typical thermogram of LecA interacting with **11** is given in Fig. 2. An analysis of the K_d values indicated that both compounds **11** and **13** were high affinity ligands for LecA with dissociation constants of 5 to 6 μM. This is 10 times better than the affinity observed for galactose, methyl galactoside or digalactoside.[15,31]

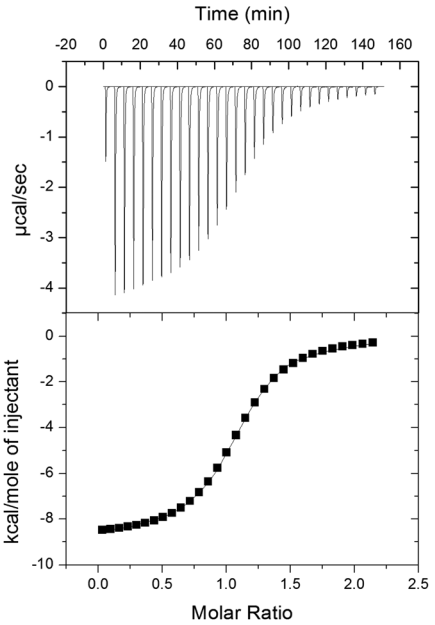


Fig. 2 Microcalorimetry data. The ITC plot (measured by VP-ITC, Microcal) obtained for the titration of LecA with compound **11**. The plot in the lower panel shows the total heat released as a function of total ligand concentration for the titration shown in the upper panel. The solid line represents the best least-square fit to experimental data using a one site model.

Interestingly, while compounds **11** and **13** were rather similar in terms of aglycon, and have close affinity for LecA, the contributions to the thermodynamics of binding were different. In both cases the interaction was driven by enthalpy, but the entropy cost of binding was significantly higher for **13** ($-T\Delta S$ = 17.1 kJ mol^{-1}) than for **11** ($-T\Delta S$ = 3.8 kJ mol^{-1}). This could be correlated to the presence of oxygen atoms in the aglycon of compound **13** that could act as hydrogen bond acceptors and therefore stabilize the conformation of some protein side chains. Commercial compounds with the aromatic group in β-configuration of galactose such as *p*-nitrophenyl β-D-galactopyranoside (**βPNPGal**), 3-carboxyumbelliferyl β-D-galactopyranoside (**CUG**) and resorufin β-D-galactopyranoside (**RG**) were also tested (Table 3). All were good ligands for LecA, although the observed dissociation constants were

Table 3 Microcalorimetry data for higher affinity compounds binding to LecA. The experiments were realized in duplicate at 298 K unless otherwise stated

Ligand	K_D (μM)	$-\Delta G$ (kJ mol^{-1})	$-\Delta H$ (kJ mol^{-1})	$T\Delta S$ (kJ mol^{-1})
αMeGal	50.0 ± 0.7	24.6	40.9 ± 0.3	16.3
βMeGal	55.7 ± 3.6	24.3	19.0 ± 0.5	−5.3
βPNPGal	26.1 ± 0.4	26.2	44.6 ± 0.3	−18.4
11	6.3 ± 0.4	29.7	33.5 ± 3.3	−3.8
13	5.4 ± 0.3	30.1	47.2 ± 1.5	−17.1
17	19.6 ± 0.3	26.9	33.4 ± 0.3	−6.5
CUG[a]	11.4[c]	28.2	37.4	−9.2
RG[b]	7.3[c]	29.3	28.5	−0.2

[a] **CUG:** 3-carboxyumbelliferyl β-D-galactopyranoside. [b] **RG:** resorufin β-D-galactopyranoside. [c] Only one experiment.

weaker than those obtained with compounds **11** and **13**. The melibioside-based compound **17** was slightly less efficient with a K_D of 19 μM.

X-Ray data

A crystal of LecA complexed with compound **11** was obtained by co-crystallization, and diffraction data were collected at a resolution of 2.15 Å (Table S1†). The asymmetric unit contained one tetramer of LecA together with four calcium ions, four molecules of **11** and 235 water molecules (Fig. 3). Each monomer of LecA adopts the β-sandwich fold that was described previously.[2] The tetrameric association is the same as the one described in previous crystal structures.[2,12,15,31] The overall structure of the lectin will therefore not be further described herein. Each binding site contains one calcium ion and one ligand that coordinates the calcium ion through oxygens *O*3 and *O*4, and establishes hydrogen bonds between the protein and all its hydroxyl groups as described in Fig. 4. One water molecule bridges hydroxyl *O*6 to the main chain and its importance in maintaining the *O*6 conformation and in the binding energy has been previously emphasized.[31]

While the galactose moiety was bound in the same orientation in the four sites of the tetramer, the naphthyl moiety displayed two different conformations in sites A and D in comparison to sites B and C. As displayed in Fig. 4E, the aromatic ring was much closer to the protein surface in chain B than in chain A. Such an orthogonal interaction between the aromatic ring of the aglycon and His50 has been observed in the crystal structure of LecA complexed with *p*-nitrophenyl β-galactopyranoside and has been described as T-stack interaction.[12] In chains B and C, a distance of 3.1 Å is measured between Cε1 of His50 and the center of mass of the first 6-member ring, which is shorter than that in chains A and D (3.8 Å).

The difference in the naphthyl group orientation between chain A/D and chain B/C is due to changes in both Φ and Ψ torsion angles at the glycosidic linkage (Φ = −84.0 ± 3.3°, Ψ = −166.6 ± 0.4° for chain A/D and Φ = −54.6 ± 6.3°, Ψ = +170.5 ± 0.1° for chain B/C). This change in the ligand conformation was correlated to a change in orientation of the Gln53 side chain. In chains B and C, the Gln53 side chain is oriented towards the ligand and establishes a hydrogen bond with the *O*6 hydroxyl of galactose, while in chains A and D, it is oriented towards the solvent. In contrast, His50 does not adopt different conformations in the different chains and the hydrogen bond between the His50 Nε2 atom and *O*6 of galactose is maintained. Comparison with the crystal structure of the LecA/PNPGal complex (PDB code 3ZYF)[12] indicates that the naphthyl group in chains B and C comes closer to the protein surface than the phenyl group in the PNP-Gal complex (a distance of 3.5 Å between Cε1 of His50 and the center of the aromatic group). As shown in Fig. 4F, this is due to the difference of conformation for the Φ torsion angle of the glycosidic linkage (Φ = −81.6 ± 3.8°, Ψ = +168.9 ± 6.0° for PNP-Gal), and also to the smaller value of the C–S–C valence angle (Θ = 104.3 ± 1.6°) compared to the C–O–C one (Θ = 109.7 ± 1.3°).

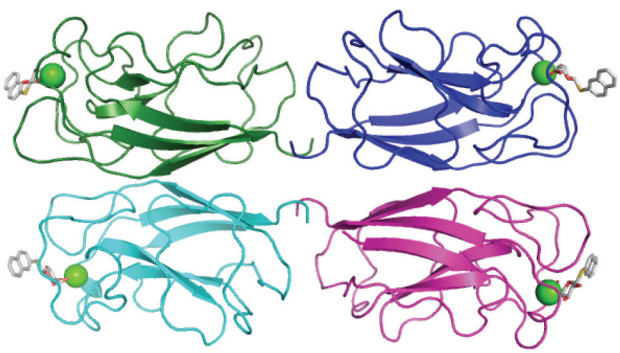


Fig. 3 Crystal structure of LecA complexed with compound **11**. Each protein chain is represented by a ribbon with different colors. Calcium ions are depicted by green spheres and the ligand by sticks.

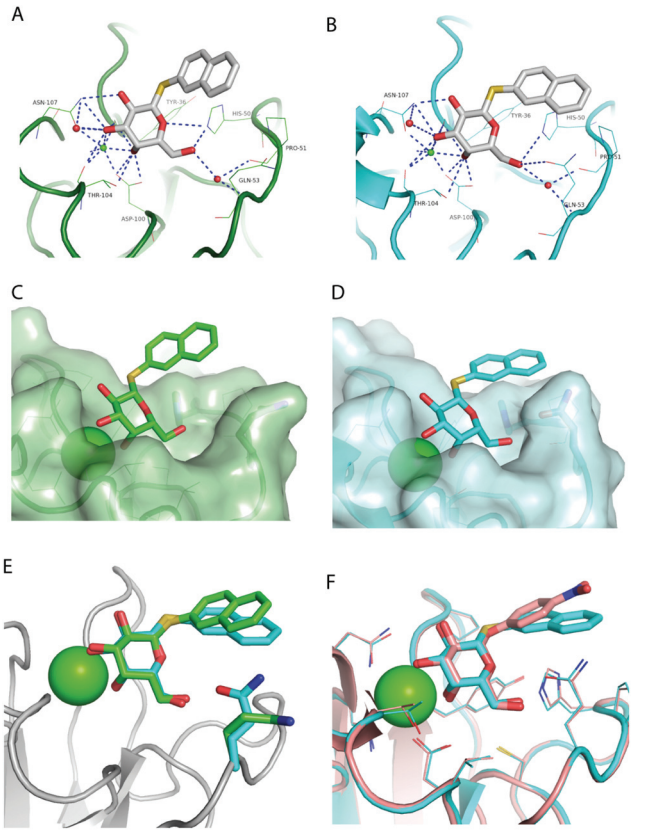


Fig. 4 Comparison of the two binding modes of **11** by LecA. A and B: compound **11** observed in the binding site of chains A and B of LecA respectively with a hydrogen bond network. C and D: the same molecules with the representation of the accessible surface for the protein. E: superimposition of the binding sites of chain A (green) and chain B (blue) with the representation of the two different conformations of Gln53. F: superimposition of the binding sites of LecA complexed with **11** (chain B) and PNP-Gal (PDB code 3ZYF).

Conclusions

The search for potent inhibitors of *P. aeruginosa* adhesion to human tissue is an active field of research. Most synthesized compounds that target LecA efficiently are multimeric,[32–36]

taking advantage of the closely located four galactose binding sites on the lectin.[37] Such compounds display high avidity for the lectins but they should result in lectin-mediated aggregation of bacteria. The resulting formation of microcolonies may be counterproductive in terms of antimicrobial strategy. Therefore, the design and synthesis of a monovalent and specific LecA ligand is a target of paramount interest. The aromatic thioglycosides described herein constitute an innovative class of inhibitors for LecA that present two advantages: the high affinity (low micromolar range) provided by the aromatic aglycon and the chemical stability in biological medium provided by the glycosidically stable thio linkage. This illustrates the strength of using intensive QSAR in glycobiology in the search for potent glycomimetic therapeutics, as we demonstrated previously for LecB. A complex trisaccharide used as a natural ligand could be replaced by a simple monosaccharide derivative.[38–40] In addition, the present investigation opens the door to seriously reconsideration of the limitations that the community has imposed on itself to strictly obey the anomeric linkage observed for naturally occurring oligosaccharide ligands while typical medicinal chemistry pharmacophores have the potential to overcome such criteria. During the creation of this manuscript, further work was published.[47]

Experimental section

General methods

All reactions in organic media were carried out under a nitrogen atmosphere using freshly distilled solvents. After work-up, organic phases were dried over anhydrous Na_2SO_4. The evolution of reactions was monitored by analytical thin-layer chromatography using silica gel 60 F254 precoated plates (E. Merck). Purifications by column chromatography were performed using silica gel 60 (40–63 μm) with the indicated eluent. Optical rotations were measured using a JASCO P-1010 polarimeter. Melting points were measured using a Fisher Jones apparatus. Roman numerals in ascending order are given to the residues from the reducing end. NMR spectra were recorded using Varian Gemini 300 and Gemini 500 spectrometers. Proton and carbon chemical shifts (δ) are reported in ppm downfield from TMS and/or with the internal reference of residual solvents. Coupling constants (J) are reported in hertz (Hz), and the following abbreviations are used: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), and broad (b). Analysis and assignments were made using COSY, DEPT, and HSQC experiments. Low-resolution (MS) and high-resolution mass spectra (HRMS) were recorded by Dr Alexandra Furtos and Karine Venne (Mass Spectrometry Laboratory, Université de Montréal, Québec, Canada).

Material. 3-Carboxyumbelliferyl β-D-galactopyranoside (**CUG**) and resorufin β-D-galactopyranoside (**RG**) were purchased from Marker Gene Technologies (Eugene, OR).

Chemistry

General procedure for phase transfer catalyzed reaction (Method A). The glycosyl bromides (1.0 eq.) were dissolved in EtOAc (1 mL per 100 mg of compound). Then, the nucleophile (2.0 eq.) was added, followed by TBAHS (1.5 eq.). Finally, the 1.0 M sodium carbonate solution (1 mL per 100 mg) was added to the reaction. The yellowish mixture was stirred for 24 h at r.t. Upon reaction completion, the reaction mixture was diluted with EtOAc (50 mL), washed twice with a saturated solution of sodium carbonate, washed once with brine, dried on anhydrous magnesium sulfate, filtered and concentrated. The crude oil was purified by silica gel chromatography (3/7 EtOAc and hexanes as the eluent) to provide the desired product.

General procedure for the Zemplén de-O-acetylation (Method B). Acetylated compounds (1.0 eq.) were dissolved in methanol (0.2 M) under a nitrogen atmosphere. Sodium methoxide 1.0 M (0.1 eq.) was added until pH 9. The reaction mixture was stirred for 18 h at r.t. Upon reaction completion, the reaction mixture was neutralized to pH 7 using Dowex 50X8 H^+ resin, filtered and concentrated to obtain the desired product.

General procedure for O-3 selective propargylation (Method C). The thiogalactoside (1.0 eq.) was dissolved in MeOH (0.2 M) under a nitrogen atmosphere. Dibutyltin oxide (1.1 eq.) was added and the reaction was heated to reflux for 3 h. Then, the solvent was removed and the crude product was dissolved in dioxane (0.2 M) under a nitrogen atmosphere. Tetrabutylammonium iodide (0.4 eq.) and propargyl bromide (5.0 eq.) were added to the mixture which was heated to reflux for 5 h. Upon reaction completion, the solvent was removed and the crude material was dissolved in pyridine (0.2 M) under a nitrogen atmosphere. Acetic anhydride (0.2 M) was added and the reaction mixture was stirred at r.t. for 18 h. The reaction mixture was diluted with EtOAc (25 mL) and washed twice with a 1.0 M HCl solution, washed once with brine, dried over anhydrous magnesium sulfate, filtered and concentrated. The crude material was purified by silica gel chromatography using EtOAc and hexanes in a 3/7 ratio as the eluent to obtain the desired product.

General procedure for the click chemistry (Method D). To a solution of **18** (1.0 eq.) in THF (0.2 M) was added copper(II) sulfate pentahydrate (0.4 eq.) dissolved in water (0.2 M). Sodium ascorbate (0.2 eq.) and methyl azidoacetate (2.0 eq.) were then added to the solution. The reaction mixture was stirred for 24 h at room temperature. Once the reaction ended, as judged by tlc, the reaction mixture was diluted with dichloromethane (10 mL) and the solution was washed with 5% EDTA and once with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The crude product was purified by silica gel flash chromatography using a 1/9 (v/v) mixture of methanol and dichloromethane to provide the desired product.

General procedure for the Glaser–Hay coupling (Method E). Propargyl ether **18** (1.0 eq.) and copper(I) chloride (0.3 eq.) were dissolved in DMF (0.2 M). Tetramethylethylenediamine (TMEDA, 0.6 eq.) was added followed by propargyl alcohol (4.0 eq.) (for the heterocoupling leading to **21**). Then, the reaction mixture was stirred for 4 h at 40 °C under a positive pressure of oxygen. Once the reaction has terminated, as

judged by tlc, 10 mL of ethyl acetate and 1.0 mL of hexanes were added. The solution was washed once with a 5% aqueous solution of EDTA, twice with water, and once with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum. The crude product was purified by silica gel flash column chromatography using a 1/1 mixture of ethyl acetate and hexanes to afford the pure product.

General procedure for the Sonogashira coupling (Method F). Dichlorobis(triphenylphosphine)palladium ($Pd(PPh_3)_2Cl_2$, 0.05 eq.), copper(I) iodide (0.025 eq.) and the corresponding aryl iodide (2.0 eq.) were dissolved in DMF (0.2 M) under a nitrogen atmosphere using a pretreatment of 5 min under ultrasound. Propargyl ether **18** (1.0 eq.) was then added, followed by triethylamine (5.0 eq.). The reaction mixture was stirred for 3 h at room temperature. Once the reaction ended, the solvent was evaporated under vacuum and the residue was dissolved in ethyl acetate (10 mL). The solution was washed once with a saturated solution of ammonium chloride, twice with water, and once with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The crude product was purified by silica gel flash column chromatography using a 3/7 (v/v) mixture of ethyl acetate and hexanes to give the series of products **25–29**.

General procedure for the hydrogenolysis (Method G). Compound **30** or **33** (1 eq.) was dissolved in MeOH to which was added 10% palladium on charcoal (10% w/w). Hydrogen gas was bubbled into the reaction mixture until starting materials disappeared as judged by tlc. The reaction mixture was filtered over a celite pad and the filtrate was concentrated under vacuum. The crude products were purified by silica gel flash column chromatography using a mixture of MeOH and DCM (1 : 9).

2-Naphthyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (4): *Method A*: Starting with compound **1** (3.0 g, 7.69 mmoles) gave **5** (3.44 g, 91%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.73–7.78 (m, 3H), 7.53 (d, 1H, J = 8.3 Hz), 7.44–7.46 (m, 1H), 5.38 (bs, 1H, $\boldsymbol{H}_4$), 5.24 (t_{app}, 1H, J = 10.0 Hz, $\boldsymbol{H}_2$), 5.03 (dd, 1H, J = 8.7 Hz, $\boldsymbol{H}_3$), 4.76 (d, 1H, J = 10.0 Hz, $\boldsymbol{H}_1$), 4.15–4.18 (m, 1H, $\boldsymbol{H}_{6a}$), 4.07–4.10 (m, 1H, $\boldsymbol{H}_{6b}$), 3.91 (t_{app}, 1H, J = 6.3 Hz, $\boldsymbol{H}_5$), 2.08 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.1, 169.9, 169.3, 133.3, 132.6, 131.8, 129.6, 129.5, 128.3, 127.6, 127.5, 126.5, 86.5, 74.4, 71.9, 67.2, 61.6, 20.8, 20.6, 20.5. The spectral data corresponded to those reported in the litterature.[41]

4-Fluorophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (5): *Method A*: Starting with compound **1** (150 mg, 0.384 mmoles) gave **6** (155 mg, 88%) as a white foam. $[\alpha]_D^{20}$ = 15.5 (c = 0.8 acetone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.51 (m, 2H), 6.95–7.01 (m, 2H), 5.35 (d, 1H, J = 3.3 Hz, $\boldsymbol{H}_4$), 5.13 (t_{app}, 1H, J = 9.8 Hz, $\boldsymbol{H}_2$), 4.99 (dd, 1H, J = 3.3 Hz, J = 6.6 Hz, $\boldsymbol{H}_3$), 4.56 (d, 1H, J = 9.8 Hz, $\boldsymbol{H}_1$), 4.02–4.17 (m, 2H, $H6_{a,b}$), 3.87 (t_{app}, 1H, J = 6.2 Hz, $\boldsymbol{H}_5$), 2.07 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.8, 170.3, 170.1, 170.0, 170.0, 169.4, 164.7, 161.4, 135.9, 135.8, 116.0, 115.7, 86.3, 74.4, 71.93, 7710, 61.5, 20.8, 20.7, 20.6; ^{19}F NMR (540 MHz, $CDCl_3$) δ −115.5; HRMS (ESI^+) $C_{20}H_{23}FO_9S$: Calcd: 481.0955 $[M + Na]^+$; Found: 481.0950 $[M + Na]^+$.

4-Methylumbellifer-7-yl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (6): *Method A*: Starting with compound **1** (100 mg, 0.256 mmoles) afforded **6** (125 mg, 94%) as a white foam. $[\alpha]_D^{20}$ = −21.3 (c = 0.81 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.57 (m, 2H), 7.28–7.32 (m, 1H), 6.28 (bs, 1H), 5.48 (d, 1H, J = 3.3 Hz, $\boldsymbol{H}_4$), 5.33 (t, 1H, J = 9.9 Hz, $\boldsymbol{H}_2$), 5.11 (dd, 1H, J = 9.9 Hz, J = 3.3 Hz, $\boldsymbol{H}_3$), 4.84 (d, 1H, J = 9.9 Hz, $\boldsymbol{H}_1$), 4.20 (d, 2H, J = 5.9 Hz, $\boldsymbol{H}_{6a+b}$), 4.04 (t, 1H, J = 5.8 Hz, $\boldsymbol{H}_5$), 2.43 (s, 3H, $\boldsymbol{CH}_3$), 2.19 (s, 3H, AcO), 2.13 (s, 3H, AcO), 2.09 (s, 3H, AcO), 1.99 (s, 3H, AcO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.1, 169.9, 169.3, 153.5, 151.8, 138.4, 125.9, 124.5, 118.9, 117.8, 115.0, 85.2, 74.8, 71.7, 67.1, 66.6, 61.8, 20.7 (2×), 20.6, 20.5, 18.5; HRMS (ESI^+) $C_{24}H_{26}O_{11}S$: Calcd: 545.1093 $[M + Na]^+$; Found: 545.1092 $[M + Na]^+$.

2-Naphthyl 2′,3′,4′,6′,2,3,6-hepta-*O*-acetyl-1-thio-β-D-lactopyranoside (7): *Method A*: Starting with compound **2** (2.0 g, 2.94 mmoles) gave **7** (1.94 g, 85%) as a white foam; $[\alpha]_D^{20}$ = −17.2 (c = 1, $CHCl_3$); mp. 126.3–127.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (bs, 1H), 7.69–7.89 (m, 3H), 7.47–7.57 (m, 3H), 5.33 (d, 1H, J = 3.5 Hz), 5.23 (t_{app}, 1H, J = 9.1 Hz), 5.06–5.12 (m, 1H), 4.89–4.97 (m, 2H), 4.74 (d, 1H, J = 9.8 Hz), 4.44 (m, 2H), 4.06–4.13 (m, 3H), 3.82–3.87 (m, 1H), 3.62–3.77 (m, 2H), 2.11–2.14 (m, 6H), 2.01–2.08 (m, 12H), 1.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 170.1, 169.8, 169.7, 169.2, 133.5, 132.9, 130.4, 128.8, 128.5, 127.8, 126.8, 126.7, 101.1, 85.4, 76.8, 76.2, 73.9, 71.1, 70.8, 70.4, 69.2, 66.8, 62.2, 61.0, 20.9, 20.7, 20.6. MS (ESI^+) $C_{36}H_{42}O_{17}S_1$: Calcd: 801.2 $[M + Na]^+$; Found: 801.3 $[M + Na]^+$.

4-Fluorophenyl 2′,3′,4′,6′,2,3,6-hepta-*O*-acetyl-1-thio-β-D-lactopyranoside (8): *Method A*: Starting with compound **2** (150 mg, 0.221 mmoles) gave **9** (83 mg, 51%) as a white foam. $[\alpha]_D^{20}$ = −1.76 (c = 1.4 acetone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.51 (m, 2H), 6.98–7.05 (m, 2H), 5.33 (d, 1H, J = 3.2 Hz, $\boldsymbol{H}_{4gal}$), 5.20 (t, 1H, J = 9.0 Hz, $\boldsymbol{H}_{3glu}$), 5.09 (dd, 1H, J = 7.8 Hz, $\boldsymbol{H}_{2gal}$), 4.94 (dd, 1H, J = 10.4 Hz, J = 3.3 Hz, $\boldsymbol{H}_{3gal}$), 4.82 (t, 1H, J = 9.5 Hz, $\boldsymbol{H}_{2glu}$), 4.51–4.58 (m, 1H, $\boldsymbol{H}_{1glu}$), 4.46 (d, 1H, J = 7.8 Hz, $\boldsymbol{H}_{1gal}$), 4.02–4.16 (m, 4H, $\boldsymbol{H}_{6a+b\ gal}$ + $\boldsymbol{H}_{6a+b\ glu}$), 3.85 (m, 1H, $\boldsymbol{H}_{5gal}$), 3.71 (t, 1H, J = 9.4 Hz, $\boldsymbol{H}_{5glu}$), 3.56–3.63 (m, 1H, $\boldsymbol{H}_{4glu}$), 2.15 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.04 (m, 9H), 1.96 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.7, 170.33, 170.18, 170.10, 169.7, 169.5, 168.9, 136.5, 116.0, 101.0, 85.0, 76.7, 75.9, 73.7, 70.9, 70.6, 70.0, 69.0, 66.5, 61.8, 60.6, 20.83, 20.7 (2×), 20.6, 20.5, 20.4; ^{19}F NMR (540 MHz, $CDCl_3$) δ −115.2; HRMS (ESI^+) $C_{32}H_{39}FO_{17}S$; Calcd: 769.1789 $[M + Na]^+$; Found: 769.1789 $[M + Na]^+$.

2-Naphthyl 2′,3′,4′,6′,2,3,4-hepta-*O*-acetyl-1-thio-β-D-melibiopyranoside (9): *Method A*: Starting with compound **3** (154 mg, 0.220 mmoles) gave **9** (115.4 mg, 67%) as a white foam. $[\alpha]_D^{20}$ = 49.4 (c = 1.1 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.78–7.87 (m, 3H), 7.48–7.56 (m, 3H), 5.23–5.33 (m, 3H), 5.15 (d, 1H, J = 3.5 Hz), 4.98–5.01 (m, 3H), 4.85 (d, 1H, J = 9.8 Hz)′ 4.15 (t_{app}, 1H, J = 6.2 Hz), 3.92–4.04 (m, 2H), 3.71–3.79 (m, 2H), 3.57(d, 1H, J = 8.9 Hz), 2.12 (s, 3H), 2.11 (s, 3H), 2.08 (s,

3H), 2.03 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 170.1, 170.0, 169.8, 169.7, 169.3, 169.2, 151.9, 137.9, 125.2, 117.6, 115.0, 96.2, 76.8, 73.6, 69.5, 68.4, 67.9, 67.8, 67.3, 66.5, 66.4, 61.5, 20.7, 20.6, 18.5; HRMS (ESI^+) $C_{36}H_{42}O_{17}S$; Calcd: 778.2147 $[M + H]^+$; Found: 801.2039 $[M + Na]^+$.

4-Methylumbellifer-7-yl 2′,3′,4′,6′,2,3,4-hepta-*O*-acetyl-1-thio-β-D-melibiopyranoside (10): *Method A*: Starting with compound **3** (150 mg, 0.221 mmoles) gave **10** (99.6 mg, 56%) as a white foam. $[\alpha]_D^{20}$ = 19.9 (c = 0.73 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, 1H, J = 8.6 Hz), 7.31–7.34 (m, 2H), 6.27 (bs, 1H), 5.26–5.32 (m, 3H), 4.99–5.14 (m, 4H), 4.90 (d, 1H, J = 10.0 Hz), 4.22 (t_{app}, 1H, J = 6.4 Hz), 4.04 (d, 2H, J = 7.0 Hz), 3.77–3.87 (m, 2H), 3.57–3.62 (m, 1H), 2.44 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm); 170.6, 170.5, 170.4, 170.3, 169.8, 169.5, 133.6, 132.9, 132.1, 129.5, 129.2, 129.0, 128.0, 127.7, 126.9, 96.5, 85.8, 76.8, 74.1, 70.3, 68.7, 68.2, 67.5, 66.9, 66.6, 61.8, 21.0, 20.9, 20.8; HRMS (ESI^+) $C_{36}H_{42}O_{19}S$; Calcd: 801.2044 $[M + Na]^+$; Found: 801.2039 $[M + Na]^+$.

2-Naphthyl 1-thio-β-D-galactopyranoside (11): *Method B*: Starting with compound **4** (3.44 g, 7.02 mmoles) provided **11** (1.98 g, 88%) as a white foam. ^{1}H NMR (600 MHz, pyridine-d_5) δ 8.92 (s, 1H), 8.16 (m, 1H), 8.00–8.08 (m, 3H), 7.67 (m, 2H), 5.61 (d, 1H, J = 9.5 Hz, $\boldsymbol{H}_1$), 4.85–4.89 (m, 2H, $\boldsymbol{H}_2$ + $\boldsymbol{H}_4$), 4.70 (dd, 1H, J = 6.8 Hz, $\boldsymbol{H}_{6a}$), 4.63 (dd, 1H, J = 5.2 Hz, $\boldsymbol{H}_{6b}$), 4.48 (dd, 1H, J = 3.2 Hz, $\boldsymbol{H}_3$), 4.43 (t_{app}, 1H, J = 6.0 Hz, $\boldsymbol{H}_5$); ^{13}C NMR (125 MHz, pyridine-d_5, δ, ppm); 134.5, 134.1, 132.6, 129.1, 128.9, 128.8, 128.3, 128.0, 127.1, 126.4, 90.0 ($\boldsymbol{C}_1$), 81.3 ($\boldsymbol{C}_5$), 76.7 ($\boldsymbol{C}_3$), 71.0 ($\boldsymbol{C}_2$), 70.4 ($\boldsymbol{C}_4$), 62.5($\boldsymbol{C}_6$); HRMS (ESI^+); Calcd: 322.0876; Found: 345.0768. Spectral and physical data correspond to those reported in the litterature.[20]

4-Fluorophenyl 1-thio-β-D-galactopyranoside (12): *Method B*: Starting with compound **5** (3.44 g, 7.02 mmoles) gave **12** (78.8 mg, 100%) as a white foam. $[\alpha]_D^{20}$ = −39.1 (c = 0.76 acetone); mp: 136.8–141.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.48–7.52 (m, 2H), 7.12–7.18 (m, 2H), 5.75 (bs, 1H), 5.13 (m, 1H, H), 4.88 (bs, 1H, $\boldsymbol{H}_4$), 4.62 (bs, 1H, $\boldsymbol{H}_1$), 4.46–4.50 (m, 2H, $\boldsymbol{H}_2$), 3.68 (m, 1H, $\boldsymbol{H}_3$), 3.39–3.48 (m, 4H); ^{13}C NMR (75 MHz, pyridine-d_5) δ 162.7, 161.1, 133.7, 133.2, 132.7 (2×), 131.3, 128.0, 127.6, 127.5, 127.4, 127.1, 126.4, 125.7, 115.9, 115.8, 87.6, 86.4, 84.2, 81.8, 79.0, 68.1, 64.9, 60.4, 56.7; ^{19}F NMR (540 MHz, $CDCl_3$) δ −114.8; HRMS (ESI^+) $C_{12}H_{15}FO_5S$; Calcd: 313.0528 $[M + Na]^+$; Found: 313.0524 $[M + Na]^+$.

4-Methylumbellifer-7-yl 1-thio-β-D-galactopyranoside (13): *Method B*: Starting with compound **6** (83.5 mg, 0.159 mmoles) gave **13** (49.8 mg, 88%) as a white foam. $[\alpha]_D^{20}$ = −54.9 (c = 0.33 in $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6); δ 7.62 (d, 1H, J = 8.6 Hz), 7.38 (s, 1H), 7.31 (d, 1H, J = 8.2 Hz), 6.28 (s, 1H), 4.75 (d, 1H, J = 9.2 Hz), 3.67–3.69 (m, 1H), 3.41–3.56 (m, 5H), 3.29–3.36 (m, 4H), 2.44 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.3, 153.7, 142.2, 126.0, 124.4, 117.7, 115.3, 113.9, 86.8, 79.9, 75.0, 69.5, 68.9, 61.1, 18.6; HRMS (ESI^+) $C_{16}H_{18}O_7S$; Calcd: 377.0673 $[M + Na]^+$; Found: 377.0669 $[M + Na]^+$.

2-Naphthyl 1-thio-β-D-lactopyranoside (14): *Method B*: Starting with compound **7** (3.44 g, 7.02 mmoles) gave **14** (1.10 g, 86%) as a white foam. $[\alpha]_D^{20}$ = −26.9 (c = 1, DMSO); mp: 217.5–218.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.48–7.50 (m, 2H), 7.10–7.13 (m, 2H), 5.40 (d, 1H, J = 5.2 Hz), 5.03 (bs, 1H), 4.72 (bs, 1H), 4.58 (bs, 1H), 4.54–4.56 (m, 2H), 4.46 (bs, 1H), 4.16 (d, 1H, J = 7.0 Hz), 3.69–3.72 (m, 1H), 3.57 (bs, 3H), 3.39–3.47 (m, 5H), 3.24–3.34 (m); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.9, 161.3, 134.0, 129.8, 116.5, 104.3, 87.4, 80.7, 79.4, 76.8, 76.1, 73.8, 72.6, 71.1, 68.7, 61.0; MS (ESI^+) $C_{22}H_{28}O_{10}S$; Calcd: 484.1 $[M]^+$; Found: 507.3 $[M + Na]^+$.

4-Fluorophenyl 1-thio-β-D-lactopyranoside (15): *Method B*: Starting with compound **8** (53 mg, 0.071 mmoles) afforded **15** (28.7 mg, 90%) as a white foam. $[\alpha]_D^{20}$ = −17.8 (c = 0.4 MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.48–7.50 (m, 2H), 7.10–7.13 (m, 2H), 5.40 (d, 1H, J = 5.2 Hz), 5.03 (bs, 1H), 4.72 (bs, 1H), 4.58 (bs, 1H), 4.54–4.56 (m, 2H), 4.46 (bs, 1H), 4.16 (d, 1H, J = 7.0 Hz), 3.69–3.72 (m, 1H), 3.57 (bs, 3H), 3.39–3.47 (m, 5H), 3.24–3.34 (m); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.9, 161.3, 134.0, 129.8, 116.5, 104.3, 87.4, 80.7, 79.4, 76.8, 76.1, 73.8, 72.6, 71.1, 68.7, 61.0; ^{19}F NMR (540 MHz, DMSO-d_6) δ −115.7; HRMS (ESI^+) $C_{18}H_{25}FO_{10}S$; Calcd: 475.1049 $[M + Na]^+$; Found: 475.1055 $[M + Na]^+$.

2-Naphthyl 1-thio-β-D-melibiopyranoside (16): *Method B*: Starting with compound **9** (87 mg, 0.112 mmoles) gave **16** (54 mg, 92%) as a white foam. $[\alpha]_D^{20}$ = 94.2 (c = 0.48 MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.91 (bs, 1H), 7.85 (d, 1H, J = 8.0 Hz), 7.81 (d, 2H, J = 8.8 Hz), 7.59 (d, 1H, J = 8.6 Hz), 7.42–7.47 (m, 2H), 5.35 (bs, 1H), 5.26 (bs, 1H), 5.17 (bs, 1H), 4.66 (d, 2H), 4.47–4.50 9 m, 2H), 4.31 (bs, 1H), 3.56–3.65 (m, H), 3.41–3.47 (m, 3H), 3.35–3.38 (m, 1H), 3.20 (t_{app}, 1H, J = 8.5 Hz), 3.06 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 133.8, 133.0, 132.1, 128.9, 128.7, 128.6, 128.1, 128.0, 127.2, 126.5, 99.5, 87.7, 79.6, 78.8, 73.0, 71.5, 70.6, 70.2, 69.4, 69.0, 67.6, 61.2; HRMS (ESI^+) $C_{22}H_{28}O_{10}S$; Calcd: 507.1303 $[M + Na]^+$; Found: 507.1298 $[M + Na]^+$.

4-Methylumbellifer-7-yl 1-thio-β-D-melibiopyranoside (17): *Method B*: Starting with compound **10** (99.6 mg, 0.123 mmoles) provided **17** (63.5 mg, 99%) as a white foam. $[\alpha]_D^{20}$ = 46.7 (c = 0.89 in MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.66 (d, 1H, J = 8.3 Hz), 7.43 (d, 1H, J = 8.4 Hz), 7.32 (bs, 1H), 6.29 (s, 1H, $\boldsymbol{H}_{allyl}$), 5.59 (bs, 1H), 5.41 (bs, 1H), 4.79 (d, 1H, J = 9.7 Hz, $\boldsymbol{H}_{4Gal}$), 4.61 (bs, 1H), 4.50–4.53 (m, 2H), 4.38 (bs, 1H), 3.48–3.61 (m, 8H), 3.37–3.39 (m, 1H), 3.22 (t_{app}, 1H, J = 8.7 Hz), 3.06–3.11 (m, 2H), 2.38 (s, 3H, ***Me***), 1.56 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.4, 153.9, 153.4, 141.6, 126.4, 124.8, 118.0, 115.8, 114.0, 99.5, 86.3, 79.6, 78.7, 73.1, 71.5, 70.5, 70.1, 69.4, 68.9, 67.4, 61.0, 18.7; HRMS (ESI^+) $C_{22}H_{28}O_{12}S$; Calcd: 539.1204 $[M + Na]^+$; Found: 539.1200 $[M + Na]^+$.

2-Naphthyl 3-*O*-propargyl-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (18): *Method C*: Starting with compound **4** (678 mg, 2.10 mmoles) gave **18** (892 mg, 88%) as a white foam. $[\alpha]_D^{20}$ = 43.1 (c = 0.6 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–8.00 (m, 1H), 7.82–7.75 (m, 3H), 7.59–7.56 (m, 1H), 7.50–7.46 (m, 2H), 5.42 (d, 1H, J = 3.4 Hz, $\boldsymbol{H}_4$), 5.14 (t, 1H, J = 9.8 Hz, $\boldsymbol{H}_2$), 4.80 (d, 1H, J = 9.8 Hz, $\boldsymbol{H}_1$), 4.23–4.12 (m, 4H, $\boldsymbol{CH}_2$ + $\boldsymbol{H}_{6a+b}$), 3.92–3.85 (m, 2H, $\boldsymbol{H}_5$ + $\boldsymbol{H}_3$), 2.44 (t, 1H, J = 2.3 Hz, $\boldsymbol{H}$–CC–CH_2), 2.15 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H); ^{13}C NMR

(75 MHz, $CDCl_3$) δ 171.2, 170.6, 170.5, 170.4 (2×), 170.3, 170.2, 169.5, 133.4, 132.6, 131.6, 129.9, 129.7, 129.6, 128.2, 127.6, 127.5, 126.5, 126.4, 86.5, 78.9, 76.7, 76.5, 75.1, 74.6, 68.4, 65.6, 62.2, 56.4, 21.0, 20.6; IR (NaCl, cm^{-1}); 3278, 3055, 2920, 2851, 2118, 1747, 1372, 1228, 1068, 1050; HRMS (ESI^+) $C_{25}H_{26}O_8S$; Calcd: 509.1257 $[M + Na]^+$; Found: 509.1252 $[M + Na]^+$.

2-Naphthyl 3-(4-(hydroxymethyl)-1*H*-1,2,3-triazole-1-methyl ester)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (19): *Method F*: Starting with compound **18** (242 mg, 0.498 mmoles) gave **19** (205 mg, 68%) as a white foam. $[\alpha]_D^{20}$ = 42.3 (*c* = 1.9 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (bs, 1H), 7.78–7.71 (m, 3H), 7.63 (s, 1H, $\boldsymbol{H}_{triazole}$), 7.54–7.50 (m, 1H), 7.47–7.40 (m, 2H), 5.45 (d, 1H, *J* = 3.2 Hz, $\boldsymbol{H}_4$), 5.15–5.09 (m, 3H, $\boldsymbol{H}_2$ + $\boldsymbol{CH}_2$–CO_2Me), 4.74–4.59 (m, 3H, H1 + $\boldsymbol{CH}_2$–C≡C–H), 4.19–4.08 (m, 2H, $\boldsymbol{H}_{6a,b}$), 3.87–3.82 (m, 1H, $\boldsymbol{H}_5$), 3.78–3.71 (m, 4H, $\boldsymbol{H}_3$ + O***Me***), 2.03–2.00 (m, 6H), 1.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 170.1, 169.4, 166.4, 144.6, 133.2, 132.3, 131.0, 129.9, 129.2, 128.0, 127.4, 127.3, 126.3, 126.2, 124.3, 86.1, 77.69, 74.4, 68.5, 66.3, 62.7, 62.0, 52.7, 50.4, 20.6, 20.5, 20.4; IR (NaCl, cm^{-1}); 3146, 3013, 2956, 1748, 1372, 1230, 1050, 752; HRMS (ESI^+) $C_{28}H_{31}N_3O_{10}S$; Calcd: 602.1812 $[M + H]^+$; Found: 602.1805 $[M + H]^+$.

2-Naphthyl 3-(4-(hydroxymethyl)-1*H*-1,2,3-triazole-1-methyl ester)-1-thio-β-D-galactopyranoside (20): *Method B*: Starting with compound **19** (189 mg, 0.314 mmoles) gave **20** (29 mg, 13%) as a white foam. $[\alpha]_D^{20}$ = 15.4 (*c* = 0.6 MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (bs, 2H), 7.81–7.90 (m, 3H), 7.46–7.55 (m, 3H), 5.41 (s, 2H), 5.12 (t_{app}, 1H, *J* = 10.0 Hz), 40.97–5.00 (m, 2H), 4.82 (bs, 1H), 4.72 (d, 1H, *J* = 12.2 Hz), 4.55 (d, 1H, *J* = 12.3 Hz), 4.10 (bs, 1H), 3.58–3.70 (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 168.3, 144.8, 133.8, 132.3, 128.9, 128.6, 128.3, 127.9, 127.2, 126.6, 125.8, 85.5, 80.2, 79.9, 69.6, 65.1, 62.2, 61.0, 53.1, 50.8, 21.3; IR (NaCl, cm^{-1}); 3146, 3013, 2956, 1748, 1372, 1230, 1050, 752; HRMS (ESI^+) $C_{22}H_{25}N_3O_7S$; Calcd: 476.1492 $[M + H]^+$; Found: 476.1486 $[M + H]^+$.

2-Naphthyl 3-*O*-hepta-2,4-diyn-1-ol-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (21): *Method E*: Starting with compound **18** (315 mg, 0.647 mmoles) gave **21** (198.6 mg, 57%) as a white foam. $[\alpha]_D^{20}$ = 58.6 (*c* = 1.1 $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.99 (bs, 1H), 7.73–7.79 (m, 3H), 7.53–7.56 (m, 1H), 7.45–7.47 (m, 2H), 5.37 (d, 2H, *J* = 3.5 Hz, $\boldsymbol{H}_4$), 5.10 (t, 1H, *J* = 10.0 Hz, $\boldsymbol{H}_2$), 4.75 (d, 1H, *J* = 10.0 Hz $\boldsymbol{H}_1$), 4.31 (bs, 2H, $\boldsymbol{CH}_2$)) 4.22 (s, 2H, $\boldsymbol{CH}_2$), 4.11–4.17 (m, 2H, $\boldsymbol{H}_{6a+b}$), 3.86 (t, 2H, *J* = 6.9 Hz, $\boldsymbol{H}_5$), 3.75 (dd, 2H, *J* = 9.4 Hz, $\boldsymbol{H}_3$), 2.13 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.98 (s, 3H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 169.5, 169.4 133.5, 132.7, 131.9, 129.8, 128.3, 127.6, 126.5, 86.5, 74.9, 70.9, 70.7, 69.6, 68.6, 65.8, 62.2, 60.3, 57.1, 51.3, 20.9, 20.5; IR (NaCl, cm^{-1}); 3146, 3013, 2956, 1748, 1372, 1230, 1050, 752; HRMS (ESI^+) $C_{28}H_{28}O_9S$; Calcd: 563.1347 $[M + Na]^+$; Found: 563.1342 $[M + Na]^+$.

Dimer 22: *Method E*: Starting with compound **18** (100.2 mg, 0.206 mmoles) gave dimer **22** (72 mg, 77%) as a white foam; ^1H NMR (600 MHz, $CDCl_3$) δ 8.00 (bs, 2H), 7.75–7.81 (m, 6H), 7.56–7.58 (m, 2H), 7.47–7.49 (m, 2H), 5.39 (d, 2H, *J* = 2.8 Hz, $\boldsymbol{H}_4$ + $\boldsymbol{H}_4'$), 5.12 (t, 2H, *J* = 9.8 Hz, $\boldsymbol{H}_2$ + $\boldsymbol{H}_2'$), 4.77 (dd, 2H, *J* = 10.0 Hz $\boldsymbol{H}_1$ + $\boldsymbol{H}_1'$), 4.25 (bs, 4H, $\boldsymbol{CH}_2$(2×)), 4.10–4.20 (m, 4H, $\boldsymbol{H}_{6a+b}$ + $\boldsymbol{H}_{6a,b}'$), 3.88 (t, 2H, *J* = 6.8 Hz, $\boldsymbol{H}_5$), 3.78 (dd, 2H, *J* = 9.4 Hz, $\boldsymbol{H}_3$), 2.15 (s, 6H, OAc(2×)), 2.04 (s, 6H, OAc(2×)), 2.00 (s, 6H, OAc(2×)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4(2×), 169.5, 133.4, 132.6, 131.7, 129.8, 129.6, 128.3, 127.6, 127.5, 126.5 (2×), 86.5, 77.3, 74.9, 74.5, 70.6, 68.3, 65.6, 62.1, 57.0, 20.9, 20.6; IR (NaCl, cm^{-1}); 3146, 3013, 2956, 1748, 1372, 1230, 1050, 752; HRMS (ESI^+) $C_{50}H_{50}O_{16}S$; Calcd: 993.2437 $[M + Na]^+$; Found: 993.2431 $[M + Na]^+$.

2-Naphthyl 3-*O*-hepta-2,4-diyn-1-ol-1-thio-β-D-galactopyranoside (23): *Method B*: Starting with compound **21** (30 mg, 0.055 mmoles) gave **23** (23 mg, 99%) as a white solid. $[\alpha]_D^{20}$ = 84.8 (*c* = 0.36 MeOH); ^1H NMR (600 MHz, DMSO-d_6) δ 8.02 (bs, 1H), 7.80–7.88 (m, 3H), 7.55–7.57 (m, 1H), 7.45–7.51 (m, 2H), 5.46 (d, 1H, *J* = 6.3 Hz, $\boldsymbol{H}_4$), 4.72–4.78 (m, 3H, $\boldsymbol{H}_2$ + $\boldsymbol{H}_1$ + ***OH***), 4.40–4.49 (q_{app}, 2H, $\boldsymbol{CH}_2$), 4.18 (bs, 2H, ***OH***), 3.94 (bs, 1H, ***OH***), 3.60–3.64 (m, 1H, $\boldsymbol{H}_3$), 3.50–3.55 (m, 3H, $\boldsymbol{H}_{6a+b}$, ***OH***), 3.40–3.42 (dd, 2H, *J* = 3.0 Hz, *J* = 5.8 Hz, $\boldsymbol{H}_5$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 133.9, 133.3, 132.0, 128.7, 128.3, 128.2, 127.8, 127.1, 126.4, 88.2, 82.5, 80.7, 79.6, 77.1, 70.2, 68.7, 68.4, 65.4, 61.0, 57.2, 49.9; IR (NaCl, cm^{-1}); 3146, 3013, 2956, 1748, 1372, 1230, 1050, 752; HRMS (ESI^+) $C_{22}H_{22}O_6S$; Calcd: 437.1042 $[M + Na]^+$; Found: 437.1037 $[M + Na]^+$.

Deprotected dimer (24): *Method B*: Starting with compound **22** (58 mg, 0.060 mmoles) gave dimer **24** (41 mg, 96%) as a white solid. $[\alpha]_D^{20}$ = 22.5 (*c* = 1.9 acetone); ^1H (600 MHz, $CDCl_3$) δ 7.98 (bs, 2H), 7.76–7.84 (m, 6H), 7.41–7.47 (m, 6H), 5.39 (d, 2H, *J* = 6.2 Hz), 4.67–4.73 (m, 4H), 4.41 (m, 4H), 3.87–3.91 (m, 2H), 3.56–3.60 (m, 2H), 3.46–3.51 (m, 8H), 3.37 (dd, 2H, *J* = 3.0 Hz, 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.9, 133.3, 132.0, 128.6, 128.3, 128.2, 127.8, 127.1, 126.4, 88.2, 82.6, 79.6, 77.6, 70.0, 68.7, 65.4, 61.0, 57.2; IR (NaCl, cm^{-1}); 3146, 3013, 2956, 1748, 1372, 1230, 1050, 752; HRMS (ESI^+) $C_{38}H_{38}O_{10}S$; Calcd: 741.1801 $[M + Na]^+$; Found: 741.1796 $[M + Na]^+$.

2-Naphthyl 3-(3-phenyl-2-propyn-1-hydroxy)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (25): *Method F*: Starting with compound **18** (130 mg, 0.267 mmoles) afforded **25** (75 mg, 77%) as a white foam. $[\alpha]_D^{20}$ = 60.5 (*c* = 0.5 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (bs, 1H), 7.83–7.76 (m, 3H), 7.61–7.57 (m, 1H0, 7.52–7.43 (m, 4H), 7.36–7.30 (m, 3H), 5.52 (d, 1H, *J* = 3.3 Hz, $\boldsymbol{H}_4$), 5.18 (t, 1H, *J* = 9.3 Hz, $\boldsymbol{H}_2$), 4.83 (d, 1H, *J* = 9.3 Hz), $\boldsymbol{H}_1$), 4.41 (s, 2H, O–$\boldsymbol{CH}_2$–CC), 4.23–4.16 (m, 2H, $\boldsymbol{H}_{6a,b}$), 3.99–3.91 (m, 2H, $\boldsymbol{H}_3$ + $\boldsymbol{H}_5$), 2.13 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 169.6, 133.4, 132.5, 131.6, 131.4, 130.0, 129.5, 128.6, 128.4, 128.3, 128.2, 127.6, 127.5, 126.5, 126.4, 122.2, 86.7, 86.4, 76.7, 74.6, 68.5, 65.8, 62.2, 57.2, 20.9, 20.7, 20.6; IR (NaCl, cm^{-1}); 3063, 2937, 2854, 1748, 1371, 1226, 1066, 1049, 757; HRMS (ESI^+) $C_{31}H_{30}O_8S$; Calcd: 585.1566 $[M + Na]^+$; Found: 585.1562 $[M + Na]^+$.

2-Naphthyl 3-(3-(2-pyridine)-2-propyn-1-hydroxy)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (26): *Method F*: Starting with compound **18** (97.2 mg, 0.200 mmoles) gave **26** (82 mg, 73%) as a white foam. $[\alpha]_D^{20}$ = 63.9 (*c* = 0.4 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (bs, 1H), 7.88–7.71 (m, 4H), 7.60–7.57 (m, 1H), 7.51–7.45 (m, 2H), 7.33–7.25 (m, 3H), 5.51 (d, 1H, *J* = 3.3 Hz, $\boldsymbol{H}_4$), 5.17 (t, 1H, *J* = 10.0 Hz, $\boldsymbol{H}_2$), 4.82 (d, 1H, *J* =

10.0 Hz, $\boldsymbol{H}_1$), 4.43 (s, 2H, $\boldsymbol{CH}_2$), 4.25–4.14 (m, 2H, $\boldsymbol{H}_{6a+b}$), 3.99–3.87 (m, 2H, $\boldsymbol{H}_3$ + $\boldsymbol{H}_5$), 2.13 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 169.5, 151.5, 148.2, 139.3, 133.4, 132.6, 131.6, 129.9, 129.6, 128.3 (3×), 128.2, 128.0, 127.6 (2×), 127.5, 127.4, 126.5, 126.4, 88.3, 86.5, 77.2, 76.5, 74.6, 68.5, 65.8, 62.2, 57.2, 21.0, 20.6; IR (NaCl, cm^{-1}); 3009, 2907, 1748, 1371, 1227, 1066, 1048; HRMS (ESI^+) $C_{30}H_{29}NO_8S$; Calcd: 564.1701 $[M + H]^+$; Found: 564.1695 $[M + H]^+$.

2-Naphthyl 3-(3-(4-methoxyphenyl)-2-propyn-1-hydroxyl)-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (27): *Method F*: Starting with compound **18** (150 mg, 0.308 mmoles) gave **27** (71.2 mg, 74%) as a white foam. $[\alpha]_D^{20}$ = 68.0 (c = 0.63 $CDCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (bs, 1H), 7.70–7.77 (m, 3H), 7.51–7.55 (m, 1H), 7.41–7.46 (m, 2H), 7.33 (d, 2H, J = 8.6 Hz), 6.79 (d, 2H, J = 8.6 Hz), 5.46 (d, 1H, J = 3.4 Hz, $\boldsymbol{H}_4$), 5.12 (t_{app}, 1H, J = 9.8 Hz, $\boldsymbol{H}_2$), 4.78 (d, 1H, J = 9.8 Hz), 4.34 (s, 2H, $\boldsymbol{CH}_2$), 4.10–4.20 (m, 2H, $\boldsymbol{H2_{a+b}}$), 3.85–3.93 (m, 2H, $\boldsymbol{H}_3$ + $\boldsymbol{H}_5$), 3.74 (s, 3H, ***MeO***), 2.07 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 169.6, 159.7, 133.3, 133.1, 132.5, 131.4, 130.0, 129.5, 128.2, 127.5, 127.4, 126.3, 113.9, 86.4, 82.8, 76.6, 74.6, 68.5, 65.9, 62.2, 57.3, 55.2, 20.9, 20.6; IR (NaCl, cm^{-1}); 3078, 2922, 1747, 1520, 1344, 1226, 749; HRMS (ESI^+) $C_{32}H_{32}O_9S$; Calcd: 615.1651 $[M + Na]^+$; Found: 615.1646 $[M + Na]^+$.

2-Naphthyl 3-(3-(4-nitrophenyl)-2-propyn-1-hydroxy)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (28): *Method F*: Starting with compound **18** (139 mg, 0.286 mmoles) gave **28** (173 mg, 61%) as a white foam. $[\alpha]_D^{20}$ = 55.6 (c = 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19–8.16 (m, 2H), 8.01 (s, 1H), 7.82–7.76 (m, 3H), 7.60–7.55 (m, 3H), 7.50–7.47 (m, 2H), 5.52 (d, 1H, J = 3.2 Hz, $\boldsymbol{H}_4$), 5.17 (t, 1H, J = 10.0 Hz, $\boldsymbol{H}_2$), 4.84 (d, 1H, J = 10.0 Hz, $\boldsymbol{H}_1$), 4.44 (s, 2H, C$\boldsymbol{H}_2$), 4.21–4.17 (m, 2H, $\boldsymbol{H}_{6a+b}$), 3.97–3.89 (m, 2H, $\boldsymbol{H}_3$ + $\boldsymbol{H}_5$), 2.13 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 169.4, 147.2, 133.3, 132.3, 131.5, 129.5, 128.2, 127.5, 127.4, 126.5, 126.4, 123.5, 89.7, 86.4, 84.7, 77.3, 76.5, 74.5, 68.5, 65.8, 62.2, 57.2, 20.9, 20.6; IR (NaCl, cm^{-1}); 3078, 2922, 1747, 1520, 1344, 1226, 749; HRMS (ESI^+) $C_{31}H_{29}NO_{10}S$; Calcd: 630.1417 $[M + Na]^+$; Found: 630.1412 $[M + Na]^+$.

2-Naphthyl 3-(3-(4-fluorophenyl)-2-propyn-1-hydroxy)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (29): *Method F*: Starting with compound **18** (139 mg, 0.286 mmoles) gave **29** (90 mg, 54%) as a white foam. $[\alpha]_D^{20}$ = 53.7 (c = 0.49 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (bs, 1H), 7.78–7.71 (m, 3H), 7.56–7.53 (m, 1H), 7.45–7.35 (m, 4H), 7.00–6.94 (m, 2H), 5.46 (d, 1H, J = 2.6 Hz, $\boldsymbol{H}_4$), 5.13 (t, 1H, J = 9.7 Hz, $\boldsymbol{H}_2$), 4.79 (d, 1H, J = 9.7 Hz, $\boldsymbol{H}_1$), 4.35 (s, 2H, O–$\boldsymbol{CH}_2$–CC), 4.21–4.10 (m, 2H, $\boldsymbol{H}_{6a,b}$), 3.96–3.83 (m, 2H, $\boldsymbol{H}_3$ + $\boldsymbol{H}_5$), 2.08 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4 (2×), 170.3, 169.5, 164.2, 160.9, 133.5, 133.4, 133.3, 132.5, 131.4, 129.9, 129.5, 128.2, 127.5, 127.4, 126.4 (2×), 115.7, 115.4, 86.3, 85.6, 84.0, 76.8, 74.5, 68.5, 65.8, 62.2, 57.2, 20.9, 20.6; ^{19}F NMR (540 MHz, $CDCl_3$) δ −113.2; HRMS (ESI^+) $C_{31}H_{29}FO_8S$; Calcd: 603.1478 $[M + Na]^+$; Found: 603.1473 $[M + Na]^+$.

2-Naphthyl 3-(3-phenyl-2-propyn-1-hydroxy)-1-thio-β-D-galactopyranoside (30): *Method B*: Starting with compound **25** (51 mg, 0.091 mmoles) gave **30** (32 mg, 82%) as a white solid. $[\alpha]_D^{20}$ = −13.5 (c = 0.4 MeOH); mp: 158.6–161.1 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (bs, 1H), 7.75–7.84 (m, 3H), 7.32–7.53 (m, 8H), 5.42 (d, 1H, J = 6.1 Hz, $\boldsymbol{H}_4$), 4.68–4.76 (m, 2H, $\boldsymbol{H}_2$ + $\boldsymbol{H}_1$), 4.49 (q, 2H, $\boldsymbol{CH}_2$), 3.96–3.99 (m, 1H, O$\boldsymbol{H}$), 3.56–3.65 (m, 1H, $\boldsymbol{H}_{6a}$), 3.43–3.54 (m, 4H, $\boldsymbol{H}_{6b}$ + $\boldsymbol{H}_5$ + O$\boldsymbol{H}$(2×)). ^{13}C NMR (75 MHz, pyridine-d_5) δ 152.2, 151.9, 151.5, 151.2, 138.2, 137.8, 137.5, 137.2, 136.8, 136.3, 135.8, 134.4, 134.0, 131.2, 130.9, 130.9, 130.5, 130.0, 129.8, 128.8, 128.1, 126.1, 125.8, 125.5, 125.2, 124.8, 91.9, 89.3, 88.2, 86.1, 83.1, 71.7, 69.4, 64.5, 60.4. HRMS (ESI^+) $C_{25}H_{24}O_5S$; Calcd: 459.1241 $[M + Na]^+$; Found: 459.1231 $[M + Na]^+$.

2-Naphthyl 3-(3-(2-pyridine)-2-propyn-1-hydroxy)-1-thio-β-D-galactopyranoside (31): *Method B*: Starting with compound **26** (82 mg, 0.145 mmoles) provided **31** (30.5 mg, 66%) as a white solid. $[\alpha]_D^{20}$ = 11.2 (c = 0.23 in MeOH); mp: 133.0–135.7 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61–8.62 (m, 1H), 8.51–8.53 (m, 1H), 7.97 (s, 1H), 7.74–7.87 (m, 4H), 7.37–7.53 (m, 4H), 4.74 (d, 1H, J = 9.6 Hz), 4.40–4.59 (m, 3H), 3.98 (d, 1H, J = 2.8 Hz), 3.61 (t, 1H, J = 9.3 Hz), 3.43–3.53 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 151.4, 148.7, 138.8, 133.2, 132.7, 131.3, 128.0, 127.6, 127.5, 127.4, 127.1, 126.4, 125.7, 123.6, 119.1, 90.0, 87.6, 82.1, 81.9, 79.0, 68.1, 64.9, 60.4, 56.7; HRMS (ESI^+) $C_{24}H_{23}NO_5S$; Calcd: 489.1347 $[M + Na]^+$; Found: 489.1341 $[M + Na]^+$.

2-Naphthyl 3-(3-(4-methoxyphenyl)-2-propyn-1-hydroxyl)-1-thio-β-D-galactopyranoside (32): *Method B*: Starting with compound **27** (68 mg, 0.114 mmoles) gave **32** (39 mg, 75%) as a white foam. $[\alpha]_D^{20}$ = −17.0 (c = 0.23 $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.79 (m, 2H), 7.28–7.56 (m, 3H), 6.88 (d, J = 7.6 Hz, 1H), 5.40 (d, 1H, J = 6.4 Hz, $\boldsymbol{H}_4$), 4.64–4.77 (m, 3H, $\boldsymbol{H}_1$ + $\boldsymbol{H}_2$ + $\boldsymbol{H}_3$), 4.46 (q, 1H, J = 16.0 Hz, C$\boldsymbol{H}_2$), 3.96 (bs, 1H, O$\boldsymbol{H}$), 3.71 (s, 3H, O***Me***), 3.54–3.66 (m, 1H, $\boldsymbol{H}_{6a}$), 3.39–3.54 (m, 4H, $\boldsymbol{H}_{6b}$ + $\boldsymbol{H}_5$ + $\boldsymbol{OH}$(2×)); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.0, 133.9, 133.6, 133.4, 131.9, 128.6, 128.3, 128.2, 128.0, 127.7, 127.1, 126.3, 114.9, 88.3, 85.7, 82.3, 79.7, 68.7, 65.6, 61.1, 57.5, 55.8; HRMS (ESI^+) $C_{26}H_{26}O_6S$; Calcd: 489.1347 $[M + Na]^+$; Found: 489.1341 $[M + Na]^+$.

2-Naphthyl 3-(3-(4-nitrophenyl)-2-propyn-1-hydroxy)-1-thio-β-D-galactopyranoside (33): *Method B*: Starting with compound **28** (105 mg, 0.172 mmoles) gave **33** (63.9 mg, 77%) as a white solid. $[\alpha]_D^{20}$ = −19.89 (c = 0.25 MeOH); mp: 175.5–180.2 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16–8.19 (m, 2H), 7.98 (bs, 1H), 7.75–7.83 (m, 3H), 7.65–7.68 (m, 2H), 7.41–7.53 (m, 3H), 5.44 (d, 1H, J = 6.4 Hz, $\boldsymbol{H}_4$), 4.69–4.76 (m, 3H, $\boldsymbol{H}_2$ + $\boldsymbol{H}_1$ + $\boldsymbol{H}_3$), 4.56 (q, 2H, $\boldsymbol{CH}_2$), 3.98 (bs, 1H, O$\boldsymbol{H}$), 3.57–3.66 (m, 1H, $\boldsymbol{H}_{6a}$), 3.44–3.54 (m, 4H, $\boldsymbol{H}_{6b}$ + $\boldsymbol{H}_5$ + O$\boldsymbol{H}$(2×)); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 161.1, 133.7, 133.2, 132.7(2×), 131.3, 128.0, 127.6, 127.5, 127.4, 127.1, 126.4, 125.7, 115.9, 115.8, 87.6, 86.4, 84.2, 81.8, 79.0, 68.1, 64.9, 60.4, 56.7; HRMS (ESI^+) $C_{25}H_{23}NO_7S$; Calcd: 504.1092 $[M + Na]^+$; Found: 504.1090 $[M + Na]^+$.

2-Naphthyl 3-(3-(4-fluorophenyl)-2-propyn-1-hydroxyl)-1-thio-β-D-galactopyranoside (34): *Method B*: Starting with compound **29** (86 mg, 0.148 mmoles) gave **34** (50 mg, 75%) as a

white solid. $[\alpha]_D^{20}$ = −14.0 (*c* = 0.74 $CHCl_3$); mp: 157.1–159.1 °C; ^{1}H NMR (600 MHz, pyridine-d_5) δ 8.01 (bs, 1H), 7.78–7.86 (m, 3H), 7.43–7.55 (m, 5H), 7.20 (t, 1H, *J* = 8.6 Hz), 5.41 (d, 1H, *J* = 6.4 Hz, H_4), 4.76 (d, 1H, *J* = 9.6 Hz, H_1), 4.71 (t, 1H, $J_{2,3}$ = 5.4 Hz, $J_{1,2}$ = 9.6 Hz, H_2), 4.69 (d, 1H, *J* = 5.4 Hz, H_3), 4.51 (q, 2H, CH_2), 3.98–4.00 (m, 1H, O*H*), 3.61–3.66 (m, 1H, H_{6a}), 3.45–3.55 (m, 4H, H_{6b} + H_5 + O*H*(2×)); ^{13}C NMR (125 MHz, pyridine-d_5) δ 162.7, 161.1, 133.7, 133.2, 132.7(2×), 131.3, 128.0, 127.6, 127.5, 127.4, 127.1, 126.4, 125.7, 115.9, 115.8, 87.6, 86.4, 84.2, 81.8, 79.0, 68.1, 64.9, 60.4, 56.7; ^{19}F NMR (540 MHz, pyridine-d_5) δ −111.0; HRMS (ESI$^+$) $C_{25}H_{23}FO_5S$; Calcd: 477.1147 $[M + Na]^+$; Found: 477.1137 $[M + Na]^+$.

2-Naphthyl 3-(3-phenyl-1-hydroxy)-1-thio-β-D-galactopyranoside (35): *Method G*: Starting with compound **30** (15 mg, 0.034 mmoles) gave **35** (12.5 mg, 85%) as a white solid. $[\alpha]_D^{20}$ = −6.2 (*c* = 0.6 $CHCl_3$); mp: 167.1–174.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.79 (m, 3H), 7.38–7.54 (m, 3H), 7.07–7.25 (m, 5H), 5.26 (d, 1H, *J* = 6.2 Hz, H_4), 4.67 (d, 2H, *J* = 9.7 Hz, H_2 + H_1), 4.46 (d, 1H, *J* = 4.6 Hz, *H*), 3.88 (s, 1H, *OH*), 3.44–3.62 (m, 6H), 3.39 (m, 2H), 3.15 (d, 1H, *J* = 8.4 Hz), 2.60 (d, 2H, *J* = 9.2 Hz, CH_2), 1.69–1.83 (m, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.7, 133.9, 133.5, 132.0, 129.0, 128.8, 128.6, 128.3, 128.2, 128.1, 127.8, 127.1, 126.4, 126.2, 88.4, 88.5, 79.8, 68.6, 65.5, 61.2, 32.2, 31.9; HRMS (ESI$^+$) $C_{25}H_{28}O_5S$; Calcd: 463.1554 $[M + Na]^+$; Found: 463.1558 $[M + Na]^+$.

2-Naphthyl 3-(3-(aniline)-2-propyn-1-hydroxyl)-1-thio-β-D-galactopyranoside (36): *Method G*: Starting with compound **33** (39.8 mg, 0.091 mmoles) gave **36** (41.4 mg, 100%) as a white solid. $[\alpha]_D^{20}$ = −31.8 (*c* = 0.16 MeOH); mp: 149.0–156.9 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (s, 1H), 7.74–7.83 (m, 3H), 7.39–7.52 (m, 3H), 6.80 (d, 2H, *J* = 8.0 Hz), 6.42 (m, 2H, *J* = 8.2 Hz), 5.23 (d, 1H, *J* = 6.2 Hz, H_4), 4.75 (bs, 1H), 4.65–4.68 (m, 2H, H_2 + H_1), 4.43 (d, 1H, *J* = 4.9 Hz, *H*), 3.85–3.88 (m, 1H, *OH*), 3.42–3.58 (m, 5H), 3.30–3.39 (m, 1H), 3.11–3.15 (m, 1H), 1.62–1.69 (m, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 146.2, 133.2, 132.8, 131.1, 128.8, 128.6, 127.9, 127.6, 127.5, 127.4, 127.1, 126.4, 125.6, 113.9, 87.7, 82.8, 79.1, 68.0, 64.8, 60.5, 31.6, 30.7; IR (NaCl, cm^{-1}); 3078, 2922, 1747, 1520, 1344, 1226, 749; HRMS (ESI$^+$) $C_{25}H_{29}NO_5S$; Calcd: 456.1845 $[M + H]^+$; Found: 456.1835 $[M + H]^+$; Calcd: 478.1663 $[M + Na]^+$; 478.1659 $[M + Na]^+$.

2-Naphthyl 3-*O*-propargyl-1-thio-β-D-galactopyranoside (37): *Method C* (*without acetylation*): Starting with compound **11** (250 mg, 0.775 mmoles) gave **37** (162.5 mg, 58%) as a white solid. $[\alpha]_D^{20}$ = +1.7 (*c* = 0.72 DMSO); mp: 144.5–152.6 °C; ^{1}H NMR (600 MHz, pyridine-d_5) δ 6.76–6.78 (m, 1H), 6.56–6.61 (m, 2H), 6.39 (s, 1H), 6.20–6.24 (m, 1H), 6.03 (s, 1H), 4.44 (bs, 4H). 4.21 (d, 1H, *J* = 9.6 Hz, H_1), 3.53–3.62 (m, 4H, H_2 + H_4 + CH_2), 3.31–3.34 (m, 1H, H_{6a}), 3.23–3.25 (m, 1H, H_{6b}), 2.99 (t, 1H, *J* = 5.8 Hz, H_5), 2.91 (dd, 1H, *J* = 3.1 Hz, *J* = 9.0 Hz, H_3), 2.19 (t, 1H, *J* = 1.9 Hz, C*H*); ^{13}C NMR (125 MHz, pyridine-d_5) δ 133.05, 132.60, 131.20, 127.9, 127.7, 127.3, 126.8, 126.5, 125.5, 124.8, 88.6, 82.6, 80.3, 79.9, 74.7, 68.4, 66.1, 61.2, 56.5; HRMS (ESI$^+$) $C_{19}H_{20}O_5S$; Calcd: 383.0928 $[M + Na]^+$; Found: 383.0930 $[M + Na]^+$.

Binding assays

ELLA (enzyme-linked lectin assay) experiments. ELLA tests were monitored using 96-well microtitre plates (Nunc Maxisorb) coated with 5 μg mL^{-1} of poly[*N*-(2-hydroxyethyl)acrylamide]α-D-galactopyranoside (Lectinity Holding, Inc.) diluted in carbonate buffer pH 9.6 (100 μL per well) for 1 hour at 37 °C. Blocking was performed for 1 h at 37 °C with 100 μL of 3% (w/v) BSA in PBS per well. Plates were then incubated at 37 °C for 1 h with 100 μL of 3 μg mL^{-1} of biotinylated LecA in the presence of serial dilutions of inhibitors diluted in 0.3% (w/v) BSA in PBS. After washing with 0.05% Tween-PBS, 100 μL of streptavidin–peroxidase conjugate (dilution 1 : 5000; Boehringer-Mannheim) was added for 1 h at 37 °C. Signal recording was made possible using 100 μL per well of 0.05 M phosphate/citrate buffer containing *O*-phenylenediamine dihydrochloride (0.4 mg mL^{-1}) and urea hydrogen peroxide (0.4 mg mL^{-1}) (Sigma-Aldrich). Reactions were stopped by the addition of 50 μL of 30% H_2SO_4 and the absorbance was read at 490 nm using a microtitre plate reader (Bio-Rad; model 680).

ITC (isothermal titration microcalorimetry) analysis. ITC experiments were performed with a VP-ITC isothermal titration microcalorimeter (Microcal; GE Healthcare) except for the PNPGal ligand that was tested on ITC200 (Microcal; GE Healthcare). Experiments were carried out at 25 °C ± 0.1 °C. Purified and lyophilized LecA and carbohydrate ligands were dissolved in the same buffer, *i.e.* 0.1 M Tris-HCl pH 7.5, 5% DMSO (dimethyl sulfoxide) and 6 μM $CaCl_2$ and degassed. Protein concentrations in the microcalorimeter cell (1.4 mL) varied from 0.05 to 0.16 mM (cell of 200 μM for the ITC200). Concentration was checked by the measurement of optical density using a theoretical molarity extinction coefficient of 28 000 (1 cm). A total of 30 injections of 10 μL of sugar solution at concentrations varying from 0.9 to 1.7 mM were added every 300 s at 310 rev min^{-1}. Integrated heat effects were analyzed by non-linear regression using a single-site binding model (Origin 7.0). The experimental data fitted to a theoretical titration curve gave the association constant (K_a) and the enthalpy of binding (ΔH). Other thermodynamic parameters such as changes in free energy (ΔG) and entropy (ΔS) were calculated from the equation $\Delta G = \Delta H - T\Delta S = -RT \ln K_a$, where *T* is the absolute temperature and *R* is the molar gas constant (8.314 J mol^{-1} K^{-1}). All experiments were performed with *c* values of $10 < c < 100$.[42]

Crystallography. LecA at 0.8 mM was incubated with 1 mM of compound **11** for 1 h prior to crystallization which was performed by the hanging drop vapor diffusion method using 1 + 1 μL drops at 20 °C. Lozenge shaped crystals were obtained from solution 2 of the Clear Strategy Screen II (Molecular Dimensions Ltd) containing 0.8 M lithium sulfate and 100 mM sodium acetate pH 4.6. 25% glycerol was added for cryoprotection prior to mounting on a litholoop (Molecular Dimensions Ltd) and freezing in liquid nitrogen. Data were collected on beamline BM14 from ESRF, Grenoble, France using a MARCCD detector. The structure was solved by molecular replacement using the coordinates of tetramer from PDB

1OKO and the Phaser program.[43] The first model was updated using ARP/wARP[44] prior to subsequent cycles of TLS and restrained refinement using REFMAC5[45] iterated with manual rebuilding in COOT.[46] Details of the model quality are given in Table 1. The coordinates and structure factor were deposited in the Protein Data bank under the code PDB 4A6S.

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