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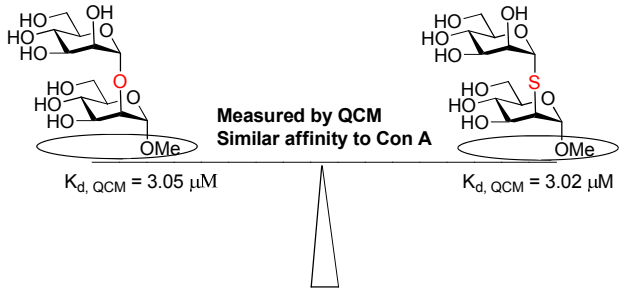
Graphical Abstract



Synthesis and Binding Affinity Analysis of α1-2- and α1-6-*O*/*S*-linked Dimannosides for the Elucidation of Sulfur in Glycosidic Bonds using Quartz Crystal Microbalance Sensors

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ABSTRACT

The role of sulfur in glycosidic bonds has been evaluated using quartz crystal microbalance methodology. Synthetic routes towards α1-2- and α1-6-linked dimannosides with *S*- or *O*-glycosidic bonds have been developed, and the recognition properties assessed in competition binding assays with the cognate lectin concanavalin A. Mannose-presenting QCM sensors were produced using photoinitiated, nitrene-mediated immobilization methods, and the subsequent binding study was performed in an automated flow-through instrumentation, and correlated with data from isothermal titration calorimetry. The recorded K_d-values corresponded well with reported binding affinities for the *O*-linked dimannosides with affinities for the α1-2-linked dimannosides in the lower micromolar range. The *S*-linked analogs showed slightly disparate effects, where the α1-6-linked analog showed weaker affinity than the *O*-linked dimannoside, as well as positive apparent cooperativity, whereas the α1-2-analog displayed very similar binding compared to the *O*-linked structure.

Keywords: Photochemistry, Thiosaccharides, CuAAC, Carbohydrates, Lectins, Biosensor, Quartz Crystal Microbalance

1. INTRODUCTION

With the increasing awareness of the role of glycans as key recognition entities in many fundamental cellular processes, more carbohydrate structures are targeted for therapeutic development, with several drugs already on the market [1–3]. Many glycans and glycoconjugates have so far been associated with cancer, viruses, bacteria and parasites, and have thus been targeted as vaccines. However, since carbohydrates are much less immunogenic than peptides, glycans conjugated with adjuvants have recently received much attention [1,4,5]. However, enzymatic degradation of the glycan-antigen remains a problem, thereby reducing the immune response, and more stable glycan analogs are needed. In this context, several types of glycans have been envisaged in which the glycosidic oxygen linkage has been exchanged for a carbon, sulfur or nitrogen atom [6–10]. Although these analogs are generally more stable, the structural modification may affect the resulting recognition effects. Not only may the interactions with the glycosidic bond change, but the overall conformation of the glycan is an important factor to consider [11].

Studies have shown that various *S*-linked disaccharides, such as thiolactose [10], thiomaltose [12], thiocellobiose [13], and glycosyldisulfides [14], display increased conformational flexibility compared to their natural analogs, and varying degrees of inhibition towards targeted enzymes. Contrary to these findings, Zhong et al. found that a series of α1-3- and α1-6-linked thiooligomannosides, derived from the natural substrate, resulted in complete loss of binding when tested against the enzyme Golgi α-mannosidase II [15]. Glycosidases have proven unable to hydrolyze thioglycosides [16–20], hypothetically due to the poor hydrogen bonding ability of the sulfur atom, thus hampering the acidic cleavage of *S,O*-acetals [21–23]. This effect is however not universal, and *O*- and *S*-linked *N*-acetylglucosamine (GlcNAc) residues have been shown to undergo cleavage with comparable efficiencies in the presence of a human *O*-GlcNAcase [24]. Although these effects are difficult to predict, thioglycosides are progressively being identified as new therapeutics [25]. For instance, recent studies explored *S*-linked glycan analogs as hydrolytically stable immunogens, used either by themselves or coupled to tetanus toxoid or bovine serum albumin for increased immunogenicity. The results showed that the glycans efficiently induced immune responses, which detected not only the *S*-linked glycan analog but also the naturally occurring *O*-linked glycan target [26–28]. Another example is morphine- and codeine-6-glucuronide (M6G and C6G), two abundant analgesic metabolites of morphine which are both more potent than morphine and display less side effects making them more attractive as analgesics. However, the bioavailability is low due to hydrolysis of the glycosidic bond, an issue which was addressed by MacDougall et al. by introducing an *S*-linkage [29]. The results showed that the thioanalogs were full agonists with M6G and that the *S*-linkage is a viable strategy for improving the pharmacological properties. Glycan thiols further present potential advantages in the *in vitro* synthesis of glycoconjugates/-proteins where site-specific glycosylation is problematic due to the many competing functional groups in natural glycans. Sulfhydryl groups present increased nucleophilicity compared to hydroxyl groups, can be oxidized to the corresponding disulfides, as well as react selectively in many other types of reactions [30–37].

Herein, the role of sulfur in specific glycosidic bonds, with respect to protein binding effects, has been evaluated using quartz crystal microbalance (QCM) instrumentation where the chips were functionalized with α-D-mannopyranoside monosaccharides [38–41]. We present the synthesis of a series of *O*- and *S*-linked dimannosides, together with subsequent affinity evaluation against lectins with known specificity towards oligomannoside glycans. The binding studies were performed as competition assays where the binding of the tested lectins towards mannose-functionalized surfaces was detected in a QCM flow-through instrumentation, which also enabled comparison of the technique with isothermal titration calorimetry (ITC). Recurring injections of solutions of lectin with the dimannoside inhibitors of varying concentration generated inhibitory response curves, which fitted well to the binding data. The resulting EC_{50}-values correlated well K_d-values estimated by ITC for the *O*-linked dimannosides.

2. RESULTS AND DISCUSSION

Two sets of disaccharides were chosen for the study (Figure 1), based on previous binding affinity studies towards the target lectin Con A [42]. α1-6-Linked dimannoside **1** has been shown to bind with relatively low affinity (similar to the affinity of methyl α-D-mannopyranoside) whereas the corresponding α1-2-linked dimannoside **3** displayed a 17-fold increase in affinity. Consequently, binding affinity analysis of the target disaccharides would illustrate the effect of sulfur in glycosidic linkages, both in high and low affinity binding events.

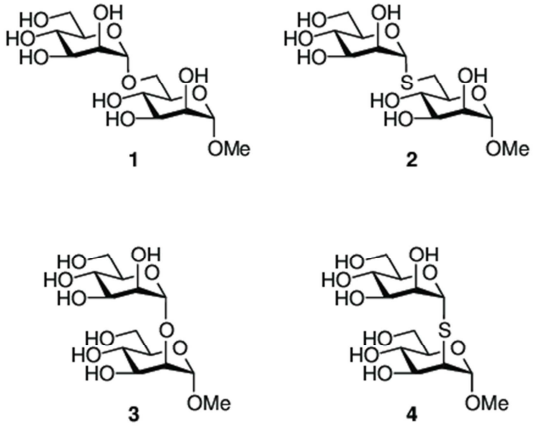


Figure 1. Dimannoside structures studied.

2.1. Synthetic design

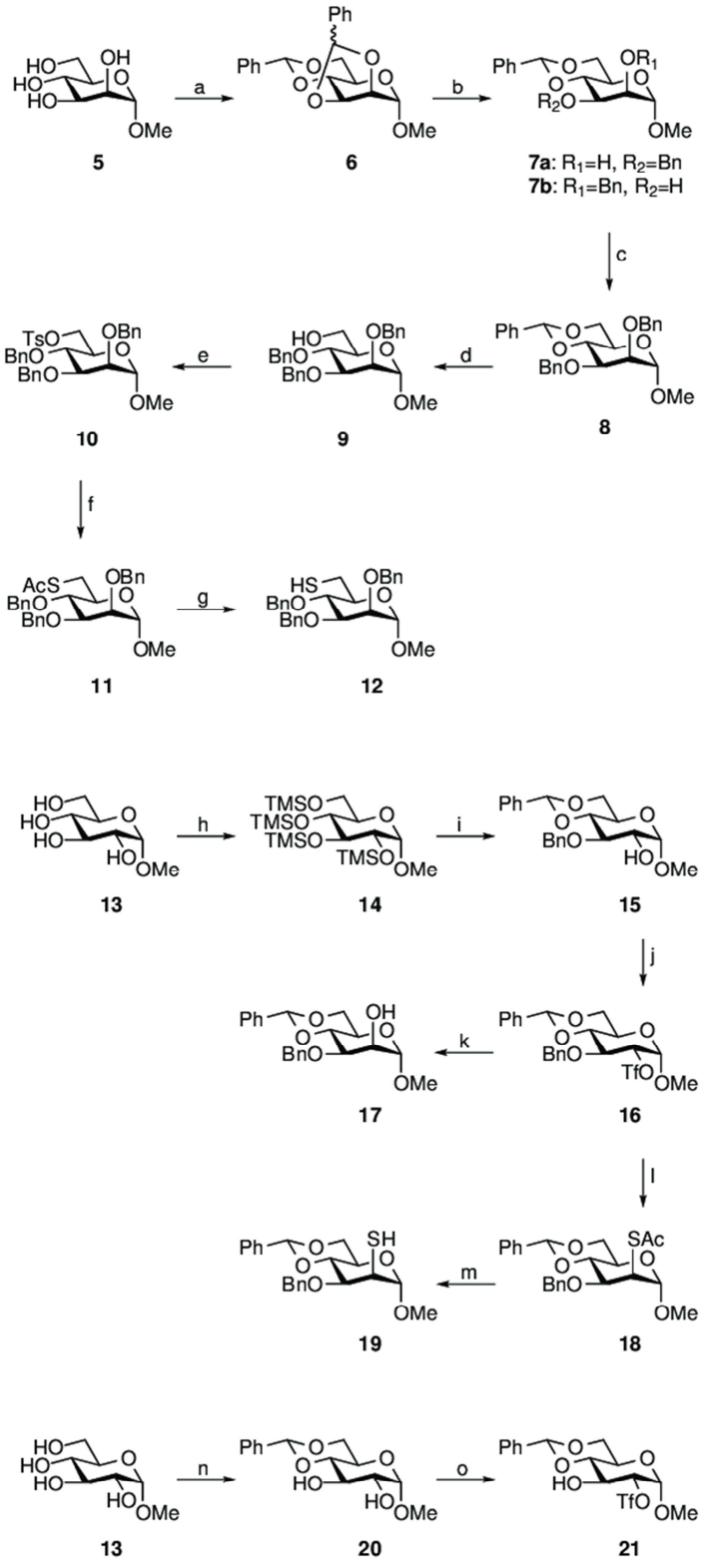
The synthetic routes towards the target disaccharides were initially designed based on glycosylation with suitable selectively protected glycosyl acceptors and thioglycosyl donors [43], where the donors were chosen based on their reported versatility in synthetic transformations and relatively mild reaction conditions needed for activation [44]. Glycosyl acceptors with one free hydroxyl or sulfhydryl group were thus designed to react with fully protected thioglycosyl donors, under *N*-iodosuccinimide-mediated and triflic acid-catalyzed conditions, to produce the corresponding fully protected disaccharides. Although glycosylation with sulfhydryl acceptors and thioglycosyl donors may potentially fail due to cross-selectivity between the acceptor sulfur entity and the donor thioether, no reports thereof could be found.

This strategy proved generally viable, but alternative strategies were also applied. Thus, glycosyl donors possessing an anomeric acetyl group were additionally designed as alternative donors for the *S*-linked disaccharide synthesis, and the synthetic strategy was subsequently further complemented with a 1-thiopyranose donor and a thioglycosyl acceptor pair.

2.2. Synthesis of glycosyl acceptors

The syntheses of selectively protected glycosyl acceptors **9** and **12** originated from commercially available methyl α-D-mannopyranoside **5** and were carried out in straightforward four/six-steps routes Scheme 1. Methyl α-D-mannopyranoside **5** was first fully protected with benzylidene groups using benzaldehyde dimethyl acetal in the presence of catalytic amounts of molecular iodine [45]. The use of iodine is an attractive alternative to the commonly used hazardous and moisture sensitive acid catalysts and the product was obtained after 3 h with a yield of 92%. The 2,3-*O*-benzylidene acetal was then selectively opened using diisobutylaluminum hydride (DIBAL-H) in hexane, leaving the more stable six-membered cyclic 4,6-*O*-acetal intact and producing a mixture of isomers **7a** and **7b** [46]. The main product was in this case isomer **7b** with the benzyl ether in the axial position. Both isomers were however used in the subsequent benzylation step; producing fully protected mannoside **8** in 93% yield over two steps. Reductive opening of the 4,6-*O*-benzylidene acetal was then achieved under copper(II) triflate ($Cu(OTf)_2$)-catalysis, successfully leaving the primary hydroxyl group unprotected and producing hydroxyl glycosyl acceptor **9** with a yield of 97% [47]. Sulfhydryl glycosyl acceptor **12** was then produced through a protection-substitution-deprotection route from glycosyl acceptor **9**. Initially, triflic anhydride was

evaluated for the leaving group transformation but discarded due to stability issues of the triflate product. Tosyl chloride (TsCl) was therefore employed giving mannoside tosylate **10** in 98% yield. The reaction was highly moisture sensitive requiring dry conditions for completion. Subsequently, the tosylate was replaced by thioacetate in an S_N2-reaction using potassium thioacetate in dimethylformamide (DMF), giving mannoside **11** in 92% yield. Finally, deacetylation produced sulfhydryl glycosyl acceptor **12** in 76% yield. The reaction proved very fast and the resulting product was highly prone to oxidation thus reducing the yield. Limiting the reaction time to 5-10 min sufficiently prevented the formation of the disulfide.



Scheme 1. Synthesis of glycosyl acceptors **9**, **12**, **17**, **19** and **21** (*Cf.* supporting information for abbreviations and details). a) $PhC(OMe)_2$, I_2, MeCN, rt, 3 h (92%); b) DIBAL-H (hexane), DCM, 0 °C, 2 h (**7a** + **7b**: 93%); c) BnBr, NaH, DMF, 0 °C-rt, 2.5 h (99%); d) $BH_3 \cdot THF$, $Cu(OTf)_2$, THF, rt, 2 h (87%); e) pyridine, MS 4 Å, TsCl, rt, 4 h (98%); f) KSAc, DMF, 60 °C, 4 h (92 %); g) NaOMe, MeOH, rt, 5-10 min, (76 %); h) TMSCl, DIPEA, DCM, 0 °C, 25 h (quant.); i) i) PhCHO, TMSOTf, -78 °C, 2 h, ii) PhCHO, Et_3SiH, -78 °C, 3 h (94%); j) pyridine, Tf_2O, DCM, -15 °C, 2.5 h (99%); k) $TBANO_2$, toluene, 70 °C, 24 h (70%); l) KSAc,

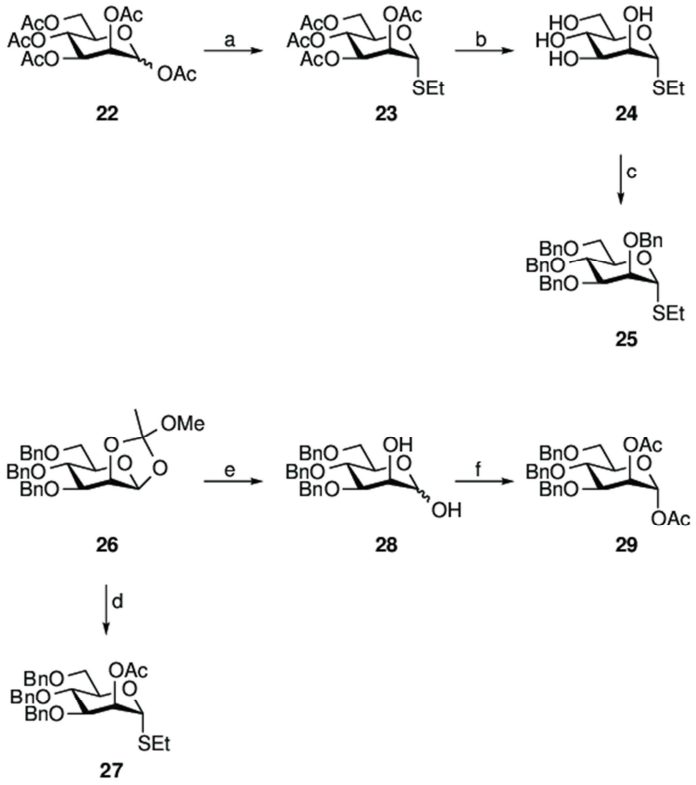
DMF, 40 °C, 16 h (89%); m) NaOMe, MeOH, rt, 5-10 min (77%); n) $PhC(OMe)_2$, TsOH, DMF, rt, 6 h (85%); o) Pyridine, Tf_2O, DCM, -20 °C, 2 h (85%).

The syntheses of glycosyl acceptors **17** and **19** started from methyl α-D-glucopyranoside **13** and were based on the key intermediate triflate **16** Scheme 1. Full protection using trimethylsilyl chloride (TMSCl) efficiently produced protected glucose derivative **14** in quantitative yield. Then, a one-pot two-step deprotection/protection reaction readily gave glucoside **15** selectively deprotected in the 2-*O*-position [48]. A 4,6-benzylidene acetal was first obtained using benzaldehyde and the Lewis acid trimethylsilyl triflate (TMSOTf), followed by reductive etherification using benzaldehyde and triethylsilane. Interestingly, Wang et al. reported the use of tetrabutylammonium fluoride (TBAF) to cleave off the final trimethylsilyl protecting-group in the 2-*O*-position [48]. However, this was deemed unnecessary since the deprotected glucoside **15** was already observed. Subsequent triflation of the hydroxyl group in the C_2-position and nitrite/thioacetate inversion would then produce the correct mannose-configuration. Triflation was easily performed using triflic anhydride in pyridine, producing compound **16** in 99% yield without the need for column purification. Inversion was then performed either with tetrabutylammonium nitrite ($TBANO_2$) producing hydroxyl glycosyl acceptor **17** in 70% yield, or with tetrabutylammonium thioacetate/potassium thioacetate (TBASAc/KSAc) producing mannoside **18** in 89% yield, according to a modified procedure by Pei et al [49]. Higher yields were generally obtained with KSAc in DMF compared to TBASAc in toluene, likely because of the feasible lower reaction temperature (40 °C instead of 70 °C) and less demanding purification. Thioacetate **18** was then treated with sodium methoxide in methanol to give sulfhydryl glycosyl acceptor **19** in 77% yield.

Following unsuccessful glycosylations using 2-thio-acceptors and different donors, the reverse strategy was instead pursued with 1-thio-α-D-mannopyranosides and corresponding 2-*O*-triflate glucoside acceptors. The best results were in this case obtained with thioglycoside acceptor **21**, prepared in two efficient steps from methyl α-D-glucopyranoside **13** according to Knapp et al [50]. Thus, the 4,6-benzylidene acetal was first produced using benzaldehyde dimethyl acetal under acidic conditions, and compound **21** obtained following pyridine-assisted triflate formation in dichloromethane at -20 °C.

2.3. Synthesis of glycosyl donors

Peracetylated and perbenzylated glycosyl donors **23** and **25** were initially envisioned for the glycosylation reactions, primarily for their facile synthesis Scheme 2. Lewis acid catalyzed coupling of ethanethiol gave glycosyl donor **23** in moderate yield, and, following Zemplén deprotection and perbenzylation, produced glycosyl donor **25** in 40% yield over two steps. As reported for several types of glycosyl donors, the electronic properties of the neighboring protecting groups significantly affect the reactivity of the activatable species, in this case the thioethyl-moeity [51–55]. For this reason, “superarmed” glycosyl donor **27** was synthesized in a straightforward five step route through key intermediate **26** Scheme 2. Orthoester **26** was synthesized according to Beignet et al [56]. starting from peracetylated D-mannose **22**. Lewis acid-promoted ring-opening under dry conditions then produced glycosyl donor **27** in 68% yield. In addition to the 1-thioethyl glycosyl donors **23**, **25** and **27**, 1-acetyl-glycosyl donor **29** was synthesized from key intermediate **26** in a two-step deprotection-protection reaction according to Ponpipom [57]. Orthoester **26** was thus hydrolyzed in aqueous acetic acid and acetylated using acetic anhydride, giving glycosyl donor **29** in 77% yield over two steps.

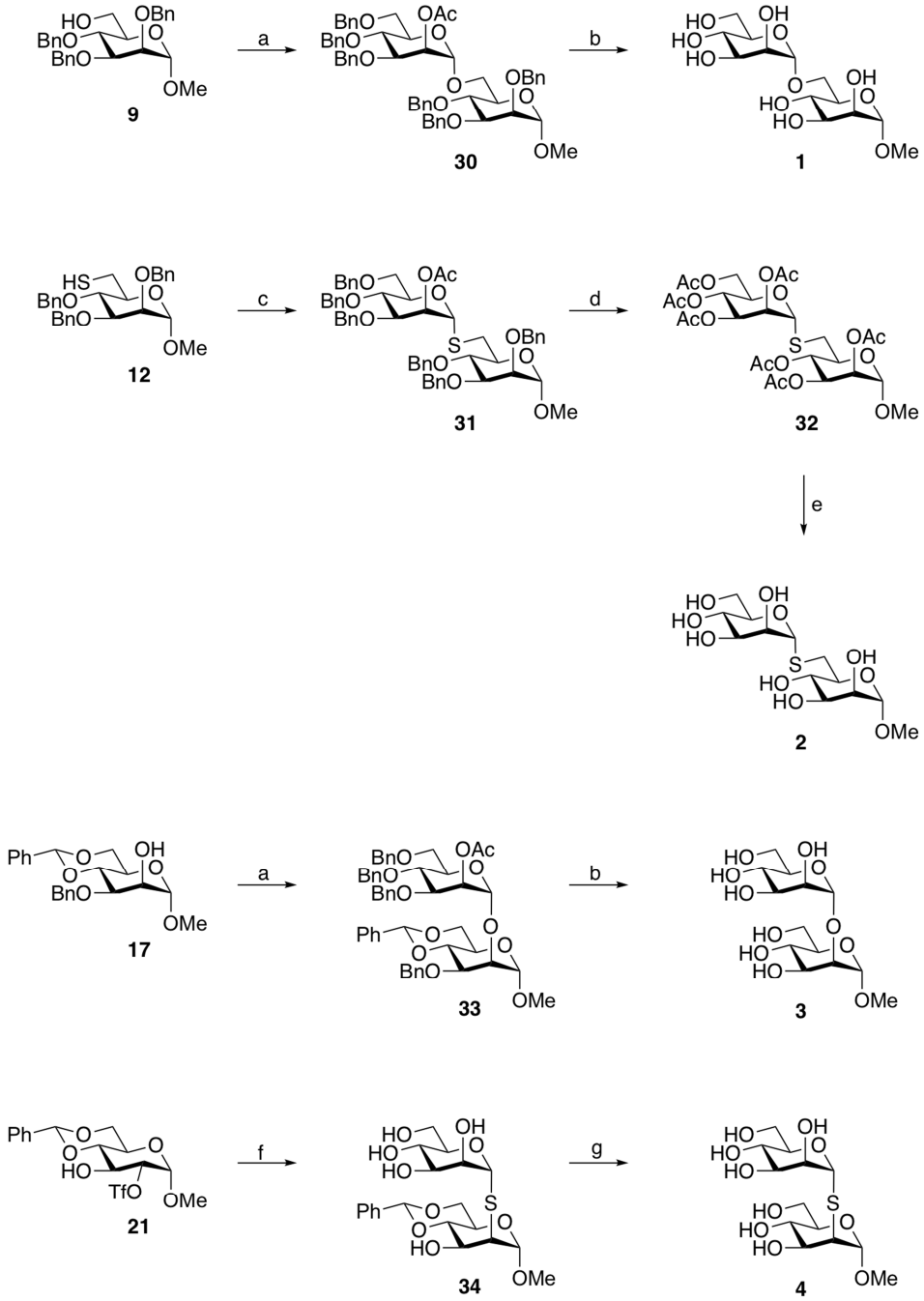


Scheme 2. Synthesis of glycosyl donors **23**, **25**, **27**, and **29** (*Cf*. supporting information for abbreviations and details); a) EtSH, $BF_3 \cdot Et_2O$, DCM, 0 °C-rt, 24 h (51%); b) NaOMe, MeOH, rt, 3 h (quant.); c) BnBr, NaH, DMF, 0 °C-rt, 4 h (40%); d) EtSH, $HgBr_2$, MeCN, 60 °C, 24 h (68%); e) AcOH:H_2O (1:2), 100 °C, 4 h; f) Ac_2O, pyridine, DMAP, rt, 1 h (77% over two steps).

2.4. Glycosylation reactions

Initially, the hydroxyl glycosyl acceptor **9** was allowed to react with the peracetylated mannosyl donor **23** under iodonium promotion, as described by Veeneman et al [43]. It was thus reported that glycosylation with a β-galactose derivative resembling mannosyl donor **23** (benzoyl instead of acetyl) underwent quick and selective formation of the β-anomer in up to 98% yield, whereas the same reaction with a β-glucose derivative resembling mannosyl donor **25** resulted in a mixture of α/β-anomers. Surprisingly, in the present case, no reaction could be observed using peracetylated mannosyl donor **23**, presumably due to the deactivating acetyl protecting groups. On the other hand, glycosylation using perbenzylated mannosyl donor **25** proceeded quickly, resulting in a complex mixture. However, electrospray ionization mass spectrometry (ESI-MS) and ^{1}H-NMR analysis confirmed the presence of the disaccharide formation as an anomeric mixture, albeit in low yield.

Glycosyl donor **27** was subsequently applied to the same reaction conditions with glycosyl acceptors **9** and **17**, successfully yielding α1-6- and α1-2-linked disaccharides **30** and **33** Scheme 3. Here, the combination of lower deactivation from the less electron withdrawing benzyl groups and the activating and directing acetyl group efficiently produced the α-anomer in short time [53,54]. Deprotection was performed in a two-step one-pot reaction, starting with deacetylation using sodium methoxide in methanol. After completion of the starting material, as determined by TLC, acetic acid was added to quench the base and the reaction mixture was stirred in the presence of palladium under hydrogen atmosphere, forming unprotected disaccharides **1** and **3** in 77% and 85% yield, respectively.



Scheme 3. Glycosylation reactions leading to dimannosides **1**-**4**. (*Cf*. supporting information for abbreviations and details); a) **27**, NIS, TfOH, DCM, MS 4 Å, -15 °C, 15 min (**30**, 80%, **33**, 39%); b) i: NaOMe, MeOH, rt, 6-24 h, ii: H_2, Pd/C, AcOH, MeOH, 24-72 h (**1**, 77%, **3**, 85%); c) **29**, $BF_3 \cdot Et_2O$, DCM, MS 3 Å, 0 °C-rt, 13 h (61%); d) i: Li, NH_3 (*l*), THF, -78 °C, 2 min, ii: pyridine, Ac_2O 17 h (85%); e) Li, MeOH, 2.5 h (94%); f) 1-thio-α-D-mannopyranose sodium salt, DMF, 50 °C, 18 h, (25%); g) DCM/MeOH/AcOH 1:1:2 (v/v), 50 °C, (76%).

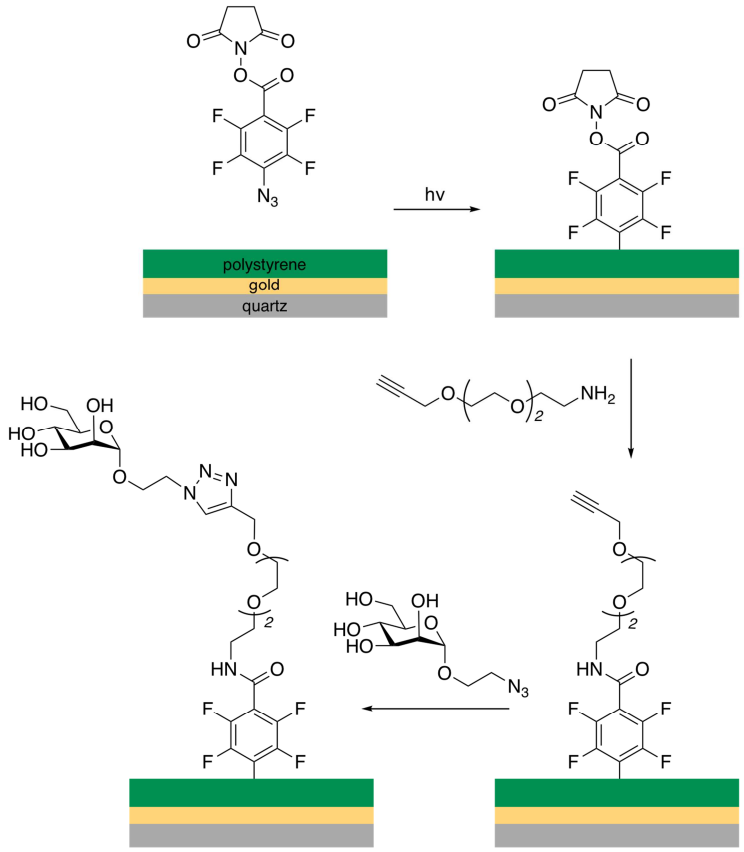
The sulfhydryl acceptors proved more challenging. Reactions under the aforementioned conditions with glycosyl acceptor **12** and glycosyl donor **27** yielded no disaccharide but instead the 1-methoxy mannoside side product, as determined by NMR-analysis. Attempts were made to pre-activate the glycosyl donor **27** by mixing it with *N*-iodosuccinimide and triflic acid prior to addition of the glycosyl acceptor, however resulting in the same product mixture. The glycosyl acceptor **12** was instead allowed to react with orthoester **26** and $HgBr_2$,

since these conditions proved successful in the formation of glycosyl donor **27**. Unfortunately, even after 24 h, no disaccharide was formed and only the corresponding disulfide as well as the hydrolyzed orthoester could be observed. The glycosyl donor **29** was subsequently evaluated according to a modified procedure by Abronina et al. Scheme 3 [58]. Using the strong Lewis acid $BF_3 \cdot Et_2O$, the desired *S*-linked disaccharide **31** was thus produced in 61% yield. Deprotection of the *S*-linked disaccharide **31** was initially evaluated using $FeCl_3$, however quickly abandoned due to purification issues. Deprotection using the same procedure as for the *O*-linked disaccharides **30** and **33** was instead attempted, and although this route initially looked promising, the resulting monosaccharide species indicated that the glycosidic linkage was too labile under these conditions. Finally, efficient deprotection was successfully achieved using Birch reduction and an acetylation-deacetylation protocol according to Cumpstey et al [32]. The protected disaccharide **31** was thus subjected to liquid ammonia containing dissolved lithium for 2 min before quenching using ammonium chloride. To simplify purification, the resulting salt mixture was subjected to acetic anhydride and pyridine overnight, giving the peracetylated disaccharide **32** in 85% yield. Subsequent deacetylation using lithium in methanol provided the desired *S*-linked disaccharide **2** in 94% yield.

The synthesis of the α1-2-*S*-linked disaccharide was initially evaluated by the same route as for the α1-6-linked analog using glycosyl donor **29** and glycosyl acceptor **19**. Unfortunately, no disaccharide was formed although both starting materials were quickly consumed. The 1-methoxy mannoside side product was instead formed together with a highly polar carbohydrate residue and benzaldehyde, indicating that the 4,6-benzylidene acetal in glycosyl acceptor **19** had been removed. Varying the reaction temperature did not result in better selectivity. Attempts to replace the 4,6-benzylidene acetal by more robust benzyl protecting groups proved unsuccessful, and an alternative route was instead devised using the reversed reactivities of the two carbohydrate moieties. The synthesis of α1-2-*S*-linked dimannoside **4** was thus performed in a 2-step route from the unprotected 1-thio-α-D-mannopyranose sodium salt [59], and triflate derivative **21**. Nucleophilic substitution of the triflate group in dimethylformamide at 50 °C for 18 h afforded dimannoside **34** in relatively low yield, showing the difficulties in efficiently forming α1-2-*S*-linked dimmanosides. Deprotection of the benzylidene moiety under mildly acidic conditions subsequently led to compound **4** in 76% yield.

2.5. Binding affinity study

The relative binding affinities of the synthesized mannosides towards the target lectin Con A were determined by competition assays using a QCM flow-through instrumentation and mannose-functionalized sensor chips, produced by methodology reported previously [39,40]. The surfaces were thus prepared using a three-step method from polystyrene-coated, gold-plated QCM crystals (Scheme 4). This involved conjugation of NHS-activated 4-azido-2,3,5,6-tetrafluorobenzoic acid to the polymer surfaces using UV-irradiation, followed by amide formation with an alkyne-containing, amine-terminated diethyleneglycol linker creating an alkyne-functionalized surface. Finally, an α-D-mannopyranosyl azide structure was coupled to the surfaces using copper-catalyzed alkyne-azide cycloaddition (CuAAC) chemistry.



Scheme 4. Functionalization of QCM sensor surfaces using photoinitiated, nitrene-mediated coupling and copper-catalyzed alkyne-azide cycloaddition.

The mannose-functionalized surfaces were subjected to injections of solutions containing Con A (2 µM) and either of the mannoside inhibitors in different concentrations. The observed reduction in measured frequency, compared to injections with pure Con A, was taken as a measure of the competitive effect of the tested mannosides. The quantitative binding was then subjected to nonlinear regression analysis using a dose-response inhibition model (equation 1, *cf*. Supporting Information).

$$Y = f_{\min} + \frac{f_{\max} - f_{\min}}{1+10^{\left(Hill\ coeff \times \left(\log(C) - log(\mathrm{EC}_{50})\right)\right)}} \qquad (1)$$

During the binding studies, an unexpected binding behavior was noticed during the injections with high inhibitor concentrations Figure 2. Control injections in the absence of lectin however showed the same behavior, indicating that the resulting frequency change emanated from an instrumental artifact due to the change of buffer composition. Subtraction of the lectin response curves with the corresponding inhibitor response curves thus resulted in the corrected binding Figure 2. Control experiments using α/β-D-galactose at varying concentrations confirmed the trend, simultaneously validating the methodology.

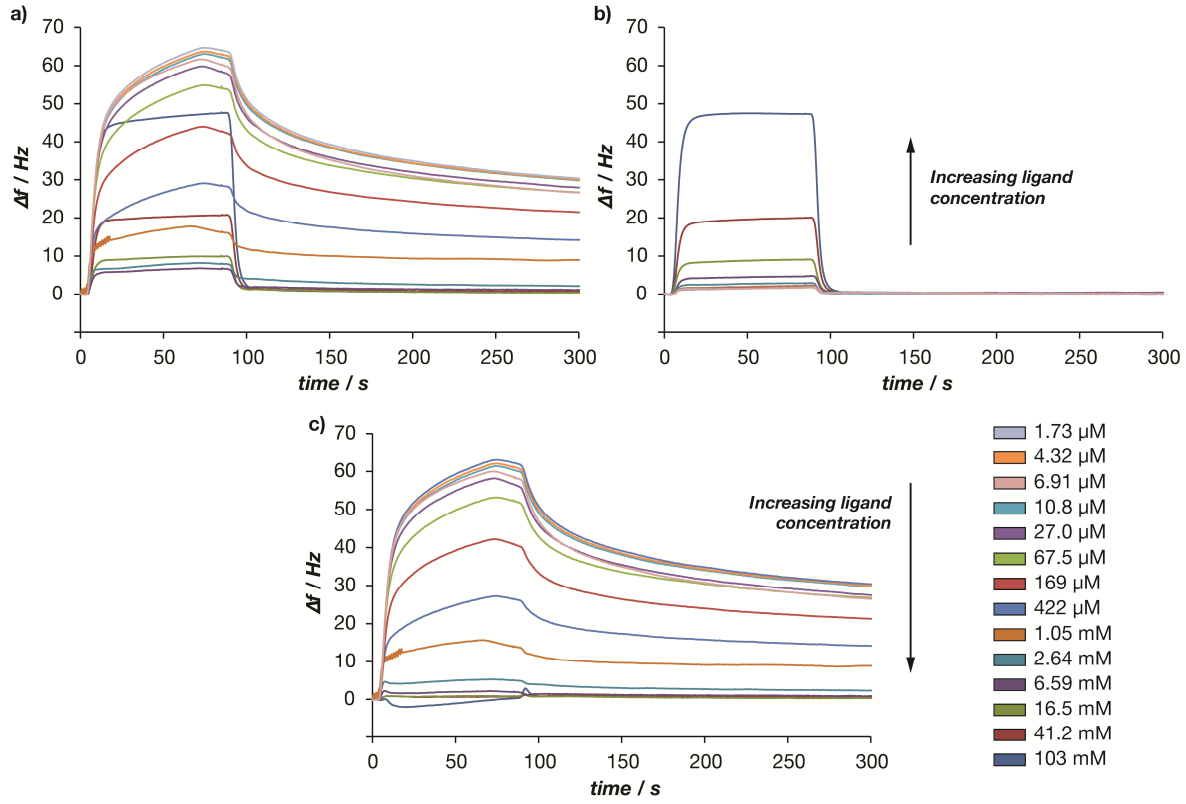


Figure 2. Example of binding analysis using QCM instrumentation. Frequency responses of: a) injections with Con A in the presence of methyl α-D-mannopyranoside **5**; b) injections with methyl α-D-mannopyranoside in the absence of Con A (higher ligand concentrations lead to higher frequency shifts); c) corrected response curves (ligand frequency effect subtracted from original frequency shifts; higher ligand concentrations lead to lower lectin binding and lower frequency shift).

The results from the binding study are presented in Figure 3 and Table 1. The stable surfaces allowed for reproducible binding over time, thus generating inhibitory response curves which fitted well to the binding data. Furthermore, the EC_{50}-values obtained for methyl α-D-mannopyranoside **5** were used as reference points, calibrating the surfaces between batches. Compound **5** was also used as reference in the affinity estimations, using a reported K_d of 122 µM [60]. As can be seen, the α1-6-*O*-linked dimannoside **1** and the α1-2-*O*-linked dimannoside **3** correlated well with previously reported K_d-values, allowing for simplified comparison between the inhibitors [60]. Contrary to previously published studies, however, the α1-6-*O*-linked dimannoside **1** showed almost twice as high inhibition as the monosaccharide in the present case. Comparison of the α1-6-linked dimannosides **1** and **2** indicated slightly weaker binding of the *S*-linked dimannoside; results that are in correlation with previous studies of *S*-linked disaccharides [10,13]. Furthermore, the results showed a steeper inhibitory response curve for compound **2**, with a corresponding Hill coefficient of 1.8, indicating apparent positive cooperativity. More in-depth analysis is required to elucidate the reason behind this effect. The α1-2-*O*-linked dimannoside **3** and the α1-2-*S*-linked analog **4** both displayed K_d-values of around 3.0 µM, making them the strongest inhibitors in the study, with 23 times higher affinities than compound **1**. No significant increase of the Hill coefficient was however observed for the α1-2-*S*-linked dimannoside.

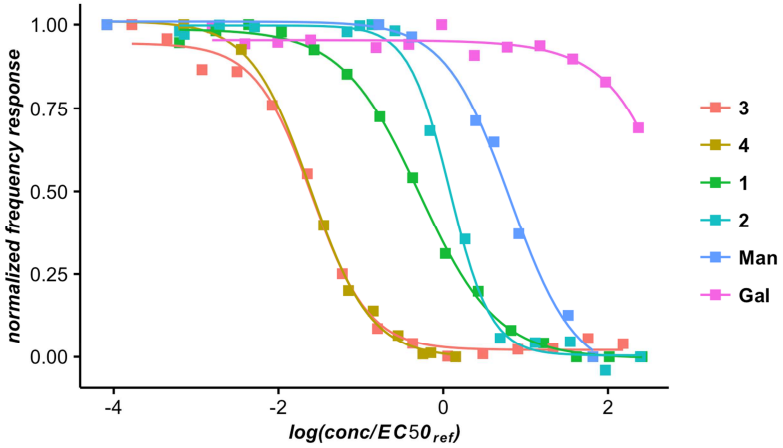


Figure 3. Inhibitory dose-response curves for interactions of dimannosides **1-4** with Concanavalin A. Data normalized with respect to frequency response, and estimated EC_{50}-values of the reference ligand ($EC_{50,ref}$) used to correlate experiments based on different surfaces for visualization purposes (*cf.* Supporting Information). Man = D-mannose; Gal = D-galactose.

Table 1. Binding data; ITC data taken from [42] and [60]; H = Hill coefficient; RP = relative potency; Man = D-mannose

Compound	$K_{d,QCM}$ (μM)	95% CI (μM)	RP_{QCM}	H_{QCM}	$K_{d,ITC}$ (μM)	RP_{ITC}
1	69.3	(58.5-80.4)	1	1	123	1
2	155	(133-182)	0.45	1.8	-	-
3	3.05	(2.4-3.55)	23	1	7.09	17
4	3.02	(2.54-3.57)	23	1.2	-	-
Man	795	(493-1280)	0.087	1	452	0.1

3. CONCLUSIONS

A series of *O*- and *S*-linked carbohydrates have been designed and synthesized for the evaluation of *S*-glycosides in lectin binding. Glycosyl acceptors possessing a single hydroxyl/sulfhydryl group were synthesized in few steps in generally high yields. The *O*-linked disaccharides were obtained with thioethyl-linked glycosyl donors under NIS/TfOH conditions. The same methodology however failed for the *S*-linked disaccharides, likely because of low selectivity between the sulfur entities. The synthesis of the α1-6-*S*-linked dimannoside was instead completed through glycosylation with a 1-*O*-acetyl-glycosyl donor under Lewis acid catalysis, and the α1-2-*S*-linked structure was formed from the reaction between the unprotected 1-thiomannose donor and a triflate acceptor. The *O*-linked disaccharides were deprotected using classical catalytic hydrogenation whereas the *S*-linked disaccharide was deprotected using Birch reduction.

A competition binding assay was performed using a QCM flow-through instrumentation with prefabricated mannose-functionalized surfaces and the model lectin Con A. The methodology proved valid and the acquired K_d-values correlated well with reported affinity values for the tested *O*-linked mannosides using isothermal titration calorimetry. The relationship between

the studied mannosides followed the expected trends, and the α1-6-*S*-linked dimannoside displayed similar quantitative inhibition as the α1-6-*O*-linked dimannoside, however with a slightly higher K_d-value and a clear apparent positive cooperativity. The highest affinities of the series were obtained for the α1-2-linked dimannosides, both of which demonstrated K_d-values in the lower micromolar range. The binding effects were in this case very similar between the *O*- and the *S*-linked structures. These data demonstrates the potential for the *S*-linked carbohydrate structures to serve as substitutes for their *O*-linked counterparts in biomedical applications.

4. EXPERIMENTAL SECTION

General Methods: All commercially available starting materials were of reagent grade and used as received. ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) data were recorded on a Bruker Avance 400 instrument at 400 MHz (^{1}H) or a Bruker DMX 500 instrument at 500 MHz (^{1}H) or 125 MHz (^{13}C). Chemical shifts are reported as δ values (ppm) with either $CHCl_3/CDCl_3$ (^{1}H: δ = 7.26, ^{13}C = 77.16) or HDO (^{1}H: δ = 4.79) as internal standards. ^{1}H peak assignments were made by first order analysis of the spectra supported by standard ^{1}H-^{1}H correlation spectroscopy (COSY). Thin layer chromatography (TLC) was performed on precoated Cromatofolios AL Silica gel 60 F254 plates (Merck). Flash column chromatography was performed on silica gel 60, 0.040-0.063 mm (SDS). Spincoating was performed using a Cookson Electronics Specialty Coating Systems Spincoater model P6708D. Photoreactions were performed at 254 nm using a photochemical reactor from Rayonet Srinivasan - Griffin. Details of all synthetic protocols are provided in the Supporting information, including a list of abbreviations of reagents and solvents.

4.1. General surface functionalization

QCM analyses were performed using flow-through Attana A200 or A100 C-Fast QCM systems. Gold-plated and polystyrene-coated [38], 10 MHz QCM crystals were obtained from Attana AB. The polystyrene surfaces were functionalized with α-D-mannopyranoside monosaccharides by Photo-Click functionalization methodologies previously developed in our laboratory [39–41]. A continuous flow of running buffer tris(hydroxymethyl)aminomethane (Tris) 10 mM or PBS 10 mM, pH 7.4, 25 μL/min) was used throughout the experiments, and samples of Con A were prepared in the same buffer. The crystals were washed/equilibrated with buffer solution prior to manipulations/measurements.

4.2. QCM analysis

Concanavalin A (Con A) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. After equilibration of the crystals in the flow-through system, they were subjected to repeated injections of BSA, followed by injections of low pH buffer (pH 1.5) and finally additional injections of BSA to fully block any non-functionalized surfaces. Binding to the surfaces was monitored by frequency logging with Attester 1.1 (Attana), and adsorption/desorption to the surface recorded as the resulting frequency shifts. Solutions of the lectins were then injected on the system, where the resulting shifts in frequency correspond to binding to the surfaces. Bound lectins were released from the surfaces between measurements by two successive injections of low pH buffer (pH 1.5). The procedure was then repeated at least two times for each carbohydrate inhibitor concentration to give an average value and determine the surface stability over time.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/

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Highlight:

1. Synthetic routes towards α1-2- and α1-6- dimannosides with S- or O-glycosidic bonds.
2. Their recognition properties assessed in competition binding assays with Con A.
3. The role of sulfur in glycosidic bonds has been evaluated using QCM methodology.
4. The binding effects were very similar between the *O*- and the *S*-linked structures.

Supporting Information

Synthesis and Binding Affinity Analysis of α1-2- and α1-6-*O/S*-linked Dimannosides for the Elucidation of Sulfur in Glycosidic Bonds using Quartz Crystal Microbalance Sensors

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Table of Contents

Abbreviations of reagents and solvents

AcOH: acetic acid; Ac_2O: acetic anhydride; BnBr: benzyl bromide; DCM: dichloromethane; DIBAL-H: diisobutylaluminum hydride; DIPEA: diisopropylethylamine; DMAP: 4-dimethylaminopyridine; DMF: dimethylformamide; Hex: hexanes; KSAc: potassium thioacetate; MeCN: acetonitrile; MS: molecular sieves; NaOMe: sodium methoxide; NIS: *N*-iodosuccinimide; OTf: triflate; rt: room temperature; $TBANO_2$: tetrabutylammonium nitrite; THF: tetrahydrofuran; TMSCl: trimethylsilyl chloride; TMSOTf: trimethylsilyl triflate; Tf_2O: triflic anhydride; TsCl: tosyl chloride; TsOH: tosylic acid

Synthesis of 6-hydroxyl and 6-sulfhydryl glycosyl acceptors

Methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside (6) (Ennis et al., 2002). Commercially available methyl α-D-mannopyranoside (**5**, 1.95 g, 10.0 mmol) was dissolved in dry acetonitrile (20 mL) and stirred at rt. Benzaldehyde dimethyl acetal (3.22 mL, 22.1 mmol) and I_2 (765 mg, 3.01 mmol) was added and the mixture was stirred at rt for 5.5 h. The mixture was then diluted with DCM and washed with water containing $Na_2S_2O_5$ (950 mg, 5.00 mmol). The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure giving 3.9 g of crude product. The crude product was separated by flash column chromatography with solvent system DCM:MeOH 12:1 giving pure compound **6** (3.43 g, 92%). TLC (Hex/EtOAc 3:1): R_f 0.51; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.55-7.55 (m, 2 H, ArH), 7.48-7.50 (m, 2 H, ArH), 7.37-7.43 (m, 6 H, ArH), 6.32 (s, 1 H, PhC*H*), 5.67 (s, 1 H, PhC*H*), 5.05 (as, 1 H, H-1), 4.66 (dd, *J* = 5.6 Hz and 8.0 Hz, 1 H, H-3), 4.36-4.42 (m, 1 H, H-6), 4.17 (d, *J* = 5.4 Hz, 1 H, H-2), 3.93 (at, *J* = 8.7 Hz, 1 H, H-4), 3.83-3.90 (m, 2 H, H-5, H-6') 3.44 (s, 1 H, OMe).

Methyl 2/3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (7a,b) (Tanaka et al., 2008). Compound **6** (985 mg, 2.66 mmol) was dissolved in dry DCM (40 mL) and stirred at 0 °C. Then $AlCl_3$ (18 mg, 0.133 mmol) was added and mixture was stirred under nitrogen while DIBAL-H (5.9

mL, 1M in hexane) was added drop wise. Mixture was then stirred at 0 °C for 2 h before quenching by addition of MeOH. The reaction mixture was then diluted with 1M HCl (aq) and extracted with DCM (2 × 50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure giving 1.03 g of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 5:1 giving pure compounds **7a** and **7b**, which could both be used in the next step (921 mg, 93%). **7a**: TLC (Hex/EtOAc 5:1): R_f 0.24; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.48-7.51 (m, 2 H, ArH), 7.28-7.41 (m, 8 H, ArH), 5.62 (s, 1 H, PhC*H*), 4.85 (d, *J* = 11.9 Hz, 1 H, PhCH_2), 4.77 (d, *J* = 1.1 Hz, 1 H, H-1), 4.72 (d, *J* = 11.9 Hz, 1 H, PhCH_2), 4.28 (dd, *J* = 4.4 Hz and 9.7 Hz, 1 H, H-6), 4.10 (at, *J* = 9.3 Hz, 1 H, H-4), 4.05-4.06 (m, 1 H, H-2), 3.91 (dd, *J* = 3.5 Hz and 9.6 Hz, 1 H, H-3), 3.87 (at, *J* = 10.0 Hz, 1 H, H-6), 3.79-3.84 (m, 1 H, H-5), 3.38 (s, 3 H, OMe), 2.64 (d, *J* = 1.2 Hz, 1 H, OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.2, 137.7, 129.1, 128.6, 128.4, 128.1, 128.0, 126.2, 101.8, 101.2, 79.0, 75.7, 73.2, 70.1, 69.1, 63.3, 55.1. **7b**: TLC (Hex/EtOAc 5:1): R_f 0.13; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.47-7.50 (m, 2 H, ArH), 7.31-7.39 (m, 8 H, ArH), 5.58 (s, 1 H, PhC*H*), 4.74-4.77 (m, 2 H, H-1, PhCH_2), 4.69 (d, *J* = 11.6 Hz, 1 H, PhCH_2), 4.26 (dd, *J* = 4.4 Hz and 9.8 Hz, 1 H, H-6), 4.06-4.10 (m, 1 H, H-3), 3.91 (t, *J* = 9.6 Hz, 1 H, H-4), 3.75-3.85 (m, 3 H, H-2, H-5, H-6'), 3.37 (s, 3 H, OMe), 2.34 (d, *J* = 8.0 Hz, 1 H, OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 137.8, 137.5, 129.3, 128.8, 128.4, 128.3, 128.1, 126.4, 102.3, 99.6, 79.7, 78.6, 73.9, 69.0, 68.9, 63.5, 55.1.

Methyl 2,3-di-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (8) (Crich et al., 2004). Compound **7a** and **7b** (865 mg, 2.32 mmol) was dissolved in dry DMF and stirred at 0 °C. Then NaH (370 mg, 9.29 mmol) was added and mixture was stirred under nitrogen for 1 h before drop wise addition of benzyl bromide (300 µL, 2.56 mmol). Mixture was stirred for 2.5 h after which it was poured on ice water and extracted with diethyl ether twice. The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure giving 1.06 g of pure product **8** (99%). TLC (Hex/EtOAc 3:1): R_f 0.47; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.50-7.51 (m, 2 H, ArH), 7.27-7.39 (m, 8 H, ArH), 5.65 (s, 1 H, PhC*H*), 4.80-4.84 (m, 2 H, PhCH_2), 4.74 (d, *J* = 12.4 Hz, 1 H, PhCH_2), 4.69 (d, *J* = 1.2 Hz, 1 H, H-1), 4.65 (d, *J* = 12.3 Hz, 1 H, PhCH_2), 4.22-4.27 (m, 2 H, H-4, H-6), 3.94 (dd, *J* = 3.3 Hz and 10.1 Hz, 1 H, H-3), 3.89 (at, *J* = 10.3 Hz, 1 H, H-6'), 3.83-3.84 (m, 1 H, H-2), 3.77 (dt, *J* = 4.6 Hz and 9.8 Hz, 1 H, H-5), 3.32 (s, 3 H, OMe). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.9, 138.3, 137.9, 129.0, 128.5, 128.4, 128.3, 128.2, 127.9, 127.6, 127.6, 126.2, 101.6, 100.7, 79.3, 76.5, 76.4, 73.8, 73.2, 69.0, 64.2, 55.0.

Methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (9) (Belakhov et al., 2004). Borane (11.2 mL, 1M in THF) was added to compound **8** (1.04 g, 2.25 mmol) under nitrogen. Reaction mixture was then stirred for 10 min until all s.m. was dissolved. Then $Cu(OTf)_2$ (41 mg, 0.112 mmol) in THF (1 mL) was added drop wise and the reaction mixture was stirred at rt for 2 h. The mixture was then cooled to 0 °C before slow addition of MeOH (3 mL). The mixture was then evaporated under reduced pressure giving 1.09 g of crude product which was separated by flash column chromatography with solvent system Hex:EtOAc 3:1 giving pure compound **9** (904 mg, 87%). TLC (Hex/EtOAc 3:1): R_f 0.09; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.27-7.37 (m, 15 H, ArH), 4.94 (d, *J* = 10.9 Hz, 1 H, PhCH_2), 4.78 (d, *J* = 12.4 Hz, 1 H, PhCH_2), 4.63-4.71 (m, 5 H, H-1, PhCH_2), 3.89 (at, *J* = 9.4 Hz, 1 H, H-4), 3.90 (dd, *J* = 3.1 Hz and 9.5 Hz, 1 H, H-3), 3.83-3.87 (m, 1 H, H-6), 3.75-3.80 (m, 2 H, H-2, H-6'), 3.62 (m, 1 H, H-5), 3.30 (s, 3 H, OMe). 1.99 (at, *J* = 6.4 Hz, 1 H, OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.6, 138.6, 138.4, 128.6, 128.5, 128.2, 128.0, 127.9, 127.9, 127.7, 99.5, 80.4, 75.3, 75.1, 74.9, 73.1, 72.4, 72.2, 62.6, 54.9.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-tosyl-α-D-mannopyranoside (10) (Kurhade et al., 2011). Compound **9** (950 mg, 2.05 mmol) was dissolved in pyridine (10 mL) and molecular sieves (4Å) were added. Mixture was then stirred at 0 °C under nitrogen for 1 h before addition of *p*-toluenesulfonyl chloride (1.37 g, 7.16 mmol). The mixture was then stirred for 4 h after which it was diluted with EtOAc and washed with 1M HCl (aq), sat. $NaHCO_3$ (aq), water and finally dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was separated by flash

column chromatography with solvent system Hex:EtOAc 9:1 giving pure compound **10** (1.24 g, 87%). TLC (Hex/EtOAc 3:1): R_f 0.53; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.75-7.77 (m, 2 H, ArH), 7.23-7.35 (m, 15 H, ArH), 7.16-7.18 (m, 2 H, ArH), 4.87 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.69 (d, *J* = 12.6 Hz, 1 H, PhC*H*$_2$), 4.63-4.66 (m, 2 H, H-1, PhC*H*$_2$), 4.54-4.59 (m, 2 H, PhC*H*$_2$), 4.45 (d, *J* = 10.9 Hz, 1 H, PhC*H*$_2$), 4.27 (dd, *J* = 1.9 Hz and 10.5 Hz, 1 H, H-6), 4.20 (dd, *J* = 5.5 Hz and 10.4 Hz, 1 H, H-6'), 3.83 (dd, *J* = 3.0 Hz and 9.2 Hz, 1 H, H-3), 3.79 (at, *J* = 9.4 Hz, 1 H, H-4), 3.71-3.74 (m, 2 H, H-2, H-5), 3.24 (s, 3 H, OMe), 2.39 (s, 3 H, Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 144.7, 138.4, 138.3, 138.2, 133.2, 129.9, 128.5, 128.5, 128.2, 128.1, 127.9, 127.9, 127.8, 127.8, 127.8, 99.0, 80.2, 75.1, 74.5, 74.2, 72.8, 72.2, 70.1, 69.4, 55.0, 21.8.

Methyl 2,3,4-tri-*O*-benzyl-6-thio-6-*S*-acetyl-α-D-mannopyranoside (11) (Cumpstey et al., 2010). Compound **10** (1.27 g, 2.12 mmol) was dissolved in dry DMF. Then KSAc (728 mg, 6.37 mmol) was added and the solution was stirred at 60°C under nitrogen for 3.5 h. The reaction mixture was then diluted with water and diethyl ether and the water phase was extracted twice with diethyl ether. The combined organic phases were washed with water, brine and dried over $MgSO_4$ and evaporated under reduced pressure giving 1.12 g of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 9:1 giving pure compound **11** (1.02 g, 92%). TLC (Hex/EtOAc 2:1): R_f 0.42; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.27-7.40 (m, 15 H, ArH), 4.94 (d, *J* = 10.8 Hz, 1 H, PhC*H*$_2$), 4.75 (d, *J* = 12.4 Hz, 1 H, PhC*H*$_2$), 4.71 (d, *J* = 12.5 Hz, 1 H, PhC*H*$_2$), 4.65-4.67 (m, 2 H, H-1, PhC*H*$_2$), 4.59-4.63 (m, 2 H, PhC*H*$_2$), 3.86 (dd, *J* = 3.1 Hz and 9.1 Hz, 1 H, H-3), 3.76-3.79 (m, 2 H, H-2, H-4), 3.69 (dt, *J* = 2.7 Hz and 8.6 Hz, 1 H, H-5), 3.58 (dd, *J* = 2.8 Hz and 13.6 Hz, 1 H, H-6), 3.09 (dd, *J* = 8.2 Hz and 13.6 Hz, 1 H, H-6'), 3.30 (s, 3 H, OMe), 2.35 (s, 3 H, SAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 195.3, 138.5, 138.4, 138.4, 128.6, 128.5, 128.5, 128.3, 128.0, 127.9, 127.8, 127.8, 127.7, 99.1, 80.2, 77.7, 75.4, 74.8, 72.9, 72.3, 71.0, 54.9, 31.4, 30.7.

Methyl 2,3,4-tri-*O*-benzyl-6-thio-α-D-mannopyranoside (12). Compound **11** (135 mg, 0.257 mmol) was dissolved in MeOH (15 mL) after which NaOMe (42 mg, 0.772 mmol) was added. Mixture was then stirred at rt under nitrogen for 5-10 min before evaporation under reduced pressure. The crude product was separated by flash column chromatography immediately, with solvent system Hex:EtOAc 9:1 giving pure compound **12** (94 mg, 76%). TLC (Hex/EtOAc 9:1): R_f 0.12; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.27-7.38 (m, 15 H, ArH), 4.96 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.77 (d, *J* = 12.4 Hz, 1 H, PhC*H*$_2$), 4.70-4.73 (m, 2 H, H-1, PhC*H*$_2$), 4.59-4.64 (m, 3 H, PhC*H*$_2$), 3.88 (dd, *J* = 3.1 Hz and 9.3 Hz, 1 H, H-3), 3.83 (t, *J* = 9.2 Hz, 1 H, H-4), 3.79-3.80 (m, 1H, H-2), 3.63 (dd, *J* = 2.3 Hz and 8.7 Hz, 1 H, H-5), 3.35 (s, 3 H, OMe), 2.88-2.93 (m, 1 H, H-6), 2.70-2.76 (m, 1 H, H-6'), 1.74 (dd, *J* = 7.0 Hz and 9.7 Hz, 1 H, SH). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.5, 138.5, 138.4, 128.6, 128.5, 128.5, 128.1, 128.0, 127.9, 127.8, 127.7, 99.2, 80.5, 77.5, 75.4, 74.8, 73.0, 72.8, 72.3, 54.9, 26.8. HRMS (ESI) m/z calcd. for $C_{28}H_{32}O_5S$ $[M+Na]^+$ 503.1868, found 503.1863.

Synthesis of 2-hydroxyl and 2-sulfhydryl glycosyl acceptors

Methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-glucopyranoside (14) (Wang et al., 2008). Commercially available 1-*O*-methyl-α-D-glucopyranoside (**13**, 1.16 g, 5.98 mmol) and DIPEA (5.2 mL, 30 mmol) was dissolved in dry DCM (30 mL) and stirred at 0 °C under nitrogen. Then, TMSCl (4.5 mL, 36 mmol) was added dropwise and reaction mixture was stirred for 25 h after which temperature had reached rt. The mixture was then evaporated after which the crude product shaken vigorously with hexane (250 mL in portions) and filtered through celite. The combined filtrate was evaporated under reduced pressure giving pure compound **14** (2.88 g, quant.). TLC (Hex/EtOAc 9:1): R_f 0.53; ^{1}H-NMR (500 MHz, $CDCl_3$): 4.61 (d, *J* = 3.6 Hz, 1 H, H-1), 3.73-3.77 (m, 2 H, H-3, H-6), 3.67 (dd, *J* = 5.4 Hz and 11.3 Hz, 1 H, H-6'), 3.50 (dd, *J* = 2.0 Hz and 5.4 Hz, 1 H, H-5), 3.47 (dd, *J* = 3.7 Hz and 9.0 Hz, 1 H, H-2), 3.43 (dd, *J* = 8.4 Hz and 9.5 Hz, 1 H, H-4), 3.34 (s, 1 H,

OMe), 0.16 (s, 9 H, Si(CH_3)$_3$), 0.16 (s, 9 H, Si(CH_3)$_3$), 0.15 (s, 9 H, Si(CH_3)$_3$), 0.12 (s, 9 H, Si(CH_3)$_3$). ^{13}C-NMR (125 MHz, $CDCl_3$): 98.8, 74.5, 73.1, 71.4, 71.2, 61.4, 53.7, 0.5, 0.1, -0.3, -1.1.

Methyl 3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (15) (Wang et al., 2008). Compound **14** (1.73 g, 3.58 mmol) and PhCHO (380 µL, 3.7 mmol) was dissolved in dry DCM (30 mL) and stirred at -78 °C under nitrogen for 30 min. Then TMSOTf (97 µL, 0.54 mmol) in DCM (1 mL) was added drop wise and the mixture was allowed to stir for 1 h. Then PhCHO (440 µL, 4.30 mmol) and Et_3SiH (630 µL, 3.9 mmol) in DCM (2 mL) was added drop wise and the mixture was stirred for another 3 h. The mixture was then heated to rt, diluted with EtOAc and washed twice with sat. $NaHCO_3$ (aq), and brine and finally dried over $MgSO_4$ and evaporated under reduced pressure giving 1.4 g of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 3:1 giving pure compound **15** (1.25 g, 94%). TLC (Hex/EtOAc 9:1): R_f 0.36; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.45-7.48 (m, 2 H, ArH), 7.23-7.38 (m, 8 H, ArH), 5.55 (s, 1 H, PhC*H*), 4.75-4.78 (m, 2 H, H-1, PhCH_2), 4.27 (dd, J = 4.6 Hz and 10.0 Hz, 1 H, H-4), 3.79-3.84 (m, 2 H, H-3, H-6), 3.69-3.76 (m, 2 H, H-2, H-5), 3.62 (at, J = 9.3 Hz, 1 H, H-6'), 3.43 (s, 3 H, OMe), 2.30 (d, J = 7.4 Hz, OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.6, 137.5, 129.1, 128.5, 128.4, 128.1, 127.8, 126.2, 101.4, 100.0, 82.1, 79.0, 74.9, 72.6, 69.2, 62.7, 55.5.

Methyl 2-*O*-trifluoromethanesulfonyl-3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-glucopyranoside (16) (Walvoort et al., 2010). Compound **15** (1.30 g, 3.49 mmol) was dissolved in dry DCM (20 mL) and stirred at -15 °C under nitrogen. Then pyridine (2.2 mL, 28 mmol) was added drop wise followed by Tf_2O (1.2 mL, 7.0 mmol) in DCM (3 mL). The reaction mixture was stirred at -15 °C for 2.5 h before it was diluted with DCM and poured on ice water. The organic phase was washed with 1M HCl (aq), sat. $NaHCO_3$ (aq), water and brine, and finally dried over $MgSO_4$ and evaporated under reduced pressure giving pure product **16** (1.74 g, 99%). TLC (Hex/EtOAc 1:1): R_f 0.86; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.45-7.48 (m, 2 H, ArH), 7.38-7.42 (m, 3 H, ArH), 7.27-7.35 (m, 5 H, ArH), 5.57 (s, 1 H, PhC*H*), 4.97 (d, J = 3.8 Hz, H-1), 4.86 (d, J = 11.0 Hz, PhCH_2), 4.77 (d, J = 11.0 Hz, PhCH_2), 4.74 (dd, J = 3.5 Hz and 9.4 Hz, 1 H, H-2), 4.32 (dd, J = 4.9 Hz and 10.3 Hz, 1 H, H-6), 4.13 (at, J = 9.4 Hz, 1 H, H-3), 3.89 (dt, J = 4.8 Hz and 10.0 Hz, 1 H, H-5), 3.77 (at, J = 10.3 Hz, 1 H, H-6'), 3.70 (at, J = 9.5 Hz, 1 H, H-4), 3.48 (s, 3 H, OMe). ^{13}C-NMR (125 MHz, $CDCl_3$): 137.5, 137.0, 129.3, 128.5, 128.5, 128.4, 128.1, 126.1, 101.7, 97.8, 83.7, 82.2, 75.4, 75.1, 68.8, 62.4, 55.9.

Methyl 3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (17) (Ennis et al., 2002). Compound **16** (496 mg, 0.984 mmol) was dissolved in dry toluene (10 mL). Then $TBANO_2$ (1.42 g, 4.92 mmol) was added and the reaction mixture was refluxed (70 °C) for 21 h. The reaction mixture was then diluted with DCM, washed twice with water and brine, and dried over $MgSO_4$ and evaporated under reduced pressure giving 820 mg of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 4:1 giving pure compound **17** (257 mg, 70%). TLC (Hex/EtOAc 4:1): R_f 0.34; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.49-7.50 (m, 2 H, ArH), 7.28-7.40 (m, 8 H, ArH), 5.62 (s, 1 H, PhC*H*), 4.85 (d, J = 11.9 Hz, PhCH_2), 4.77 (as, H-1), 4.71 (d, J = 11.9 Hz, PhCH_2), 4.28 (dd, J = 4.0 Hz and 9.6 Hz, 1 H, H-6), 4.05-4.13 (m, 2 H, H-2, H-4), 3.91 (dd, J = 3.5 Hz and 9.5 Hz, 1 H, H-3), 3.87 (at, J = 10.1 Hz, 1 H, H-6'), 3.81 (dt, J = 4.4 Hz and 9.5 Hz, 1 H, H-5), 3.38 (s, 3 H, OMe), 2.65 (as, 1 H, OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.2, 137.7, 129.1, 128.6, 128.4, 128.1, 128.0, 126.2, 101.8, 101.2, 79.0, 75.7, 73.2, 70.1, 69.1, 63.3, 55.1.

Methyl 2-thio-2-*S*-acetyl-3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (18) (Cumpstey et al., 2010). Compound **16** (1.12 g, 2.22 mmol) was dissolved in dry DMF (25 mL) and stirred at rt. Then KSAc (1.27 g, 11.1 mmol) was added and mixture was stirred at at 40 °C under nitrogen for 16 h. The reaction mixture was then diluted with diethyl ether and water and the water phase was extracted with diethyl ether twice. The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure giving 1.53 g of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 9:1 giving pure

compound **18** (851 mg, 89%). TLC (Hex/EtOAc 6:1): R_f 0.20; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.49-7.50 (m, 2 H, ArH), 7.24-7.40 (m, 8 H, ArH), 5.59 (s, 1 H, PhC*H*), 4.73 (as, H-1), 4.68 (d, *J* = 12.2 Hz, PhC*H*$_2$), 4.63 (d, *J* = 12.2 Hz, PhC*H*$_2$), 4.38 (ad, *J* = 5.0 Hz, 1 H, H-2), 4.29 (dd, *J* = 5.0 Hz and 9.9 Hz, 1 H, H-3), 4.24 (dd, *J* = 4.2 Hz and 9.9 Hz, 1 H, H-6), 3.85 (dt, *J* = 4.4 Hz and 9.8 Hz, 1 H, H-5), 3.79 (at, *J* = 10.2 Hz, 1 H, H-6'), 3.70 (at, *J* = 9.6 Hz, 1 H, H-4), 3.36 (s, 3 H, OMe), 2.40 (s, 3 H, SAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 194.3, 138.1, 137.5, 129.1, 128.4, 128.3, 127.8, 127.7, 126.2, 102.6, 101.8, 80.6, 78.2, 72.9, 72.1, 69.0, 63.9, 55.3, 48.2, 30.9, 29.9.

Methyl 2-thio-3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (19). Compound **18** (105 mg, 0.250 mmol) was dissolved in MeOH (5 mL) after which NaOMe (40 mg, 0.750 mmol) was added. Mixture was then stirred at rt under nitrogen for 10 min before evaporation under reduced pressure. The crude product was separated by flash column chromatography immediately, with solvent system Hex:EtOAc 10:1 giving pure compound **19** (75 mg, 77%). TLC (Hex/EtOAc 10:1): R_f 0.19; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.50-7.53 (m, 2 H, ArH), 7.30-7.42 (m, 8 H, ArH), 5.65 (s, 1 H, PhC*H*), 4.79-4.81 (m, 2 H, H-1, PhC*H*$_2$), 4.72 (d, *J* = 12.3 Hz, PhC*H*$_2$), 4.21-4.31 (m, 2 H, H-4, H-6), 4.14 (dd, *J* = 4.7 Hz and 9.6 Hz, 1 H, H-3), 3.86-3.91 (m, 2 H, H-5, H-6'), 3.66 (at, *J* = 5.5 Hz, 1 H, H-2), 3.38 (s, 3 H, OMe), 2.01 (d, *J* = 6.3 Hz, 1 H, SH). HRMS (ESI) m/z calcd. for $C_{21}H_{24}O_5S$ $[M+Na]^+$ 411.1242, found 411.1234.

Synthesis of glycosyl donors

Ethyl 1-thio-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (23) (Saksena et al., 2002). α/β-D-Mannose pentaacetate (**22**, 786 mg, 2.01 mmol) and ethanethiol (450 µL, 6.0 mmol) was dissolved in dry DCM (20 mL) and stirred at 0 °C under nitrogen. Then, $BF_3{\cdot}Et_2O$ (2.0 mL, 16 mmol) in DCM (5 mL) was added drop wise. The reaction mixture was then allowed to reach rt and after 24 h, the reaction was quenched by diluting it with DCM and pouring the mixture on ice water. The organic phase was washed with sat. $NaHCO_3$ (aq), water and brine, and finally dried over $MgSO_4$ and evaporated under reduced pressure giving 526 mg of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 2:1 giving pure compound **23** (402 mg, 51%). TLC (Hex/EtOAc 1:1): R_f 0.31; ^{1}H-NMR (500 MHz, $CDCl_3$): 5.34 (dd, *J* = 1.5 Hz and 3.2 Hz, 1 H, H-2), 5.29-5.33 (m, 2 H, H-1, H-4), 5.27 (dd, *J* = 3.3 Hz and 9.9 Hz, 1 H, H-3), 4.40 (ddd, *J* = 2.2 Hz, 5.3 Hz and 9.5 Hz, 1 H, H-5), 4.32 (dd, *J* = 5.5 Hz and 12.2 Hz, 1 H, H-6), 4.10 (dd, *J* = 2.3 Hz and 12.3 Hz, 1 H, H-6'), 2.58-2.71 (m, 2 H, SC*H*$_2$CH$_3$), 2.17 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.31 (t, *J* = 7.4 Hz, 3 H, SCH$_2$C*H*$_3$). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.8, 170.2, 169.9, 169.9, 82.5, 71.4, 69.7, 69.1, 66.6, 62.6, 25.6, 21.1, 20.9, 20.9, 20.8, 14.9.

Ethyl 1-thio-α-D-mannopyranoside (24) (Saksena et al., 2002). Compound **23** (902 mg, 2.30 mmol) was dissolved in MeOH (20 mL) after which NaOMe (137 mg, 2.53 mmol) was added. Mixture was then stirred at rt under nitrogen for 3 h after which the solvent was evaporated under reduced pressure, giving pure product **24** (525 mg, quant.). ^{1}H-NMR (500 MHz, D_2O): 5.32 (d, *J* = 1.1 Hz, 1 H, H-1), 4.04 (dd, *J* = 1.6 Hz and 3.4 Hz, 1 H, H-2), 4.00 (ddd, *J* = 2.3 Hz, 6.3 Hz and 9.6 Hz, 1 H, H-5), 3.88 (dd, *J* = 2.3 Hz and 12.3 Hz, 1 H, H-6), 3.75-3.79 (m, 2 H, H-3, H-6'), 3.66 (t, *J* = 9.8 Hz, 1 H, H-4), 2.61-2.73 (m, 2 H, SC*H*$_2$CH$_3$), 1.27 (t, *J* = 7.4 Hz, 3 H, SCH$_2$C*H*$_3$). ^{13}C-NMR (125 MHz, D_2O): 84.5, 72.0, 71.2, 67.4, 73.2, 61.0, 25.5, 14.3.

Ethyl 1-thio-2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (25) (Saksena et al., 2002). Compound **24** (70 mg, 0.311 mmol) was dissolved in dry DMF (4 mL) and stirred at 0 °C under nitrogen for 30 min. Then BnBr (160 µL, 1.4 mmol) was added followed by slow addition of NaH (60% in mineral oil, 124 mg, 3.11 mmol). The reaction mixture was then stirred vigorously for 4 h before it was diluted with diethyl ether and water and the water phase was extracted with diethyl ether twice. The combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure giving 153 mg of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 7:1 giving pure compound **25** (73 mg, 40%). TLC

(Hex/EtOAc 3:1): R_f 0.29; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.24-7.39 (m, 18 H, ArH), 7.16-7.18 (m, 2 H, ArH), 5.40 (as, 1 H, H-1), 4.88 (d, *J* = 10.8 Hz, PhC*H*$_2$), 4.73 (d, *J* = 12.5 Hz, PhC*H*$_2$), 4.65-4.68 (m, 2 H, PhC*H*$_2$), 4.54-4.60 (m, 2 H, PhC*H*$_2$), 4.49-4.52 (m, 2 H, PhC*H*$_2$), 4.13 (dd, *J* = 4.4 Hz and 10.0 Hz, H-4), 4.03 (t, *J* = 9.7 Hz, H-3), 3.80-3.85 (m, 3 H, H-2, H-5, H-6), 3.71 (dd, *J* = 1.2 Hz and 11.0 Hz, H-6'), 2.52-2.67 (m, 2 H, SC*H*$_2$CH$_3$), 1.24 (t, *J* = 7.4 Hz, 3 H, SCH$_2$C*H*$_3$). ^{13}C-NMR (125 MHz, $CDCl_3$): 138.7, 138.5, 138.4, 138.3, 128.5, 128.4, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 82.0, 80.5, 76.5, 75.3, 75.2, 73.5, 72.2, 72.2, 72.1, 69.3, 25.5, 15.1.

Ethyl 1-thio-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (27) (Düffels et al., 2000). Compound **26**, synthesized in five steps from D-mannose according to Beignet *et al.* (Beignet et al., 2004), (2.00 g, 3.94 mmol) was dissolved in dry MeCN (20 mL). Then molecular sieves (4Å) were added and the mixture was stirred at rt under nitrogen for 1.5 h. Then, ethanethiol (970 µL, 13 mmol) and $HgBr_2$ (71 mg, 0.20 mmol) in dry MeCN (1 mL) was added to the reaction mixture. The reaction mixture was then stirred at 60 °C under nitrogen for 24 h after which it was diluted with DCM and washed with sat. $NaHCO_3$ (aq) and brine, and finally dried over $MgSO_4$ and evaporated under reduced pressure giving 1.6 g of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 4:1 giving pure compound **27** (1.44 g, 68%). TLC (Hex/EtOAc 4:1): R_f 0.28; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.25-7.36 (m, 13 H, ArH), 7.15-7.17 (m, 2 H, ArH), 5.43 (dd, *J* = 1.5 Hz and 2.6 Hz, 1 H, H-2), 5.32 (d, *J* = 1.2 Hz, 1 H, H-1), 4.85 (d, *J* = 10.8 Hz, PhC*H*$_2$), 4.67-4.70 (m, 2 H, PhC*H*$_2$), 4.46-4.53 (m, 3 H, PhC*H*$_2$), 4.16 (dd, *J* = 3.4 Hz and 9.1 Hz, H-5), 3.89-3.95 (m, 2 H, H-3, H-4), 3.84 (dd, *J* = 4.3 Hz and 11.0 Hz, H-6), 3.68 (dd, *J* = 2.0 Hz and 10.8 Hz, H-6'), 3.69 (dd, *J* = 1.6 Hz and 10.8 Hz, H-6'), 2.55-2.69 (m, 2 H, SC*H*$_2$CH$_3$), 2.16 (s, 3 H, OAc), 1.28 (t, *J* = 7.4 Hz, 3 H, SCH$_2$C*H*$_3$). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.6, 138.5, 138.3, 137.9, 128.7, 128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 127.1, 82.6, 78.7, 75.3, 74.7, 73.6, 72.0, 72.0, 70.7, 69.0, 25.6, 21.3, 15.0.

1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (29) (Mayer and Schmidt, 1999). Compound **26** (2.30 g, 4.54 mmol) was dissolved in AcOH (35 mL) and water (18 mL) was added. The reaction mixture was then refluxed (100 °C) for 4 h after which the solvent was evaporated under reduced pressure giving a yellow syrup. The crude product **28** was then dissolved in pyridine (20 mL) and Ac_2O (4.3 mL, 45 mmol) was added. The reaction mixture was stirred at rt for 2 h after which the solvent was evaporated under reduced pressure giving 2.4 g of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 5:1 giving pure compound **29** (1.87 g, 77% over two steps). TLC (Hex/EtOAc 3:1): R_f 0.32; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.26-7.36 (m, 13 H, ArH), 7.16-7.18 (m, 2 H, ArH), 6.12 (d, *J* = 2.0 Hz, H-1), 5.36 (at, *J* = 2.4 Hz, H-2), 4.86 (d, *J* = 10.6 Hz, PhC*H*$_2$), 4.73 (d, *J* = 11.1 Hz, PhC*H*$_2$), 4.68 (d, *J* = 12.0 Hz, PhC*H*$_2$), 4.55 (d, *J* = 11.1 Hz, PhC*H*$_2$), 4.50-4.52 (m, 2 H, PhC*H*$_2$), 3.95-4.00 (m, 2 H, H-3, H-4), 3.80-3.87 (m, 2 H, H-5, H-6), 3.69 (dd, *J* = 1.6 Hz and 10.8 Hz, H-6'), 2.17 (s, 3 H, OAc), 2.07 (s, 3 H, OAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.3, 168.5, 138.3, 137.8, 128.6, 128.5, 128.5, 128.3, 128.1, 128.0, 128.0, 127.9, 127.8, 91.5, 77.8, 75.5, 74.0, 73.9, 73.7, 72.1, 68.6, 67.7, 21.1, 21.1.

Glycosylation reactions

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (30). Compound **9** (311 mg, 0.670 mmol) and compound **27** (467 mg, 0.870 mmol) was dissolved in dry DCM (10 mL). Then molecular sieves (4Å) were added and the reaction mixture was stirred at -10 °C under nitrogen for 45 min. Then NIS (451 mg, 2.01 mmol) was added and the reaction vessel was shielded with aluminum foil. Then TfOH (9 µL, 0.1 mmol) in dry DCM (1 mL) was added drop wise. After 30 min, the reaction mixture was diluted with DCM and water. Then $Na_2S_2O_5$ (380 mg, 2.01 mmol) was added and the mixture was shaken until no red color could be observed. The organic phase was washed twice with sat. $NaHCO_3$ (aq) and brine, and finally dried over $MgSO_4$ and evaporated under reduced pressure giving 648 mg of crude product. The crude product was separated by flash column chromatography with solvent system

Hex:EtOAc 3:1 giving pure compound **30** (503 mg, 80%). TLC (Hex/EtOAc 3:1): R_f 0.19; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.20-7.38 (m, 28 H, ArH), 7.12-7.14 (m, 2 H, ArH), 5.46-5.47 (m, 1 H, H-2'), 4.95 (d, *J* = 1.4 Hz, 1 H, H-1'), 4.91 (d, *J* = 11.3 Hz, 1 H, PhC*H*$_2$), 4.85 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.70-4.71 (m, 3 H, H-1, PhC*H*$_2$), 4.63-4.66 (m, 2 H, PhC*H*$_2$), 4.56-4.61 (m, 2 H, PhC*H*$_2$), 4.42-4.49 (m, 4 H, PhC*H*$_2$), 3.95 (dd, *J* = 3.3 Hz and 9.4 Hz, 1 H, H-3'), 3.84-3.91 (m, 4 H, H-4'), 3.78-3.80 (m, 2 H, H-2), 3.66-3.72 (m, 3 H), 3.59 (dd, *J* = 1.4 Hz and 10.7 Hz, 1 H), 3.25 (s, 3 H, OMe), 2.14 (s, 3 H, OAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.5, 138.8, 138.6, 138.6, 138.5, 138.4, 138.0, 128.5, 128.5, 128.5, 128.4, 128.4, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 98.9, 98.1, 80.4, 77.7, 75.2, 75.1, 74.8, 74.7, 74.4, 73.5, 72.8, 72.2, 71.5, 71.5, 71.1, 68.9, 68.7, 66.9, 54.8, 21.3. HRMS (ESI) m/z calcd. for $C_{57}H_{62}O_{12}$ $[M+Na]^+$ 961.4139, found 961.4133.

Methyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (1) (Sandström et al., 2004). Compound **30** (289 mg, 0.308 mmol) was dissolved in MeOH (10 mL). Then NaOMe (9 mg, 0.2 mmol) in MeOH (0.5 mL) was added and the reaction mixture was stirred at rt under nitrogen for 24 h. Then AcOH (70 µL, 1.2 mmol) and Pd (10% on C) (160 mg, 0.15 mmol) was added to and the reaction mixture was stirred at rt under hydrogen for 72 h. Then, the reaction mixture was filtered through celite and rinsed with MeOH. The combined filtrate was evaporated under reduced pressure giving 138 mg of crude product. The crude product was separated by flash column chromatography with solvent system DCM:MeOH 3:1 giving pure compound **1** (84 mg, 77%). TLC (DCM/MeOH 3:1): R_f 0.10; ^{1}H-NMR (500 MHz, D_2O): 4.90 (d, *J* = 1.6 Hz, 1 H, H-1'), 4.74 (d, *J* = 1.6 Hz, 1 H, H-1), 3.96-3.99 (m, 2 H, H-2'), 3.93-3.94 (m, 1 H, H-2), 3.89 (dd, *J* = 2.0 Hz and 12.0 Hz, 1 H), 3.84 (dd, *J* = 3.5 Hz and 9.4 Hz, 1 H), 3.68-3.77 (m, 6 H, H-3), 3.65 (at, *J* = 9.5 Hz, 1 H), 3.40 (s, 3 H, OMe). ^{13}C-NMR (125 MHz, D_2O): 101.6, 100.1, 73.3, 71.4, 71.3, 71.2, 70.6, 70.5, 67.3, 67.1, 66.2, 61.5, 55.4.

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-6-thio-α-D-mannopyranoside (31). Compound **12** (167 mg, 0.348 mmol) and compound **29** (242 mg, 0.452 mmol) was dissolved in dry DCM (10 mL). Then molecular sieves (3Å) were added and the reaction mixture was stirred at 0 °C under nitrogen for 15 min. Then $BF_3{\cdot}Et_2O$ (160 µL, 1.5 mmol) in DCM (0.5 mL) was added drop wise. The reaction mixture was then stirred for 13 h after which it was diluted with DCM and poured on ice water. The organic phase was washed with sat. $NaHCO_3$ (aq) and brine, and finally dried over $MgSO_4$ and evaporated under reduced pressure giving 385 mg of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 6:1 giving pure compound **31** (1.44 g, 61%). TLC (Hex/EtOAc 4:1): R_f 0.39; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.24-7.38 (m, 28 H, ArH), 7.15-7.16 (m, 2 H, ArH), 5.51-5.52 (m, 1 H, H-2'), 5.41 (as, 1 H, H-1'), 4.92 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.84 (d, *J* = 10.8 Hz, 1 H, PhC*H*$_2$), 4.71-4.72 (m, 3 H, H-1, PhC*H*$_2$), 4.64-4.68 (m, 2 H, PhC*H*$_2$), 4.58-4.60 (m, 3 H, PhC*H*$_2$), 4.46-4.51 (m, 2 H, PhC*H*$_2$), 4.41 (d, *J* = 12.0 Hz, 1 H, PhC*H*$_2$), 4.14 (dd, *J* = 1.9 Hz and 8.2 Hz, 1 H), 3.92-3.99 (m, 2 H, H-3'), 3.85-3.87 (m, 2 H), 3.82 (dd, *J* = 3.7 Hz and 10.9 Hz, 1 H), 3.73-3.77 (m, 2 H, H-2, H-5), 3.61 (dd, *J* = 1.2 Hz and 10.7 Hz, 1 H), 3.32 (s, 3 H, OMe), 3.09 (dd, *J* = 2.1 Hz and 13.6 Hz, 1 H, H-6$_a$), 2.84 (dd, *J* = 7.4 Hz and 13.6 Hz, 1 H, H-6$_b$), 2.15 (s, 3 H, OAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.4, 138.6, 138.6, 138.5, 138.4, 138.4, 137.9, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 99.0, 83.1, 80.3, 78.8, 77.4, 75.4, 75.2, 74.6, 74.6, 73.5, 72.8, 72.3, 72.1, 72.0, 71.0, 70.5, 68.9, 55.0, 33.0, 21.3. HRMS (ESI) m/z calcd. for $C_{57}H_{62}O_{11}S$ $[M+Na]^+$ 977.3911, found 977.3905.

Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-6-thio-α-D-mannopyranoside (32). Compound **31** (201 mg, 0.211 mmol) was dissolved in dry THF (4 mL) and added to a prepared solution of NH_3 (*l*) (~10 mL condensed from gas) containing dissolved Li (100 mg, 15 mmol) at -78 °C under nitrogen. The reaction mixture was stirred for 2 min before quenching by addition of NH_3Cl (until blue color disappeared). The reaction mixture was then allowed to reach rt and the solvent evaporated at 1 atmosphere. After evaporation, the reaction mixture was dried under vacuum for 2 h before the addition of pyridine (30 mL) and Ac_2O (12 mL)

giving a milky slurry. The reaction mixture was then stirred at rt under nitrogen for 22 h. After that, the reaction mixture was diluted with DCM and washed with water, 6 M HCl (aq) and brine. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure giving 263 mg of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 4:1 1:1 giving pure compound **32** (117 mg, 85%). TLC (Hex/EtOAc 1:1): R_f 0.44; ^{1}H-NMR (500 MHz, $CDCl_3$): 5.21-5.36 (m, 7 H, H-1, H-2, H-3, H-4, H-2', H-3', H-4'), 4.26 (d, J = 1.6 Hz, 1 H, H-1'), 4.37 (ddd, J = 2.2 Hz, 5.0 Hz and 9.5 Hz, 1 H, H-5'), 4.31 (dd, J = 5.0 Hz and 12.2 Hz, 1 H, H-6$_a$'), 4.09 (dd, J = 2.1 Hz and 12.3 Hz, 1 H, H-6$_b$'), 3.94 (ddd, J = 2.9 Hz, 7.4 Hz and 9.6 Hz, 1 H, H-5), 3.42 (s, 3 H, OMe), 2.85 (dd, J = 2.8 Hz and 14.0 Hz, 1 H, H-6$_a$), 2.72 (dd, J = 7.4 Hz and 14.0 Hz, 1 H, H-6$_b$), 2.17 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.05 (s, 6 H, OAc), 1.99 (s, 6 H, OAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.8, 170.3, 170.1, 170.0, 170.0, 169.9, 169.9, 98.6, 82.7, 77.4, 71.0, 69.6, 69.6, 69.5, 69.3, 69.0, 68.9, 66.3, 62.4, 55.6, 32.1, 21.1, 21.0, 20.9, 20.9, 20.8, 20.8. HRMS (ESI) m/z calcd. for $C_{27}H_{38}O_{17}S$ [M+Na] $^+$ 689.1722, found 689.1715.

Methyl α-D-mannopyranosyl-(1→6)-6-thio-α-D-mannopyranoside (2). Compound **32** (218 mg, 0.393 mmol) was dissolved in MeOH (10 mL) and stirred at rt under nitrogen. Then Li (5.5 mg, 0.79 mmol) dissolved in MeOH (1 mL) was added to the reaction mixture and the mixture was stirred for 2.5 h before evaporation giving the pure product **2** (137 mg, 94%). ^{1}H-NMR (500 MHz, D_2O): 5.34 (d, J = 1.1 Hz, 1 H, H-1'), 4.72 (d, J = 1.4 Hz, 1 H, H-1), 4.06 (dd, J = 1.3 Hz and 3.3 Hz, 1 H, H-2'), 4.00 (ddd, J = 2.1 Hz, 6.0 Hz and 9.9 Hz, 1 H, H-5'), 3.93 (dd, J = 1.6 Hz and 3.3 Hz, 1 H, H-2), 3.88 (dd, J = 2.2 Hz and 12.3 Hz, 1 H, H-4'), 3.70-3.81 (m, 4 H, H-3, H-5, H-3', H-6$_a$'), 3.67 (at, J = 9.7 Hz, 1 H, H-6$_b$'), 3.62 (at, J = 9.5 Hz, 1 H), 3.41 (s, 3 H, OMe), 3.17 (dd, J = 2.3 Hz and 14.0 Hz, 1 H, H-6$_a$), 3.80 (dd, J = 8.4 Hz and 14.0 Hz, 1 H, H-6$_b$). ^{13}C-NMR (125 MHz, D_2O): 101.6, 85.1, 73.9, 72.3, 71.7, 71.6, 71.1, 70.5, 70.1, 67.7, 61.5, 55.4, 32.2. HRMS (ESI) m/z calcd. for $C_{13}H_{24}O_{10}S$ [M+Li] $^+$ 379.1245, found 379.1235.

Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (33). Compound **17** (168 mg, 0.451 mmol) and compound **27** (314 mg, 0.870 mmol) was dissolved in dry DCM (15 mL). Then molecular sieves (4Å) were added and the reaction mixture was stirred at -10 °C under nitrogen for 20 min. Then NIS (406 mg, 1.80 mmol) was added and the reaction vessel was shielded with aluminum foil. Then TfOH (6 µL, 0.07 mmol) in dry DCM (0.5 mL) was added drop wise. After 30 min, the reaction mixture was diluted with DCM and water. Then $Na_2S_2O_5$ (340 mg, 1.80 mmol) was added and the mixture was shaken until no red color could be observed. The organic phase was washed twice with sat. $NaHCO_3$ (aq) and brine, and finally dried over $MgSO_4$ and evaporated under reduced pressure giving 405 mg of crude product. The crude product was separated by flash column chromatography with solvent system Hex:EtOAc 6:1 giving pure compound **33** (150 mg, 39%). TLC (Hex/EtOAc 6:1): R_f 0.36; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.50-7.52 (m, 2 H, ArH), 7.23-7.40 (m, 21 H, ArH), 7.16-7.18 (m, 2 H, ArH), 5.63 (s, 1 H, PhC*H*), 5.59 (dd, J = 1.6 Hz and 3.0 Hz, 1 H, H-2'), 5.12 (d, J = 1.3 Hz, 1 H, H-1'), 4.86 (d, J = 10.7 Hz, 1 H, PhCH_2), 4.83 (d, J = 12.3 Hz, 1 H, PhCH_2), 4.77 (d, J = 11.0 Hz, 1 H, PhCH_2), 4.74 (d, J = 1.5 Hz, 1 H, H-1$_b$), 4.64-4.67 (m, 2 H, PhCH_2), 4.56 (d, J = 11.0 Hz, 1 H, PhCH_2), 4.83 (d, J = 12.0 Hz, 1 H, PhCH_2), 4.77 (d, J = 11.0 Hz, 1 H, PhCH_2), 4.23 (dd, J = 4.6 Hz and 10.0 Hz, 1 H), 4.01-4.05 (m, 3 H, H-3', H-2), 3.89-3.94 (m, 2 H), 3.72-3.85 (m, 5 H), 3.23 (s, 3 H, OMe), 2.14 (s, 3 H, OAc). ^{13}C-NMR (125 MHz, $CDCl_3$): 170.2, 138.7, 138.4, 138.3, 138.1, 137.8, 129.0, 128.5, 128.5, 128.3, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.6, 126.3, 101.7, 100.9, 100.3, 79.1, 78.2, 76.7, 75.7, 75.5, 74.6, 73.6, 73.0, 72.1, 72.0, 69.2, 69.0, 68.6, 63.9, 54.9, 21.3. HRMS (ESI) m/z calcd. for $C_{50}H_{54}O_{12}$ [M+Na] $^+$ 869.3513, found 869.3507.

Methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (3) (Sandström et al., 2004). Compound **33** (224 mg, 0.265 mmol) was dissolved in MeOH (15 mL). Then NaOMe (14 mg, 0.27 mmol) in MeOH (0.5 mL) was added and the reaction mixture was stirred at rt under nitrogen for 6 h. Then AcOH (60 µL, 1.1 mmol) and Pd (10% on C) (280 mg, 0.27 mmol) was added to and the

reaction mixture was stirred at rt under hydrogen for 24 h. Then, the reaction mixture was filtered through celite and rinsed with MeOH. The combined filtrate was evaporated under reduced pressure giving 178 mg of crude product. The crude product was separated by flash column chromatography with solvent system DCM:MeOH 3:1 giving pure compound **3** (80 mg, 85%). TLC (DCM/MeOH 3:1): R_f 0.09; ^{1}H-NMR (500 MHz, D_2O): 5.01 (d, J = 1.6 Hz, 1 H, H-1'), 4.99 (d, J = 1.5 Hz, 1 H, H-1), 4.06 (dd, J = 1.7 Hz and 3.2 Hz, 1 H, H-2'), 3.95 (dd, J = 1.7 Hz and 3.3 Hz, 1 H, H-2), 3.82-3.90 (m, 4 H, H-3, H-3'), 3.57-3.78 (m, 6 H), 3.89 (s, 3 H, OMe). ^{13}C-NMR (125 MHz, D_2O): 102.9, 100.0, 79.2, 73.9, 73.2, 70.9, 70.8, 70.6, 67.6, 67.5, 61.8, 61.6, 55.4.

Methyl α-D-mannopyranosyl-(1→2)-2-thio-(*R*)-4,6-*O*-benzylidene-α-D-mannopyranoside (34). 1-Thio-α-D-mannopyranose sodium salt, prepared according to Pei et al. (Pei et al., 2006), (180 mg, 1.1 eq.) and compound **21**, prepared in two steps from methyl α-D-glucopyranoside **13** according to Knapp et al. (Knapp et al., 1990), (316 mg, 1.0 eq.) were added to dry DMF (2 mL). The mixture was stirred at 50 °C under nitrogen atmosphere for 18 hours. After removal of the solvent, the residue was directly purified by flash chromatography with solvent system $CHCl_2$/MeOH 10:1 giving compound **34** (94 mg, 25%). TLC (DCM/MeOH 5:1): R_f 0.62; The compound decomposed in solution and was used directly in the next step.

Methyl α-D-mannopyranosyl-(1→2)-2-thio-α-D-mannopyranoside (4). Compound **34** (80 mg) was dissolved in DCM/MeOH/MeCOOH (1:1:2, 2 mL), and the mixture was stirred at 50 °C under nitrogen atmosphere overnight. The solvent was removed under reduced pressure and co-evaporated with toluene. The residue was directly purified by flash chromatography with solvent system $CHCl_3$/MeOH 3:1 giving compound **4** (49 mg, 76%). TLC (DCM/MeOH 3:1): R_f 0.50; ^{1}H NMR (600 MHz, D_2O) δ = 5.41 (s, 1 H, H-1'), 5.00 (s, 1 H, H-1), 4.19 (dd, J = 9.5, 4.7, 1 H, H-3), 4.14 (dd, J = 3.3, 1.4, 1 H, H-2'), 4.05 – 4.00 (m, 1 H, H-5'), 3.93 (dd, J = 12.3, 2.1, 1 H, H-6a'), 3.89 (dd, J = 12.3, 2.1, 1 H, H-6a), 3.81 – 3.74 (m, 3 H, H-3', H-6b, H-6b'), 3.69 – 3.62 (m, 2 H, H-5, H-4'), 3.54 (t, J = 9.7, 1 H, H-4), 3.47 (d, J = 4.7, 1 H, H-2), 3.40 (s, 3 H, OMe). ^{13}C NMR (151 MHz, D_2O) δ = 102.3, 87.6, 74.2, 73.4, 72.0, 71.5, 70.0, 68.4, 67.8, 61.6, 61.3, 55.4, 52.8. HRMS (ESI) m/z calcd. for $C_{13}H_{24}O_{10}S$ $[M+Na]^+$ 395.0982, found: 395.0959.

Binding analysis

Binding analyses were performed using a QCM flow-through instrumentation (Attana). The functionalized QCM sensors were initially equilibrated to produce a stable baseline signal, and then subjected to BSA injections to block nonspecific binding. Competitive binding was subsequently recorded as shifts in frequency, corresponding to resulting protein surface binding, by injections of solutions containing Con A (2 μM) and the respective ligands at different concentrations (*cf*. Figure 2a). Between individual recordings, bound protein was released by successive injections of low pH buffer (pH 1.5). Frequency effects emanating from the ligand (*cf.* Figure 2b) were subtracted, resulting in corrected binding curves (*cf*. Figure 2c), from which the quantitative binding was extracted and subjected to nonlinear regression analysis using a dose-response inhibition model (equation 1).

$$Y = f_{min} + \frac{f_{max} - f_{min}}{1 + 10^{\left(Hill\ coeff \times \left(\log(C) - \log(EC_{50})\right)\right)}} \qquad (1)$$

Y represents the frequency response (Hz), f_{min} and f_{max} are the minimum and maximum frequencies recorded (Hz), *Hill coeff* is the Hill coefficient, C is the concentration (M) number, and EC_{50} is the half maximal effective concentration (M) number. The Hill coefficient is generally considered a measure of potential cooperativity effects in proteins composed of several subunits (Gesztelyi et al., 2012). K_d-values were subsequently estimated using the Cheng-Prusoff relationship (Cheng and Prusoff, 1973), using the reported value of 122 μM of the reference compound (methyl α-D-mannopyranoside; Mandal et al., 1994). For visualization purposes, the data from the respective experiments, including the resulting fitted competition binding curves, were normalized with

respect to frequency response, and the respective estimated EC_{50}-values of the reference ligand ($EC_{50,ref}$) were used to correlate experiments based on different surfaces (*cf*. Figure 3).

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NMR spectra

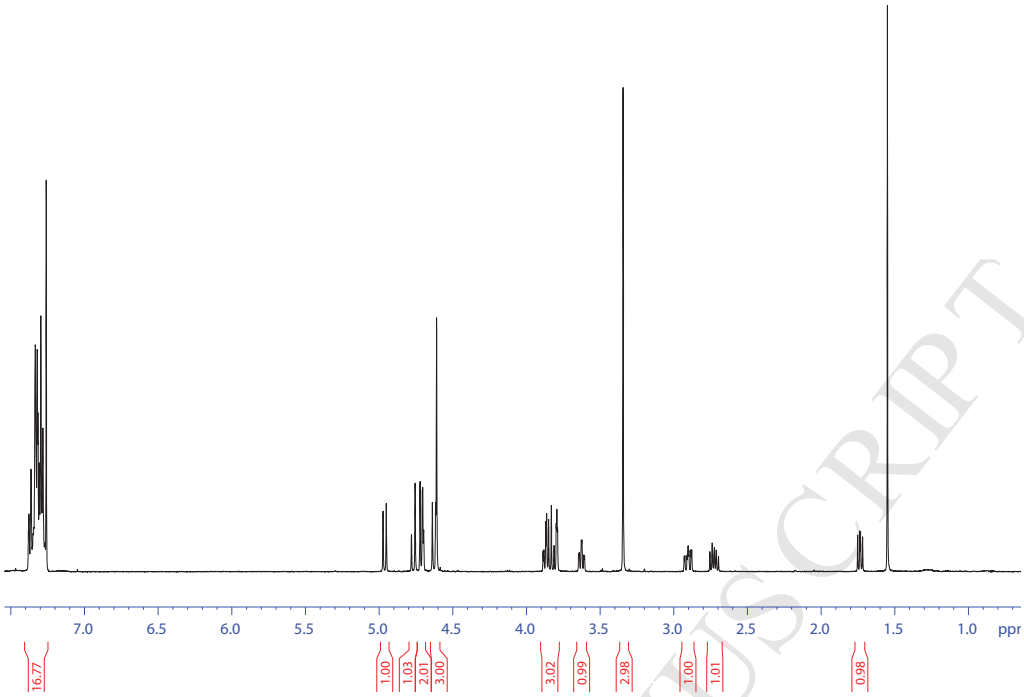

*Figure S1. ^{1}H NMR ($CDCl_3$) for methyl 2,3,4-tri-O-benzyl-6-thio-α-D-mannopyranoside (**12**)*

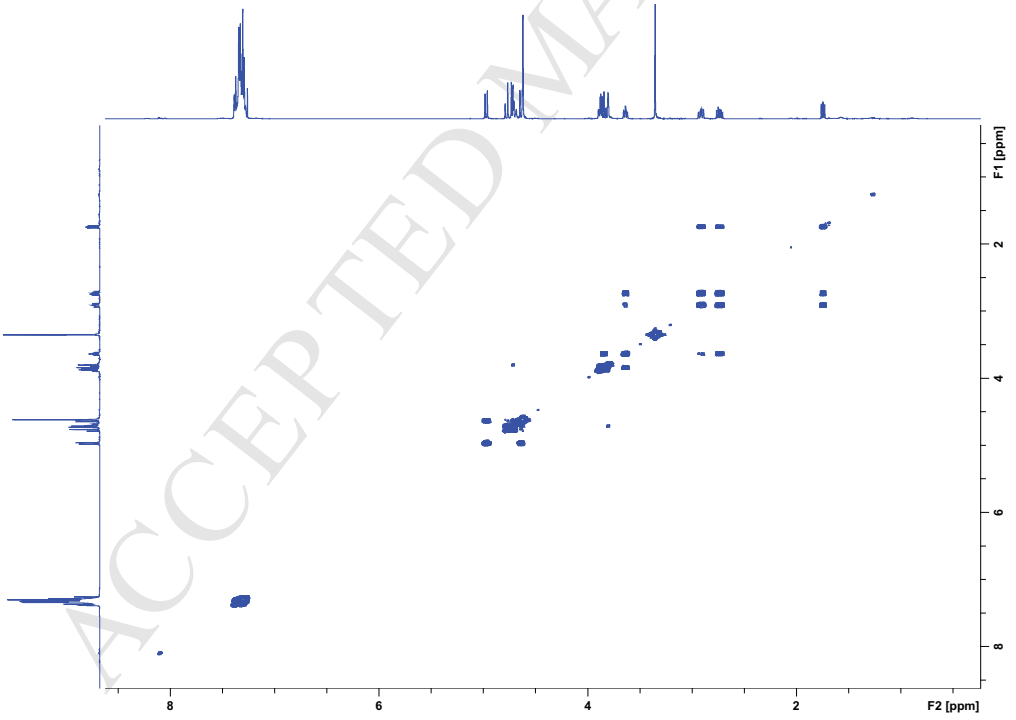

*Figure S2. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for methyl 2,3,4-tri-O-benzyl-6-thio-α-D-mannopyranoside (**12**)*

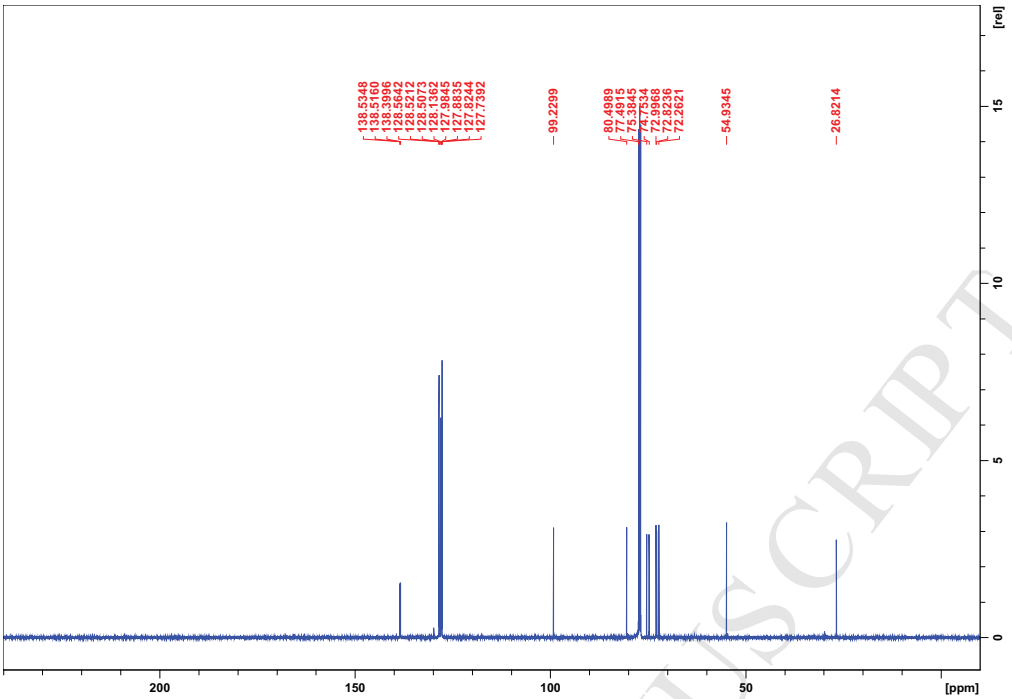

*Figure S3. ^{13}C NMR ($CDCl_3$) for methyl 2,3,4-tri-O-benzyl-6-thio-α-D-mannopyranoside (**12**)*

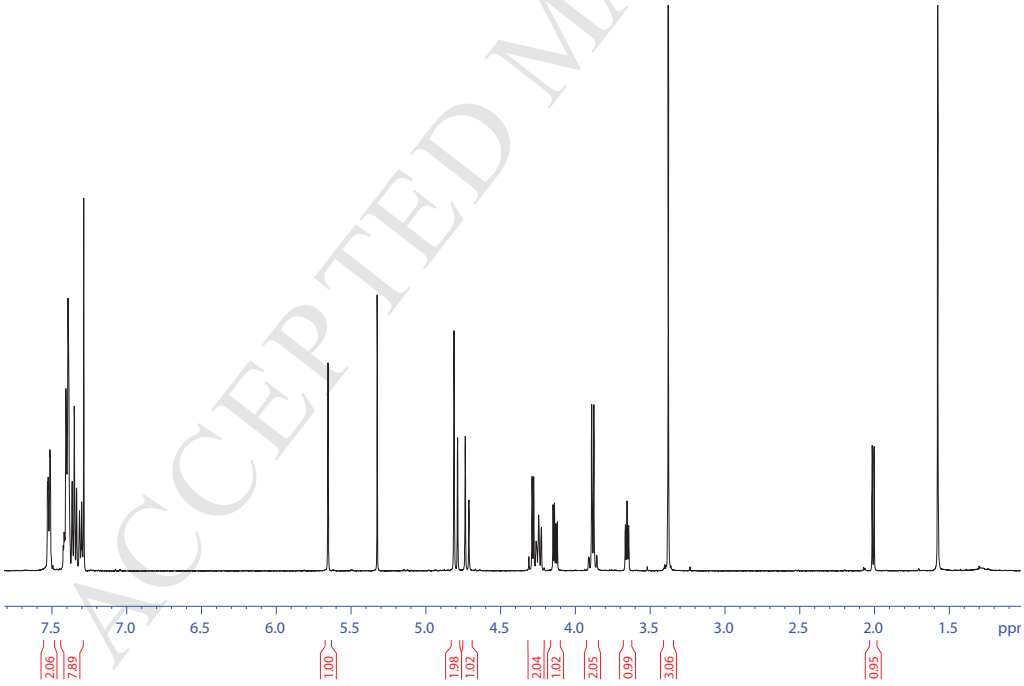

*Figure S4. ^{1}H NMR ($CDCl_3$) for methyl 2-thio-3-O-benzyl-(R)-4,6-O-benzylidene-α-D-mannopyranoside (**19**)*

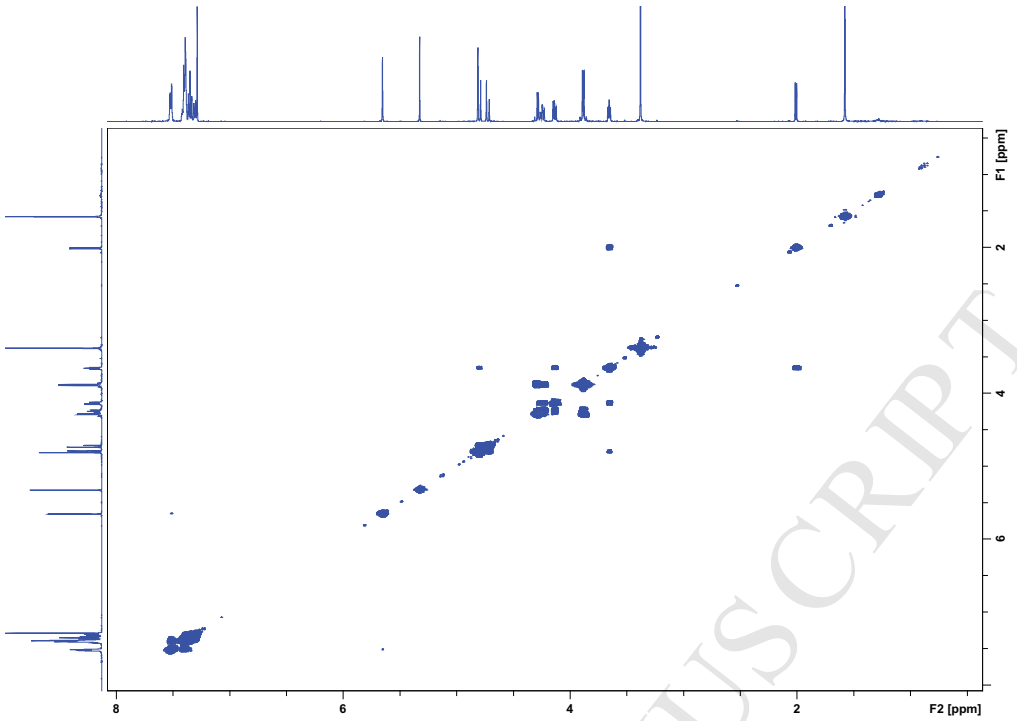

*Figure S5. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for methyl 2-thio-3-O-benzyl-(R)-4,6-O-benzylidene-α-D-mannopyranoside (**19**)*

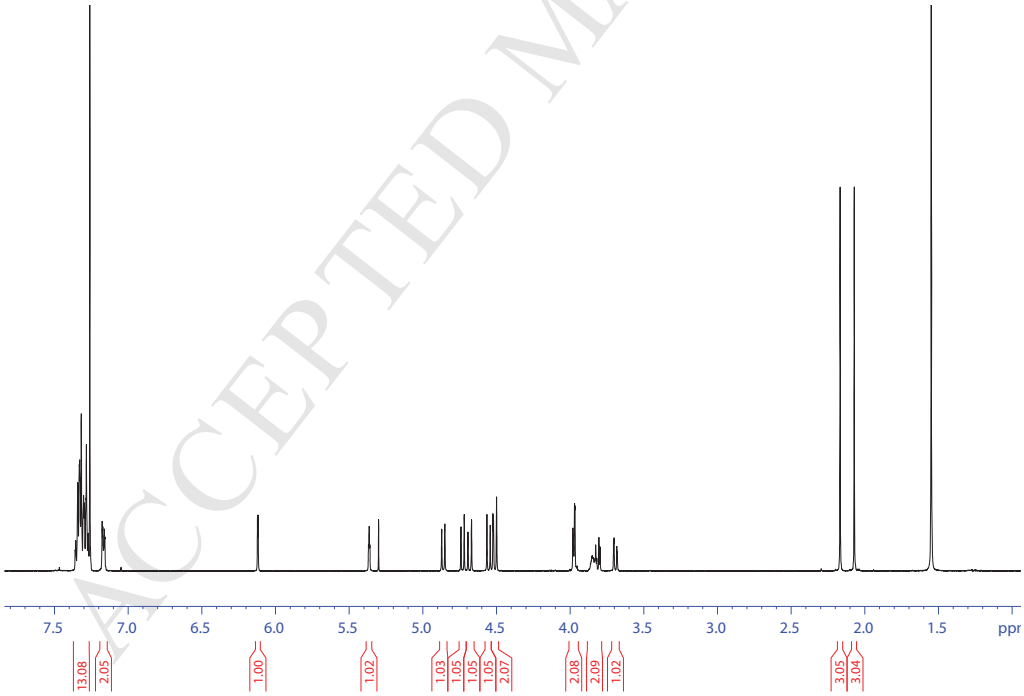

*Figure S6. ^{1}H NMR ($CDCl_3$) for 1,2-di-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**29**)*

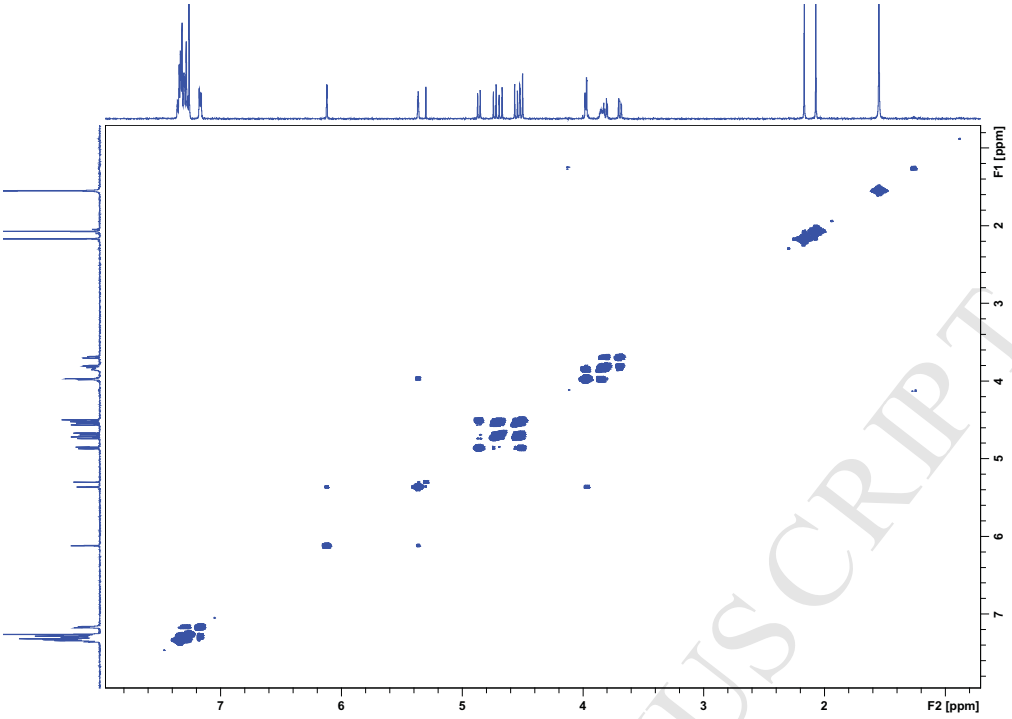

*Figure S7. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for 1,2-di-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**29**)*

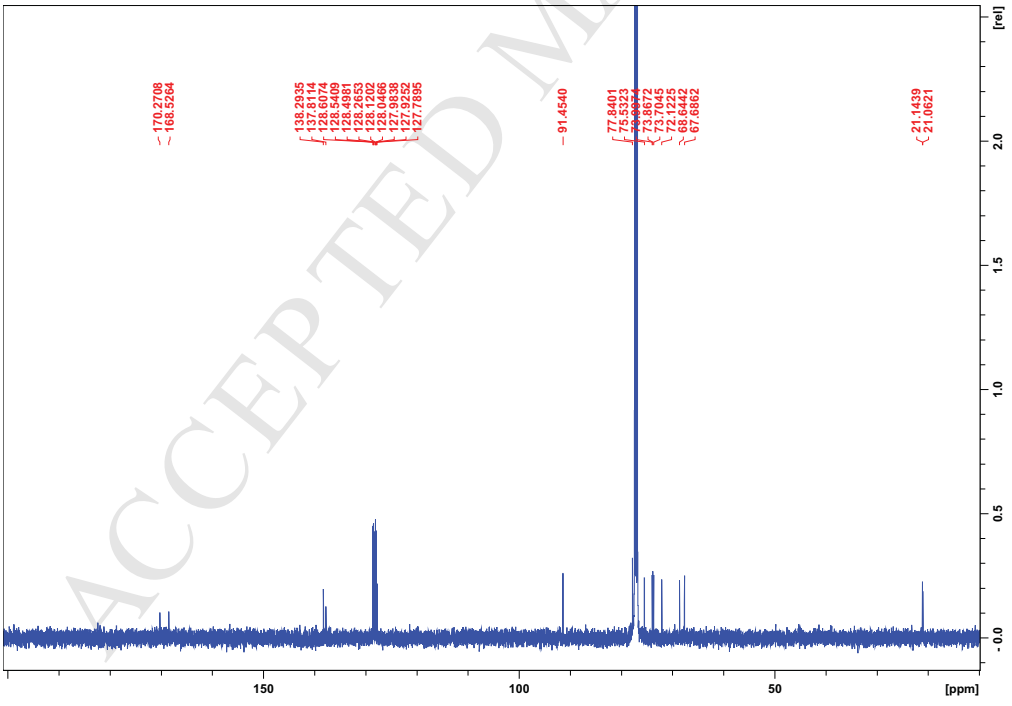

*Figure S8. ^{13}C NMR ($CDCl_3$) for 1,2-di-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**29**)*

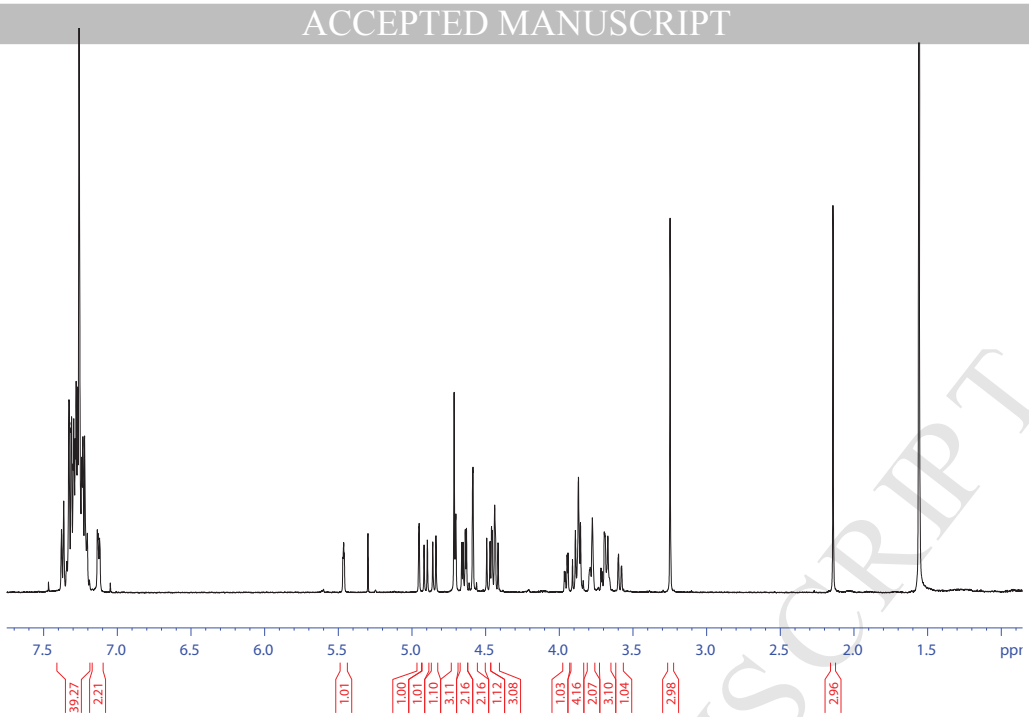

*Figure S9. ^{1}H NMR ($CDCl_3$) for ethyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**30**)*

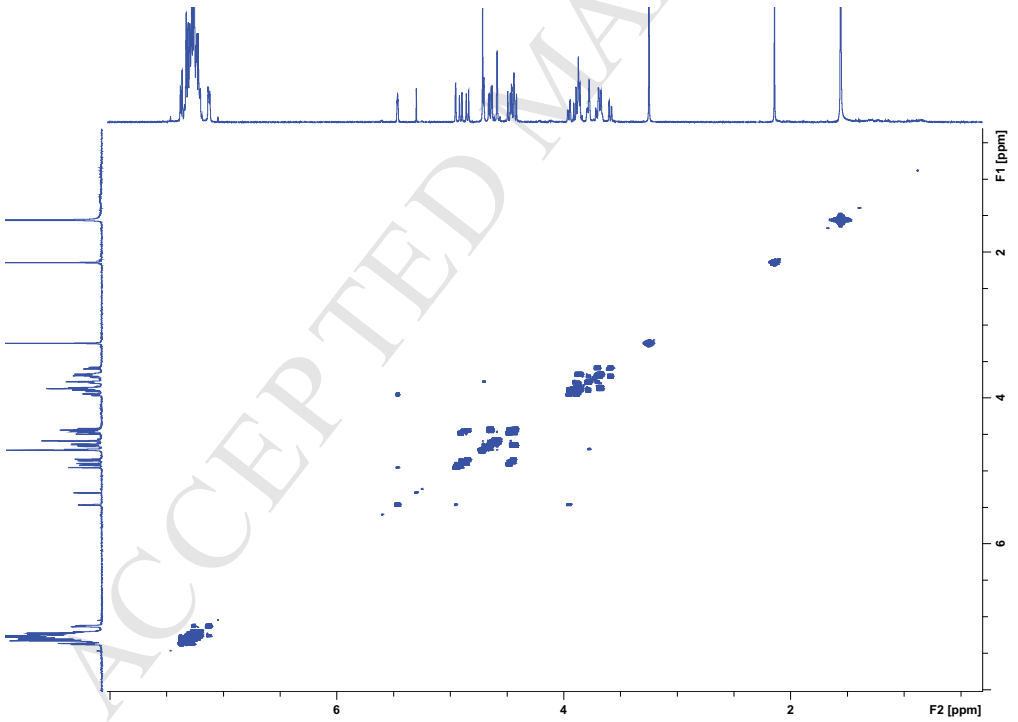

*Figure S10. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for ethyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**30**)*

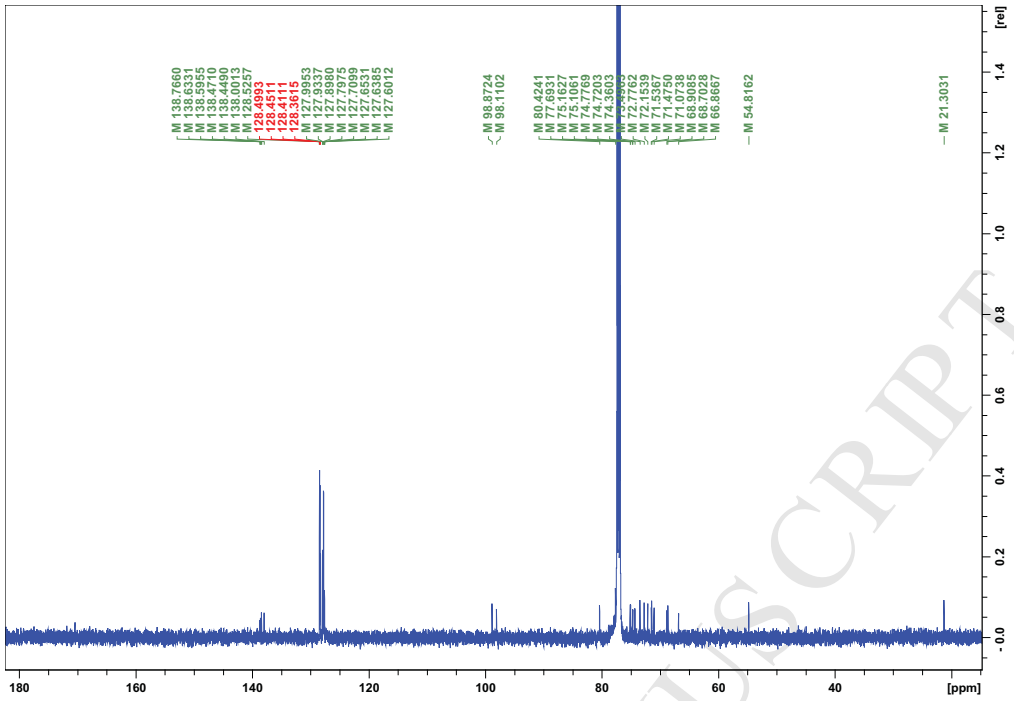

*Figure S11. ^{13}C NMR ($CDCl_3$) for ethyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (**30**)*

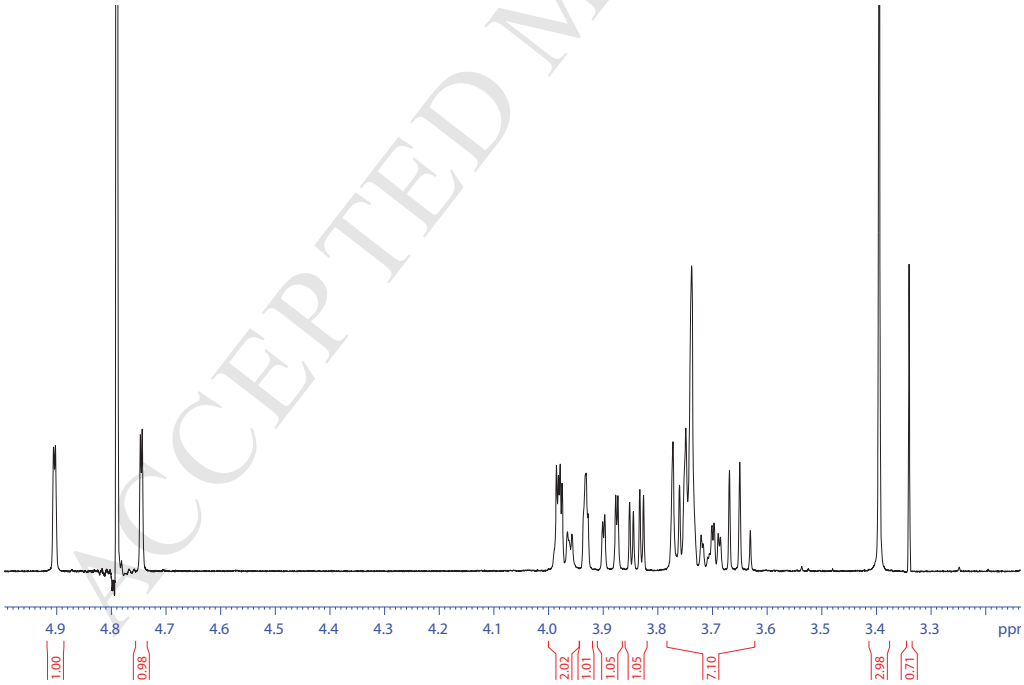

*Figure S12. ^{1}H NMR (D_2O) for methyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**1**)*

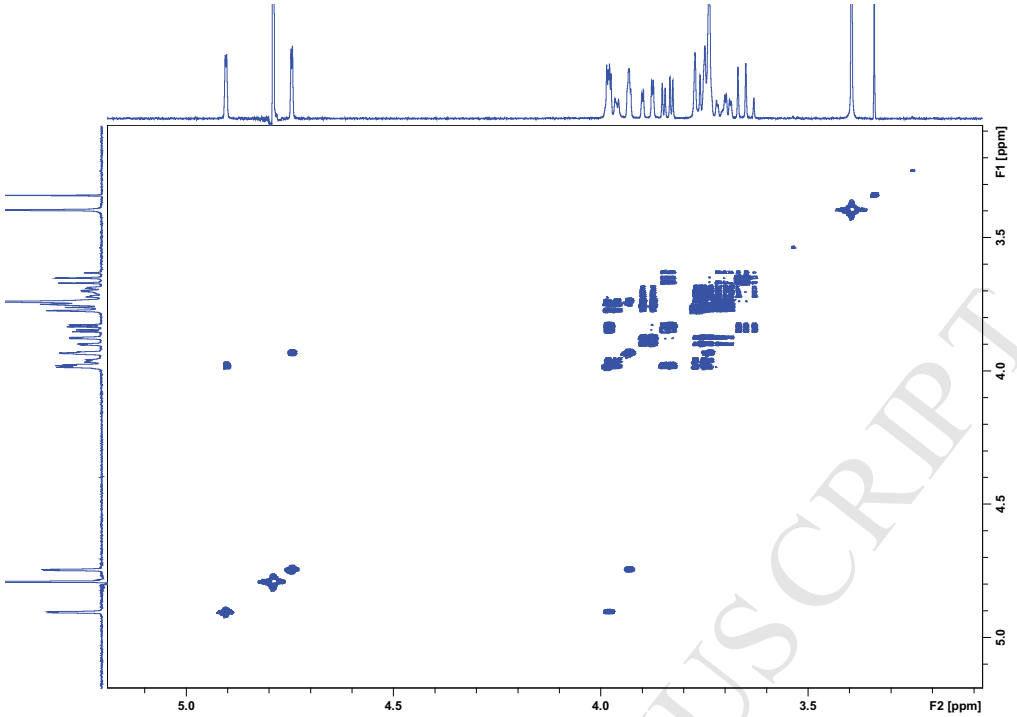

*Figure S13. ^{1}H-^{1}H COSY NMR (D_2O) for methyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**1**)*

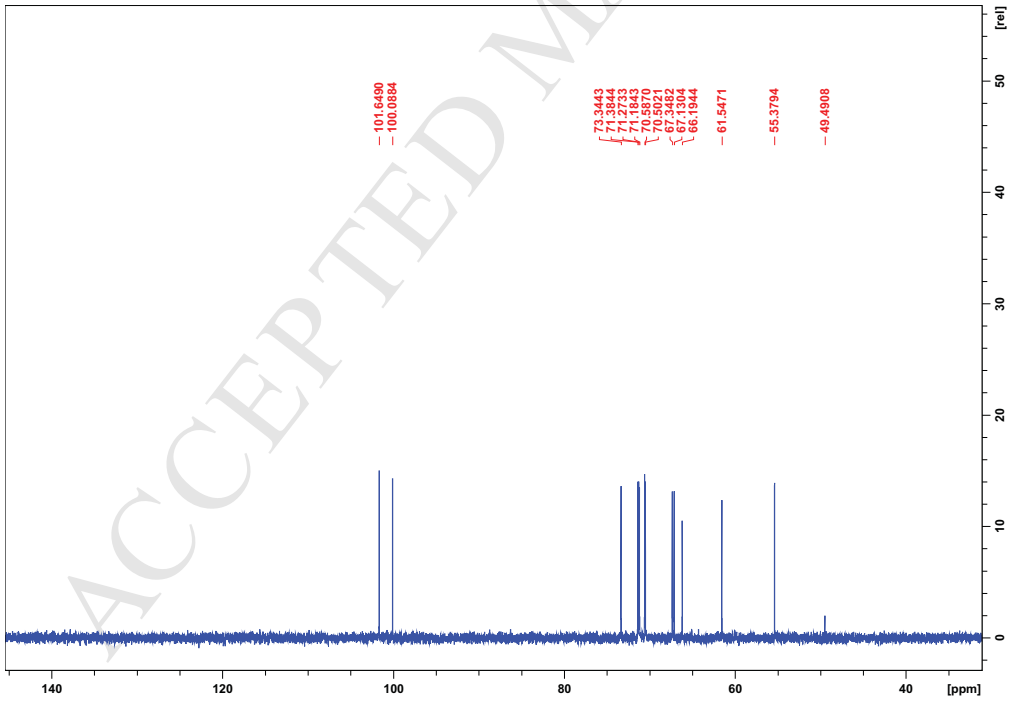

*Figure S14. ^{13}C NMR (D_2O) for methyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**1**)*

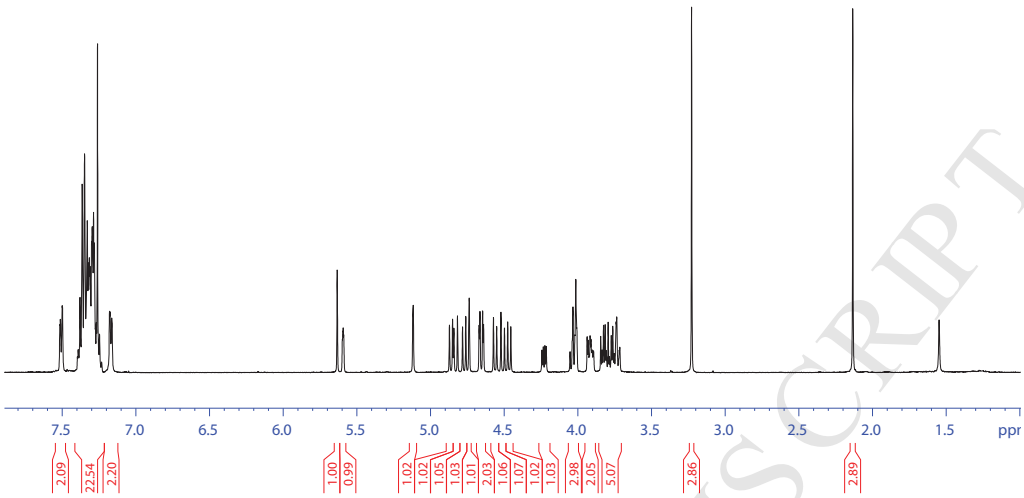

*Figure S15. ^{1}H NMR ($CDCl_3$) for methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3-O-benzyl-(R)-4,6-O-benzylidene-α-D-mannopyranoside (**33**)*

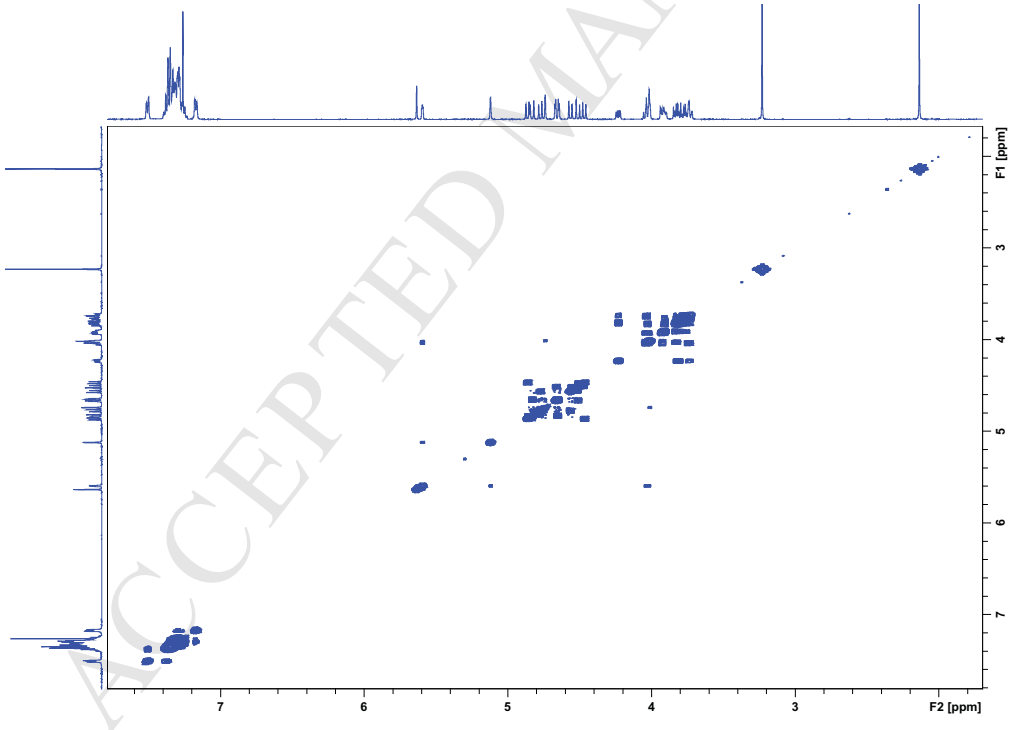

*Figure S16. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3-O-benzyl-(R)-4,6-O-benzylidene-α-D-mannopyranoside (**33**)*

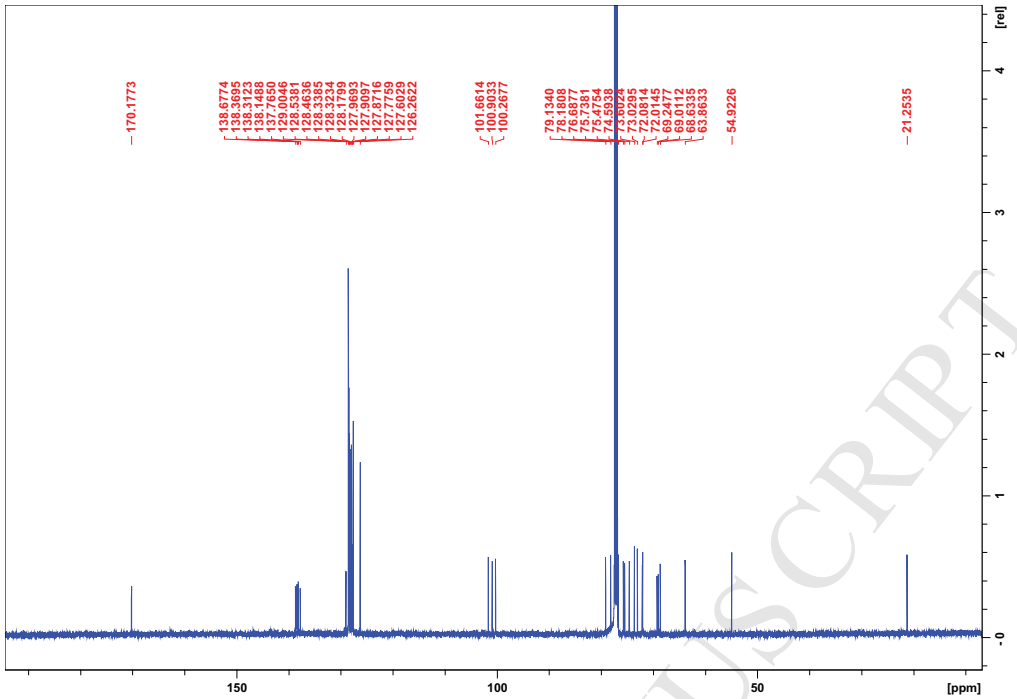

*Figure S17. ^{13}C NMR ($CDCl_3$) for methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3-O-benzyl-(R)-4,6-O-benzylidene-α-D-mannopyranoside (**33**)*

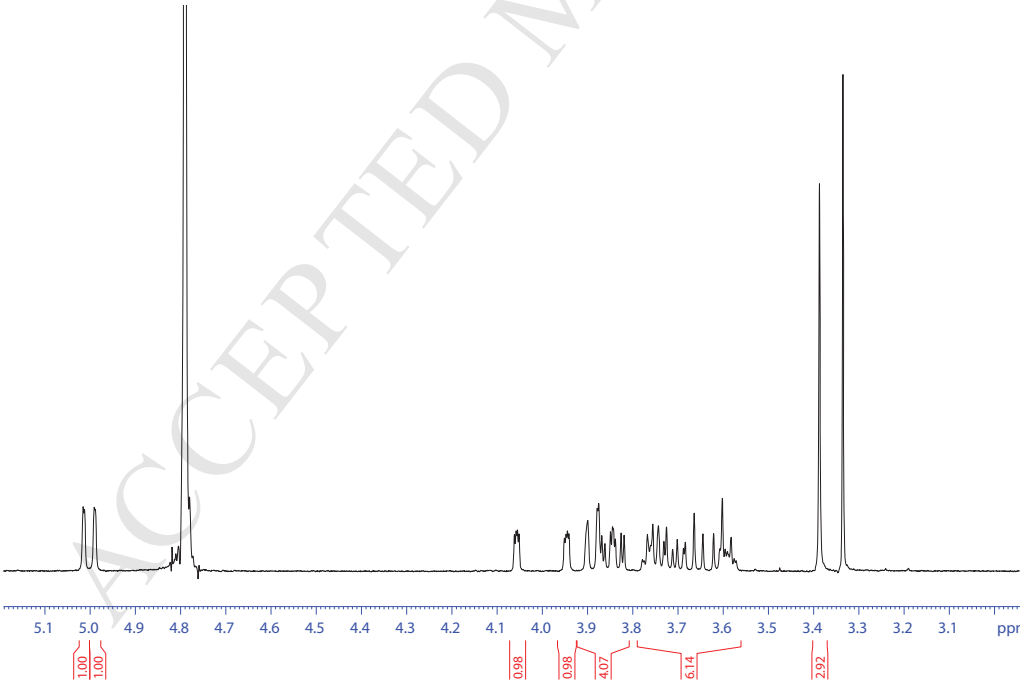

*Figure S18. ^{1}H NMR (D_2O) for methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (**3**)*

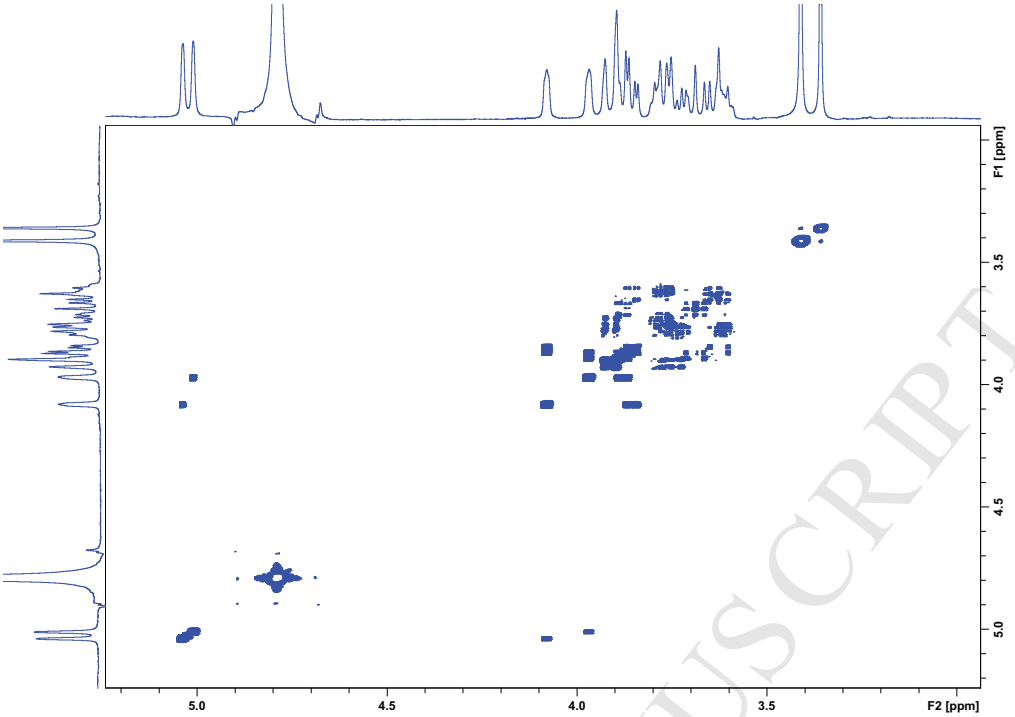

*Figure S19. ^{1}H-^{1}H COSY NMR (D_2O) for methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (**3**)*

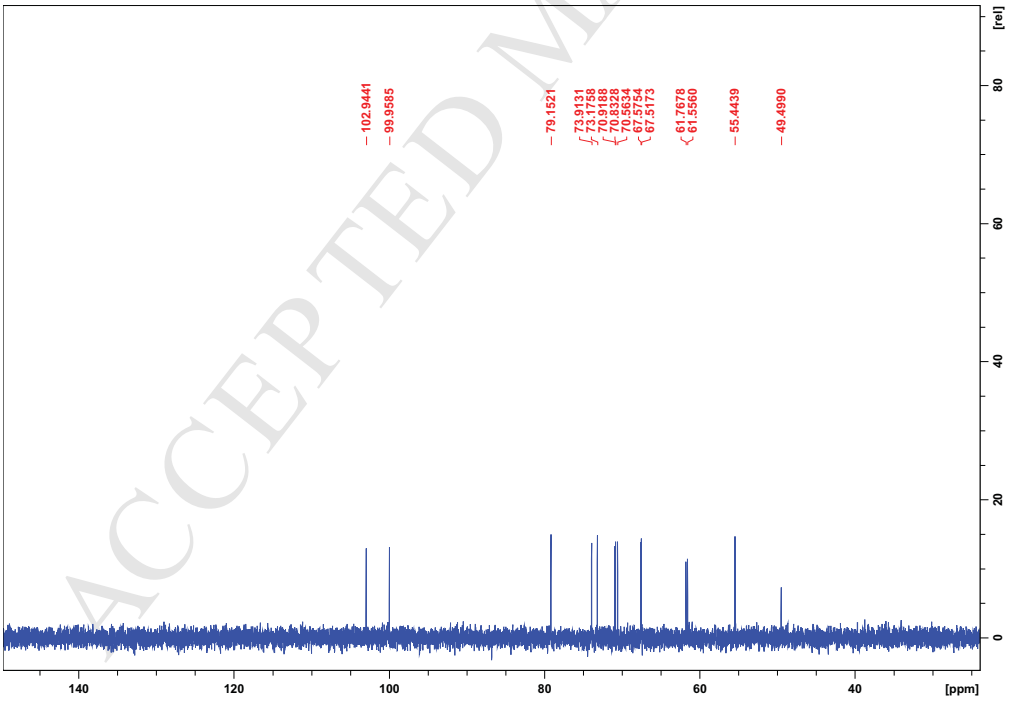

*Figure S20. ^{13}C NMR (D_2O) for methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (**3**)*

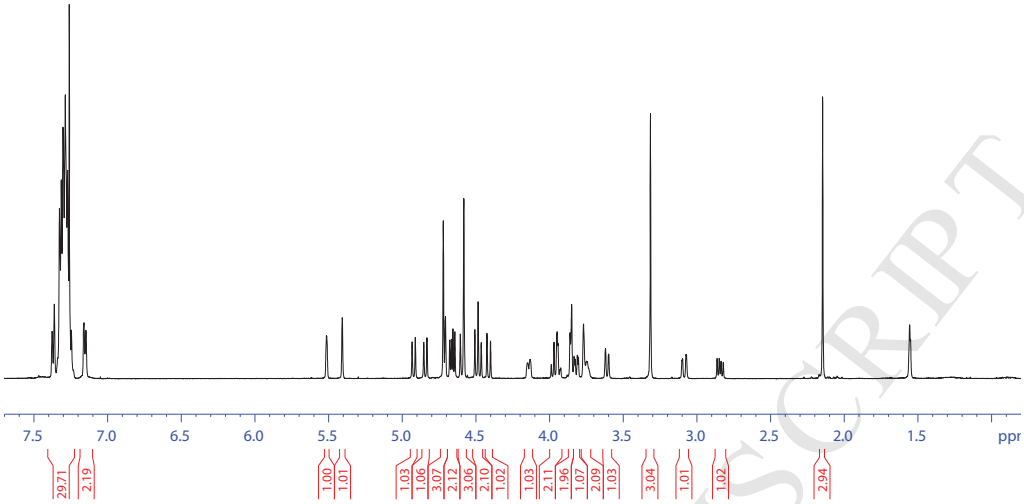

*Figure S21. ^{1}H NMR ($CDCl_3$) for methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-6-thio-α-D-mannopyranoside (**31**)*

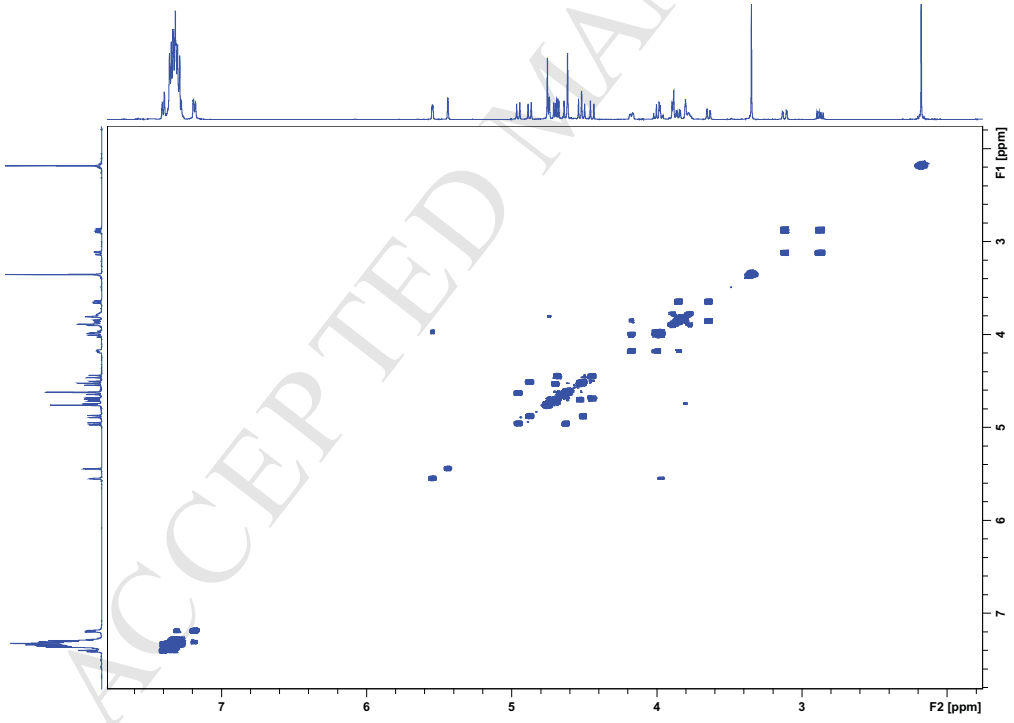

*Figure S22. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-6-thio-α-D-mannopyranoside (**31**)*

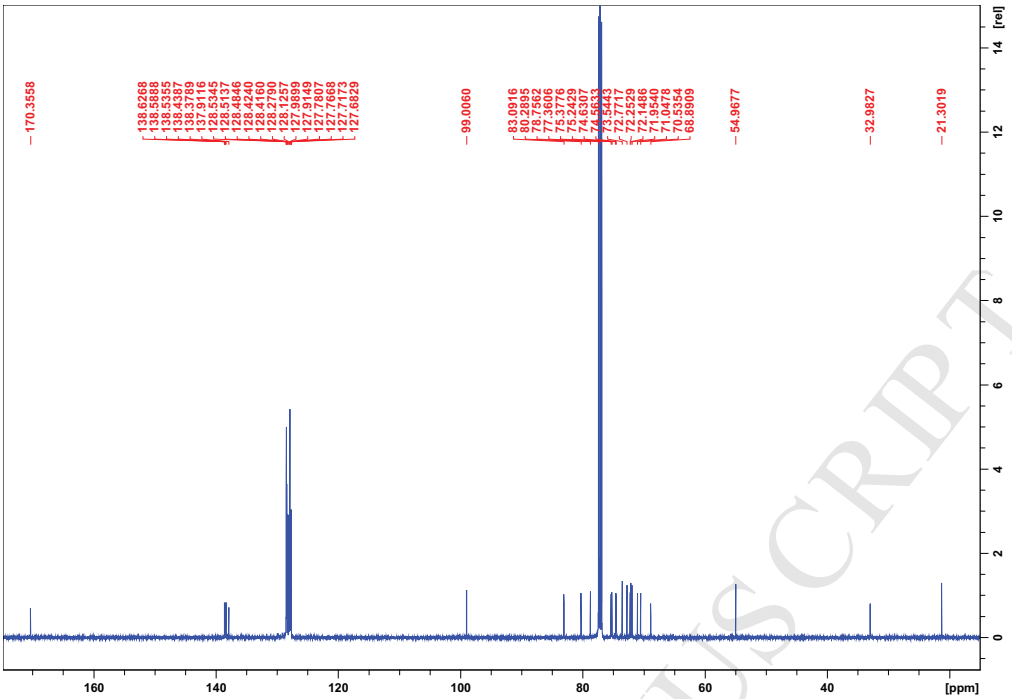

*Figure S23. ^{13}C NMR ($CDCl_3$) for methyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-6-thio-α-D-mannopyranoside (**31**)*

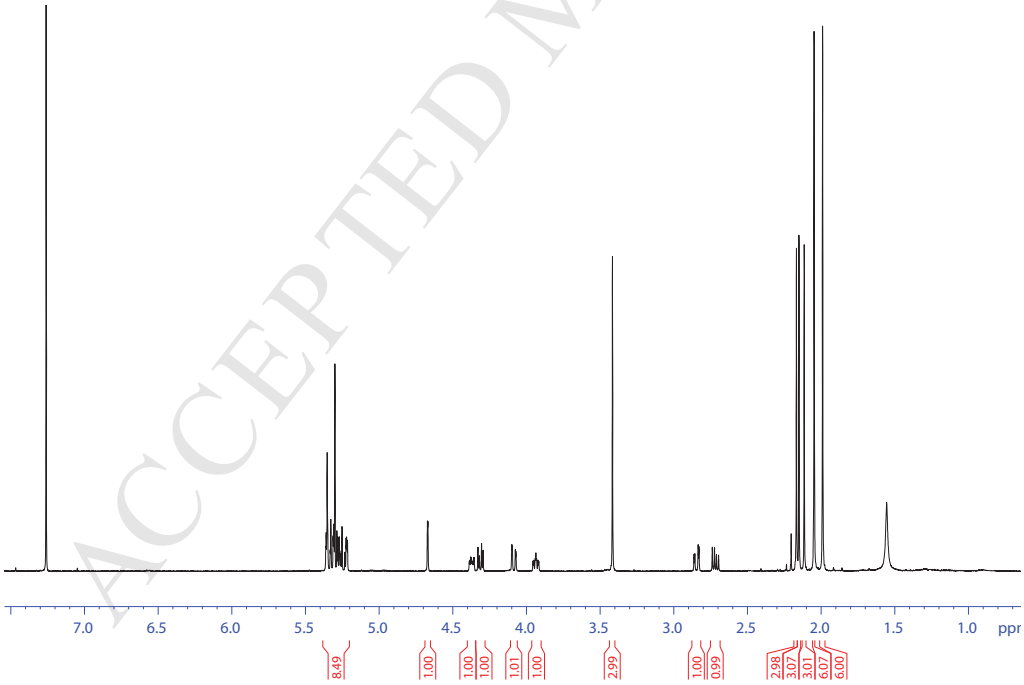

*Figure S24. ^{1}H NMR ($CDCl_3$) for methyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranoside (**32**)*

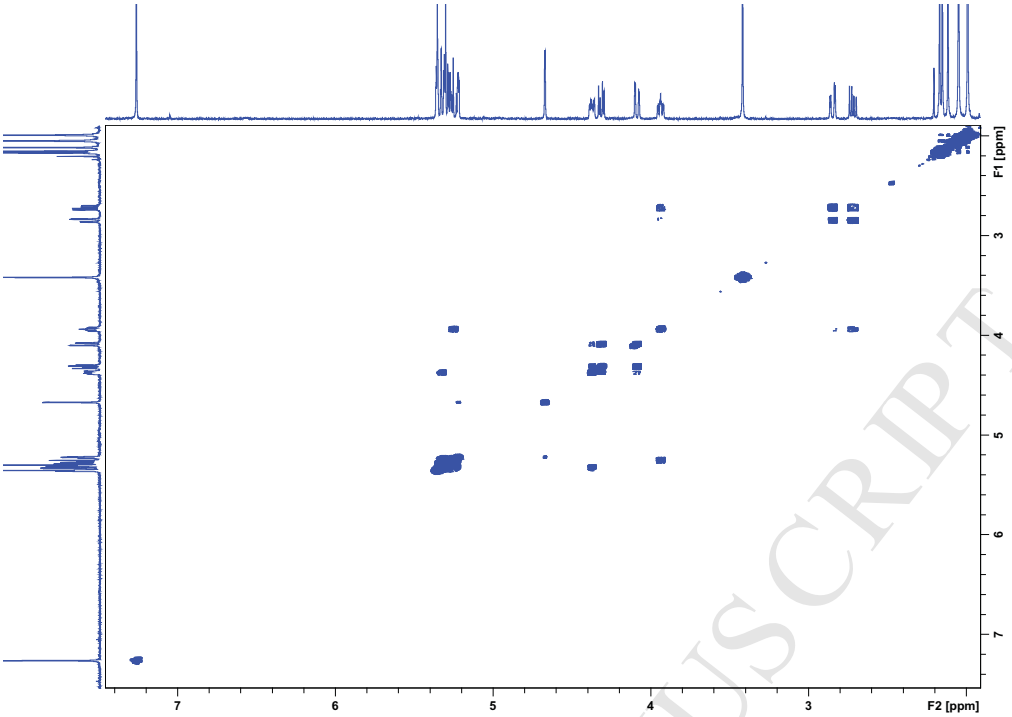

*Figure S25. ^{1}H-^{1}H COSY NMR ($CDCl_3$) for methyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranoside (**32**)*

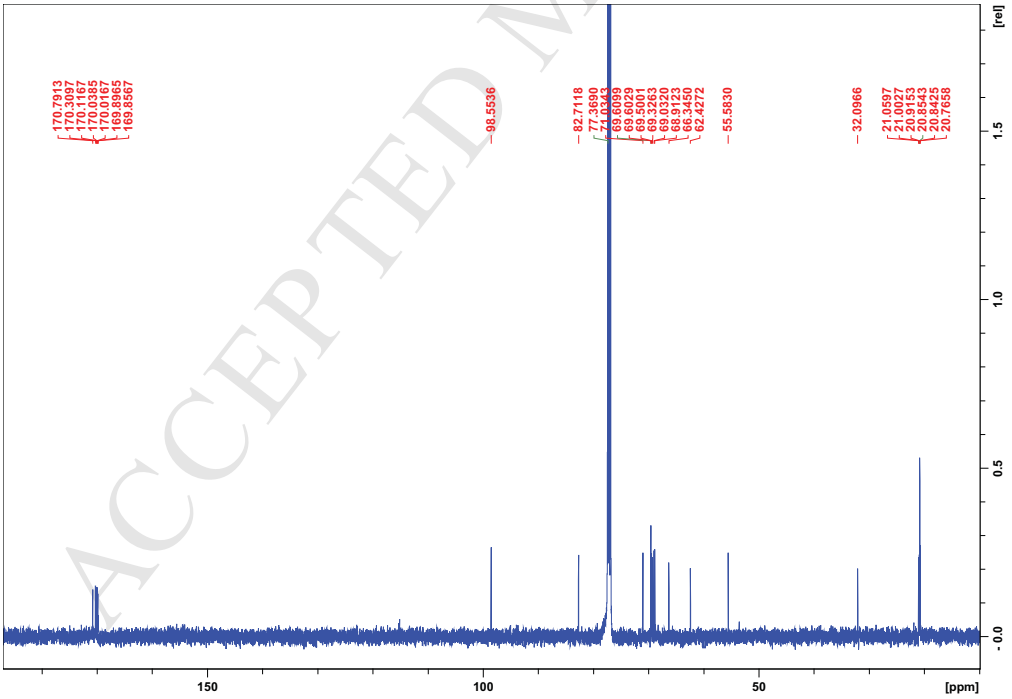

*Figure S26. ^{13}C NMR ($CDCl_3$) for methyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-acetyl-6-thio-α-D-mannopyranoside (**32**)*

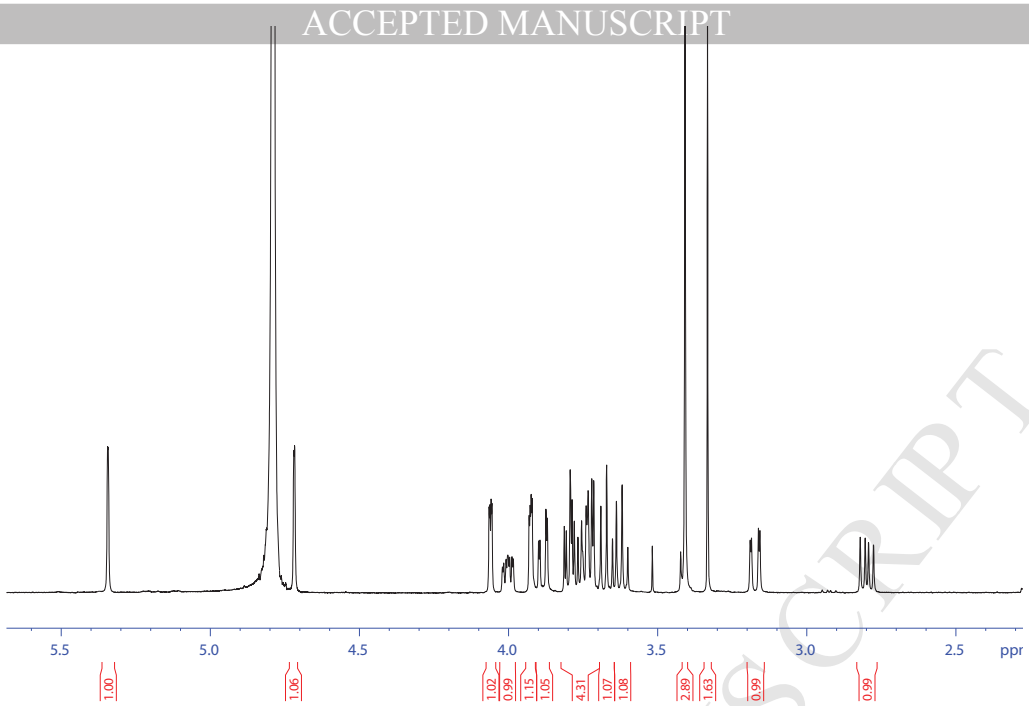

*Figure S27. ^{1}H NMR (D_2O) for methyl α-D-mannopyranosyl-(1→6)-6-thio-α-D-mannopyranoside (**2**)*

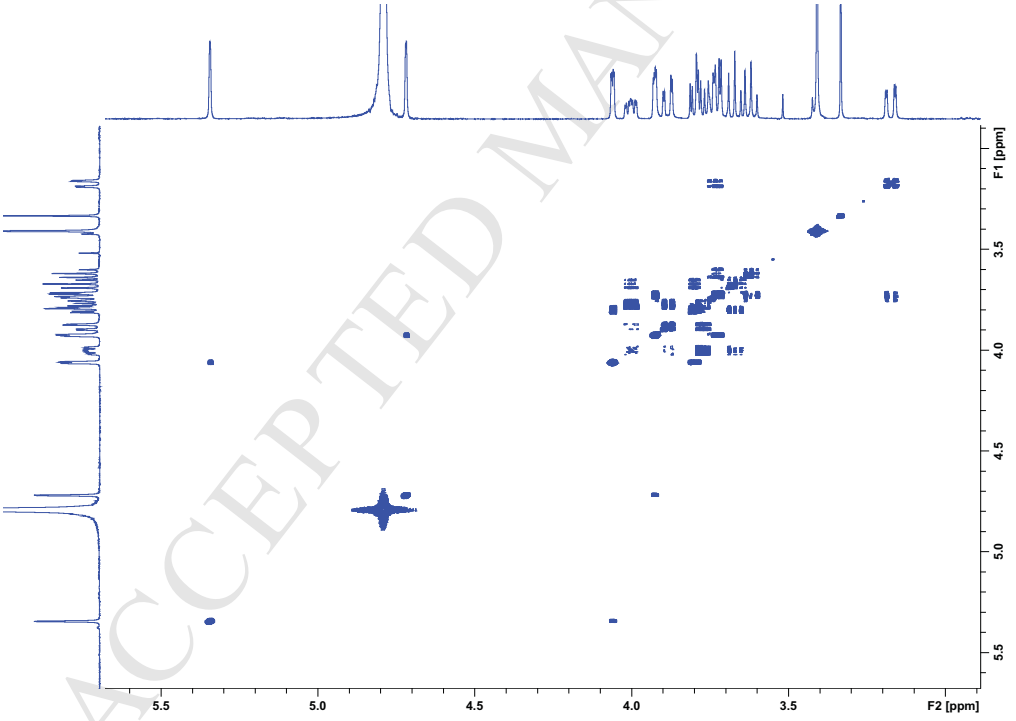

*Figure S28. ^{1}H-^{1}H COSY NMR (D_2O) for methyl α-D-mannopyranosyl-(1→6)-6-thio-α-D-mannopyranoside (**2**)*

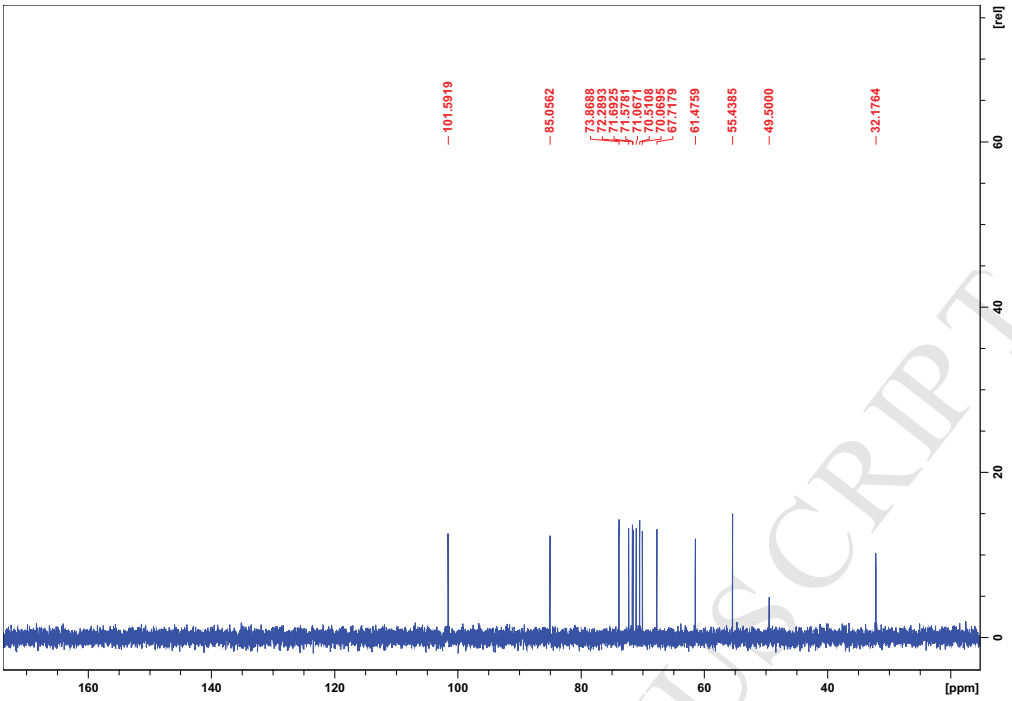

*Figure S29. ^{13}C NMR (D_2O) for methyl α-D-mannopyranosyl-(1→6)-6-thio-α-D-mannopyranoside (**2**)*