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Synthesis of a library of variously modified 4-methylumbelliferyl xylosides and a structure–activity study of human β4GalT7†

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Proteoglycans (PGs) are complex macromolecules that are composed of glycosaminoglycan (GAG) chains covalently attached to a core protein through a tetrasaccharide linker. The biosynthesis of PGs is complex and involves a large number of glycosyltranferases. Here we present a structure–activity study of human β4GalT7, which transfers the first Gal residue onto a xyloside moiety of the linkage region. An efficient and regiocontrolled synthesis of a library of modified analogs of 4-methylumbelliferyl xyloside (XylMU) is reported herein. Hydroxyl groups at the position C-2, C-3 or C-4 have been epimerized and/or replaced by a hydrogen or a fluorine, while the anomeric oxygen was replaced by either a sulfur or a sulfone. The effect of these compounds on human β4GalT7 activity *in vitro* and on GAG biosynthesis *in cellulo* was then evaluated.

1 Introduction

Proteoglycans (PGs) consist of linear polysaccharide chains called glycosaminoglycans (GAGs), covalently attached to serine residues of a core protein through a tetrasaccharide linker (Fig. 1a). PGs are mainly located in the extracellular matrix and at the cell plasma membranes, and are also found intracellularly. There are two major classes of GAG chains: chondroitin sulfate (CS)/dermatan sulfate (DS) and heparin/heparan sulfate (HS). Because of the structural diversity and anionic characteristics of GAG chains, PGs interact with signaling proteins, growth factors, immuno-modulators and a variety of ligands, and thus play an important role in numerous physiological processes, such as cell differentiation, proliferation and migration, and tissue morphogenesis and architecture.[1] However, deregulation of PG metabolism is involved in degenerative pathologies such as cancer,[2] osteoarticular and cardiovascular disorders,[3] as well as genetic diseases, like the Ehlers–Danlos syndromes.[4]

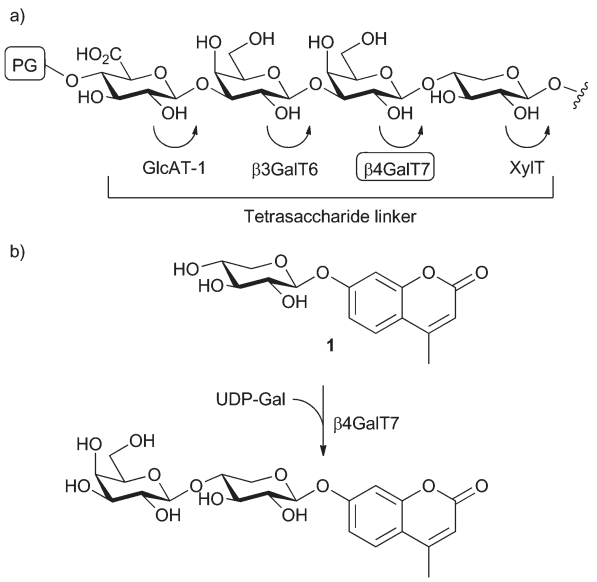

Fig. 1 (a) Biosynthesis of the tetrasaccharide linker of HS and CS/DS PGs. (b) Galactosylation of XylMU **1** to form Gal-XylMU.

PG biosynthesis is initiated by the formation of a tetrasaccharide linker, GlcA(β1–3)Gal(β1–3)Gal(β1–4)Xylβ– on the PG core protein, which is used as a primer for the elongation of the CS/DS and HS chains. Among the enzymes involved,

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β1,4-galactosyltransferase 7 (β4GalT7) catalyses the transfer of the first Gal residue of the linkage region from the activated sugar UDP-galactose (UDP-Gal) onto the xylose residue (Fig. 1a).[5] Because all GAGs share the same stem core tetrasaccharide, β4GalT7 is a key enzyme and a prime target for the study of GAG biosynthesis and the development of specific therapeutic agents. Interestingly, GAG biosynthesis can be modulated by β-D-xylosides carrying a hydrophobic aglycon, such as 4-methylumbelliferyl β-D-xylopyranoside (XylMU, **1**). Hydrophobic xyloside derivatives can act as acceptor substrates of β4GalT7 and initiate GAG chain biosynthesis (Fig. 1b).[6] Several chemical syntheses of xyloside analogs as potential inhibitors of GAG synthesis have also been reported. Tsuzuki *et al.*[7a] and Garud *et al.*[7b] have described the synthesis of triazole analogs which were able to inhibit GAG formation with various efficiencies depending on the hydrophobicity of the aglycone group. Siegbahn *et al.*[8] reported a collection of xylosides linked to different aromatic aglycons with anomeric oxygen or sulfur. The ability of modified xylosides to modulate β4GalT7 enzymatic efficiency depends on the nature of the substitution and its position on xylose, and on the anomeric center.

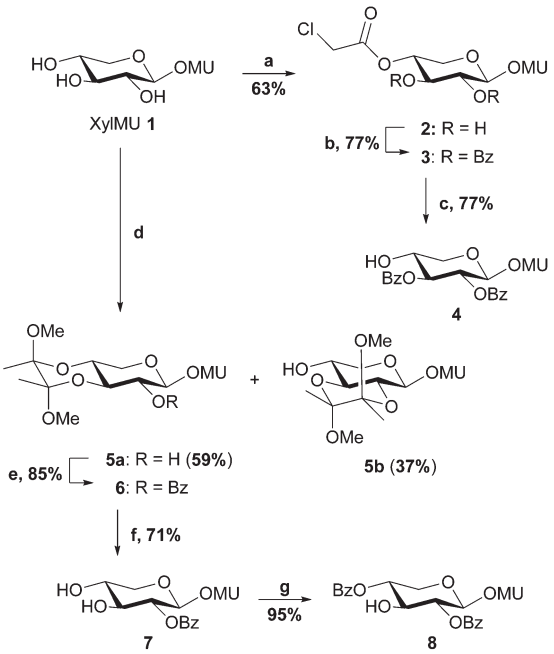
We recently reported a structure-guided approach for the design of β4GalT7 inhibitors.[9] That study showed that the aglycon 4-methylumbelliferyl (MU) produced better xylosides in terms of *in vitro* enzymatic efficiency and *in cellulo* GAG priming, in comparison with naphthyl xylosides for instance. This observation could be explained, at least in part, by the structural organisation of the β4GalT7 active site comprising His[195] whose nitrogen backbone was predicted to interact with the carbonyl group of the coumarinyl molecule. We have also shown that 4-methylumbelliferyl 4-deoxy-4-fluoro-xyloside (4F-XylMU **18**) inhibited β4GalT7 activity *in vitro* and GAG priming in recombinant cells.[9] Here, we present an efficient and regiocontrolled synthesis and the functional evaluation of the effect of a large library of variously modified analogs of XylMU **1** on the β4GalT7 activity, using a double screening strategy: (i) we tested the compounds towards the *in vitro* activity of the purified recombinant human β4GalT7, and (ii) we determined their potency to regulate the glycanation of the core protein of decorin, used as a model PG *in cellulo*.

2 Results

2.1 Synthesis

Starting from XylMU **1**, our goal was to isolate each OH group of the xylose moiety and then perform chemical modifications (fluorination, deoxygenation). Although **1** was commercially available, it was prepared as reported in the literature.[10]

2.1.1 Isolation of the OH groups at C-2, C-3 and C-4. Isolation of each one of the three hydroxyl groups of D-xylose can prove difficult, as they are all secondary, equatorial and therefore similar in reactivity. Hence, there is no general protecting group strategy for all positions of D-xylose. We thus used different strategies to selectively isolate each hydroxyl group of XylMU **1** (Scheme 1).



Scheme 1 Reagents and conditions: (a) (i) Bu_2SnO (1.5 eq.), iPrOH, reflux, 3 h, (ii) $ClCH_2C(O)Cl$ (1 eq.), CH_2Cl_2, 0 °C, 1 h; (b) BzCl (3 eq.), CH_2Cl_2, pyridine, 0 °C → rt, 12 h; (c) thiourea (5 eq.), MeOH, reflux, 6 h; (d) 2,2,3,3-tetramethoxybutane (2 eq.), $(MeO)_3CH$ (4 eq.), CSA (0.05 eq.), MeOH, reflux, 12 h; (e) BzCl (1.1 eq.), Et_3N, DMAP, CH_2Cl_2, 0 °C → rt, 12 h; (f) 95% TFA, CH_2Cl_2, rt, 1 h; (g) BzCl (1.5 eq.), Me_2SnCl_2 (0.05 eq.), DIPEA, THF, rt, 2 h.

Protected xyloside **4**, having the free C4-OH, was prepared according to the literature[11] using selective dibutyltin-mediated chloroacetylation of **1** followed by subsequent benzoylation. After dechloroacetylation with thiourea in methanol, **4** was isolated in 37% yield in four steps.

In order to isolate the C2-OH, we used butanediacetal (BDA) as a protecting group, which has already been employed to protect xylosides, leading to mixtures of 2,3- and 3,4-protected sugars.[8c,12] Introduction of the BDA group was achieved by refluxing a mixture of **1** in methanol for 12 h, in the presence of 2,2,3,3-tetramethoxybutane (TMB), trimethylorthoformate and a catalytic amount of camphor sulfonic acid (CSA).[13] The two separable BDA-protected xylosides **5a** (3,4-protected) and **5b** (2,3-protected) were obtained in a 3 : 2 ratio in 96% yield, and allowed us to isolate the C2- and C4-OH groups in one step.

Furthermore, we took advantage of the preparation of 3,4-protected xyloside **5a** to isolate the C3-OH. First, **5a** was benzoylated to give **6** in 85% yield, and then the BDA group was removed using 95% TFA to afford **7** in 71% yield. Finally, selective benzoylation of the C4-OH with benzoyl chloride in the presence of Me_2SnCl_2 led to derivative **8** with the free C3-OH in 95% yield.[14]

Having isolated the OH groups at C-2, C-3 and C-4, we performed either fluorination (two epimers) or deoxygenation of these positions.

2.1.2 Chemical modifications at C-4. The fluoro- and deoxy-analogs at C-4 were prepared from either 2,3-di-*O*-ben-

zoylated xyloside **4** or 2,3-BDA-protected xyloside **5b**, both with the free C4-OH.

Fluorination reactions were envisaged using diethylaminosulfur trifluoride (DAST). This reagent has been extensively applied to the fluorination of carbohydrates, converting alcohols to fluorides with overall inversion of stereochemistry.[15] Therefore, in order to introduce an equatorial fluoride at C-4, the corresponding OH group on precursors **4** and **5b** had to be first inverted.

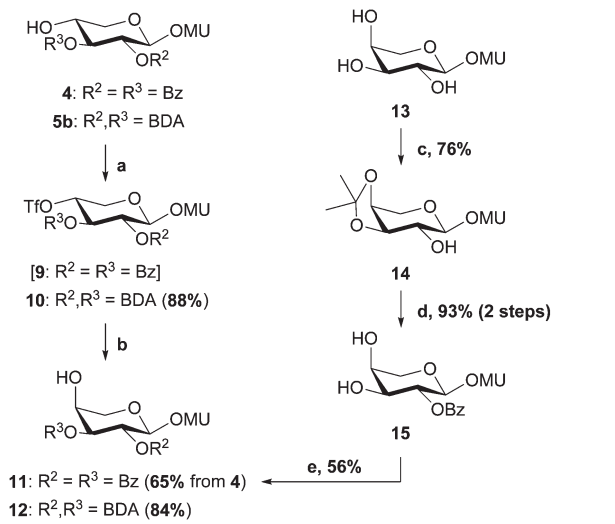
Epimerization of C4-OH was achieved by using the Lattrell–Dax reaction,[16] *i.e.* displacement of a 4-*O*-triflyl group by tetrabutylammonium nitrite (TBAN) (Scheme 2). By reaction with trifluoromethanesulfonic anhydride, triflates **9** and **10** were prepared from **4** and **5b**, respectively. While triflate **9** had to be used rapidly after a quick work-up to avoid the possible displacement of the neighboring benzoyl group at C-3, triflate **10** could be isolated in 88% yield after flash chromatography. Consecutive inversion was carried out with TBAN in toluene at room temperature to afford arabinosides **11** and **12**, with the free C4-OH, in 65% yield from **4** and 74% yield from **5b**, respectively.

Since this inversion step corresponds to the conversion of a D-xyloside to an L-arabinoside, we also thought of working directly on L-arabinose (Scheme 2). Although it was commercially available, 4-methylumbelliferyl α-L-arabinopyranoside **13** was prepared in the laboratory by glycosylation of peracetylated α-L-arabinopyranosyl bromide with 4-methylumbelliferone (66% yield), followed by transesterification under the Zemplén conditions (81% yield), using the same procedures as those described for the synthesis of XylMU **1**.[10] Arabinoside **13** was then protected at C-3 and C-4 with an isopropylidene acetal, to give **14** in 76% yield. Next, benzoylation of the free C2-OH and removal of the isopropylidene[17] afforded diol **15** in 93% yield in two steps. Finally, the OH group at C-3 was selectively benzoylated at low temperature (−35 °C) to afford **11** in 40% yield in four steps. This revealed to be a more efficient and straightforward route to obtain our desired precursor the free axial C4-OH. In comparison, **11** was prepared from xyloside **4** in 24% yield in six steps, while BDA-protected arabinoside **12** was obtained from xyloside **5b** in 27% yield in three steps.

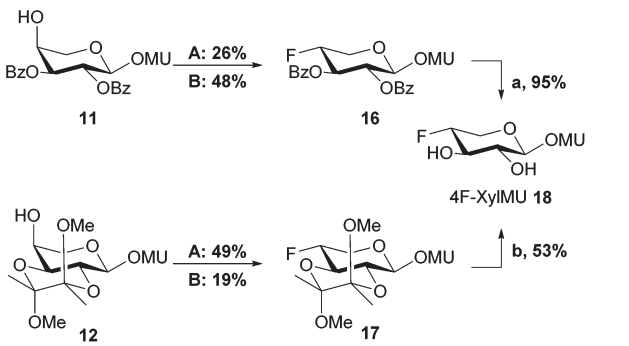
Arabinosides **11** and **12**, with the free C4-OH, were then engaged in fluorination reactions to introduce an equatorial fluorine atom (Scheme 3).

Reaction of **11** and **12** with DAST in dichloromethane, from −40 °C to room temperature (method A), afforded the desired 4-deoxy-4-fluoro-xylosides **16** and **17** in 26% and 49% yields, respectively. When the reaction was carried out in diglyme at 100 °C (method B), as described in the literature for the synthesis of 2-deoxy-2-fluoro-D-glucose,[18] xyloside **16** was obtained in a better yield of 48%, while xyloside **17** was obtained in a lower 19% yield. **16** was then deprotected with sodium methoxide to afford 4F-XylMU **18** in 95% yield, while the BDA group on **17** was removed with 95% TFA, to give **18** in 53% yield.

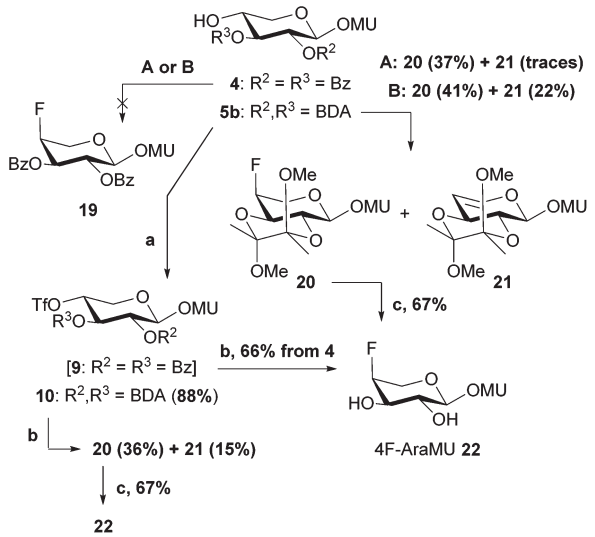
When method A or B was applied to fluorinate xyloside **4**, only traces of the desired 4-deoxy-4-fluoro-arabinoside **19** were observed (Scheme 4). However, starting from BDA-protected xyloside **5b**, fluorination with DAST afforded the targeted fluorinated arabinoside **20**, along with the elimination product **21**. Upon deprotection of the BDA group, 4F-AraMU **22** was obtained from **20** in 67% yield. As fluorination of xyloside **4** with DAST failed, we decided to use instead a triflate/fluoride tandem sequence, with tetrabutylammonium fluoride (TBAF) as fluorinating agent (Scheme 4). 2,3-Di-*O*-benzoylated triflate **9** was successfully fluorinated by reaction with an excess of TBAF in acetonitrile at 75 °C. To our surprise, it also resulted in the removal of the benzoyl groups, which directly afforded desired 4F-AraMU **22** in 66% yield from **4**. Such lability of ester groups in the presence of TBAF has already been reported.[19] We also tested this method for the fluorination of BDA-protected xyloside **5b**, which led to arabinoside **20** in 36% yield and the elimination product **21** in 15% yield, a result comparable to those obtained with the fluorination with DAST. The BDA group was then removed to afford **22** in 67% yield.



Scheme 2 Reagents and conditions: (a) Tf_2O (1.5 eq.), CH_2Cl_2, pyridine, 0 °C, 10 min; (b) $TBANO_2$ (5 eq.), toluene, rt, 20 min; (c) 2-methoxypropene (4 eq.), ACS, pyridine, rt, 2 h; (d) BzCl (1.1 eq.), Et_3N, DMAP, CH_2Cl_2, rt, 3 h and then 70% AcOH, 70 °C, 1 h; (e) BzCl (1.1 eq.), pyridine, −35 °C, 4 h.



Scheme 3 Reagents and conditions: *method A*: DAST (4.5 eq.), CH_2Cl_2, −40 °C → rt, 4 h; *method B*: DAST (3 eq.), diglyme, 100 °C, 7 min; (a) MeONa, MeOH, rt, 30 min; (b) TFA/H_2O, rt, 2 h.



Scheme 4 Reagents and conditions: *method A*: DAST (4.5 eq.), CH_2Cl_2, −40 °C → rt, 4 h; *method B*: DAST (3 eq.), diglyme, 100 °C, 7 min; (a) Tf_2O (1.5 eq.), CH_2Cl_2, pyridine, 0 °C, 10 min; (b) TBAF (9.5 eq.), MeCN, 75 °C, 1 h; (c) TFA/H_2O, rt, 2 h.

The deoxygenation of xylopyranosides by the reaction of xanthates intermediates with Bu_3SnH and AIBN in a Barton McCombie radical deoxygenation reaction has been reported.[8c] A similar methodology was used to deoxygenate xylosides **4** and **5b** (Scheme 5). First, thionocarbonates **23** and **24** were prepared from **4** and **5b** in 68% and 76% yield, respectively, by reaction with *O*-phenyl chlorothionoformate. Then, Barton–McCombie radical deoxygenation of **23** and **24** in presence of Bu_3SnH and ACBN in toluene at reflux afforded 4-deoxy-xylosides **25** and **26** in 88% and 62% yield, respectively. After deprotection, 4H-XylMU **27** was obtained in 87% yield from **25** and in 33% yield from **26**.

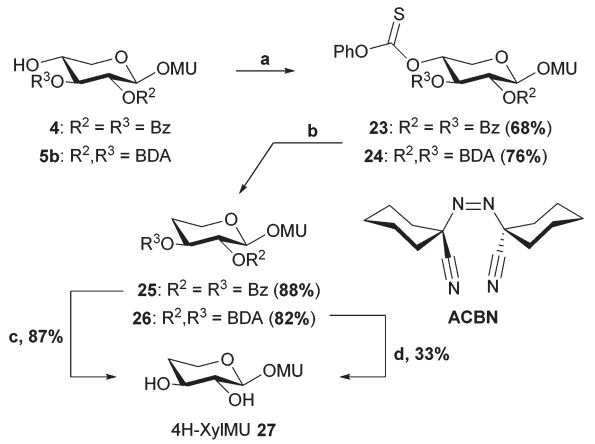
In conclusion, 4F-XylMU **18** and 4F-AraMU **22**, as well as 4H-XylMU **27**, were successfully prepared from either 2,3-di-*O*-benzoylated xyloside **4** or BDA-protected xyloside **5b**. Although no thorough conformational study was performed, analysis of the NMR coupling constants suggest that β-glycosides **18** ($J_{1,2}$ = 6.9 Hz), **22** ($J_{1,2}$ = 7.3 Hz) and **27** ($J_{1,2}$ = 7.2 Hz) are in the normal $^4C_1$ chair conformation. Interestingly, the use of BDA as protecting group locked the carbohydrate ring in this $^4C_1$ chair conformation during the syntheses starting from **5b**, while the use of benzoyl groups induced a deformation of the chair, as most of the intermediates starting from **4** were not in a normal chair conformation ($J_{1,2}$ < 5 Hz) until the final deprotection restored the $^4C_1$ conformation.

2.1.3 Chemical modifications at C-2. The fluoro- and deoxy-analogs at C-2 were prepared from BDA-protected xyloside **5a**, with the free C2-OH.

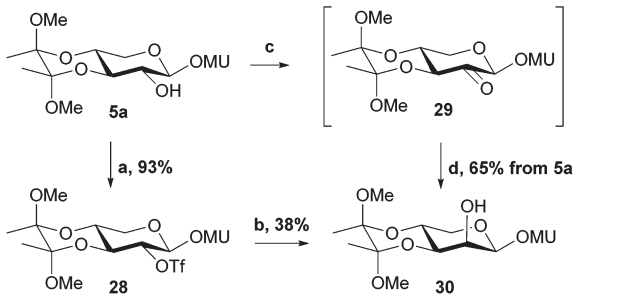
Here again, the C2-OH on **5a** had first to be inversed in order to introduce an equatorial fluorine atom. However, when we used the triflate/TBAN reaction sequence, lyxoside **30** was obtained in low 36% yield from triflate **28** (Scheme 6). We thus decided to investigate another method to epimerize the C2-OH. Manner *et al.* have recently reported a Swern oxidation–epimerization-reduction sequence to selectively epimerize any position in isopropylidene-protected xylopyranosides, thus forming arabinosides, ribosides or lyxosides.[20] Following their strategy, ketone **29** was prepared from xyloside **5a** by Swern oxidation, and then reduced *in situ* with sodium borohydride to afford lyxoside **30** in an overall 65% yield.

We then carried out fluorination reactions on both BDA-protected lyxoside **30** and xyloside **5a** (Scheme 7). Fluorination of **30** with DAST using method A afforded xyloside **31** in 54% yield, while method B gave only 21%. Subsequent removal of the BDA group afforded 2F-XylMU **33** in 77% yield. Fluorination of **5a** with DAST only led to recovery of the starting material. However, fluorination of the corresponding triflate **28** with an excess of TBAF afforded desired lyxoside **32** in 39% yield, which was then deprotected to give 2F-LyxoMU **34** in 88% yield.

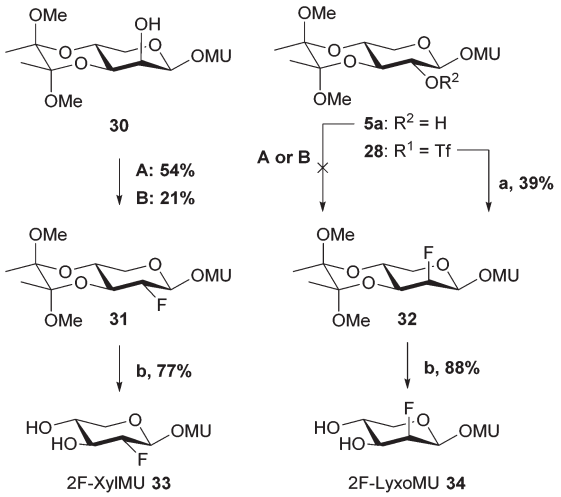
Derivative **5a** was also engaged in deoxygenation reaction (Scheme 8). Thionocarbonate **35**, prepared from **5a** in 59% yield, was deoxygenated using Barton–McCombie reaction to give 2-deoxy derivative **36** in 44% yield over two steps.



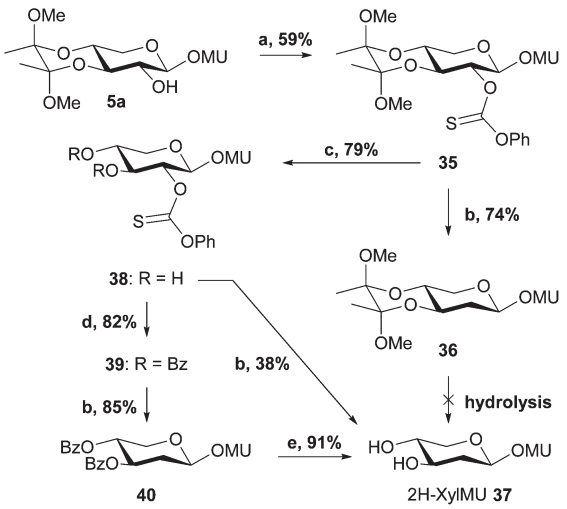
Scheme 5 Reagents and conditions: (a) PhO(CS)Cl (3.2 eq.), CH_2Cl_2, pyridine, rt, 1.5 h; (b) Bu_3SnH (1.5 eq.), ACBN, benzene, 80 °C, 3 h; (c) MeONa, MeOH, rt, 30 min; (d) TFA/H_2O, rt, 2 h.



Scheme 6 Reagents and conditions: (a) Tf_2O (1.5 eq.), CH_2Cl_2, pyridine, 0 °C, 10 min; (b) $TBANO_2$ (5 eq.), toluene, rt, 20 min; (c) 2 M oxalyl chloride (3 eq.), DSMO (6 eq.), CH_2Cl_2, −78 °C, 2 h and then DIPEA (10 eq.), −78 °C → rt, 30 min; (d) $NaBH_4$ (2.7 eq.), MeOH, 0 °C, 4 h.



Scheme 7 Reagents and conditions: *method A*: DAST (4.5 eq.), CH_2Cl_2, −40 °C → rt, 4 h; *method B*: DAST (3 eq.), diglyme, 100 °C, 7 min. (a) TBAF (9.5 eq.), MeCN, 75 °C, 1 h; (b) TFA/H_2O, rt, 2 h.



Scheme 8 Reagents and conditions: (a) PhO(CS)Cl (3.2 eq.), CH_2Cl_2, pyridine, rt, 1.5 h; (b) Bu_3SnH (1.5 eq.), ACBN, benzene, 80 °C, 3 h; (c) 95% TFA, CH_2Cl_2, rt, 10 min; (d) BzCl (3 eq.), pyridine, rt, 12 h; (e) MeONa, MeOH, rt, 30 min.

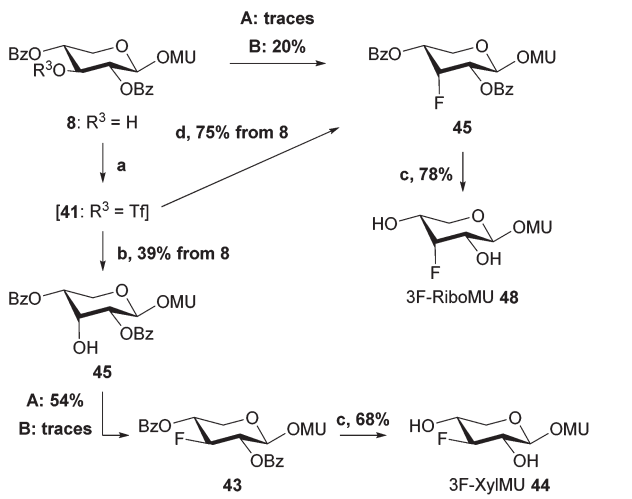
However, removal of the BDA group in acidic conditions (95% TFA) led to the degradation of **36** by hydrolysis of the aglycon. We thus revised our synthetic strategy and decided to remove the BDA group before the deoxygenation. Deprotection of thionocarbonate **35** afforded diol **38** in 79% yield. However, subsequent radical deoxygenation led to 2H-XylMU **37** in only 38% yield after isolation from a complex mixture. Therefore, we decided to reprotect diol **38** with basic-labile benzoyl groups to give **39** in 82% yield, which was deoxygenated to afford deoxy-derivative **40** in better 85% yield. Consecutive deprotection of **40** using sodium methoxide afforded 2H-XylMU **37** in 91% yield, without any degradation. While this three-step route was longer than the direct deoxygenation of **38**, the overall yield was better (63%).

In conclusion, 2F-XylMU **33** and 2F-LyxoMU **34**, as well as 2H-XylMU **37**, were successfully prepared from xyloside **5a**. In particular, the synthesis of **37** highlighted the incompatibility of acid-labile protecting groups with 2-deoxy derivatives. Analysis of the NMR coupling constants indicates that β-glycosides **33** ($J_{1,2}$ = 7.2, $J_{1,F}$ = 3.8 Hz), **34** ($J_{1,2}$ = 1.9, $J_{1,F}$ = 10.9 Hz), and **37** ($J_{1,2}$ = 7.3 and 2.8 Hz) are in a $^4C_1$ conformation, as it was observed on C4-modified analogs, with the same conclusion regarding the influence of BDA and benzoyl groups on the conformation of the synthetic intermediates.

2.1.4 Chemical modifications at C-3. The fluoro- and deoxy-analogs at C-3 were prepared from xyloside **8**, with the free C3-OH.

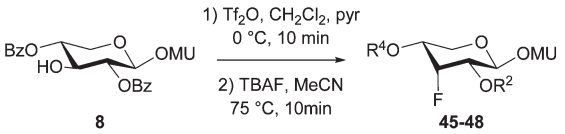
In order to introduce an equatorial fluorine atom, the C3-OH was first epimerized (Scheme 9). Triflate **41** was prepared from **8** and then reacted with TBAN to give riboside **42** in 39% yield over two steps. Next, fluorination of **42** with DAST using method A led to xyloside **43** in 54% yield, while reaction using method B only yielded traces of the desired product. Finally, deprotection afforded 3F-XylMU **44** in 68% yield.

Direct fluorination of **8** with DAST only led to traces of desired riboside **45** when method A was used. Using method B, the product was obtained in 20% yield. On the other hand, reaction of triflate **41** with an excess of TBAF directly yielded desired 3F-RiboMU **48**. However, the compound was isolated with inseparable TBAF-derived salts that could not be removed by purification. Since the use of an excess of TBAF (9.5 equiv.) only led to impure deprotected product **48** (Table 1, entry 1), we decided to test the reaction with 4.5 equiv. of TBAF. After 10 min at 75 °C, TLC showed complete conversion, and a separable mixture of di- and mono-benzoylated 3-deoxy-3-



Scheme 9 Reagents and conditions: *method A*: DAST (4.5 eq.), CH_2Cl_2, −40 °C → rt, 4 h; *method B*: DAST (3 eq.), diglyme, 100 °C, 7 min. (a) Tf_2O (1.5 eq.), CH_2Cl_2, pyridine, 0 °C, 10 min; (b) $TBANO_2$ (5 eq.), toluene, rt, 20 min; (c) MeONa, MeOH, rt, 30 min; (d) TBAF (1.1 eq.), MeCN, 75 °C, 10 min.

Table 1 Removal of Bz groups during fluorination of **8** with TBAF



8 **45-48**

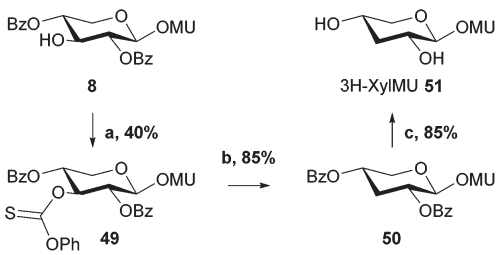
		Yield (%)			
Entry	TBAF (eq.)	**45** R^2 = H R^4 = Bz	**46** R^2 = Bz R^4 = H	**47** R^2 = H R^4 = Bz	**48** R^2 = H R^4 = H
1	9.5	—	—	—	nd
2	4.5	19	16	23	—
3	1.1	75	—	—	—

fluoro-ribosides was isolated (Table 1, entry 2). Use of only 1.1 equiv. of TBAF led to desired 2,4-di-*O*-benzoylated riboside **45** in 75% yield as the major product, with only traces of the mono-protected derivatives. 3F-RiboMU **48** was then obtained in 78% yield after deprotection.

Finally, radical deoxygenation of thionocarbonate **49**, prepared from **8** in 40% yield, afforded 3-deoxy-xyloside **50** in 85% yield, which was subsequently deprotected to give 3H-XylMU **51** in 85% yield (Scheme 10).

In conclusion, 3F-XylMU **44** and 3F-RiboMU **48**, as well as 3H-XylMU **51**, were successfully prepared from xyloside **8**. Regarding the conformation of these β-glycosides, NMR data support that xyloside **44** ($J_{1,2}$ = 7.3 Hz) is in a $^4C_1$ conformation, while riboside **48** ($J_{1,2}$, = 4.1 Hz) and xyloside **51** ($J_{1,2}$ < 1 Hz) are not. It should be noted that xyloside **8** ($J_{1,2}$ 4.4 Hz) was already in a twisted conformation, probably due to the presence of the benzoyl groups at C-2 and C-4, and that was the case for all the intermediates during the syntheses of the three analogs. However, while the final deprotection restored the normal chair conformation for xyloside **44**, it surprisingly did not for riboside **48** and xyloside **51**.

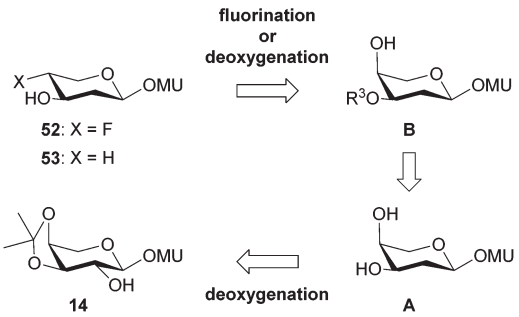
2.1.5 Synthesis of analogs modified at two positions. While synthesizing analogs modified at C-2, C-3 or C-4, we wanted to prepare an example of xyloside modified at two positions, to further study how it would affect the enzymatic affinity. We thereby report the synthesis of 2,4-dideoxy-4-fluoro-xyloside **52** and 2,4-dideoxy-xyloside **53**.



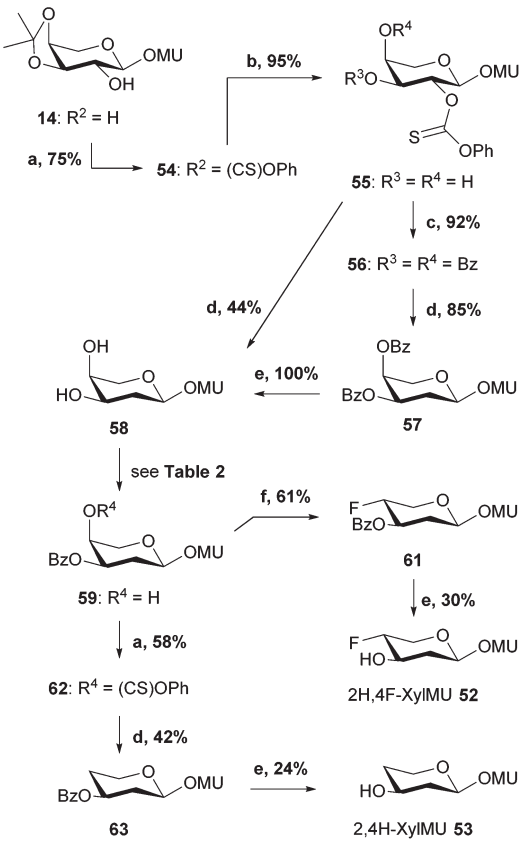
Scheme 10 Reagents and conditions: (a) PhO(CS)Cl (3.2 eq.), CH_2Cl_2, pyridine, rt, 1.5 h; (b) Bu_3SnH (1.5 eq.), ACBN, benzene, 80 °C, 3 h; (c) MeONa, MeOH, rt, 30 min.

Our retrosynthetic strategy was to first deoxygenate the position 2, and then either fluorinate or deoxygenate the position 4 (Scheme 11). Arabinoside **14** was selected as precursor because it had the following characteristics: (a) the C2-OH was already differentiated; (b) the equatorial C3-OH and the axial C4-OH were differentiable; (c) the C4-OH was already in the right conformation to introduce an equatorial fluorine atom.

As presented on Scheme 12, thionocarbonate **54** was first prepared in 75% yield from **14**. The isopropylidene group was then removed to give diol **55** in 95% yield. Direct deoxygena-



Scheme 11 Retrosynthesis of 2,4-modified xylosides **52** and **53**.



Scheme 12 Reagents and conditions: (a) PhO(CS)Cl (3.2 eq.), CH_2Cl_2, pyridine, rt, 1.5 h; (b) 95% TFA, CH_2Cl_2, rt, 10 min; (c) BzCl (3 eq.), pyridine, rt, 12 h; (d) Bu_3SnH (1.5 eq.), ACBN, benzene, 80 °C, 3 h; (e) MeONa, MeOH, rt, 30 min; (f) DAST (3 eq.), diglyme, 100 °C, 7 min.

tion of **55** afforded the 2-deoxy-arabinoside **58** in 44% yield after isolation from a complex mixture, while benzoylation of **55** followed by deoxygenation and deprotection afforded **58** in better 78% yield over three steps.

The synthesis of intermediate **59**, precursor of our targeted analogs, required the selective benzoylation of the C3-OH. According to the NMR data, diol **58** was in a normal chair conformation, which should allow to differentiate the equatorial C3-OH over the axial C4-OH. Various methods were thus tested, as shown in Table 2. While benzoylation of **58** in pyridine at −35 °C did not work (entry 1), reaction in presence of Me_2SnCl_2 led to a mixture of mono-protected products in 3 : 1 ratio in favour of **59**, but with a low conversion (entry 2). A better conversion was obtained using dibutyltin-mediated benzoylation (entry 4), although the selectivity was only 2 : 1. Upon purification, **59** could be isolated in 51% yield. Having isolated the C4-OH, **59** was either fluorinated or deoxygenated.

Fluorination with DAST in diglyme afforded 2,4-dideoxy-4-fluoro-xyloside **61** in 51% yield. However, final deprotection led to 2H,4F-XylMU **52** in only 30% yield, due to the sensitivity of the compound upon neutralization of the sodium methoxide with acidic resin.

Deoxygenation of thionocarbonate **62**, prepared from **59** in 58% yield, afforded 2,4-dideoxy-derivative **63** in 42% yield. Final deprotection led to 2,4H-XylMU **53** in only 24% yield, the compound being even more sensitive than **52** to traces of acid.

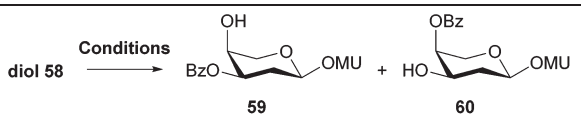
In conclusion, starting with arabinoside **14** as a common precursor, we were able to prepare two analogs modified at C-2 and C-4. 2H,4F-XylMU **52** was obtained in 4% over nine steps, while 2,4H-XylMU **53** was obtained in 2% yield over ten steps. Moreover, it should be noted that, according to the NMR, β-glycosides **52** ($J_{1,2}$ = 5.0 and 3.7 Hz) and **53** ($J_{1,2}$ = 3.6 and <1 Hz) seem to be in a twisted chair conformation. In addition to the chemical modifications, this might have a critical impact on the recognition of these molecules by β4GalT7, and therefore on the enzymatic activity.

2.1.6 Synthesis of analogs modified at the anomeric center. It has already been reported that xylosides with *S*-glycosidic bonds generally initiate GAG priming comparable to the corresponding *O*-xylosides.[21] For example, Jacobsson *et al.* investigated a series of 1-thio-β-xylopyranosides that showed strong priming of relatively short GAG chains.[22] Siegbahn *et al.* showed that sulfur analog **65**, as well as sulfone analog **66**, were galactosylated by β4GalT7 more efficiently than the corresponding *O*-xyloside **64** (Fig. 2).[8a] Furthermore, **66** was able to efficiently prime GAG chains in cell studies. Therefore, in order to investigate the effect of replacing the anomeric oxygen with sulfur, we decided to prepare analogs **67–74** (Fig. 2). As the first results of the biological tests on C-2, C-3 and C-4 modified *O*-xylosides were suggesting that the best inhibition was obtained when replacing the C4-OH by a fluorine atom, we decided to synthesize sulfur analogs **69** and **70**, as well as sulfone analogs **73** and **74**.

XylSMU **67** and AraSMU **68** were prepared by glycosylation of the corresponding peracetylated bromides **75** and **76** with 7-mercapto-4-methylcoumarin, followed by transesterification in the Zemplén conditions (Scheme 13). Furthermore, oxidation of the sulfur prior to the transesterification afforded sulfone analogs **79** and **80** which gave, after deprotection $XylSO_2MU$ **71** and $AraSO_2MU$ **72**.

4F-XylSMU **69** and 4F-$XylSO_2MU$ **73** were synthesized from AraSMU **68** using the same strategy as described for the corresponding *O*-xylosides (Scheme 14). The C4-OH was first isolated in four steps to give arabinoside **84** in 44%, which was then fluorinated with DAST using the best conditions identified in the *O*-series to give xyloside **85** in 51% yield. **85** was then either directly deprotected to afford 4F-XylSMU **69** in 63% yield, or oxidized and then deprotected to give 4F-$XylSO_2MU$ **73**. However, while oxidation of **85** successfully afforded sulfone analog **86** in 87% yield, subsequent treatment with sodium methoxide led to the formation of a significant side product identified as glycal **87**, which competed with the deprotection of **86**, lowering the yield of **73** to 36% yield. This could be explained by the high acidity of the anomeric proton neighbouring the sulfone group.

Table 2 Selective benzoylation of position 3 of 2-deoxy derivative **58**



Entry	Conditions	Yield[a] (%) 59	60
1	BzCl, pyr −35 °C, 4 h	—	—
2	BzCl, Me_2SnCl_2 DIPEA, THF, rt, 1 h	33	10
3	(1) Bu_2SnO, $CHCl_3$/MeOH 1 : 1 60 °C, 4 h (2) BzCl, CH_2Cl_2	51	25

[a] Determined by NMR.

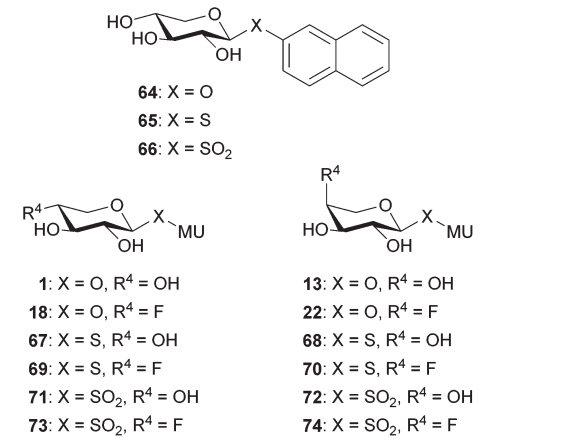
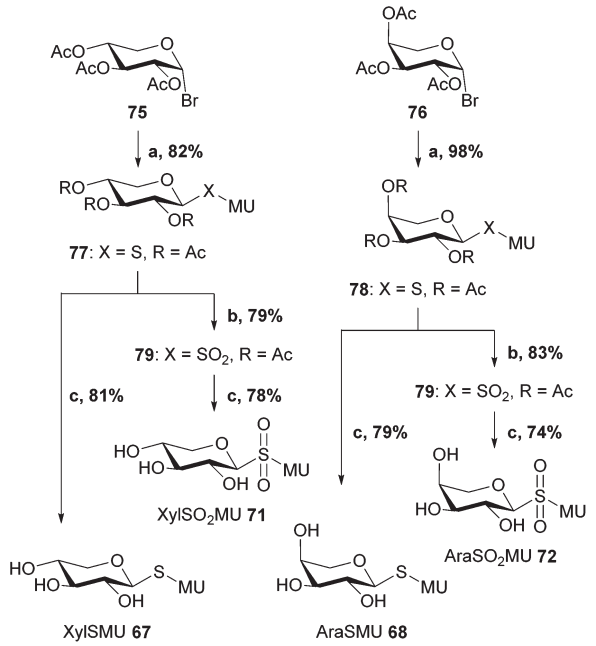
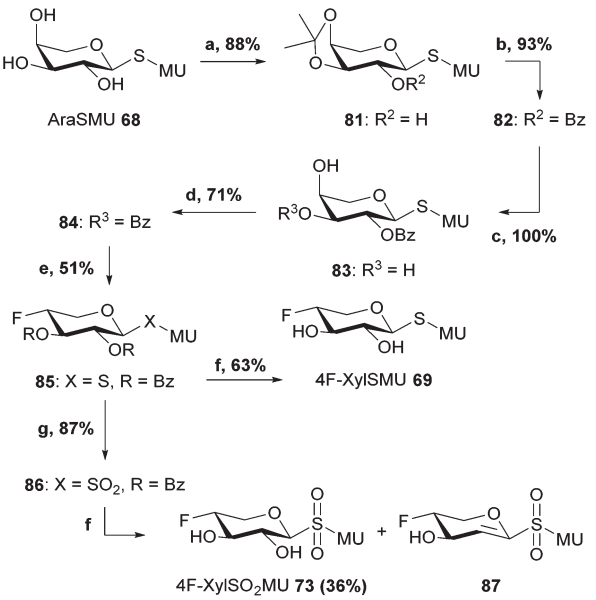


Fig. 2 Modifications at the anomeric center: reported analogs **64–66**, XylMU **1**, synthesized analogs **13–22** and our targeted sulfur and sulfone analogs **67–74**.

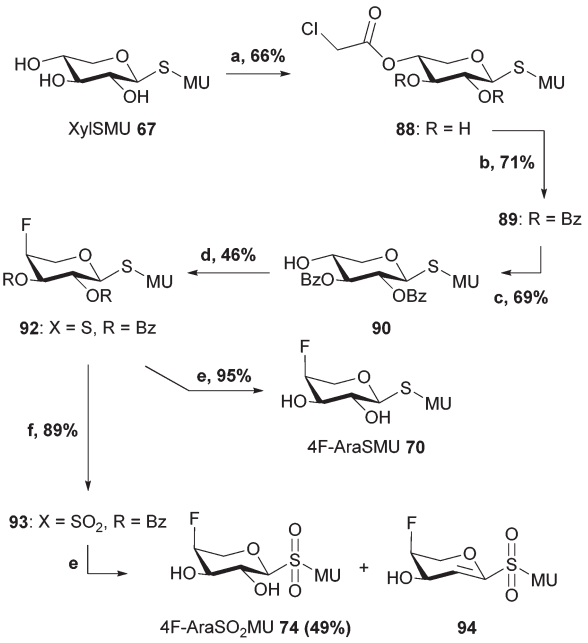


Scheme 13 Reagents and conditions: (a) 7-mercapto-4-methylcoumarin (1.5 eq.), K_2CO_3, acetone, rt, 12 h; (b) mCPBA (4 eq.), CH_2Cl_2, 0 °C, 2 h; (c) MeONa, MeOH, rt, 30 min.



Scheme 14 Reagents and conditions: (a) 2-methoxypropene (4 eq.), ACS, pyridine, rt, 2 h; (b) BzCl (3 eq.), pyridine, CH_2Cl_2, rt, 12 h; (c) 95% TFA, rt, 30 min; (d) BzCl (1.1 eq.), CH_2Cl_2, pyridine, −15 °C, 2 h; (e) DAST (3 eq.), diglyme, 100 °C, 7 min; (f) MeONa, MeOH, rt, 30 min; (g) *m*CPBA (4 eq.), CH_2Cl_2, 0 °C, 2 h.

4F-AraSMU **70** and 4F-AraSO$_2$MU **74** were synthesized from XylSMU **67** using the same strategy as described in the *O*-series (Scheme 15). The C4-OH was first isolated using a chloroacetate to give **90** in 32% yield over four steps. Synthesis of the triflate followed by fluorination with TBAF afforded arabinoside



Scheme 15 Reagents and conditions: (a) (i) Bu_2SnO (1.5 eq.), iPrOH, reflux, 3 h, (ii) $ClCH_2C(O)Cl$ (1 eq.), CH_2Cl_2, 0 °C, 1 h; (b) BzCl (3 eq.), CH_2Cl_2, pyridine, 0 °C → rt, 12 h; (c) thiourea (5 eq.), MeOH, reflux, 6 h; (d) (i) Tf_2O (1.5 eq.), CH_2Cl_2, pyridine, 0 °C, 10 min, (ii) TBAF (1.1 eq.), MeCN, 75 °C, 20 min; (e) MeONa, MeOH, rt, 30 min; (f) *m*CPBA (4 eq.), CH_2Cl_2, 0 °C, 2 h.

92 in 46% yield (two steps). **92** was then either directly deprotected to afford **70** in 95% yield, or oxidized and then deprotected to give **74**. Oxidation of **92** gave sulfone analog **93** in 89% yield, which was then treated with sodium methoxide. Again, we observed the competitive formation of glycal **94**, and **74** was obtained in only 49% yield.

In conclusion, sulfur analogs **67**–**70** and sulfone analogs **71**–**74** were synthesized in good yields. On a conformational aspect, all these molecules are in normal chair conformation.

In summary, we prepared a library of 19 analogs of XylMU **1** (Fig. 3), which were tested as substrates and/or inhibitors for β4GalT7.

2.2 Biochemical and biological screening of modified xylosides

In order to identify lead compounds with biological effects from the series of analogs obtained by chemical synthesis, a two-step screening process was set up. This procedure included: (1) an *in vitro* assay to assess the potency of the compounds to act as substrates, inhibitors or activators of the purified recombinant human β4GalT7 using XylMU **1** as a reference acceptor substrate, and (2) an *in cellulo* screening test to identify compounds capable of modulating (initiating or inhibiting) galactosyltransferase activity in cultured HeLa cells, taken as model cells expressing β4GalT7 endogenously. In the second step, the inhibitory potency of the effector molecule was monitored through its capacity to reduce the formation of

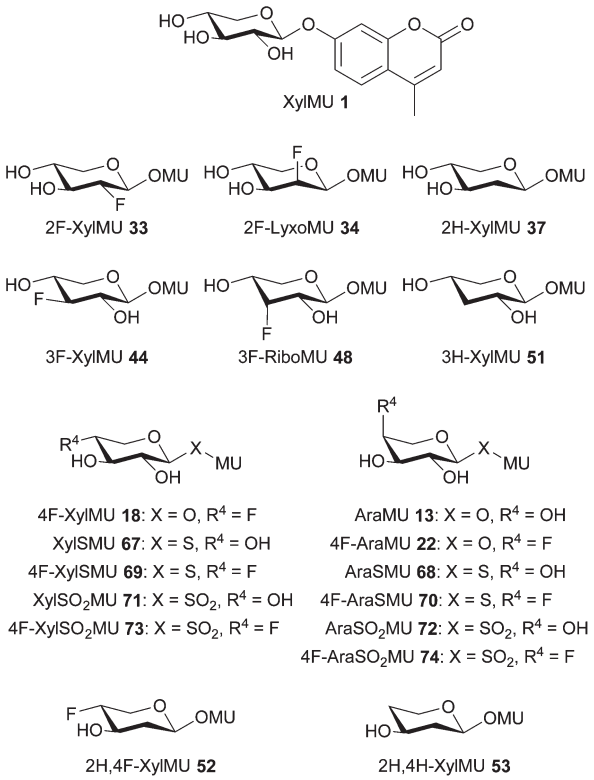


Fig. 3 Summary of the synthesized analogs of XylMU **1**.

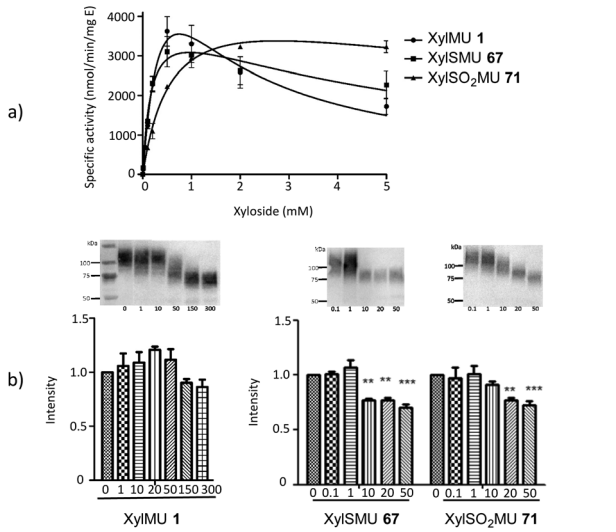


Fig. 4 (a) Effects of thio- and sulfonyl xylosides **67** and **71** on β4GalT7 activity. Assays using purified recombinant 6His-GST-hβ4GalT7ΔNt60 were performed as described in the experimental data section. Kinetic curves of purified recombinant enzyme towards XylSMU **67** and $XylSO_2MU$ **71** were determined using nonlinear least-squares regression analysis of the data fitted to substrate inhibition rate equation. The results are the mean values ± S.E. of three independent measurements on assays performed in duplicate. (b) Effects of XylSMU **67** and $XylSO_2MU$ **71** on decorin core protein glycosylation in HeLa cells. HeLa cells are stably expressing the human recombinant decorin core protein. The decorin glycosylation level was monitored by western blotting, as described in the experimental data section, and then quantified using ImageJ software. Immunoblot analyses of the decorin core protein glycanation level are shown as inserts. The results are the mean values ± S.E. of three independent measurements. Statistical analysis was carried out by a Student's *t*-test with $^{**}p < 0.01$ and $^{***}p < 0.001$ *versus* decorin glycosylation in the presence of DMSO.

GAG chains onto the core protein of decorin, chosen as a model PG.

The relative rates of enzymatic activation/inhibition and β4GalT7 kinetic parameters were determined *in vitro* as described in the experimental data section and are all reported in Table 3 and Fig. 4. Immunoblots from the *in cellulo* screening step are shown in Fig. 4 and in ESI.†

2.2.1 Modifications at the anomeric position. Since modifications at the anomeric center have been shown to significantly influence the capacity of xylosides to act as primers of GAG synthesis,[8a,21d] thio- and sulfonyl derivatives of XylMU **1** were tested as substrates for the recombinant β4GalT7. Since it was previously shown that thioxylosides exhibit interesting pharmacological properties,[23] developing such derivatives could be an attractive option to treat pathological situations involving a GAG defect.

Table 3 Kinetic parameters of β4GalT7 towards C2-modified analogs. Kinetic parameters of purified recombinant 6His-GST-hβ4GalT7ΔNt60 towards modified analogs were determined as described in the experimental data section. The results are the mean values of three independent measurements ± S.D. on assays performed in duplicate. ND indicates that no kinetic constant could be determined using an excess of glycoside

Analogs	K_m (mM)	k_{cat} (min^{-1})	Efficiency (min^{-1} mM^{-1})
XylMU **1**	0.34 ± 0.04	195.4 ± 14.9	570.0
2F-XylMU **33**	>10	ND	ND
2F-LyxoMU **34**	>10	ND	ND
2H-XylMU **37**	3.06 ± 0.23	133.2 ± 20.9	44.3
XylSMU **67**	0.11 ± 0.007	155.6 ± 17.7	1426.8
$XylSO_2MU$ **71**	0.15 ± 0.041	194.0 ± 41.1	1253.3

Thio and sulfonyl derivatives of **1** acted as better substrates than the *O*-xyloside acceptor. Indeed, XylSMU **67** and $XylSO_2MU$ **71** respectively displayed enzymatic efficiencies about 2.5 and 2.2 times greater than that obtained with **1**, mainly due to apparent lower K_m values (3 and 2 fold, respectively) (Table 3). These results are in agreement with those obtained with their corresponding naphthyl counterparts.[8a] Interestingly, detailed kinetic analyses showed that these compounds did not display Michaelis–Menten profile but substrate inhibition kinetics (Fig. 4a), which could be attributed to an allosteric control mechanism. Such regulation mechanism has been previously observed for UDP-glucuronosyltransferases involved in drug metabolism,[24] but to our knowledge, has not been described for glycosyltransferases responsible for GAG synthesis. Importantly, *in cellulo* tests indicated that both **67** and **71** efficiently competed with the GAG-substitution of the decorin core protein, inducing a reduction in band intensity and a shift towards lower molecular weights as their concentration increased (Fig. 3b). In this respect, **67** was particularly

efficient since a concentration of 10 μM was sufficient to produce a band shift comparable to that produced by **1** at 150 μM (Fig. 3b).

In conclusion, substituting the *O*-linker of **1** by either a sulfur atom or a sulfone group provides a new interesting class of xylosidic substrates that efficiently compete with the GAG-substitution of decorin *in cellulo*. The biochemical/biological properties of these molecules deserve to be further investigated in a therapeutic context.

2.2.2 Modifications at the C-4 position. Since it has been reported by us and others that 4F-derivatives display interesting inhibitory properties,[7a,9,25] we decided to test the capacity of the C4-fluorinated analogs of the thio- and sulfonyl-xylosides to modulate β4GalT7 activity. 4F-XylSMU **69**, and to a lesser extent 4F-XylSO_2MU **73**, reduced the galactosylation of XylMU **1** catalyzed by the purified β4GalT7 by about 20% (Fig. 5d) and was thus a weaker inhibitor compared to 4F-XylMU **18**.[9] Comparable results were observed *in cellulo*, as no significant change of the glycosylation level of the decorin core protein occurred in the presence of **69** (see the ESI†).

Altogether, these results show that, under our experimental conditions, although their unsubstituted counterparts XylSMU **67** and XylSO_2MU **71** act as "good" substrates and competitors of β4GalT7, both 4F-XylSMU **69** and 4F-XylSO_2MU **73** derivatives were only moderate effectors of the enzyme.

It has to be noted that the deoxy analog 4H-XylMU **27** is also a weak effector of the enzyme (Fig. 5b).

2.2.3 Arabinosides. In order to assess whether L-arabinosides are also able to modulate the β4GalT7 activity, AraMU **13**, AraSMU **68** and AraSO_2MU **72** were tested as potential acceptor substrates or inhibitors for β4GalT7.

In contrast to its corresponding naphthyl-derivative,[8b] AraMU **13** moderately enhanced the β4GalT7 activity, with an increase of up to 20% of the galactosylation rate of XylMU **1** in the presence of 5 mM of **13** (Fig. 5c). AraSMU **68** led to a modest increase (about 13%) of the relative enzymatic β4GalT7 activity when used at 2.5 mM (Fig. 5c). However, the latter compound precipitated at concentrations higher than 2.5 mM and was thus not suitable for further *in vitro* assays. AraSO_2MU **72** operated as a versatile but weak effector depending on its concentration: 2.5 mM of the molecule slightly enhanced the β4GalT7 activity (about 7%), while 5 mM resulted in a decrease of about 18% of the galactosyltransferase activity (Fig. 5c). However, the molecular mechanism by which these arabinosides, and especially **72**, modulate the β4GalT7 activity has not yet been characterized. It can be suggested that replacing the naphthyl group by 4-methylumbelliferyl as an aglycon produces subtle conformational changes in the structure of the overall conjugate, thus affecting its capacity to interact with the enzyme. This conjecture should be ascertained through advanced enzymatic assays coupled with structural analyses of the compounds in complex with the protein. Furthermore, it should be noted that none of these arabinosides were able to compete with the glycosylation process of the decorin core protein in cells (see the ESI†).

The ability of C4-fluorinated L-arabinosides to activate or inhibit the galactosylation of XylMU **1** by β4GalT7 was also investigated. 4F-AraMU **22** displayed significant inhibitory properties. About 18% relative inhibition was reached at 2.5 and 5 mM of **22** (Fig. 5b). Unlike **22**, 4F-AraSO_2MU **74** displayed activator properties *in vitro*. About 13% and 22% relative increases of enzymatic activity were observed for the latter compound at 2.5 and 5 mM, respectively (Fig. 4d). It is reasonable to assume that activation from exogenous effectors arises as a consequence of a positive allosteric control mechanism, which has not yet been described for human glycosyltransferases involved in GAG synthesis. Such a hypothesis is corroborated by the substrate inhibition kinetics described above for the thio- and sulfonyl derivatives of **1**. Structural investigations are currently in progress to discover the β4GalT7 allosteric binding site(s). However, 4F-AraSO_2MU **74** presented no activating effect on the glycosylation of the decorin core protein *in cellulo*, possibly because this compound is not lipophilic enough to modulate the β4GalT7 activity in live cells (see the ESI†). 4F-AraSMU **70** showed no effect on β4GalT7 activity (Fig. 5d), indicating that the C4-fluorinated thio- and sulfonyl-L-arabinosides did not show better inhibitory potency than their O-counterpart. Further advanced enzymatic studies

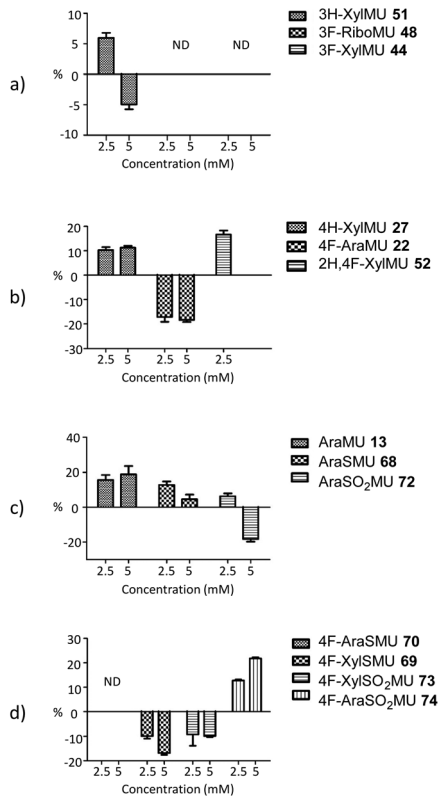


Fig. 5 Effects of modified xylosides on β4GalT7 activity. Assays using purified recombinant 6His-GST-hβ4GalT7ΔNt60 were performed in the presence of fixed **1** (0.3 mM) and UDP-Gal (1 mM) and two concentrations of modified xylosides (2.5 mM and 5 mM). Activities are presented compared to activity of XylMU **1**. The results are the mean values ± S.E. of three independent measurements on assays performed in duplicate. ND indicates that no effect could be detected using an excess of modified xylosides.

would be useful to determine whether the inhibitions observed for the C4-fluorinated L-arabinosides **22** and **74** follow a competitive inhibition model, taking into account the high flexibility of the β4GalT7 structure.[26] Furthermore, none of these C4-fluorinated derivatives were able to affect the glycosylation process of the decorin core protein in cells (see the ESI†). Altogether, these results indicate that epimerisation of the hydroxyl group at the C4 position and replacement by a fluorine mostly prevent the xyloside analog from binding to the active site and acting as substrates or inhibitors of β4GalT7.

2.2.4 Modifications at the C-2 position. The role and effects of xylose 2-*O*-modifications represent a major biological issue in the GAG biology field since phosphorylation at this position is a key regulatory mechanism of linker formation and GAG chain synthesis initiation.[27] We have previously reported that C2-phosphorylation of xylose critically affects the capacity of a xyloside analog to be a substrate of the recombinant β4GalT7.[27b] Thus, in this study, we have examined the effects of various 2-*O*-modifications on the β4GalT7 activity.

It was clearly seen that 2-fluoro compounds were not suitable substrates for the enzyme, as both 2F-XylMU **33** and 2F-LyxoMU **34** showed very low *in vitro* galactosyltransferase activity, with a K_m value higher than 10 mM (Table 3). In addition, 2H-XylMU **37** was a weak substrate, with an enzymatic efficiency about 13 times lower than that obtained for XylMU **1** (Table 3). These results were confirmed *in cellulo*, as none of these C2-modified glycosides efficiently competed with the glycosylation process of the decorin core-protein (see the ESI†). These results corroborate our previous findings, indicating that the C2-phosphorylated analog of 7-methoxy-2-naphthyl xyloside was not a substrate for the recombinant β4GalT7.[27b] They also emphasize that the integrity of the C2 position is essential for a xyloside to bind the active site and to act as a substrate of β4GalT7.

Finally, it was shown that, similarly to 4F-XylSO_2MU **74**, 2H,4F-XylMU **52** exhibited activator properties at 2.5 mM concentration, with an increase of about 17% with respect to the control activity (Fig. 5b). Unfortunately, **52** precipitated at concentrations higher than 2.5 mM under aqueous conditions, rendering it unsuitable for further *in vitro* assays. Nonetheless, because of its small molecular mass and its hydrophobic properties, it was worth checking its activating properties *in cellulo*. Indeed, **52** enhanced the glycosylation level of the decorin core protein, since a marked band shift towards higher molecular mass could be observed as the concentration of **52** was increased (see the ESI†). Thus, unlike either 4F-XylSMU **69** or 4F-AraSO_2MU **74**, 2H,4F-XylMU **52** may deserve to be further considered for its biological activity, being one of the few β4GalT7 activators reported to date with potential efficacy in cells.

2.2.5 Modifications at the C-3 position. We finally determined whether substitutions onto the C3 position of XylMU **1** would give rise to novel effectors of β4GalT7. To this end, 3F-XylMU **44**, 3F-RiboMU **48** and 3H-XylMU **51** were synthesized. However, none of the fluorinated derivatives **44** and **48** affected the β4GalT7 activity *in vitro* (Fig. 5a) or were able to compete with the decorin core protein glycosylation *in cellulo* (see the ESI†). These results somewhat differ from those reported for 3-deoxy-3-fluoro-naphthyl xyloside, which was shown to inhibit β4GalT7,[8b] but may result from the use of 4-methylumbelliferone as an aglycon. Advanced structural investigations are in progress to decipher the precise role of the aglycon in the potency of xylosides to affect *in vitro* or *in cellulo* β4GalT7 activity, which has been highlighted by many previous studies.[8a,21d,28] 3H-XylMU **51** acted as a weak β4GalT7 effector. Regarding the *in vitro* enzyme activity (Fig. 5a), **51** displayed around 5% relative activation rate for 2.5 mM and less than 5% relative inhibition rate for 5 mM, whereas no effect of this compound could be observed *in cellulo* (see the ESI†).

Altogether, these results clearly show that C3-modified analogs of XylMU **1** should not be considered as precursors for β4GalT7 effector candidates, as substituting the equatorial C3 OH-group mainly generated compounds that did not affect the *in vitro* galactosyltransferase activity or the *in cellulo* decorin glycosylation. These results are in good agreement with the impact of the C3-substituent on the conformational equilibrium of the xylopyranoside moiety and therefore on the potency of the conjugates to bind and affect the enzyme activity.[29]

3 Conclusions

Based on our previous work that demonstrated that 4-methylumbelliferone (MU) as an aglycon fits well into the β4GalT7 active site,[9] we designed a rational approach to produce analogs of XylMU **1** targeting this enzyme to be used as enzymatic modulators and/or cellular effectors for GAG initiation. To this end, we prepared a large chemolibrary of glycosides modified at C-2, C-3 and C-4 by epimerisation, deoxygenation and fluorination. We also designed an original two-step approach to test these compounds: (i) *in vitro* as substrates and/or inhibitors of the purified recombinant human β4GalT7 and (ii) *in cellulo* as modulators of GAG synthesis.

Several key points have emerged from this study. Importantly, by combining a sulfur atom or a sulfone group at the anomeric center and MU as an aglycon, we designed an interesting new class of glycosides that acted as substrates *in vitro* and as efficient competitors of GAG synthesis *in cellulo*. These β4GalT7 modulators open up avenues towards the development of GAG-based drugs for pathological situations involving GAG disruption of this metabolic pathway.

We have expounded, for the first time described here for a GAG synthesizing glycosyltransferase, that human β4GalT7 responds to allosteric control mechanisms. Indeed, 2H,4F-XylMU **52** moderately, but significantly, activated β4GalT7 *in vitro* and *in cellulo*. It is, to our knowledge, the first described molecule targeting β4GalT7 that is capable of potentiating GAG initiation synthesis *in cellulo*. On the other hand, we show that the thio and sulfonyl analogs of XylMU **1** exhibited typical substrate inhibition kinetics. These results point to

an allosteric regulation of human β4GalT7, either positive or negative depending on the structure of the xyloside. Structural investigations of human β4GalT7 in complex with these xyloside analogs are currently in progress to identify such possible allosteric binding site(s).

A precise picture of the consequences of modifying the hydroxyl group at different positions of the xylose moiety was also drawn. The 2-*O*-modified glycosides are very weak acceptor substrates, mainly due to a reduced apparent affinity for β4GalT7. This observation is likely to be attributed to the loss of hydrogen bonding between the hydroxyl group at this position and the neighboring carboxylic acid Asp228, in agreement with the structure of human β4GalT7[26b] and our model of the XylMU **1** binding site.[9] This is a major result from a biological point of view. It confirms the assumption that the phosphorylated form of xylose, known to occur *in vivo*, is unlikely to be a substrate of human β4GalT7, in striking contrast to the following glycosyltransferase in the pathway β3GalT6, which displays a strong preference towards C2 phosphorylated xylose.[27a] This result reinforces the crucial role of xylose C2 substitution as a key regulation mechanism of β4GalT7 activity and GAG synthesis initiation.

The 3-*O*-modified glycosides cannot be further considered as modulators of β4GalT7 as very weak activatory or inhibitory effects have been shown under our experimental conditions. The importance of this position can be attributed either to the loss of a hydrogen bond with Asp228 or to the presence of alternatives, such as screw conformers. Indeed, the importance of conformational effects in modified analogs, in particular at the C3 position, has been emphasized in previous studies.[8c]

The C4 position emerges as the most suitable position to target in order to modulate (activate or inhibit) β4GalT7 activity and GAG priming in cells, as the highest percentage of activation or inhibition was shown with 2H,4F-XylMU **52** and 4F-AraSO$_2$MU **74** as positive effectors of β4GalT7, and 4F-XylSMU **69** and 4F-AraMU **22** as β4GalT7 inhibitors. These results corroborate previous studies by us[9] and others[7a,b,8a,b] indicating that replacing the C4-OH group by a fluorine is a suitable strategy for the design of inhibitors of β4GalT7. However, it is important to highlight that the activatory and inhibitory potencies of these C4-modified analogs remain relatively modest. This supports the idea of a narrow active site comprising a network of hydrogen bonds between the hydroxyl groups of the xylose moiety and carboxylic acid amino acid residues that does not tolerate well changes at any position. We thus also contributed to resolving the question whether GAG priming is possible from analogs with xylose modifications, as reported in earlier investigations on modified benzyl-D-xylopyranosides,[30] or whether xylose modifications alter the capacity of the analogs to bind and affect the β4GalT7, as more recently postulated.[8c] Our results support the second hypothesis.

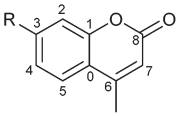
Altogether, we have designed and exhibited good substrates and competitors of GAG synthesis *in cellulo*, activator xyloside analogs of β4GalT7 capable of stimulating GAG synthesis *in cellulo*, and new allosteric control mechanisms in the regulation of β4GalT7. These findings open up new avenues towards chemical biology tools to manipulate GAG synthesis and to intervene in pathological situations involving GAG alterations.

4 Experimental data

4.1 Synthesis

Solvents were dried by standard methods, and molecular sieves were activated prior to use by heating for 4 h at 500 °C. Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–25 °C with a PerkinElmer 341 polarimeter. ^{1}H, ^{19}F (^{1}H-decoupled) and ^{13}C NMR spectra were recorded at 25 °C using a Bruker NanoBay 400 instrument, with Me$_4$Si as an internal standard, unless otherwise stated. High-resolution mass spectra (ESI HRMS) were recorded on a Bruker maXis mass spectrometer by the "Fédération de Recherche ICOA/CBM" (FR 2708) platform. Flash-silica chromatography was performed on Silica gel 60 (0.040–0.063 mm, Merck, Darmstadt). The reactions were monitored by TLC on coated aluminium sheets (Silica gel 60 GF$_{254}$, Merck), and spots were detected under UV light and by charring with a 95 : 5 mixture of ethanol and sulfuric acid.

For the NMR data, 4-methylumbelliferyl aglycon group atoms are numbered as indicated below:



4.1.1 Typical procedure for the preparation of triflates. Alcohol (1 mmol) was dissolved in freshly distilled CH_2Cl_2 (2 mL) and pyridine (0.48 mL, 6 mmol), and then cooled to 0 °C while stirring under argon. Trifluoromethylsulfonic anhydride (0.13 mL, 1.5 mmol) was added dropwise, and stirring was continued for 10 minutes. The reaction mixture was then diluted with CH_2Cl_2, washed with cold 1 N HCl and sat. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The triflate was either purified by flash chromatography (BDA-protected products) or directly engaged in subsequent reactions (Bz-protected products).

4.1.2 Typical procedure for the epimerization with TBANO$_2$. Triflate (1 mmol) was dissolved in dry toluene (2 mL) and solid tetrabutylammonium nitrite (1.44 g, 5 mmol) was added. After stirring for 20 min at rt, the reaction mixture was evaporated. The residue was dissolved in CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered and concentrated. Epimerized alcohol was obtained after flash chromatography.

4.1.3 Typical procedure for the fluorination with DAST. *Method A*: A stirred solution of alcohol (1 mmol) in freshly distilled CH_2Cl_2 (2 mL) was cooled to −40 °C under Ar. DAST (0.6 mL, 4.5 mmol) was then added, and the solution was left to stir for 4 h, warming to rt. The reaction was then quenched

with MeOH and diluted with CH_2Cl_2. The crude mixture was washed with sat. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The desired fluoro product was obtained after flash chromatography. *Method B*: To a solution of alcohol (1 mmol) in diglyme (2.5 mL) was added DAST (0.36 mL, 3 mmol). The mixture was then stirred at 100 °C for 7 min, cooled to 0 °C, and quenched with MeOH. The crude mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ and water (5×). The dried solution ($MgSO_4$) was co-evaporated with water (2×), toluene (2×) and CH_2Cl_2. The residue was then purified by flash chromatography to afford the desired fluoro product.

4.1.4 Typical procedure for the fluorination with TBAF. Triflate (1 mmol) was dissolved in freshly distilled acetonitrile (9 mL), and to this tetrabutylammonium fluoride (1 M solution in THF, 9.5 mL, 9.5 mmol) was added. The reaction mixture was heated at reflux for 1 h, cooled to rt and concentrated. The residue was dissolved in CH_2Cl_2, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The desired fluoro product was obtained after flash chromatography.

4.1.5 Typical procedure for the preparation of thionocarbonates. To a solution of alcohol (1 mmol), pyridine (1.6 mL, 20 mmol) and DMAP (124 mg, 1 mmol) in dry CH_2Cl_2 (6 mL) at 0 °C under Ar was added *O*-phenyl chlorothionoformate (0.44 mL, 3.2 mmol), and the solution was stirred at rt for 1.5 h. The reaction mixture was then diluted with CH_2Cl_2, washed with water, 1 M HCl (only Bz-protected products), sat. $NaHCO_3$ and water, dried over $MgSO_4$, filtered and concentrated. The crude was then purified by flash chromatography to afford the desired thionocarbonate.

4.1.6 Typical procedure for the radical deoxygenation. Argon was bubbled for 1 h at rt into a solution of the thionocarbonate (0.33 mmol), Bu_3SnH (145 μL, 0.5 mmol) and ACBN (50 mg) in benzene (5 mL). The reaction mixture was stirred at 80 °C for 3 h, cooled to rt and concentrated. The residue was then purified by flash chromatography to afford the desired deoxygenated product.

4.2 Selected experimental data

4.2.1 4-Methylumbelliferyl 2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-xylopyranoside (5b). A suspension of **1** (300 mg, 0.97 mmol) in a solution of 2,2,3,3-tetramethoxybutane (0.215 mL, 1.94 mmol) and trimethyl orthoformate (0.43 mL, 3.88 mmol) in methanol (5 mL) was treated with camphorsulfonic acid (12 mg, 0.05 mmol), and the mixture was refluxed overnight. The cooled reaction mixture was then treated with powdered $NaHCO_3$ (0.5 g), filtered and concentrated. Flash chromatography (CH_2Cl_2/acetone 12 : 1 + 0.1% Et_3N) afforded the protected carbohydrates **5a** (241 mg, 59%) and **5b** (152 mg, 37%) as pale yellow foams. **5b:** $[\alpha]_D$ +128.6 (c 0.73 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.51 (1 H, d, $J_{4,5}$ 8.6, H-5 MU), 6.98 (2 H, m, H-2 MU, H-4 MU), 6.18 (1 H, d, $J_{7,Me}$ 1.2, H-7 MU), 5.21 (1 H, d, $J_{1,2}$ 7.1, H-1), 4.13 (1 H, m, H-5eq), 3.98 (1 H, m, H-4), 3.80 (2 H, m, H-2, H-3), 3.49 (2 H, dd, $J_{5ax,5eq}$ 11.7, $J_{4,5ax}$ 9.0, H-5ax), 3.37, 3.34 (6 H, 2 s, OMe BDA), 2.53 (1 H, d, $J_{4,OH}$ 3.2, 4-OH), 2.41 (3 H, d, Me MU), 1.38, 1.35 (6 H, 2 s, Me BDA); δ_C (100 MHz, $CDCl_3$) 161.1, 159.6, 154.8, 152.3 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.5 (1 C, C-5 MU), 115.1 (1 C, C-0 MU), 113.7 (1 C, C-4 MU), 112.8 (1 C, C-7 MU), 104.5 (1 C, C-2 MU), 99.8, 99.6 (2 C, C_{quat} BDA), 98.6 (1 C, C-1), 72.7, 68.7 (2 C, C-2, C-3), 67.3 (1 C, C-4), 66.5 (1 C, C-5), 48.1, 48.0 (2 C, OMe BDA), 18.7 (1 C, Me MU), 17.6 (2 C, Me BDA); ESI^+ HRMS $[M + H]^+$ *m*/*z* 423.1649 calcd for $C_{21}H_{27}O_9$, found: 423.1647.

4.2.2 4-Methylumbelliferyl 2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-α-L-arabinopyranoside (12). Alcohol **5b** (0.15 g, 0.35 mmol) was subjected to the general procedures for the preparation of triflate. Flash chromatography (CH_2Cl_2/acetone 98 : 2 + 0.1% Et_3N) afforded triflate **10** (171 mg, 88%) as a white foam. $[\alpha]_D$ −145.5 (c 0.96 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.54 (1 H, d, $J_{4,5}$ 8.4, H-5 MU), 6.98 (2 H, m, H-2 MU, H-4 MU), 6.21 (1 H, d, $J_{7,Me}$ 1.5, H-7 MU), 5.33 (1 H, d, $J_{1,2}$ 7.1, H-1), 5.02 (1 H, m, H-4), 4.32 (1 H, dd, $J_{5ax,5eq}$ 13.0, $J_{4,5ax/eq}$ 5.1, H-5ax/eq), 4.13 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 8.4, H-3), 3.94 (1 H, dd, H-2), 3.82 (1 H, dd, $J_{4,5ax/eq}$ 6.1, H-5ax/eq), 3.37, 3.32 (6 H, 2 s, OMe BDA), 2.43 (3 H, d, Me MU), 1.37, 1.35 (6 H, 2 s, Me BDA); δ_C (100 MHz, $CDCl_3$) 160.9, 159.2, 154.8, 152.1 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.6 (1 C, C-5 MU), 115.4 (1 C, C-0 MU), 113.5 (1 C, C-4 MU), 113.1 (1 C, C-7 MU), 104.7 (1 C, C-2 MU), 100.1, 100.0 (3 C, C_{quat} BDA, CF_3), 98.4 (1 C, C-1), 82.4 (1 C, C-4), 68.6, 68.5 (2 C, C-2, C-3), 64.0 (1 C, C-5), 48.2, 48.1 (2 C, OMe BDA), 18.7 (1 C, Me MU), 17.4 (2 C, Me BDA); δ_F (376 MHz, $CDCl_3$) −74.4 (s); ESI^+ HRMS $[M + H]^+$ *m*/*z* 555.1142 calcd for $C_{22}H_{26}F_3O_{11}S$, found: 555.1146.

Triflate **10** (171 mg, 0.31 mmol) was then subjected to the general procedure for the epimerization with TBAN. Flash chromatography (CH_2Cl_2/acetone 12 : 1 + 0.1% Et_3N) afforded **12** (109 mg, 84%) as a white foam. M.p. 180 °C (from EP/EtOAc); $[\alpha]_D$ −86.5 (c 0.99 in $CHCl_3$); δ_H (250 MHz, $CDCl_3$) 7.45 (1 H, d, $J_{4,5}$ 8.2, H-5 MU), 6.94 (2 H, m, H-2 MU, H-4 MU), 6.13 (1 H, d, $J_{7,Me}$ 1.4, H-7 MU), 5.09 (1 H, d, $J_{1,2}$ 7.9, H-1), 4.18 (1 H, dd, $J_{2,3}$ 10.3, H-2), 4.08 (1 H, dd, $J_{5ax,5eq}$ 12.9, $J_{4,5ax/eq}$ 2.0, H-5ax/eq), 3.97 (1 H, m, H-4), 3.84 (1 H, dd, $J_{3,4}$ 3.1, H-3), 3.72 (1 H, dd, $J_{4,5ax/eq}$ 1.7, H-5ax/eq), 3.31, 3.26 (6 H, 2 s, OMe BDA), 2.36 (3 H, d, Me MU), 1.32, 1.328 (6 H, 2 s, Me BDA); δ_C (100 MHz, $CDCl_3$) 161.1, 159.7, 154.8, 152.4 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.4 (1 C, C-5 MU), 114.9 (1 C, C-0 MU), 114.0 (1 C, C-4 MU), 112.7 (1 C, C-7 MU), 104.3 (1 C, C-2 MU), 100.4, 99.9 (2 C, C_{quat} BDA), 98.6 (1 C, C-1), 69.5 (1 C, C-3), 67.2 (1 C, C-5), 67.0 (1 C, C-4), 66.4 (1 C, C-2), 48.1, 47.9 (2 C, OMe BDA), 18.7 (1 C, Me MU), 17.7, 17.6 (2 C, Me BDA); ESI^+ HRMS $[M + H]^+$ *m*/*z* 423.1649 calcd for $C_{21}H_{27}O_9$, found: 423.1648.

4.2.3 4-Methylumbelliferyl 4-deoxy-4-fluoro-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-xylopyranoside (17). Alcohol **12** (50 mg, 0.12 mmol) was subjected to the general procedure for the fluorination with DAST (method A). Flash chromatography (toluene/EtOAc 4 : 1 + 0.1% Et_3N) afforded **17** (25 mg, 49%) as a white foam. $[\alpha]_D$ −163.1 (c 1 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.53 (1 H, d, $J_{4,5}$ 8.6, H-5 MU), 7.00 (2 H, m, H-2 MU, H-4 MU), 6.19 (1 H, d, $J_{7,Me}$ 1.3, H-7 MU), 5.39 (1 H, d, $J_{1,2}$ 6.8, H-1), 4.77 (1 H, dm, $J_{4,F}$ 52.2, H-4), 4.16 (2 H, m, H-3, H-5ax/eq), 3.91 (1 H, ddd, $J_{2,3}$ 11.1, $J_{2,F}$ 1.0, H-2), 3.79 (1 H, m, H-5ax/eq), 3.37,

3.36 (6 H, 2 s, OMe BDA), 2.42 (3 H, d, Me MU), 1.38, 1.35 (6 H, 2 s, Me BDA); δ_C (100 MHz, $CDCl_3$) 160.9, 159.5, 154.8, 152.2 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.5 (1 C, C-5 MU), 115.1 (1 C, C-0 MU), 113.5 (1 C, C-4 MU), 112.9 (1 C, C-7 MU), 104.7 (1 C, C-2 MU), 99.7, 99.5 (2 C, C_{quat} BDA), 98.6 (1 C, C-1), 83.6 (1 C, d, $J_{4,F}$ 183.1, C-4), 69.9 (1 C, d, $J_{3,F}$ 22.3, C-3), 68.0 (1 C, d, $J_{2,F}$ 9.7, C-2), 64.4 (1 C, d, $J_{5,F}$ 27.2, C-5), 48.1, (2 C, OMe BDA), 18.7 (1 C, Me MU), 17.6, 17.5 (2 C, Me BDA); δ_F (376 MHz, $CDCl_3$) −190.4; ESI^+ HRMS $[M + H]^+$ *m*/*z* 425.1606 calcd for $C_{21}H_{26}FO_8$, found: 425.1603.

4.2.4 4-Methylumbelliferyl 4-deoxy-4-fluoro-β-D-xylopyranoside (18). A mixture of **17** (80 mg, 0.19 mmol) in 95% TFA (1 mL) was stirred at rt for 2 h. The reaction mixture was then co-evaporated with water (3×), toluene (2×) and CH_2Cl_2. Flash chromatography (CH_2Cl_2/MeOH 9 : 1) afforded **18** (31 mg, 53%) as a white foam. M.p. 208 °C (from MeOH); $[\alpha]_D$ −62.9 (c 1.42 in $CHCl_3$/MeOH 1 : 1); δ_H (400 MHz, $CDCl_3$/MeOD 1 : 1) 7.64 (1 H, d, $J_{4,5}$ 8.9, H-5 MU), 7.07 (1 H, dd, $J_{2,4}$ 2.4, H-4 MU), 7.03 (1 H, d, H-2 MU), 6.20 (1 H, d, $J_{7,Me}$ 1.7, H-7 MU), 5.09 (1 H, d, $J_{1,2}$ 6.9, H-1), 4.50 (1 H, dm, $J_{4,F}$ 49.7, H-4), 4.16 (1 H, ddd, $J_{5ax,5eq}$ 12.3, $J_{5eq,F}$ 7.5, $J_{4,5eq}$ 5.2, H-5eq), 3.77 (1 H, ddd, $J_{3,F}$ 16.5, $J_{2,3} = J_{3,4}$ 8.5, H-3), 3.66 (1 H, ddd, $J_{5ax,F}$ 8.4, $J_{4,5ax}$ 6.5, H-5ax), 3.60 (1 H, dd, H-2), 2.45 (3 H, d, Me MU); δ_C (100 MHz, $CDCl_3$/MeOD 1 : 1) 162.1, 160.1, 154.6, 153.9 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.9 (1 C, C-5 MU), 115.0 (1 C, C-0 MU), 113.8 (1 C, C-4 MU), 111.9 (1 C, C-7 MU), 104.0 (1 C, C-2 MU), 100.8 (1 C, C-1), 89.3 (1 C, d, $J_{4,F}$ 180.7, C-4), 73.7 (1 C, d, $J_{3,F}$ 19.0, C-3), 72.3 (1 C, d, $J_{2,F}$ 8.6, C-2), 62.7 (1 C, d, $J_{5,F}$ 28.2, C-5), 18.0 (1 C, Me MU); δ_F (376 MHz, $CDCl_3$/MeOD 1 : 1) −198.8; ESI^+ HRMS $[M + H]^+$ *m*/*z* 311.0925 calcd for $C_{15}H_{16}FO_6$, found: 311.0924.

4.2.5 4-Methylumbelliferyl 4-deoxy-4-fluoro-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-α-L-arabinopyranoside (20). The compound was prepared from either **5b** or **10**. From **5b**: Alcohol **5b** (40 mg, 0.09 mmol) was subjected to the general procedure for the fluorination with DAST (method B). Flash chromatography (toluene/EtOAc 4 : 1 + 0.1% Et_3N) afforded **20** (16 mg, 41%) and elimination product **21** (22%). From **10**: Triflate **10** (0.114 g, 0.21 mmol) was subjected to the general procedure for the fluorination with TBAF. **20** (32 mg, 36%) was isolated along with **21** (15%). **20**: $[\alpha]_D$ −59.9 (c 0.98 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.52 (1 H, d, $J_{4,5}$ 8.7, H-5 MU), 7.01 (2 H, m, H-2 MU, H-4 MU), 6.20 (1 H, d, $J_{7,Me}$ 1.3, H-7 MU), 5.18 (1 H, d, $J_{1,2}$ 7.9, H-1), 4.81 (1 H, dm, $J_{4,F}$ 52.2, H-4), 4.30 (1 H, dd, $J_{5ax,5eq} = J_{5eq,F}$ 12.1, $J_{4,5eq}$ 1.7, H-5eq), 4.25 (1 H, dd, $J_{2,3}$ 10.6, H-2), 3.94 (1 H, ddd, $J_{3,F}$ 29.2, $J_{3,4}$ 2.5, H-3), 3.81 (1 H, dd, $J_{5ax,F}$ 34.6, $J_{4,5ax}$ < 1, H-5ax), 3.39, 3.33 (6 H, 2 s, OMe BDA), 2.42 (3 H, d, Me MU), 1.42, 1.35 (6 H, 2 s, Me BDA); δ_C (100 MHz, $CDCl_3$) 161.0, 159.6, 154.8, 152.3 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.4 (1 C, C-5 MU), 115.1 (1 C, C-0 MU), 114.1 (1 C, C-4 MU), 112.9 (1 C, C-7 MU), 104.2 (1 C, C-2 MU), 100.5, 99.9 (2 C, C_{quat} BDA), 98.6 (1 C, C-1), 86.5 (1 C, d, $J_{4,F}$ 184.8, C-4), 68.4 (1 C, d, $J_{3,F}$ 17.2, C-3), 66.3 (1 C, d, $J_{2,F}$ 1.6, C-2), 65.8 (1 C, d, $J_{5,F}$ 20.4, C-5), 48.2, 48.0 (2 C, OMe BDA), 18.7 (1 C, Me MU), 17.6 (2 C, Me BDA); δ_F (376 MHz, $CDCl_3$) −204.1; ESI^+ HRMS $[M + H]^+$ *m*/*z* 425.1606 calcd for $C_{21}H_{26}FO_8$, found: 425.1607.

4.2.6 4-Methylumbelliferyl 4-deoxy-4-fluoro-α-L-arabinopyranoside (22). 20 (47 mg, 0.1 mmol) was subjected to the same procedure as that described for the preparation of **18** using 95% TFA. Flash chromatography afforded **22** (21 mg, 67%). $[\alpha]_D$ +1.0 (c 1.02 in $CHCl_3$/MeOH 1 : 1); δ_H (400 MHz, $CDCl_3$/MeOD 1 : 1) 7.60 (1 H, d, $J_{4,5}$ 8.8, H-5 MU), 7.06 (1 H, dd, $J_{4,5}$ 8.8, $J_{2,4}$ 2.4, H-4 MU), 7.02 (1 H, d, H-2 MU), 6.17 (1 H, d, $J_{7,Me}$ 1.5, H-7 MU), 4.98 (1 H, d, $J_{1,2}$ 7.3, H-1), 4.79 (1 H, dm, $J_{4,F}$ 48.9, H-4), 4.19 (1 H, ddd, $J_{5ax,5eq}$ 13.3, $J_{5ax,F}$ 12.2, $J_{4,5ax}$ 2.5, H-5ax), 3.81 (3 H, m, H-2, H-3, H-5eq), 2.42 (3 H, d, Me MU); δ_C (100 MHz, $CDCl_3$/MeOD 1 : 1) 162.1, 160.2, 154.6, 153.8 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.8 (1 C, C-5 MU), 114.9 (1 C, C-0 MU), 113.9 (1 C, C-4 MU), 111.9 (1 C, C-7 MU), 103.9 (1 C, C-2 MU), 100.7 (1 C, C-1), 88.7 (1 C, d, $J_{4,F}$ 179.2, C-4), 71.6 (1 C, d, $J_{3,F}$ 18.3, C-3), 70.7 (1 C, d, $J_{2,F}$ 1.3, C-2), 64.3 (1 C, d, $J_{5,F}$ 20.3, C-5), 18.1 (1 C, Me MU); δ_F (376 MHz, $CDCl_3$/MeOD 1 : 1) −206.2; ESI^+ HRMS $[M + H]^+$ *m*/*z* 311.0925 calcd for $C_{15}H_{16}FO_6$, found: 311.0927.

4.2.7 4-Methylumbelliferyl 2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-4-*O*-(phenylthio)thionocarbonyl β-D-xylopyranoside (24). Alcohol **5b** (0.468 g, 1.1 mmol) was subjected to the general procedure for the preparation of thionocarbonate. Flash chromatography (petroleum ether/EtOAc 2 : 1 + 0.1% Et_3N) afforded **24** (466 mg, 76%) as a white foam. $[\alpha]_D$ −174.9 (c 1.01 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.53 (1 H, d, $J_{4,5}$ 8.6, H-5 MU), 7.44 (2 H, m, o-Ph), 7.32 (1 H, m, p-Ph), 7.15 (2 H, m, m-Ph), 7.01 (2 H, m, H-2 MU, H-4 MU), 6.20 (1 H, d, $J_{7,Me}$ 1.7, H-7 MU), 5.49 (1 H, m, H-4), 5.42 (1 H, d, $J_{1,2}$ 6.7, H-1), 4.42 (1 H, dd, $J_{5ax,5eq}$ 13.4, $J_{4,5ax/eq}$ 4.7, H-5ax/eq), 4.23 (1 H, dd, $J_{2,3}$ 10.8, $J_{3,4}$ 8.3, H-3), 4.03 (1 H, dd, H-2), 3.85 (1 H, dd, $J_{4,5ax/eq}$ 4.9, H-5ax/eq), 3.39, 3.33 (6 H, 2 s, OMe BDA), 2.43 (3 H, d, Me MU), 1.39, 1.36 (6 H, 2 s, Me BDA); δ_C (100 MHz, $CDCl_3$) 194.1 (1 C, C═S), 161.1, 159.5, 154.8, 153.3, 152.3 (5 C, C_{quat} Ph, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 129.6 (2 C, o-Ph), 126.7 (1 C, p-Ph), 125.5 (1 C, C-5 MU), 121.8 (1 C, m-Ph), 115.1 (1 C, C-0 MU), 113.6 (1 C, C-4 MU), 112.9 (1 C, C-7 MU), 104.6 (1 C, C-2 MU), 99.7 (2 C, C_{quat} BDA), 98.7 (1 C, C-1), 79.6 (1 C, C-4), 68.7 (1 C, C-2), 68.3 (1 C, C-3), 63.4 (1 C, C-5), 48.2, 48.1 (2 C, OMe BDA), 18.7 (1 C, Me MU), 17.6, 17.5 (2 C, Me BDA); ESI^+ HRMS $[M + H]^+$ *m*/*z* 559.1632 calcd for $C_{28}H_{31}O_{10}S$, found: 559.1629.

4.2.8 4-Methylumbelliferyl 4-deoxy-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-β-D-xylopyranoside (26). 24 (0.46 g, 0.82 mmol) was subjected to the general procedure for the radical deoxygenation. Flash chromatography (petroleum ether/EtOAc 2 : 1 + 0.1% Et_3N) afforded **26** (206 mg, 62%) as a white foam. $[\alpha]_D$ −86.9 (*c* 1.08 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.50 (1 H, d, $J_{4,5}$ 8.6, H-5 MU), 6.98 (2 H, m, H-2 MU, H-4 MU), 6.17 (1 H, s, H-7 MU), 5.10 (1 H, d, $J_{1,2}$ 7.8, H-1), 4.12 (1 H, m, H-5ax/eq), 3.91 (1 H, m, H-3), 3.75 (1 H, dd, $J_{2,3}$ 9.6, H-2), 3.66 (1 H, m, H-5ax/eq), 3.38, 3.31 (6 H, 2 s, OMe BDA), 2.41 (3 H, s, Me MU), 1.89 (2 H, m, H-4), 1.34 (6 H, s, Me BDA); δ_C (100 MHz, $CDCl_3$) 161.1, 159.8, 154.8, 152.4 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.4 (1 C, C-5 MU), 114.9 (1 C, C-0 MU), 113.9 (1 C, C-4 MU), 112.7 (1 C, C-7 MU), 104.2 (1 C, C-2 MU), 99.9, 99.8 (2 C, C_{quat} BDA), 98.6 (1 C, C-1), 71.4 (1 C, C-2), 67.4 (1 C, C-3), 62.9 (1 C, C-5), 48.1, 47.9 (2 C, OMe BDA), 29.6

(1 C, C-4), 18.7 (1 C, Me MU), 17.7, 17.6 (2 C, Me BDA); ESI^+ HRMS $[M + H]^+$ *m/z* 407.1400 calcd for $C_{21}H_{27}O_8$, found: 407.1698.

4.2.9 4-Methylumbelliferyl 4-deoxy-β-D-xylopyranoside (27). **26** (50 mg, 0.12 mmol) was subjected to the same procedure as that described for the preparation of **18.** Flash chromatography (CH_2Cl_2/MeOH 9 : 1) afforded the deprotected compound **27** (11 mg, 33%). M.p. 185 °C (from EP/EA); $[\alpha]_D$ −41.0 (c 1, MeOH/$CHCl_3$ 3 : 1); δ_H (400 MHz, MeOD/$CDCl_3$ 3 : 1) 7.63 (1 H, d, $J_{4,5}$ 8.7, H-5 MU), 7.07 (1 H, dd, $J_{2,4}$ 2.4, H-4 MU), 7.02 (1 H, d, H-2 MU), 6.19 (1 H, d, $J_{7,Me}$ 1.3, H-7 MU), 4.94 (1 H, d, $J_{1,2}$ 7.2, H-1), 4.04 (1 H, ddd, $J_{5ax,5eq}$ 12.0, $J_{4ax,5eq}$ 4.9, $J_{4eq,5eq}$ 2.4, H-5eq), 3.73 (1 H, ddd, $J_{3,4ax}$ 10.8, $J_{2,3}$ 8.6, $J_{3,4eq}$ 5.0, H-3), 3.67 (1 H, ddd, $J_{4ax,5ax}$ 11.8, $J_{4eq,5ax}$ 2.4, H-5ax), 2.45 (3 H, d, Me MU), 2.03 (1 H, dddd, $J_{4ax,4eq}$ 13.3, H-4eq), 1.75 (1 H, dddd, H-4ax); δ_C (100 MHz, MeOD/$CDCl_3$ 3 : 1) 162.2, 160.4, 154.6, 153.9 (4 C, C-1 MU, C-3 MU, C-6 MU, C-8 MU), 125.8 (1 C, C-5 MU), 114.8 (1 C, C-0 MU), 113.8 (1 C, C-4 MU), 111.8 (1 C, C-7 MU), 103.8 (1 C, C-2 MU), 101.0 (1 C, C-1), 74.6 (1 C, C-2), 70.6 (1 C, C-3), 61.6 (1 C, C-5), 32.3 (1 C, C-4), 18.1 (1 C, Me MU); ESI^+ HRMS $[M + H]^+$ *m/z* 293.1019 calcd for $C_{15}H_{17}O_6$, found: 293.1018.

4.3 Biological assays

4.3.1 Expression vector construction. The human β4GalT7 sequence (GenBank® nucleotide sequence accession number NM_007255) was cloned by PCR amplification from a placenta cDNA library (Clontech), as described previously.[26b] For bacterial expression, a truncated form of β4GalT7 was expressed as a fusion protein of a 6His tag and glutathione S-transferase (6His-GST-hβ4GalT7). A sequence lacking the codons of the first 60 N-terminal amino acids was amplified from the full-length cDNA and subcloned into NcoI and NotI sites of pETM30 to produce the plasmid pETM30-hβ4GalT7ΔNt60. For the *in cellulo* analysis of decorin core protein glycosylation, the human decorin cDNA sequence (GenBank® accession number NM_001920.3) was cloned by PCR amplification from a placenta cDNA library and the full-length cDNA sequence was inserted into pcDNA3.1(+) to produce pcDNA-decorinHis.[31]

4.3.2 Bacterial expression and purification of the soluble form of hβ4GalT7ΔNt60. To express 6His-GST-hβ4GalT7ΔNt60, *E. coli* Rosetta2 (DE3) bacteria were transformed with the pETM30-hβ4GalT7ΔNt60 plasmid. The recombinant bacteria were then selected based on kanamycin resistance (50 μg mL^{-1}). A single recombinant colony was inoculated into fresh Luria–Bertani (LB) medium and cultured overnight at 37 °C on an orbital shaker (160 rpm). The overnight culture was transferred into fresh LB medium, supplemented with 50 μg mL^{-1} kanamycin and incubated at 37 °C until the OD_{600} value reached 0.6. The expression of hβ4GalT7DNt60 was induced by the addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma-Aldrich) to the bacterial suspension that was then incubated overnight at 25 °C under continuous shaking (160 rpm). The bacterial cells were then harvested by centrifugation at 6000*g* for 20 min at 4 °C. The pellets were resuspended in a lysis buffer (50 mM sodium phosphate, pH 6.9, 300 mM NaCl, 10 mM DTT, 20 μM $MnCl_2$, 0.5% Triton, 1 mM EDTA, and 5% (v/v) glycerol). The suspended cells were lysed using a French press at 25 kpsi. Soluble proteins were collected from the supernatant after centrifugation for 30 min at 12 000*g*. The supernatant was supplemented with endonuclease (Pierce Universal Nuclease, Thermo Fisher) and 2 mM $MnCl_2$. After incubation (1 hour, 4 °C), the supernatant was clarified by filtration (0.2 μm Supor® Membrane; PALL-Life Science) and applied onto a 5 mL glutathione Sepharose column (GSTrap 4B; GE Healthcare) connected to an AKTA Prime Plus instrument (GE Healthcare). Proteins were eluted as 2 mL fractions using an elution buffer (50 mM Tris-base, pH 8.0, 10 mM reduced glutathione and 150 mM NaCl). The protein purity of the eluted fractions was evaluated by 12% (w/v) SDS-PAGE analysis, followed by staining with Coomassie Brilliant Blue. Fractions containing the pure protein were concentrated and used to determine the kinetic parameters of the enzyme. The protein concentration was measured using the Bradford technique.[32]

4.3.3 Determination of the *in vitro* kinetic parameters of hβ4GalT7ΔNt60. Kinetic constants, *i.e.* the Michaelis constant (K_m) and the catalytic constant (k_{cat}), were determined towards modified xylosides by incubating the purified 6His-GST-hβ4GalT7ΔNt60 (100 ng) with increasing concentrations of xylosides (0–5 mM) for 30 min at 37 °C, in the presence of a fixed concentration of UDP-Gal (1 mM) and 10 mM $MnCl_2$, in a Bis-Tris buffer (50 mM, pH 6.5). The incubation mixture was then centrifuged at 10 000*g* for 1 min at 4 °C. The samples were analyzed by high performance liquid chromatography (HPLC) with a reverse phase C18 analytical column (xBridge, 4.6 × 150 mm, 5 μm, Waters) using Waters equipment (Alliance Waters e2695) coupled to a UV detector (Shimadzu SPD-10A). Kinetic parameters were determined by nonlinear least-squares regression analysis of the data fitted to the Michaelis–Menten rate equation or the substrate inhibition rate equation using the curve-fitter program of GraphPad Prism 5 (GraphPad Software, USA).

4.3.4 *In vitro* competition assays of hβ4GalT7ΔNt60 activity by modified xylosides. The *in vitro* inhibitory activities of modified xylosides were evaluated using 100 ng of purified protein incubated for 30 min at 37 °C in a Bis-Tris buffer (50 mM, pH 6.5), 10 mM $MnCl_2$ and 1 mM UDP-Gal in the presence of a fixed concentration of the XylMU acceptor substrate (0.3 mM) and various concentrations of the xyloside analogs (0, 2.5 and 5 mM). The amount of the reaction product (Gal-XylMU) was measured by HPLC, as described above. Inhibition or activation percentages were obtained from enzymatic activity determined in the presence or absence of modified xylosides.

4.3.5 *In cellulo* analysis of decorin core protein glycosylation. For the eukaryotic expression of human decorin, HeLa cells (American Type Culture Collection, ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen), penicillin (100 units per ml)–streptomycin (100 mg ml^{-1}), and 1 mM glutamine. The pcDNA-decorinHis plasmid was trans-

fected into HeLa cells grown to 80% confluency, using PolyEthylenImine (PEI, Polysciences, Inc.) according to the manufacturer's recommendations, and selected with geneticin (G-418, 1 mg ml^{-1}, Sigma). Recombinant cells were cultured in a 6 well-plate in DMEM complete medium, starved for 24 h, and supplemented with modified xylosides (0–300 μM). After 72 h, the cell medium was collected, concentrated by centrifugation at 4 °C for 45 min at 7000*g* using an Amicon Ultra4 3 MWCO concentrating system (Merck, Millipore, Germany) and analyzed by SDS-PAGE (10 μg of protein per lane). The glycosylation level of the decorin core protein was monitored by immunoblotting using a 1 : 500 dilution of a primary polyclonal anti-human decorin antibody (R&D Systems) and a 1 : 5000 dilution of a secondary anti-mouse antibody coupled to horseradish peroxidase (Cell Signaling). The expression level of glycosylated decorin in cells treated with 0.6% (v/v) DMSO (xyloside diluent) was used as a control. The expression level of glycosylated decorin in cells treated with 300 μM XylMU was used as a positive competition control.

Conflicts of interest

There are no conflicts to declare.

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