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Synthesis, conformation and biology of naphthoxylosides

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ABSTRACT

Proteoglycans (PG) are polyanionic proteins consisting of a core protein substituted with carbohydrate chains, that is, glycosaminoglycans (GAG). The biosynthesis of GAG can be manipulated by simple xylosides carrying hydrophobic aglycons, which can enter the cell and initiate the biosynthesis. While the importance of the aglycon is well investigated, there is far less information on the effect of modifications in the xylose residue.

We have developed a new synthetic protocol, based on acetal protection and selective benzylation, for modification of the three hydroxyl groups in xylose. Thus we have synthesized twelve analogs of 2-naphthyl β-D-xylopyranoside (XylNap), where each hydroxyl group has been epimerized or replaced by methoxy, fluoro, or hydrogen.

To gain more information about the properties of xylose, conformational studies were made on some of the analogs. It was found that the $^4C_1$ conformation is highly predominant, accompanied by a nonnegligible population of the $^2S_0$ conformation. However, deoxygenation at C3 results in a large portion of the $^1C_4$ conformation.

The GAG priming ability and proliferation activity of the twelve analogs, were investigated using a matched pair of human breast fibroblasts and human breast carcinoma cells. None of the analogs initiated the biosynthesis of GAG, but an inhibitory effect on endogenous PG production was observed for analogs fluorinated or deoxygenated at C4. From our data it seems reasonable that all three hydroxyl groups in XylNap are essential for the priming of GAG chains and for selective toxicity for tumor cells.

1. Introduction

Outer surfaces of tumor cells are covered by a variety of glycosaminoglycan chains (GAG) that are involved in the pathobiology of all stages of cancer progression. Thereby, they can affect cancer cell proliferation, tumor invasion and metastasis, and cancer stem cell differentiation.[1–3] These long unbranched polyanionic carbohydrate chains are covalently attached to proteins as proteoglycans (PG). Two classes of GAG chains, namely condroitin sulfate/dermatan sulfate (CS/DS) and heparan sulfate (HS) are coupled to the protein by xylose, a highly unusual carbohydrate in mammalian cells. The biosynthesis of GAG starts by coupling of xylose to a serine residue of the protein followed by the formation of a linker tetrasaccharide (i.e., GlcAβ1–3Galβ1–3Galβ1–4Xylβ–). The linker tetrasaccharide is further elongated by addition of repeating disaccharides, for example, –4GlcAβ1–4GlcNAcα1– for heparan sulfate (HS) or –4GlcAβ1–3GalNAcβ1– for chondroitin sulfate/dermatan sulfate (CS/DS). The growing chain is later on modified by epimerization, deacetylation and sulfation reactions, resulting in extensive structural diversity (Fig. 1).

The knowledge on structure recognition of the antiproliferative GAG is still scarce due to a lack of effective molecular tools to identify and correlate specific structures with functions. The structure of PG/GAG, as well as the expression of enzymes involved in their biosynthesis and degradation varies in normal and tumor cells and there is ample evidence indicating an importance of GAG chain structure for the control of cell proliferation and on aggressive tumor phenotype. For example, Sasisekharan and co-workers previously showed that GAG fragments, released from tumor cell surface, inhibit tumor growth and colonization in vitro as well as in vivo by affecting tumor cell proliferation, apoptosis and neovascularization.[4]

A problem with using GAGs as anti-tumor substances is the limited uptake of these highly polyanionic macromolecules. However, simple xylosides carrying hydrophobic and uncharged aglycons can enter cells and serve as primers for GAG formation, independently of core protein synthesis.[5,6] The xylosides initiate GAG synthesis by serving as acceptors in the first galactosylation step. The

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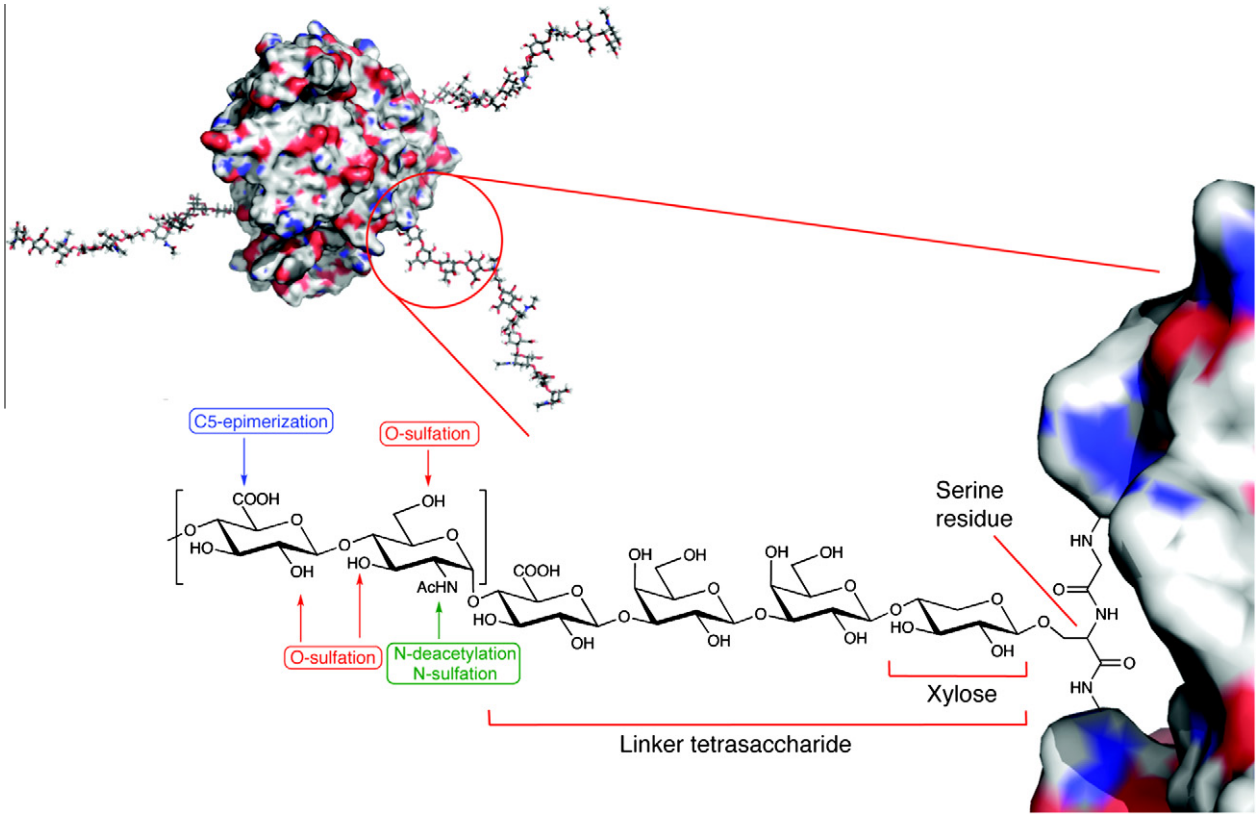


Figure 1. Proteoglycans are composed of glycosaminoglycan chains (GAG) linked to a core protein by the highly unusual carbohydrate xylose.

composition of GAG assembled on the xyloside primers depend mainly on the structure of the aglycon, which may reflect selective partitioning of primers into different intracellular compartments or into different branches of biosynthetic pathways. In most cases, priming of CS/DS dominates, and synthesis of free HS chains is low or undetectable. Increased yields of HS, which has proven to be important for anti-tumor therapy, can be obtained when the aglycon of the xylosides comprises aromatic, polycyclic structures, such as naphthol derivatives. These xyloside-primed GAG chains can be retained inside the cells, but are usually secreted into the medium, and show interesting biological properties, such as activation of basic fibroblast growth factor, antithrombotic effects, and growth inhibition of transformed cells.[7–11] We have previously reported that the GAG priming XylNapOH (**1a**, Fig. 2) selectively inhibits growth of tumor cells in vitro as well as in vivo.[12] Treatment with this xyloside at a pharmacologically relevant dose reduced the average tumor load by up to 97% in a SCID mice model.[10]

In a recent publication we used radiolabeled xylosides to investigate the mechanism of these tumor selective xylosides.[13] We could thus show that tumor cells treated with XylNapOH, secrete specific GAG chains. These GAG chains are then re-internalized by both normal and cancer cells, and further on transported to the nuclei where they induce apoptosis. We also showed that XylNapOH treatment lowers the level of histone H3 acetylation selectively in bladder and breast carcinoma cells without affecting the expression of histone H3. The down-regulation of histone H3 acetylation makes the transcription of DNA impossible and the cells are forced into apoptosis, which is an explanation for the toxic effects shown by these molecules. In contrary, XylNapOH-primed GAG chains from normal cells, or GAG chains from the analogous XylNap (**1b**, Fig. 2), are not internalized and do not cause growth retardation. We propose that, in vivo, the antiproliferative XylNapOH-primed GAG chains produced by tumor cells inhibit tumor growth in an autocrine fashion by formation of antiproliferative GAG chains on the xyloside pro-drug, while surrounding normal cells produce no antiproliferative GAG chains.

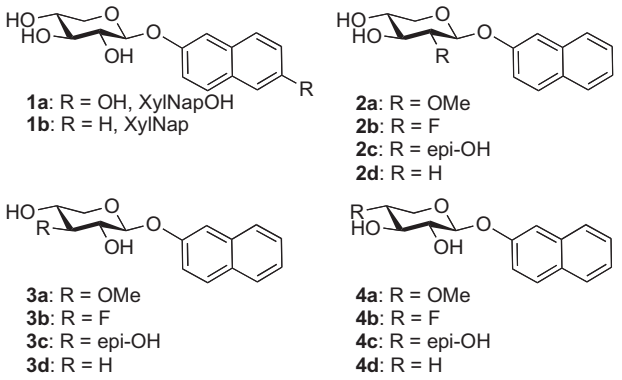


Figure 2. Structures of XylNapOH, XylNap and analogs.

While the importance of the aglycon has been investigated in a number of publications,[12,14–22] there is far less information on the effect of modifications in the xylose residue. In an article from 1996, Esko et al. investigated a series of modified benzyl β-D-xylosides. These compounds were tested for priming of GAG chains in Chinese hamster ovary (CHO) cells. Apart from the 3-fluoro- as well as the 2- and 3-methoxy analogs, all compounds failed to initiate priming.

Being a pentose, rather than a hexose like glucose or galactose, xylose poses unusual difficulties in selective protection of the three hydroxyl groups and there is a need for uniform methods for the

synthesis of analogs. The three hydroxyl groups are all secondary, equatorial and thus of very similar reactivity. In the 70's, Chalk et al. reported the order of reactivity of hydroxyl groups in methyl β-D-xylopyranoside on sulfonylation in pyridine as 4-OH > 3-OH > 2-OH.[23] Contradictory results were published by Kondo a decade later, who stated that tosylation of methyl β-D-xylopyranoside gave the order of reactivity 4-OH > 2-OH > 3-OH.[24] Kishimoto et al. observed the same trend while studying the reactivity towards a β-4-*O*-type quinone methide.[25] Later on, Helm and Ralph described a method to introduce ester-type protecting groups in xylose in a selective manner using Bu_2SnO to direct the reactivity.[26] They obtained mainly the 4-*O*-protected compounds of the β-anomer while the α-anomer gave the 2-*O*-protected species. The stannylidene complexes of β-D-xylopyranosides can also be used to obtain selective chloroacetylation of 4-OH.[27] Another way to selectively protect the 4-OH is to use 2,3-anhydro-ribopyranoside derivatives. Derivatization of the 4-position, and subsequent opening of the 2,3-anhydro ring provides xylosides with 2- and 3-position unprotected.[28]

It is also possible to use cyclic acetals such as isopropylidene ketal[26] or butanediacetal (BDA)[29], which have been used to protect xylosides, leading to two major products: 2,3- and 3,4-protected sugars. Acetals are widely used as protecting groups but their acid-lability might cause compatibility problems in some reactions. Silicon protecting groups could alternatively be used, but they are known to migrate under acidic conditions, leading to undesired products.[30,31] In addition, silicon groups are not stable towards fluoride ions. The *tert*-butyl-diphenyl silyl (TBDPS) was successfully employed in monoprotection of a trimethylsilylethyl xyloside, giving mainly the 4-*O*-protected compound.[32] However, a general protective group strategy for all positions of xylose is not presently available.

The aims of this work are: (i) to pin-point the requirements of the xylose moiety for specific cell toxicity and GAG priming in a matched normal and cancer cell pair, that is, CCD1095-SK (human breast fibroblasts), HCC70 (human breast carcinoma cells); (ii) to form a baseline for comparison of effects shown by naphthoxylosides modified either in the sugar residue or the aromatic moiety; (iii) to develop methodology for selective manipulations on xylose and (iv) to investigate how modifications in the sugar residue affect the conformation, and thereby properties, of naphthoxylosides. Thus, each of the hydroxyl groups in **1b** was replaced by three different functionalities (OMe, F, deoxy) while others remained unchanged. In addition, we synthesized the epimerized products, thus altogether twelve new compounds (Fig. 2).

2. Results and discussion

2.1. Synthesis

Three different strategies were evaluated for the introduction of protective groups in xylose: BDA, partial benzylation and bulky silyl groups (i.e., TBDPS).

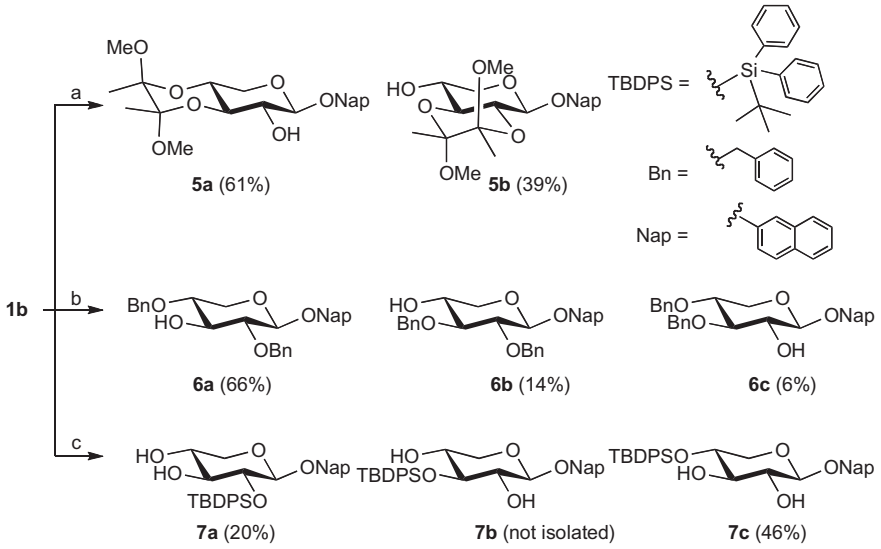
Standard conditions for introduction of the BDA group, that is, butane-2,3-dione, a catalytic amount of CSA, and $CH(OMe)_3$ in methanol, gave two inseparable mixtures of free 2-OH and 4-OH with different stereochemistry of the BDA.[33] The conditions were changed to 2,2,3,3-tetramethoxybutane[34] and a catalytic amount of $BF_3{\cdot}OEt_2$ in acetonitrile, which gave the two BDA-protected derivatives **5a** and **5b** in a 3:2 ratio in quantitative yield, without mixed stereochemistry in the acetal (Scheme 1).

Direct, regioselective phase transfer benzylation using BnBr, QI and KOH (10%) in CH_2Cl_2, resulted in a separable mixture of mainly di-protected compounds. 2-Naphthyl 2,4-di-*O*-benzyl-β-D-xylopyranoside (**6a**) was obtained in 66%, 2-naphthyl 2,3-*O*-di-benzyl-β-D-xylopyranoside (**6b**) in 14% yield and 2-naphthyl 3,4-di-*O*-benzyl-β-D-xylopyranoside (**6c**) in 6% yield.

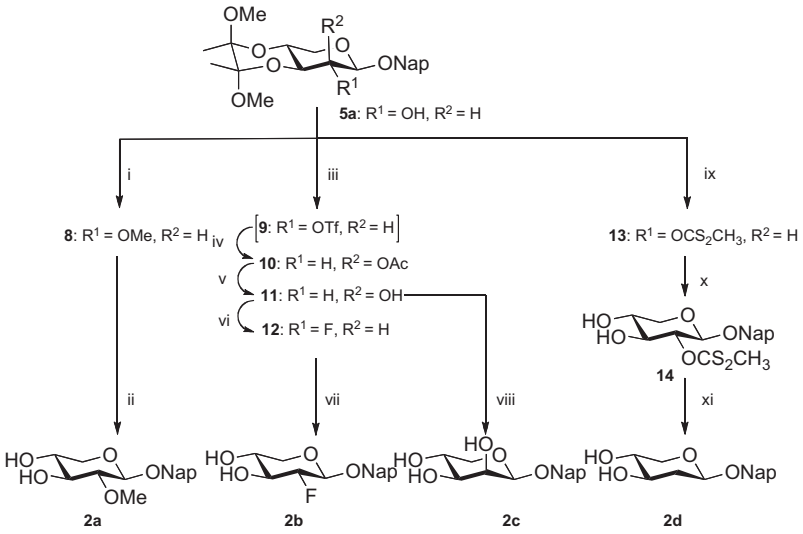
Finally, TBDPS protection, using TBDPSCl, Et_3N, and DMAP in CH_2Cl_2, gave mainly compound **7c** with the TBDPS group in position 4 along with compound **7a** and only trace amounts of compound **7b**.

Since the silyl protective group strategy was not very successful, this route was abandoned. The BDA group is acid labile and in some cases a loss of final product was observed in the deprotection step due to cleavage of the naphthyl moiety. Because of the low yield of **6c** in the benzylation reaction, we, therefore, decided to use the benzyl group strategy for position 3 (Scheme 3) and 4 (Scheme 4) and to use the BDA route for position 2 (Scheme 2).

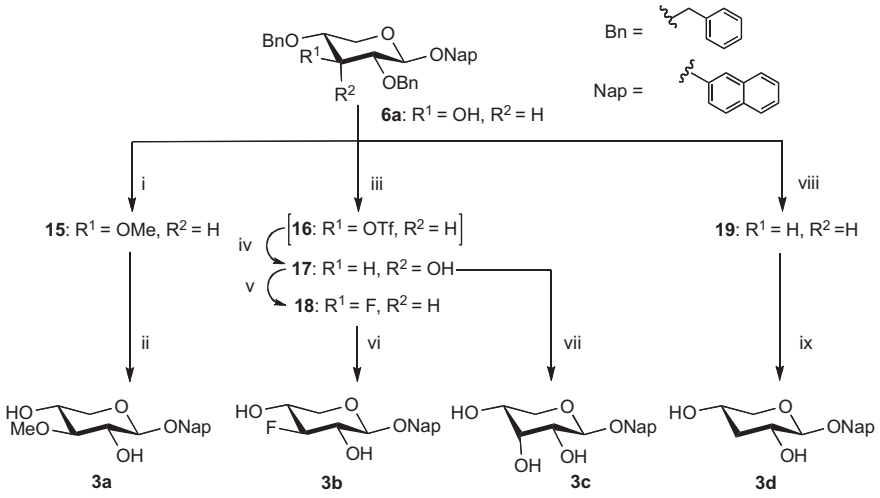
Subsequently, chemical transformations leading to target molecules were performed. The most straightforward was the synthesis of the methoxy derivatives and a similar procedure was employed for each of the positions. The unprotected hydroxyl group was deprotonated using sodium hydride and methyl iodide was introduced to give the 2-OMe (**8**), 3-OMe (**15**) and 4-OMe compounds



Scheme 1. Protective group strategies for xylose. Reagents and conditions: (a) 2,2,3,3-Tetramethoxybutane (2.2 equiv), $BF_3{\cdot}OEt_2$ (cat.), CH_3CN, o.n.; (b) BnBr (2.2 equiv), QI (2 equiv), KOH (10% aq), CH_2Cl_2, rt, 16 h; (c) TBDPSCl (2 equiv), Et_3N (2 equiv), DMAP (1 equiv), CH_2Cl_2, 65 h.



Scheme 2. Transformation of position 2. Reagents and conditions: (i) MeI (2 equiv), NaH (2 equiv), DMF, 0 °C, N_2, 16 h, **8** (89%); (ii) 95% TFA/CH_2Cl_2 1:1, 15 min, **2a** (quant.); (iii) Tf_2O (2 equiv), pyridine (4 equiv), CH_2Cl_2, −78 to 0 °C, N_2, 2.5 h; (iv) CsOAc (5 equiv), DMF, 50 °C, 3 d, **10** (82% over two steps); (v) 0.05 M NaOMe, 2.5 h, **11** (90%); (vi) DAST (2 equiv), CH_2Cl_2, 40 °C, N_2, 2 h, **12** (42%); (vii) 95% TFA/CH_2Cl_2 1:7, 15 min, **2b** (90%); (viii) 95% TFA/CH_2Cl_2 1:7, 15 min, **2c** (21%); (ix) (a) NaH (2 equiv), imidazole (cat.), THF, 0 °C to rt, 45 min; (b) CS_2 (9 equiv), N_2, rt, 1 h; (c) MeI (3 equiv), rt, N_2, 21 h, **13** (80%); (x) 95% TFA/CH_2Cl_2 1:1, 15 min, **14** (86%);(xi) AIBN (cat.), *n*-Bu_3SnH (1.5 equiv), toluene, 1 h, **2d** (30%).



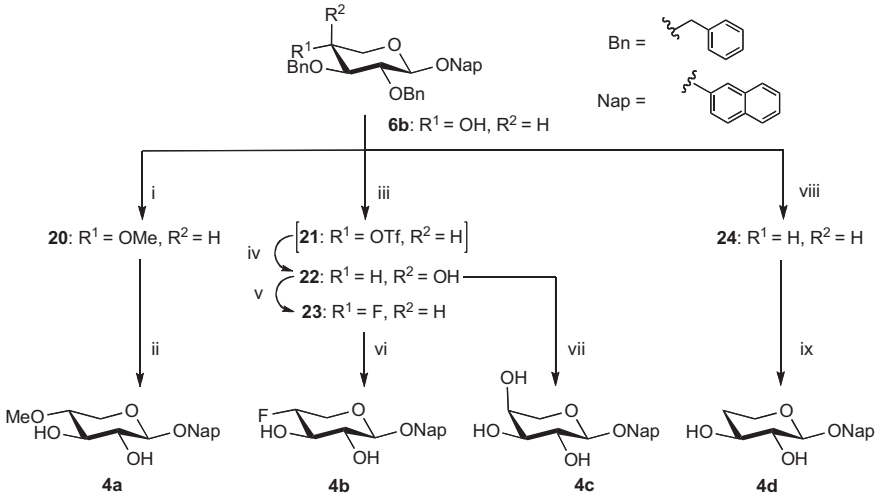
Scheme 3. Transformation of position 3. Reagents and conditions: (i) MeI (2 equiv), NaH (2 equiv), DMF, 0 °C, N_2, 16 h **15** (98%); (ii) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **3a** (94%) (iii) Tf_2O (1.5 equiv), pyridine (3 equiv), CH_2Cl_2, −10 °C, N_2, 2 h; (iv) QNO_2 (4 equiv), DMF, 50 °C, N_2, 16 h, **17** (54%); (v) DAST (2.5 equiv), CH_2Cl_2, −40 °C, N_2, 1 h; MeOH, 20 min, **18** (47%); (vi) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **3b** (94%); (vii) 10% Pd/C (HCl (21.7 equiv), DMF, H_2, rt, **3c** (73%); (viii) (a) NaH (2 equiv), imidazole, THF, 0 °C–rt, 45 min; (b) CS_2 (10 equiv), 1 h, N_2, rt, 1 h; (c) MeI (3 equiv), rt, N_2, 16 h; (d) AIBN (0.8 equiv), *n*-Bu_3SnH (15 equiv), toluene, Δ, 40 min, **19** (68%); (ix) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **3d** (85%).

(**20**).[35] The yields of these one-step procedures were almost quantitative.

The fluoro- and epi-analogs were obtained from analogous reaction sequences. First, **6a** and **6b** were reacted with trifluoromethane sulfonic anhydride. The resulting triflates (**16** and **21**) were inverted to hydroxyl groups in the presence of quaternary nitrite salt in DMF at 50 °C[36] to give **17** and **22** in 54% and 50% yield, respectively. For position 2, a reaction using QNO_2 gave only 24% of the desired product **11**. Thus the inversion was performed using CsOAc instead of the quaternary nitrite salt, to give the acetate **10** in 82% over two steps.[37] The acetate was then hydrolyzed using Zemplén conditions to give compound **11**. To form the fluorinated derivatives **12** (42%), **18** (47%), and **23** (41%), the above reaction sequence was extended by the addition of DAST.[38] Interestingly, depending on the position, the temperature of the reaction had to be modified. The 3-F compound was obtained after reacting at −40 °C, whereas formation of the 2-F and 4-F required heating to +40 °C. This diversity suggests different reactivity for each of the groups, which may be caused by conformation.

Deoxygenation reactions were performed using a two-step method, that is, xanthate formation followed by Barton–McCombie radical deoxygenation.[35] Xanthates were synthesized from carbon disulfide and methyl iodide that were reacted with deprotonated hydroxide in positions 2, 3 or 4. Yields varied from modest to good. These intermediates were then deoxygenated using AIBN and *n*-Bu_3SnH in toluene at reflux.

For position 2, the xanthate was removed after the deprotection since the regular order led to degradation of the material, indicating that the xylopyranoside is more acid-labile when the hydroxyl group in position 2 is removed.



Scheme 4. Transformation of position 4. Reagents and conditions: (i) MeI (2 equiv), NaH (2 equiv), DMF, 0 °C, N_2, 16 h; (ii) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **4a** (66%); (iii) Tf_2O (1.5 equiv), pyridine (3 equiv), CH_2Cl_2, −10 °C, N_2, 2 h; (iv) QNO_2 (4 equiv), DMF, 50 °C, N_2, 16 h **22** (50%); (v) DAST (2.5 equiv), CH_2Cl_2, −40 °C, N_2, 1 h; MeOH, 20 min, **23** (41%); (vi) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **4b** (61%); (vii) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **4c** (33%); (viii) (a) NaH (2 equiv), imidazole, THF, 0 °C to rt, 45 min; (b) CS_2 (10 equiv), 1 h, N_2, rt, 1 h; (c) MeI (3 equiv), rt, N_2, 16 h; (d) AIBN (0.8 equiv), *n*-Bu_3SnH (15 equiv), toluene, Δ, 40 min, **24** (93%); (ix) 10% Pd/C, HCl (21.7 equiv), DMF, H_2, rt, **4d** (45%).

The BDA protecting group was removed by treatment with 95% TFA in CH_2Cl_2 for a short time and gave compounds **2a**–**d** in modest to quantitative yields. The benzylated compounds were deprotected using 10% Pd/C (Degussa type) under atmospheric pressure of hydrogen[39] to give **3a**–**d** and **4a**–**d** in modest to high yields.

2.2. Conformation

1D ^1H and ^{13}C experiments together with a number of 2D ^1H, ^1H- and ^1H, ^{13}C-correlated experiments were used to assign ^1H and ^{13}C NMR chemical shifts of xylosides **1a**, **1b**, **2a**, **3a**, **3b**, **3d**, **4a** and the monodeprotonated form of **1a**, **1a** (**Na**), in methanol as a solvent. The ^1H NMR chemical shifts were subsequently refined together with $^nJ_{HH}$ coupling constants using a total lineshape analysis as implemented in the PERCH NMR spin simulation software.[40] The ^1H and ^{13}C chemical shifts of the xylose residues in **1a**, **1a** (**Na**), **1b**, **2a**, **3a**, **3b**, **3d** and **4a** are compiled in Table 1. The chemical shifts are similar but changes due to substituents, atomic replacement or deoxygenation lead to conspicuous differences. The $^nJ_{HH}$ coupling constants differ suggesting conformational changes, both small and large, between the seven xylosides.

Analysis of the conformational space available to the xylosides is herein based on three-bond $^1H,^1H$ spin–spin coupling constants. To this end molecular mechanics models were built in canonical ring conformations and subsequently energy minimized. A hexopyranose residue may be described by a pseudorotational wheel

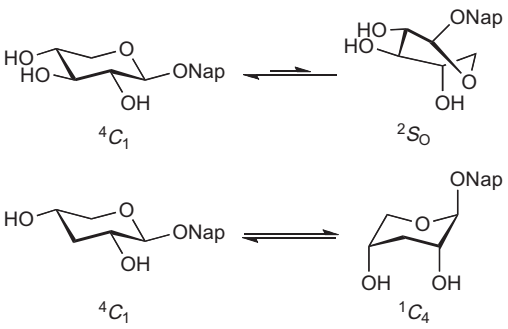
Table 1
δ_H, $^nJ_{HH}$, δ_C of xylosides in methanol-d_4 at 37 °C and sugar ring populations. For methylene groups the ^1H chemical shift and $^3J_{HH}$ of the pro-*R* proton is given prior to that of the pro-*S* proton

Compound	1	2	3	4	5	Me	$^4C_1$	$^2S_0$	$^1C_4$
1a	4.942	3.479	3.450	3.596	3.959, 3.404		94.7	5.3	0
	7.530	9.082	8.805	5.356, 10.180	−11.477				
	103.51	74.87	77.81	71.14	66.97				
1a (**Na**)	4.865	3.456	3.437	3.585	3.948, 3.363		94.0	6.0	0
	7.419	9.032	8.704	5.332, 10.128	−11.443				
	104.08	74.96	77.87	71.22	66.94				
1b	5.029	3.508	3.471	3.611	3.977, 3.447		93.7	6.3	0
	7.492	9.104	8.803	5.355, 10.125	−11.454				
	103.05	74.83	77.78	71.11	66.99				
2a	5.079	3.199	3.483	3.616	3.959, 3.416	3.695	94.4	5.6	0
	7.451	9.067	8.876	5.391, 10.100	−11.451				
	103.07	84.70	77.36	71.12	66.93	61.10			
3a	5.041	3.575	3.199	3.668	3.955, 3.444	3.683	92.4	7.6	0
	7.387	8.902	8.611	5.320, 9.966	−11.485				
	103.03	74.38	87.27	70.72	66.86	60.91			
3b[a]	5.064	3.742	4.346	3.865	3.999, 3.467		95.9	4.1	0
	7.530	8.805	8.553	5.719, 10.274	−11.667				
	102.50	73.25	97.81	69.41	65.76				
3d[b]	5.271	3.802	1.828, 2.353	3.856	4.002, 3.545		48.0	0	52.0
	4.513	7.081, 4.046	6.873, 3.860	3.132, 5.652	−11.608				
	101.60	68.40	35.47	66.05	68.26				
4a	5.024	3.527	3.545	3.307	4.154, 3.421	3.519	90.6	9.4	0
	7.325	8.933	8.030	5.161, 9.765	−11.565				
	102.93	74.84	76.79	80.78	64.39	59.02			

[a] $^2J_{F,H3}$ = 52.427 Hz, $^3J_{F,H2}$ = 13.391 Hz, $^3J_{F,H4}$ = 13.275 Hz, $^1J_{F,C3}$ = 184.1 Hz.
[b] $^2J_{H3(pro\text{-}R),H3(pro\text{-}S)}$ = −13.538 Hz, $^4J_{H3(pro\text{-}R),H5(pro\text{-}S)}$ = −1.174 Hz, $^4J_{H3(pro\text{-}S),H5(pro\text{-}R)}$ = −1.162 Hz.

and the itinerary between different conformations in describing the conformational space available to it.[41] Since xylopyranose is lacking the primary hydroxymethyl group present in glucose one may anticipate enhanced ring flexibility and several conformations need to be considered, in particular the $^4C_1$ conformation, various skew conformations and the $^1C_4$ conformation. However, the boat, half-chair and envelope conformations are higher in potential energy and will not be considered in the present analysis.[42] The three-state equilibrium between the two chair conformers and one of the skew conformers for each of the six skew conformers was analyzed using the five $^3J_{HH}$ coupling constants in the pyranose ring. For the xylosides **1a**, **1b**, **2a**, **3a**, **3b** and **4a** the $^4C_1$ conformation is highly predominant and of the skew conformations only a contribution of the $^2S_0$ conformation gave a better agreement between the $^nJ_{HH}$ coupling constants from the molecular mechanics models and the experimentally determined ones, suggesting its presence in a conformational equilibrium, which has also been proposed for β-D-xylopyranose based on modeling with the MM3 program.[43] Notably, there exists a nonnegligible population of the $^2S_0$ conformation in **1a**, **1a** (**Na**), **1b**, **2a**, **3a**, **3b** and **4a**, that is, >4% up to almost 10%, with an evident increase in population as the methoxy group substitution is shifted away from the anomeric center. We conclude that the $^2S_0$ conformation is present in equilibrium with the $^4C_1$ conformation (Scheme 5) in the seven studied xylosides (Table 1). The conformational equilibrium of the 3-deoxy-analogue **3d** was also investigated, using seven $^3J_{HH}$ coupling constants in the pyranose ring. Interestingly, a two-state equilibrium between $^4C_1$ and $^1C_4$ is then present in which the two conformations are equally populated (Scheme 5 and Table 1).

For conformational analysis the xylopyranosides were dissolved in methanol, which like the hydroxyl groups of the solute molecules can act as both hydrogen bond donors and acceptors. Except for the deoxygenations the structural modifications retain hydrogen-bonding abilities. In methyl α- and β-D-xylopyranoside the solvent structure and hydroxyl group conformational preferences in methanol have been investigated by molecular dynamics simulations.[44] The simulations showed solvent structuring around the solute molecule with the hydroxyl groups of methanol donating hydrogen bonds to the hydroxyl groups of the methyl xylopyranosides. The hydroxyl groups of the solute molecules are arranged in a counter-clockwise orientation, that is, towards the glycosidic oxygen. This structural network will upon substitution or epimerization of the xyloside hydroxyl groups be perturbed and can be altered to different extent. The relative importance of the anomeric effect, 1,3-diaxial interactions of electronegative groups and potential intraresidue hydrogen bonds may subsequently be ascertained by future molecular dynamics simulations. The present investigation has shown that conformational equilibria need to be considered for the β-D-xylopyranosides and analogs thereof.



Scheme 5. Conformational behavior of xylose analogs **1b** (top) and **3d** (bottom).

Table 2
Antiproliferative activity and retention times for compounds **1b**–**4d**. The antiproliferative activity was recorded towards CCD1095-SK cells and HCC70 cells and expressed as ED_{50}-values (μM)

Compound	Modification	Antiproliferative activity (ED_{50}, μM)		Retention time (min)
		CCD1095-SK	HCC70	
1b	—	243	91	24.66 ± 0.022
2a	OMe	55	127	31.13 ± 0.030
2b	F	12	22	32.57 ± 0.023
2c	Epi	50	363	23.61 ± 0.004
2d	Deoxy	19	228	29.57 ± 0.002
3a	OMe	17	145	27.22 ± 0.016
3b	F	72	94	28.66 ± 0.009
3c	Epi	182	178	27.95 ± 0.015
3d	Deoxy	79	179	30.06 ± 0.052
4a	OMe	93	135	29.20 ± 0.007
4b	F	32	37	28.64 ± 0.075
4c	Epi	48	371	22.85 ± 0.011
4d	Deoxy	285	284	26.97 ± 0.058

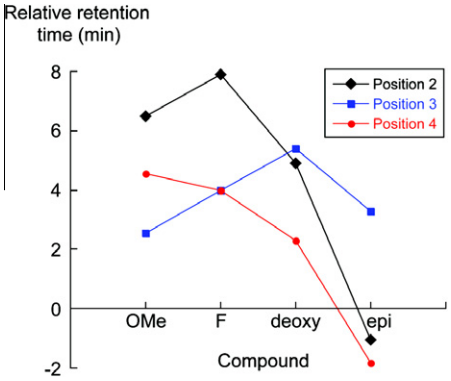


Figure 3. Relative gradient HPLC retention times of compounds **2a**–**4d** compared to the unmodified **1b** (the retention time for **1b**, 24.66 min, has been subtracted from the actual retention times).

Gradient HPLC retention times can be used to substitute log *P* values.[45] The gradient HPLC retention times for the compounds were measured using a C-18 column and a mobile phase of water (0.1% TFA) with a gradient of MeCN from 1 min increasing by 1.2% per minute. The retention times were measured for three separate runs per compound, and the calculated mean retention times are presented in Table 2. To visualize the polarities of the compounds, the gradient HPLC retention times relative to the unmodified **1b** were plotted (Fig. 3). In general, the exchange of a hydroxyl group for methoxy, fluorine or hydrogen, results in less polar compounds. However, the analogs epimerized in position 2 or 4 are more polar, which indicates that the hydroxyl groups are more available for interaction with water. In contrary, modifications in position 3 do not give large changes in polarity and the epimerized analog **3c** is actually less polar than the methoxy analog.

2.3. Biology

For determination of GAG priming and proliferation activity, a matched pair of normal breast fibroblasts (CCD-1095Sk) and human breast carcinoma cells (HCC70 cells) were used. Untreated breast carcinoma cells secreted large sized PG and medium sized GAG into the culture medium. Treatment with 2-naphthyl β-D-xylopyranoside (**1b**) or 2-(6-hydroxynaphthyl) β-D-xylopyranoside (**1a**) initiated biosynthesis of free, regular-size GAG chains that were secreted into the medium as expected. Furthermore, the size of GAG chains produced by **1a** was smaller compared to the size of

GAG chains produced by **1b** to some extent. In contrary, the twelve analogous compounds (**2a**–**4d**) used in this study did not induce priming of GAG chains.

As previously reported β-D-xylosides with hydrophobic aglycones can initiate synthesis of GAG chains in most cell types, and at sufficiently high xyloside concentration endogenous PG synthesis is competitively inhibited. We observed a small inhibitory effect on endogenous PG production for compounds **4b** and **4d** (33% and 24%, respectively, Fig. 4).

During the formation of the linker tetrasaccharide, the xylose residue is galactosylated at C4 (3Galβ1–4Xylβ). If the acceptor hydroxyl group at C4 is missing, as in the case of **4b** and **4d**, the GAG chain elongation can be anticipated to decline. Kuberan and coworkers have published a series of 4-deoxy-4-fluoro-xylosides with various triazole-based aglycons with the aim of finding a selective GAG inhibitor.[46,47] By comparing the elution profile of PG/GAG extracts from untreated CHO cells with the elution profiles of PG/GAG extracts from CHO cells that were treated with 4-deoxy-4-fluoro-xylosides, it was found that some of the xylosides were GAG inhibitors, showing inhibition by up to ~90% after treatment at 1 mM of the xylosides.

To further investigate the inhibitory effect shown by naphthoxylosides fluorinated in position 4 we incubated HCC70 cells with the GAG primer **1a**, which gave a strong priming of GAG chains (Fig. 5, solid black line). With the addition of 1 equiv (0.1 mM) of the 4-fluoro analog **4b**, the amount of GAG chains decreased by 23% (Fig. 5, dotted red line).

To establish the antiproliferative effects of these compounds, **2a**–**4d** were added to the growth medium at increasing concentrations, and cell proliferation was recorded using a crystal violet method.[9,10] The inhibitory effect of the compounds are expressed as ED_{50} (M) scored after 96 h of exposure (Table 2).

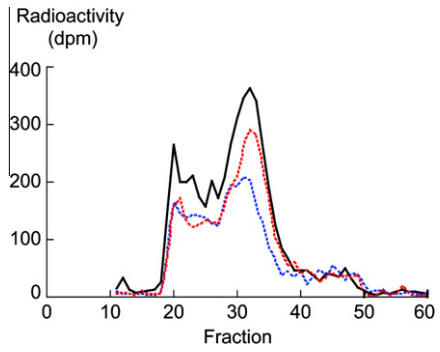


Figure 4. Priming of PG and GAG in HCC70 cells incubated with 0.1 mM **4b** (dotted blue line) or 0.1 mM **4d** (dotted red line). The solid line shows the result for untreated cells.

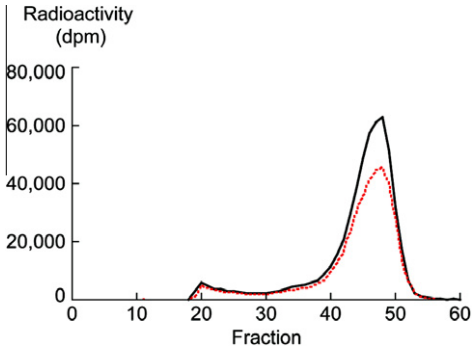


Figure 5. Priming of GAG in HCC70 cells incubated with **1a** (solid black line) and equimolar amounts of **1a** and **4b** (dotted red line).

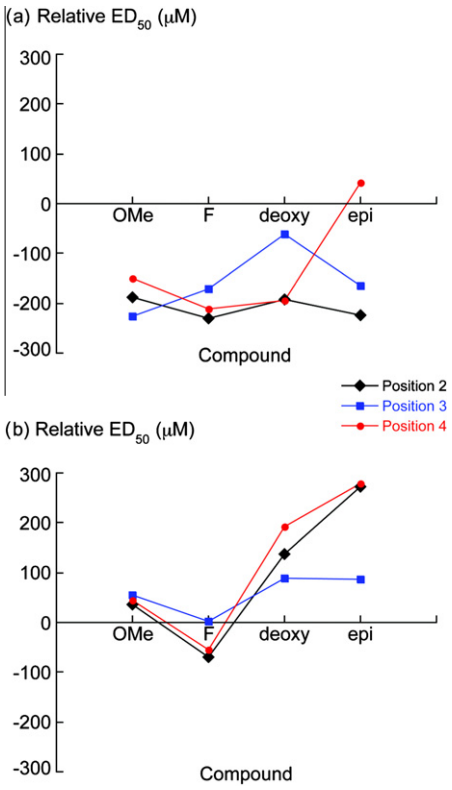


Figure 6. Relative antiproliferative activity of compounds **2a**–**4d** in (a) CCD1095-SK (normal) cells and (b) HCC70 (tumor) cells.

To compare the antiproliferative effects of these compounds in the two cell lines, the ED_{50} value for the reference compound (XylNap, **1b**) was subtracted from the ED_{50} value for each compound and the relative ED_{50} values were plotted (Fig. 6). Thus, negative values indicate a stronger antiproliferative effect compared to the reference **1b**.

In general, the normal cells showed a higher sensitivity towards these compounds, compared to the reference **1b** but no structure-activity relationships could be deduced from these data. In contrary, tumor cells were less sensitive and only the three fluoro analogs showed toxicity comparable to **1b**.

We have earlier observed a correlation between toxicity of various xylosylated dihydroxynaphthalenes and the polarity, measured as gradient HPLC retention times. However, we could not find any correlations between the ED_{50} values found for these compounds and their retention times.

3. Conclusions

We have developed a new synthetic protocol, based on acetal protection and selective benzylation, for modification of all three positions in xylose. Using this protocol we have synthesized analogs where each hydroxyl group has been epimerized or replaced with methoxy, fluoro or hydrogen.

We have investigated the conformational behavior of some of these compounds and found that they usually adopt a normal $^4C_1$-conformation, accompanied by a nonnegligible population of the $^2S_0$ conformation. However, deoxygenation at position 3 results in a significant amount of the $^1C_4$-conformation. The conformational flexibility of the compounds epimerized at C2 and at C3, that is, **2c** and **3c**, may be even more complex as suggested by a preliminary analysis of NMR data and these conformational equilibria will be addressed in a future study.

We have earlier shown that some monoxylosylated dihydroxynaphthalenes, especially 2-(6-hydroxynaphthyl) β-D-xylopyranoside (XylNapOH, **1a**), show selective toxicity versus tumor cells and that the selective activity is dependent on priming of soluble GAG chains, especially heparan sulfate. With this investigation we conclude that it seems difficult to reestablish the tumor selective antiproliferative activity by modifying the xylose residue in naphthoxylosides. None of the tested analogs **2a**–**4d**, showed priming of soluble GAG chains and consequently no selective toxicity.

Furthermore, our data indicate that normal and tumor cells react differently to these compounds, with the normal cells being more sensitive. The only compounds that showed increased toxicity in tumor cells were the fluorinated analogs. Some of these fluorinated analogs were shown to be inhibitors of the biosynthesis of PG as well as of the biosynthesis of soluble GAG chains primed from XylNap.

4. Experimental section

4.1. Synthesis

NMR spectra were recorded with a Bruker Avance II operating at 294 K. ^{1}H NMR spectra were assigned using COSY (2D homonuclear shift correlation) with a gradient selection. Chemical shifts are given in ppm downfield from Me_4Si, with reference to residual $CHCl_3$ (7.26) or MeOH-d_4 (3.31). Coupling constant values are given in Hz. Mass spectra were recorded on Micromass Q-Tof micro™. Optical rotations were measured on Perkin Elmer instruments, Model 341 polarimeter. Agilent 1100 Series HPLC with Thermo Scientific ODS-2 HYPERSIL C_{18} column was used to record retention times for log *P* correlation. Reactions were monitored by TLC using alumina plates coated with silica gel and visualized using UV light or by charring with *para*-anisaldehyde. Preparative chromatography was performed with silica gel (35–70 μm, 60 Å). All the solvents were dried prior to use unless otherwise stated. The purchased reagents were used without further purification.

Synthesis and physical characterization of compound **1b** has been described.[17] A simplified synthetic route was used that comprised treatment of a mixture of 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose and 2-naphthol in CH_2Cl_2 with $BF_3{\cdot}OEt_2$ followed by de-*O*-acetylation. The analyses of the products were in accordance with published data.

4.1.1. 2-Naphthyl 2-methoxy-β-D-xylopyranoside (2a)

Compound **8** (150 mg, 0.37 mmol) was dissolved in CH_2Cl_2 (8 mL) and 95% TFA (8 mL) was added. After 15 min, the red solution was evaporated and the remaining crude was dissolved in Et_2O (10 mL) and washed twice with $NaHCO_3$ (sat. aq). The aqueous layers were extracted with Et_2O and the combined organic layers were dried over $MgSO_4$, filtered, concentrated, and chromatographed (CH_2Cl_2/MeOH 20:1) yielding **2a** (quant.). $[\alpha]_D^{20}$ −28° (*c* 0.78, CH_3OH); ^{1}H NMR (MeOH-d_4): δ 7.79 (m, 3H, ArH), 7.40 (m, 3H, ArH), 7.23 (dd, 1H, *J* = 8.9, 2.5, ArH), 5.08 (d, 1H, *J* = 7.5, C1–H), 3.96 (dd, 1H, *J* = 11.3, 5.2, C5–H), 3.70 (s, 3H, OCH_3), 3.62 (ddd, 1H, *J* = 10.1, 8.9, 5.3, C4–H), 3.48 (t, 1H, *J* = 9.0, C3–H), 3.42 (dd, 1H, *J* = 11.3,10.2, C5–H), 3.18 (dd, 1H, *J* = 9.0, 7.5, C2–H); ^{13}C NMR (MeOH-d_4) δ 156.4, 135.9, 131.3, 130.5, 128.6, 128.1, 127.5, 125.3, 119.8, 111.8, 102.9, 84.7, 77.3, 71.1, 66.9, 61.2; HRMS calcd for $C_{16}H_{18}NaO_5^+$ [M+Na]: 313.1052; found: 313.2138.

4.1.2. 2-Naphthyl 2-deoxy-2-fluoro-β-D-xylopyranoside (2b)

This was prepared from **12** as described for **2a** but with 1 mL TFA instead of 8 mL (90%). $[\alpha]_D^{20}$ −23° (*c* 0.56, CH_3OH); ^{1}H NMR (MeOH-d_4): δ 7.82–7.75 (m, 3H, ArH), 7.47–7.41 (m, 1H, ArH), 7.39–7.33 (m, 2H, ArH), 7.23 (dd, 1H, *J* = 8.9, 2.5, ArH), 5.29 (dd, 1H, *J* = 7.4, 3.5, C1–H), 4.29 (ddd, 1H, *J* = 51.2, 8.5, 7.5, C2–H), 3.98 (ddd, 1H, *J* = 11.3, 5.2, 1.2, C5–H), 3.72 (dt, 1H, *J* = 15.4, 8.8, C3–H), 3.67 (dt, 1H, *J* = 10.0, 5.2, C4–H), 3.49 (dd, 1H, *J* = 11.3, 10.1, C5–H); ^{13}C NMR (MeOH-d_4): δ 156.1, 135.8, 131.4, 130.5, 128.6, 128.2, 127.5, 125.4, 119.8, 112.0, 100.3 (d, *J* = 23.5 Hz), 93.4 (d, *J* = 186.9 Hz), 75.2 (d, *J* = 18.2 Hz), 70.7 (d, *J* = 7.8 Hz), 66.9; HRMS calcd for $C_{15}H_{16}FO_4^+$ [M+H]: 279.1033; found: 279.1066.

4.1.3. 2-Naphthyl β-D-lyxopyranoside (2c)

This was prepared from **11** as described for **2a** but with 1 mL TFA instead of 8 mL (21%). $[\alpha]_D^{20}$ −99° (*c* 0.6, CH_3OH); ^{1}H NMR (MeOH-d_4): δ 7.81–7.73 (m, 3H, ArH), 7.46 (d, 1H, *J* = 2.4, ArH), 7.45–7.32 (m, 2H, ArH), 5.45 (d, 1H, *J* = 2.5, C1–H), 4.14–4.08 (m, 2H, C2–H and C5–H), 3.87 (ddd, 1H, *J* = 9.7, 6.1, 3.5, C4–H), 3.78 (dd, 1H, *J* = 6.5, 3.5, C3–H), 3.44 (dd, 1H, *J* = 11.7, 5.9, C5–H); ^{13}C NMR (MeOH-d_4): δ 156.5, 135.9, 131.2, 130.3, 128.6, 128.1, 127.4, 125.2, 120.1, 111.9, 99.9, 73.5, 69.6, 69.3, 63.9; HRMS calcd for $C_{15}H_{16}NaO_5^+$ [M+Na]: 299.0895; found: 299.0882.

4.1.4. 2-Naphthyl 2-deoxy-β-D-threopyranoside (2d)

n-Bu_3SnH (0.061 mL, 0.23 mmol) in toluene (3 mL) was heated to reflux. **14** (55 mg, 0.15 mmol) in toluene (7 mL) was then added dropwise, followed by AIBN (2.5 mg, 0.015 mmol). The mixture was then stirred for 1 h, after which it was evaporated and chromatographed (CH_2Cl_2/MeOH 20:1) to give **2d** (11.5 mg, 30%). $[\alpha]_D^{20}$ −62° (*c* 0.28, CH_3OH); ^{1}H NMR (MeOH-d_4): δ 7.80–7.72 (m, 3H, ArH), 7.45–7.38 (m, 1H, ArH), 7.36–7.31 (m, 2H, ArH), 7.21 (dd, 1H, *J* = 8.9, 2.4, ArH), 5.44 (dd, 1H, *J* = 7.8, 2.6, C1–H), 4.05 (dd, 1H, *J* = 11.4, 4.3, C5–H), 3.76–3.68 (m, 1H, C3–H), 3.57–3.48 (m, 1H, C4–H), 3.42 (dd, 1H, *J* = 11.4, 8.5, C5–H), 2.37 (ddd, 1H, *J* = 13.0, 4.7, 2.7, C2–H), 1.82 (ddd, 1H, *J* = 13.0, 9.8, 7.9 Hz, C2–H); ^{13}C NMR (MeOH-d_4): δ 156.2, 135.9, 131.2, 130.3, 128.6, 128.0, 127.4, 125.2, 119.9, 111.6, 98.8, 71.7, 71.2, 66.0, 38.3; HRMS calcd for $C_{15}H_{16}NaO_4^+$ [M+Na]: 283.0946; found: 283.0976.

4.1.5. 2-Naphthyl 3-methoxy-β-D-xylopyranoside (3a)

10% Pd/C (Degussa type) (14 mol %) (91 mg, 0.04 mmol) was suspended in DMF (8 mL) and purged with H_2 vacuum-pumped into the reaction vessel. HCl (37%) (21.7 equiv, 0.206 mL, 6.73 mmol) in DMF (8 mL) was added and H_2 was re-introduced. The resulting mixture was pre-conditioned for 10 min after which time the substrate (144 mg, 0.31 mmol) was added in DMF (15 mL) followed by H_2. The mixture was then stirred for 4 h. The reaction was then quenched with Et_3N (0.288 mL), filtered through Celite washing with EtOAc. The crude mixture was washed twice with water, extracted from EtOAc. Combined organic layers were washed with brine and dried over $MgSO_4$, evaporated and purified by flash chromatography (heptane/EtOAc, 3:1 to 1:1) yielding **3a** (94%). $[\alpha]_D^{20}$ −37° (*c* 0.2, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.84–7.52 (m, 3H, ArH), 7.51–7.24 (m, 4H, ArH), 5.05 (d, 1H, *J* = 7.5, C1–H), 3.96 (dd, 1H, *J* = 11.4, 5.4, C5–H), 3.69 (s, 3H, C3–OCH_3), 3.63–3.68 (m, 1H, C4–H), 3.57 (dd, 1H, *J* = 8.9, 7.5, C2–H), 3.46 (dd, 1H, *J* = 11.4, 10.1, C5–H), 3.20 (t, 1H, *J* = 8.9, C3–H); ^{13}C NMR (MeOH-d_4) 156.6, 131.3, 130.4, 128.6, 128.1, 127.4, 125.3, 120.0, 111.9, 102.9, 87.3, 74.4, 70.7, 66.9, 61.1; HRMS calcd for $C_{16}H_{18}O_5Na^+$ [M+Na]: 313.1046; found: 313.1210.

4.1.6. 2-Naphthyl 3-deoxy-3-fluoro-β-D-xylopyranoside (3b)

This was prepared from **18** as described for **3a** (94%). $[\alpha]_D^{20}$ −23° (*c* 0.3, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.87–7.75 (m, 3H, ArH), 7.50–7.24 (m, 4H, ArH), 5.07 (d, 1H, *J* = 7.6, C1–H), 4.35 (dt, 1H, *J* = 52.5, 8.7, C3–H), 4.00 (ddd, 1H, *J* = 11.4, 6.1, 5.6, C5–H), 3.82–3.92 (m, 1H, C4–H), 3.74 (ddd, 1H, *J* = 16.4, 8.7, 7.6, C2–H), 3.48 (ddd, 1H, *J* = 11.4, 10.3, 1.0, C5–H); ^{13}C NMR (MeOH-d_4) δ 135.8, 131.4, 130.4, 128.6, 128.1, 127.4, 125.4, 120.0, 112.0, 102.3 (*J* = 11.3),

97.8 (J = 183.4), 73.2 (J = 18.3), 69.4 (J = 18.0), 65.7 (J = 9.4); HRMS calcd for $C_{15}H_{15}FO_4Na^+$ [M+Na]: 301.0847; found: 301.1021.

4.1.7. 2-Naphthyl β-D-ribopyranoside (3c)

This was prepared from **17** as described for **3a** (73%). $[\alpha]_D^{20}$ −124° (c 1.1, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.80–7.74 (m, 3H, ArH), 7.46–7.41 (m, 2H, ArH), 7.37–7.33 (m, 1H, ArH), 7.24 (dd, 1H, J = 9.0, 2.5, ArH), 5.59 (d, 1H, J = 4.0, C1–H), 4.08 (app. t,1H, J = 3.2, C3–H), 3.79–3.91 (m, 4H, C2–H, C4–H and C5–H); ^{13}C NMR (MeOH-d_4) δ 155.9, 135.9, 131.2, 130.4, 128.6, 128.1, 127.4, 125.2, 119.8, 111.8, 100.5, 72.4, 70.4, 68.3, 65.6; HRMS calcd for $C_{15}H_{16}O_5Na^+$ [M+Na]: 299.0895; found: 299.1111.

4.1.8. 2-Naphthyl 3-deoxy-β-D-erythropyranoside (3d)

This was prepared from **19** as described for **3a** (85%). $[\alpha]_D^{20}$ −116° (c 1.7, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.83–7.74 (m, 3H, ArH), 7.48–7.24 (m, 4H, ArH), 5.26 (d, 1H, J = 4.8, C1–H), 3.85 (m, 1H, C4–H), 4.00 (ddd, 1H, J = 11.5, 3.2, 1.0, C5–H), 3.80 (ddd, 1H, J = 8.0, 4.8, 3.9, C2–H), 3.54 (ddd, 1H, J = 11.5, 5.9, 1.1, C5–H), 2.35 (dtd, 1H, J = 13.4, 3.9, 1.2, C3–H), 1.81 (dtd, 1H, J = 13.4, 8.0, 0.8, C3–H); ^{13}C NMR (MeOH-d_4) δ 156.1, 135.9, 131.2, 130.4, 128.6, 128.1, 127.4, 125.2, 120.0, 111.7, 101.6, 68.4, 68.3, 66.0, 35.7; HRMS calcd for $C_{15}H_{16}O_4Na^+$ [M+Na]: 283.1310; found: 283.1357.

4.1.9. 2-Naphthyl 4-methoxy-β-D-xylopyranoside (4a)

This was prepared from **20** as described for **3a** (66%). $[\alpha]_D^{20}$ −54° (c 1.5, MeOH) ^{1}H NMR (MeOH-d_4) δ 7.79–7.75 (m, 3H, ArH), 7.45–7.25 (m, 4H, ArH), 5.02 (d, 1H, J = 7.4, C1–H), 4.16 (dd, 1H, J = 11.4, 5.0, C5–H), 3.52 (s, 1H, OCH_3), 3.50–3.56 (m, 2H, C2–H and C3–H), 3.42 (dd, 1H, J = 11.4, 9.9, C5–H), 3.27–3.33 (m, 1H, C4–H); ^{13}C NMR (MeOH-d_4) 156.6, 135.8, 130.3, 128.6, 128.1, 127.4, 125.3, 120.0, 111.9, 102.8, 80.8, 76.8, 74.8, 64.4, 59.1; HRMS calcd for $C_{16}H_{18}O_5Na^+$ [M+Na]: 313.1052; found: 313.1026.

4.1.10. 2-Naphthyl 4-deoxy-4-fluoro-β-D-xylopyranoside (4b)

This was prepared from **23** as described for **3a** (61%). $[\alpha]_D^{20}$ −35° (c 0.3, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.80–7.76 (m, 3H, ArH), 7.46–7.25 (m, 4H, ArH), 5.12 (d, 1H, J = 7.3, C1–H), 4.49 (dddd, 1H, J = 50.3, 13.6, 8.1, 5.4, C4–H), 4.17 (dt, 1H, J = 11.7, 5.4, C5–H), 3.80–3.66 (m, 2H, C5–H and C3–H), 3.56 (ddd, 1H, J = 9.4, 7.3, 0.6, C2–H); 13C NMR (MeOH-d_4) δ 156.5, 135.8, 131.3, 130.4, 128.6, 128.1, 127.4, 125.3, 120.0, 112.0, 102.6, 91.0 (d, J = 180.1), 75.6 (d, J = 18.8), 74.2 (d, J = 9.1), 63.9 (d, J = 28.6); HRMS calcd for $C_{15}H_{15}FO_4Na$+ [M+Na]: 301.0847; found: 301.0865

4.1.11. 2-Naphthyl α-L-arabinopyranoside (4c)

This was prepared from **22** as described for **3a** (33%). $[\alpha]_D^{20}$ −4° (c 0.6, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.80–7.74 (m, 3H, ArH), 7.45–7.27 (m, 4H, ArH), 5.02 (d, 1H, J = 7.1, C1–H), 3.99 (dd, 1H, J = 12.4, 2.8, C5–H), 3.91–3.93 (m, 1H, C4–H), 3.88 (dd, 1H, J = 9.1, 7.1, C2–H), 3.80 (dd, 1H, J = 12.4, 1.5, C5–H), 3.68 (dd, 1H, J = 9,1, 3.5, C3–H); ^{13}C NMR (MeOH-d_4) 156.7, 135.8, 131.2, 130.3, 128.6, 128.1, 127.4, 125.2, 120.1, 111.9, 102.8, 74.2, 72.3, 69.7, 67.2; HRMS calcd for $C_{15}H_{16}O_5Na^+$ [M+Na]: 299.0890; found: 299.0900.

4.1.12. 2-Naphthyl α-L-threopyranoside (4d)

This was prepared from 24 as described for 3a (45%). $[\alpha]_D^{20}$ −16° (c 0.5, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.79–7.74 (m, 3H, ArH), 7.45–7.26 (m, 4H, ArH), 4.98 (d, 1H, J = 7.3, C1–H), 4.01 (ddd, 1H, J = 12.0, 4.9, 2.3, C5–H), 3.67–3.75 (m, 2H, C3–H and C5–H), 3.43 (dd, 1H, J = 8.7, 7.3, C2–H), 2.00 (app. dp, 1H, J = 13.1, 2.3, C4–H), 1.70 (dddd, 1H, J = 17.0, 13.1, 10.9, 4.9, C4–H); 13C NMR (MeOH-d_4) 156.8, 135.9, 131.3, 130.7, 130.4, 128.7, 128.1, 127.4, 125.3, 120.1, 111.9, 102.9, 76.4, 72.2, 62.6, 34.2; HRMS calcd for C15H16O4Na+ [M+Na]: 283.0941; found: 283.0933.

4.1.13. (1*R*, 3*S*, 4*S*, 6*R*, 9*S*, 10*R*)-10-Hydroxy-3,4-dimethoxy-3,4-dimethyl-9-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (5a) and(1*S*, 3*R*, 4*R*, 6*R*, 7*S*, 10*R*)-10-hydroxy-3,4-dimethoxy-3,4-dimethyl-7-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (5b)

Compound **1b** (800 mg, 2.90 mmol) was dissolved in dry CH_3CN (40 mL) under N_2 atmosphere. 2,2,3,3-Tetramethoxybutane (1.010 mL, 5.79 mmol) was added to the solution, followed by addition of $BF_3{\cdot}OEt_2$ (0.10 mL, 0.84 mmol) and the solution got dark brown. After 18 h, Et_3N (4 mL, 29 mmol) was added and the dark brown color partly disappeared. The mixture was then concentrated and purified by flash chromatography (heptane/EtOAc 2:1). **5a** and **5b** were obtained as white crystals in 61% and 39%, respectively. **5a**: $[\alpha]_D^{20}$ 81° (c 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.82–7.74 (m, 3H, ArH), 7.49–7.43 (m, 1H, ArH), 7.42–7.36 (m, 2H, ArH), 7.28 (dd, 1H, J = 9.0, 2.5, ArH), 5.04 (d, 1H, J = 7.3, C1–H), 4.01 (dd, 1H, J = 10.8, 4.9, C5–H), 3.95–3.86 (m, 2H, C4–H and C2–H), 3.79 (t, 1H, J = 9.7, C3–H), 3.60 (t, 1H, J = 10.6, C5–H), 3.34 (s, 3H, OCH_3), 3.30 (s, 3H, OCH_3), 2.49 (d, 1H, J = 2.4, C2–OH), 1.40 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 154.7, 134.3, 130.3, 129.8, 127.8, 127.4, 126.6, 124.7, 119.1, 112.0, 102.6, 100.0, 99.7, 72.3, 71.4, 65.7, 64.6, 48.2, 48.2, 17.9, 17.8; HRMS calcd for $C_{21}H_{26}NaO_7^+$ [M+Na]: 413.1576; found: 413.2185. **5b**: $[\alpha]_D^{20}$ −103° (c 0.98, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.79–7.73 (m, 3H, ArH), 7.47–7.42 (m, 1H, ArH), 7.40–7.34 (m, 2H, ArH), 7.24 (dd, 1H, J = 8.9, 2.4, ArH), 5.28 (d, 1H, J = 7.2, C1–H), 4.16 (dd, 1H, J = 11.6, 5.3, C5–H), 4.04–3.96 (m, 1H, C4–H), 3.87–3.77 (m, 2H, C2–H and C3–H), 3.50 (dd, 1H, J = 11.7, 9.1, C5–H), 3.39 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 2.28 (d, 1H, J = 2.9, C4-OH), 1.48 (s, 3H, CH_3), 1.43 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 154.9, 134.4, 130.2, 129.5, 127.8, 127.3, 126.5, 124.5, 119.4, 111.8, 99.9, 99.8, 99.5, 73.0, 69.1, 67.7, 66.6, 48.2, 48,2, 17.8, 17.8; HRMS calcd for $C_{21}H_{26}NaO_7^+$ [M+Na]: 413.1576; found: 413.2185.

4.1.14. 2-Naphthyl 2,4-di-*O*-benzyl-β-D-xylopyranoside (6a), 2-naphthyl 2,3-di-*O*-benzyl-β-D-xylopyranoside (6b), and 2-naphthyl 3,4-di-*O*-benzyl-β-D-xylopyranoside (6c)

Compound **1b** (3.00 g, 0.011 mol) was dissolved in CH_2Cl_2 (150 mL) while stirring and BnBr (2.84 mL, 0.024 mol), QI (8.02 g, 0.022 mol) and KOH (10%) (150 mL) were added to this solution. Stirring continued for 22 h after which time the reaction mixture was transferred into a separating funnel and extracted from CH_2Cl_2 (2 × 100 mL). The organic layers were washed with brine (200 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude mixture was purified by column chromatography eluting with heptane/EtOAc gradient from 16:1 to 4:1. Di-protected compounds were obtained in following yields: 3-OH (**6a**) (66%), 4-OH (**6b**) (14%), and 2-OH (**6c**) (6%). Compound **6a:** $[\alpha]_D^{20}$ −30° (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.8–7.7 (m, 3H, ArH), 7.5–7.2 (m, 14H, ArH), 5.15 (d, 1H, J = 7.4, C1–H), 5.05 (d, 1H, J = 11.4, CH_2Ph), 4.83 (d, 1H, J = 11.4, CH_2Ph), 4.80 (d, 1H, J = 11.8, CH_2Ph), 4.67 (d, 1H, J = 11.8, CH_2Ph), 4.05 (dd, 1H, J = 11.6, 5.1, C5–H), 3.82 (td, 1H, J = 9.2, 2.2, C3–H), 3.66–3.60 (m, 1H, C4–H), 3.59 (dd, 1H, J = 9.2, 7.4, C2–H), 3.43 (dd, 1H, J = 11.6, 9.5, C5–H); ^{13}C NMR ($CDCl_3$) δ 154.9, 138.2, 134.4, 129.8, 128.7, 128.3, 128.1, 128.0, 127.80, 127.3, 124.6, 111.5, 102.0, 81.0, 75.8, 74.9, 73.4, 64.3; HRMS calcd for $C_{29}H_{28}O_5Na^+$ [M+Na]: 479.1834; found: 479.2127. Compound **6b**: $[\alpha]_D^{20}$ −11° (c 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.81–7.75 (m, 3H, ArH), 7.48–7.23 (m, 14H, ArH), 5.32 (d, 1H, J = 5.9, C1–H), 4.98 (d, 1H, J = 11.2, CH–Ph), 4.94 (d, 1H, J = 11.6, CH–Ph), 4.81 (d, 1H, J = 11.2, CH–Ph), 4.70 (d, 1H, J = 11.6, CH–Ph), 4.16 (dd, 1H, J = 11.6, 4.2, C5–H), 3.84–3.77 (m, 2H, C2–H and C4–H), 3.62 (app. t, 1H, J = 7.3, C3–H), 3.49 (dd, 1H, J = 11.6, 7.9, C5–H), 2.54 (d, 1H, J = 5.2, C4-OH); ^{13}C NMR ($CDCl_3$) δ 129.8, 128.8, 128.7, 128.3, 128.2, 128.0, 127.8, 127.3, 126.6, 124.5,

119.0, 111.3, 100.9, 81.2 79.4, 74.5, 74.5, 68.9, 64.5; HRMS calcd for $C_{29}H_{28}O_5Na^+$ [M+Na]: 479.1834; found: 479.1845. Compound **6c**: $[\alpha]_D^{20}$ −69° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.80–7.72 (m, 3H, ArH), 7.46–7.30 (m, 13H, ArH), 7.23 (dd, 1H, *J* =8.9, 2.5, ArH), 5.31 (d, 1H, *J* = 5.1, C1–H), 4.90, 4.82 (ABq, 1H each, *J* = 11.5, CH_2–Ph), 4.70 (s, 2H, CH_2–Ph), 4.15 (dd, 1H, *J* = 12.0, 3.5, C5–H), 3.93 (m, 1H, C2–H), 3.77 (t, 1H, *J* = 6.4, C3–H), 3.70 (dt, 1H, *J* = 6.4, 3.5, C4–H), 3.58 (dd, 1H, *J* = 12.0, 6.5, C5–H), 3.07 (d, 1H, *J* = 6.2, C2–OH); ^{13}C NMR ($CDCl_3$) δ 154.8, 138.5, 137.7, 134.4, 130.0, 129.6, 128.7, 128.7, 128.3, 128.0, 128.0, 128.0, 127.8, 127.3, 126.6, 124.5, 119.1, 111.2, 100.4, 79.1, 76.2, 73.8, 72.7, 70.7, 61.7; HRMS calcd for $C_{29}H_{28}O_5Na^+$ [M+Na]: 479.1834; found: 479.1740.

4.1.15. (1*R*, 3*S*, 4*S*, 6*R*, 9*R*, 10*S*)-10-methoxy-3,4-dimethoxy-3,4-dimethyl-9-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (8)

Compound **8** was obtained from **5a** using the same method as for **15** (89%). $[\alpha]_D^{20}$ 89° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.83–7.77 (m, 3H, ArH), 7.50–7.45 (m, 1H, ArH), 7.42–7.37 (m, 2H, ArH), 7.28 (dd, 1H, *J* = 8.9, 2.5, ArH), 5.06 (d, 1H, *J* = 7.1, C1–H), 4.02–3.89 (m, 2H, C5–H and C4–H), 3.80 (t, 1H, *J* = 9.7, C3–H), 3.72 (s, 3H, C2-OCH_3), 3.57 (t, 1H, *J* = 10.3, C5–H), 3.51 (dd, 1H, *J* = 9.2, 7.6, C2–H), 3.36 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 1.41 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ155.1, 134.4, 130.2, 129.7, 127.8, 127.3, 126.5, 124.6, 119.2, 111.8, 102.8, 99.7, 99.7, 80.2, 72.9, 65.8, 64.2, 60.9, 48.2, 48.1, 18.0, 17.7; HRMS calcd for $C_{22}H_{28}NaO_7^+$ [M+Na]: 427.1733, found: 427.1716.

4.1.16. (1R, 3S, 4S, 6R, 9R, 10R)-10-acethoxy-3,4-dimethoxy-3,4-dimethyl-9-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (10)

Compound **5a** (150 mg, 0.38 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to −78 °C under N_2, while stirring. Pyridine (0.125 mL, 1.5 mmol) was added, followed by dropwise addition of Tf_2O (0.130 mL, 0.77 mmol). The temperature was raised to 0 °C. After 3 h, the reaction mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ (sat. aq), and dried over Na_2SO_4. Once filtered, solvents were evaporated and the remaining crude triflate was submitted to subsequent reaction. The crude was dissolved in DMF (8 mL) and CsOAc (185 mg, 0.96 mmol) was introduced under nitrogen atmosphere, while stirring. The resulting mixture was heated to 50 °C for three days. After that time it was allowed to cool to rt and solvents were evaporated. The crude was purified by flash chromatography eluting with heptane/EtOAc (4:1), which gave **10** in 82% yield over the two steps. $[\alpha]_D^{20}$ 73° (*c* 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.80–7.72 (m, 3H, ArH), 7.45 (dt, 1H, *J* = 6.9, *J* = 1.3, ArH), 7.39–7.34 (m, 1H, ArH), 7.33 (d, 1H, *J* = 2.4, ArH), 7.18 (dd, 1H, *J* = 8.9, 2.5, ArH), 5.65 (dd, 1H, *J* = 3.1, 1.1, C2–H), 5.28 (d, 1H, *J* = 1.1, C1–H), 4.24 (dt, 1H, *J* = 10.4, 5.1, C4–H), 4.12 (dd, 1H, *J* = 11.0, 5.1, C5–H), 3.87 (dd, 1H, *J* = 10.2, 3.1, C3–H), 3.58 (t, 1H, *J* = 10.9, C5–H), 3.29 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 2.28 (s, 3H, OAc), 1.31 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 170.7, 154.6, 134.3, 130.2, 129.7, 127.8, 127.3, 126.6, 124.6, 119.0, 111.4, 100.4, 99.9, 98.5, 69.4, 69.1, 64.8, 63.0, 48.3, 48.1, 21.3, 17.9, 17.8; HRMS calcd for $C_{23}H_{28}NaO_8^+$ [M+Na]: 455.1682; found: 455.2603.

4.1.17. (1R, 3S, 4S, 6R, 9R, 10R)-10-hydroxy-3,4-dimethoxy-3,4-dimethyl-9-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (11)

Compound **10** (58 mg, 0.13 mmol) was dissolved in 0.05 M NaOMe (5 mL). The solution was stirred for 2.5 h, after which Amberlite IR 120 H^+ was added until pH was neutral. The mixture was then filtered and concentrated under reduced pressure to give **11** (47 mg) in 90% yield. $[\alpha]_D^{20}$ 109° (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ7.81–7.74 (m, 3H, ArH), 7.48–7.43 (m, 1H, ArH), 7.41–7.36 (m, 2H, ArH), 7.27 (dd, 1H, *J* = 9.0, 2.4, ArH), 5.23 (d, 1H, *J = 0.9, C1–H), 4.34 (dt, 1H, J* = 10.4, *J* = 5.1, C4–H), 4.29 (s, 1H, C2–H), 4.08 (dd, 1H, *J* = 10.9, 5.2, C5–H), 3.79 (dd, 1H, *J* = 10.1, 2.8, C3–H), 3.53 (t, 1H, *J* = 10.8, C5–H), 3.32 (s, 3H, OCH_3), 3.29 (s, 3H, OCH_3), 2.59 (d, 1H, *J* = 2.0, C2–OH), 1.40 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 154.5, 134.3, 130.2, 129.7, 127.8, 127.3, 126.6, 124.7, 119.0, 111.5, 100.6, 99.9, 99.4, 70.7, 69.9, 64.7, 62.5, 48.3, 48.1, 17.9, 17.9; HRMS calcd for $C_{21}H_{26}NaO_7^+$ [M+Na]: 413.1576; found: 413.2404.

4.1.18. (1R, 3S, 4S, 6R, 9R, 10S)-10-fluoro-3,4-dimethoxy-3,4-dimethyl-9-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (12)

Compound **12** was obtained from **11** in 42% using analogous method as for compound **18**. For this compound, the reaction mixture was heated to +40 °C instead of being cooled. $[\alpha]_D^{20}$ 104° (*c* 0.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.82−7.75 m, 3H, ArH), 7.49–7.36 (m, 3H, ArH), 7.28 (dd, 1H, *J* = 8.9, 2.4, ArH), 5.21 (dd, 1H, *J* = 7.0, 4.8, C1–H), 4.61 (ddd, 1H, *J* = 52.1, 9.2, 7.0, C2–H), 4.05–3.89 (m, 3H, C3–H, C4–H and C5–H), 3.61 (dd, 1H, *J* = 10.8, 9.9, C5–H), 3.34 (s, 3H, OCH_3), 3.31 (s, 3H, OCH_3), 1.34 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 154.7, 134.3, 133.1, 129.8, 127.8, 127.4, 126.3, 119.3, 112.1, 100.6 (d, *J* = 24.4), 100.0, 99.8, 89.2 (d, *J* = 189.9), 71.2 (d, *J* = 18.3), 65.2 (d, *J* = 8.0), 64.2, 48.3, 48.2, 17.8, 17.7; HRMS calcd for $C_{21}H_{25}FNaO_6^+$ [M+Na]: 415.1533; found: 415.1556.

4.1.19. (1R, 3S, 4S, 6R, 9R, 10S)-3,4-dimethoxy-3,4-dimethyl-10-(mehtylthio)thiocarbonyloxy-9-(2-naphthyl)oxy-2,5,8-trioxabicyclo[4.4.0]decane (13)

NaH (42 mg, 1.06 mmol) and imidazole (2 cristals) were added to **5a** (206 mg, 0.53 mmol) in THF (8 mL) under N_2 atmosphere. The mixture was stirred for 1 h, and CS_2 (0.285 mL, 4.75 mmol) was introduced. After 18 h, MeI (0.082 mL, 1.32 mmol) was added. An hour later, the mixture was diluted with CH_2Cl_2, and washed with water, HCl (0.2 M), $NaHCO_3$ (sat. aq), and water. The organic phase was then dried over $MgSO_4$, filtered, concentrated and chromatographed (heptane/EtOAc 2:1) to give **13** (203 mg, 80%). $[\alpha]_D^{20}$ 120° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.80–7.73 (m, 3H, ArH), 7.48–7.42 (m, 1H, ArH), 7.40–7.35 (m, 2H, ArH), 7.19 (dd, 1H, *J* = 8.9, 2.4, ArH), 6.21–6.14 (m, 1 H, C2–H), 5.35 (d, 1H, *J* = 6.4, C1–H), 4.13–4.02 (m, 3H, C3–H, C4–H and C5–H), 3.60–3.69 (m, 1H, C5–H), 3.31 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 2.60 (s, 1H, SCH_3), 1.35 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$): δ: 215.3, 155.0, 134.3, 130.3, 129.7, 127.8, 127.3, 126.6, 124.7, 119.4, 112.0, 101.2, 100.0, 99.8, 79.1, 70.8, 65.4, 64.2, 48.2, 48.0, 19.6, 17.8, 17.7; HRMS calcd for $C_{23}H_{28}NaO_7S_2^+$ [M+Na]: 503.1174; found: 503.1158.

4.1.20. 2-Naphthyl2-O-(methylthio)thiocarbonyl-β-D-threopyranoside (14)

This compound was prepared from **12** as described for **2a** (86%). $[\alpha]_D^{20}$ 16° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.81–7.74 (m, 3H, ArH), 7.49–7.44 (m, 1H, ArH), 7.43–7.37 (m, 2H, ArH), 7.21 (dd, 1H, *J* = 8.8, 2.5, ArH), 5.99 (dd, 1H, *J* = 6.9, 5.4, C2–H), 5.45 (d, 1H, *J* = 5.3, C1–H), 4.27 (dd, 1H, *J* = 12.0, 4.0, C5–H), 4.02–3.97 (m, 1H, C3–H), 3.93–3.88 (m, 1H, C4–H), 3.61 (dd, 1H, *J* = 12.0, *J* = 7.1, C5–H), 2.88 (d, 1H, *J* = 5.2, C2–H), 2.63 (s, 3H, SCH_3); ^{13}C NMR ($CDCl_3$): δ 216.6, 154.3, 134.3, 130.3, 129.9, 127.8, 127.4, 126.8, 124.9, 119.0, 111.8, 98.8, 79.5, 73.1, 69.5, 63.8, 19.8; HRMS calcd for $C_{17}H_{18}NaO_5S_2^+$ [M+Na]: 389.0493; found: 389.0494.

4.1.21. 2-Naphthyl 2,4-di-O-benzyl-3-methoxy-β-D-xylopyraoside (15)

6a (200 mg, 0.44 mmol) was dissolved in freshly distilled DMF (5 mL) under N_2 atmosphere and cooled to 0 °C while stirring. NaH (35 mg, 0.88 mmol) was then added portion-wise and stirring

continued for further 15 min at sub-ambient temperatures. Subsequently, MeI (0.055 mL, 0.88 mmol) was introduced and the mixture was left to stir for 18 h warming to rt. The reaction mixture was then quenched using NH_4Cl sat. and extracted from CH_2Cl_2 (2×10 mL). Combined organic layers were washed with brine (15 mL) and dried over $MgSO_4$, filtered and concentrated under reduced pressure. Compound **15** was obtained in 98% yield after flash chromatography (heptane/EtOAc 5:1). $[\alpha]_D^{20}$ −14° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.83–7.72 (m, 3H, ArH), 7.5–7.2 (m, 14H, ArH), 5.12 (d, 1H, *J* = 7.5, C1–H), 5.01 (d, 1H, *J* = 11.1, CH_2–Ph), 5.01 (d, 1H, *J* = 11.1, CH_2–Ph), 4.85 (d, 1H, *J* = 11.1, CH_2–Ph), 4.79 (d, 1H, *J* = 11.7, CH_2–Ph), 4.67 (d, 1H, *J* = 11.7, CH_2–Ph), 4.02 (dd, 1H, *J* = 11.6, 5.3, C5–H), 3.71 (s, 3H, C3–OCH_3), 3.67–3.60 (m, 2H, C2–H and C4–H), 3.44 (dd, 1H, *J* = 8.9, 7.9, C3–H), 3.39 (dd, 1H, *J* = 11.7, 9.8, C5–H); ^{13}C NMR ($CDCl_3$) δ 154.9, 138.3, 138.2, 134.3 130.0, 129.6, 128.5, 128.4, 128.2, 127.9, 127.8, 127.8, 127.7, 127.2, 126.5, 124.4, 119.0, 111.4, 102.1, 85.5, 81.5, 77.5, 75.1, 73.4, 64.1, 61.3, HRMS calcd for $C_{30}H_{30}O_5Na^+$ [M+Na]: 493.1991; found: 493.2007.

4.1.22. 2-Naphthyl 2,4-di-O-benzyl-β-D-ribopyranoside (17)

6a (100 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to −10 °C under N_2, while stirring. Tf_2O (0.055 mL, 0.33 mmol) was then added dropwise followed by pyridine (0.053 mL, 0.66 mmol). After 2 h the reaction mixture was quenched with HCl (0.5 N) (2 mL), washed with $NaHCO_3$ (sat.) (7 mL), brine (10 mL) and dried over Na_2SO_4. Once filtered, solvents were evaporated and the remaining crude triflate was submitted to subsequent reaction. The crude (116 mg, 0.22 mmol) was dissolved in DMF (8 mL) and QNO_2 (254 mg, 0.88 mmol) was introduced under nitrogen atmosphere, while stirring. The resulting mixture was heated to 50 °C for 16 h. After that time it was allowed to cool to rt and H_2O (20 mL) was added. The solution was extracted from EtOAc (3×30 mL), dried over $MgSO_4$ and evaporated. The crude was purified by flash chromatography eluting with heptane/EtOAc (3:1), which gave **17** in 54% yield over the two steps. $[\alpha]_D^{20}$ −29° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.80–7.74 (m, 3H, ArH), 7.47–7.30 (m, 13H, ArH), 7.22 (dd, 1H, *J* = 8.9, 2.5, ArH), 5.63 (d, 1H, *J* = 5.6, C1–H), 4.93 (d, 1H, *J* = 11.9, CH–Ph), 4.79 (d, 1H, *J* = 11.9, CH–Ph), 4.69 (d, 1H, *J* = 12.00, CH–Ph), 4.62 (d, 1H, *J* = 12.0, CH–Ph), 4.32 (dd, 1H, *J* = 7.8, 3.4, C3–H), 3.96 (dd, 1H, *J* = 11.7, 7.5, C5–H), 3.86 (dd, 1H, *J* = 11.7, 3.9, C5–H), 3.69–3.65 (m, 2H, C2–H and C4–H), 2.79 (d, 1H, *J* = 4.7, C3-OH); ^{13}C NMR ($CDCl_3$) δ 154.8, 138.0, 137.9, 134.5, 130.0, 129.7, 128.7, 128.7, 128.6, 128.6, 128.1, 128.0, 127.9, 127.9, 127.8, 127.3, 126.6, 124.5, 119.0, 111.1, 98.2, 77.0, 74.3, 73.5, 71.9, 67.4, 61.3; HRMS calcd for $C_{29}H_{28}O_5Na^+$ [M+Na]: 479.1834; found: 479.2127.

4.1.23. 2-Naphthyl 2,4-di-O-benzyl-3-deoxy-3-fluoro-β-D-xylopyranoside (18)

Compound **17** (108 mg, 0.24 mmol) was dissolved in CH_2Cl_2 (4 mL) and cooled to −40 °C, under N_2, while stirring. DAST (0.078 mL, 0.59 mmol) was then added. The reaction was monitored by TLC (heptane/EtOAc, 3: 1) which indicated its completion after 1 h. It was quenched with MeOH (2 mL) and stirred for additional 10 min. The crude mixture was washed with $NaHCO_3$ (sat.) (10 mL), brine (20 mL) and dried over $MgSO_4$. Purification by flash chromatography eluting with heptane/EtOAc (8:1) allowed **18** in 47% yield. $[\alpha]_D^{20}$ 4° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.81–7.74 (m, 3H, ArH), 7.48–7.21 (m, 14H, ArH), 5.12 (d, 1H, *J* = 7.4, C1–H), 4.97 (d, 1H, *J* = 11.4, CH–Ph), 4.89 (d, 1H, *J* = 11.4, CH–Ph), 4.84 (d, 1H, *J* = 11.8, CH–Ph), 4.72 (dt, 1H, *J* = 51.8, 8.5, C3–H), 4.67 (d, 1H, *J* = 11.8, CH–Ph), 4.06 (dt, 1H, *J* = 11.9, 5.6, C5–H), 3.87–3.75 (m, 2H, C2–H and C4–H), 3.41 (ddd, 1H, *J* = 11.9, 9.9, 0.7, C5–H); ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ 154.8, 138.0, 137.8, 134.4, 130.2, 129.8, 128.7, 128.7, 128.5, 128.2, 128.2, 128.0, 127.8, 127.3, 126.7, 119.0, 111.6, 101.6 (d, *J* = 11.7), 96.7 (d, *J* = 186.4), 79.5 (d, *J* = 18.2), 75.4 (d, *J* = 17.2), 74.8, 73.4 (d, *J* = 1.7), 63.2 (d, *J* = 9.6); HRMS calcd for $C_{29}H_{27}FO_4Na^+$ [M+Na]: 481.1786; found: 481.1812.

4.1.24. 2-Naphthyl 2,4-di-O-benzyl-3-deoxy-β-D-erythropyranoside (19)

Compound **6a** (198 mg, 0.43 mmol) and imidazole (3 cristals) were dissolved in THF (10 mL) under N_2 atmosphere. The resulting solution was cooled to 0 °C while stirring and NaH (21 mg, 0.87 mmol) was added. The mixture was stirred for 1 h, warming to rt, and CS_2 (0.259 mL, 4.30 mmol) was introduced. After another hour MeI (0.080 mL, 1.29 mmol) was injected. The reaction progress was monitored by TLC (heptane/EtOAc, 3:1) and it indicated its completion after one more hour. The solvents were removed under reduced pressure and the crude was submitted to further transformations without purification. The resulting crude was dissolved in toluene (10 mL) and AIBN (57 mg, 0.35 mmol) and *n*-Bu_3SnH (1.74 mL, 6.45 mmol) were added. The mixture was brought to reflux for 30 min and evaporated. The resulting crude was purified by flash chromatography eluting with heptane/EtOAc gradient 14:1 to 10:1, allowing **19** in 68% yield. $[\alpha]_D^{20}$ −32° (*c* 2.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.82–7.77 (m, 3H, ArH), 7.50–7.27 (m, 14H, ArH), 5.32 (d, 1H, *J* = 5.9, C1–H), 4.89 (d, 1H, *J* = 11.8, CH–Ph), 4.80 (d, 1H, *J* = 11.8, CH–Ph), 4.66 (d, 1H, *J* = 11.8, CH–Ph), 4.59 (d, 1H, *J* = 11.8, CH–Ph), 4.10 (ddd, 1H, *J* = 11.3, 4.0, 1.7, C5–H), 3.70–3.65 (m, 2H, C2–H and C4–H), 3.59 (dd, 1H, *J* = 11.3, 7.4, C5–H), 2.50 (dtd, 1H, *J* = 13.1, 4.6, 1.7, C3–H), 1.90 (dt, 1H, *J* = 13.1, 9.3, C3–H); ^{13}C NMR ($CDCl_3$) δ 154.8, 138.5, 138.4, 134.5, 129.7, 128.7, 128.6, 128.0, 128.0, 127.9, 127.8, 127.4, 126.6, 124.5, 119.1, 111.2, 101.6, 74.2, 72.5, 71.3, 71.2, 66.2, 33.2; HRMS calcd for $C_{29}H_{28}O_4Na^+$ [M+Na]: 463.1885; found: 463.0302.

4.1.25. 2-Naphthyl 2,3-di-O-benzyl-4-methoxy-β-D-xylopyranoside (20)

This was prepared from **6b** as described for **15** (quant.). $[\alpha]_D^{20}$ 9° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.82–7.76 (m, 3H, ArH), 7.50–7.25 (m, 14H, ArH), 5.19 (d, 1H, *J* = 7.3, C1–H), 5.04 (d, 1H, *J* = 11.0, CH–Ph), 4.87–4.93 (m, 2H, CH–Ph), 4.87 (d, 1H, *J* = 11.0, CH–Ph), 4.17 (dd, 1H, *J* = 11.5, 5.0, C5–H), 3.75 (dd, 1H, *J* = 9.0, 7.3, C2–H), 3.65 (dd, 1H, *J* = 9.0, 8.4, C3–H), 3.55 (s, 3H, OCH_3), 3.50–3.56 (m, 1H, C4–H), 3.41 (dd, 1H, *J* = 11.5, 9.5, C5–H); ^{13}C NMR ($CDCl_3$) δ 155.2, 135.8, 131.3, 130.4, 128.6, 128.1, 127.4, 125.3, 120.0, 111.6, 102.4, 81.5, 83.6, 80.0, 75.6, 75.4, 63.8, 59.2; HRMS calcd for $C_{30}H_{30}O_5Na^+$ [M+Na]: 493.1991, found: 493.2011.

4.1.26. 2-Naphthyl 2,3-di-O-benzyl-α-L-arabinopyranoside (22)

This was prepared from **6b** as described for **17** (50%.). $[\alpha]_D^{20}$ −25° (*c* 0.28, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.83–7.76 (m, 3H, ArH), 7.50–7.27 (m, 14H, ArH), 5.20 (d, 1H, *J* = 6.5, C1–H), 5.02 (d, 1H, *J* = 11.2, CH–Ph), 4.86 (d, 1H, *J* = 11.2, CH–Ph), 4.83 (d, 1H, *J* = 11.7, CH–Ph), 4.74 (d, 1H, *J* = 11.7, CH–Ph), 4.14 (dd, 1H, *J* = 12.4, 3.8, C5–H), 4.07–4.02 (m, 2H, C2–H and C4–H), 3.73 (dd, 1H, *J* = 8.1, 3.6, C3–H), 3.63 (dd, 1H, *J* = 12.4, 2.0, C5–H); ^{13}C NMR ($CDCl_3$) δ 155.1, 138.3, 137.9, 134.4, 129.6, 128.7, 128.6, 128.3, 128.2, 128.0, 128.0, 127.8, 127.3, 126.5, 124.5, 119.3, 111.5, 101.09, 79.3, 77.8, 75.1, 72.7, 66.2, 64.7; HRMS calcd for $C_{29}H_{28}O_5Na^+$ [M+Na]: 479.1829; found: 479.1849.

4.1.27. 2-Naphthyl 2,3-di-O-benzyl-4-deoxy-4-fluoro-β-D-xylopyranoside (23)

Compound **23** was obtained using the method described for **12**. The compound was purified using flash chromatography, but some impurities remained and it was not possible to eliminate them. $[\alpha]_D^{20}$ +2° (*c* 2.0, $CHCl_3$) ^1H NMR ($CDCl_3$): δ 7.82–7.75 (m, 3H, ArH), 7.50–7.30 (m, 14H, ArH), 7.25–7.22 (m, 1H, ArH), 5.27 (d, 1H, *J* = 6.7, C1–H), 4.98 (d, 1H, *J* = 11.2, CH_2–Ph), 4.93–4.67 (m, 4H,

CH_2–Ph and C4–H), 4.26–4.18 (m, 1H, C5–H), 3.89–3.73 (m, 2H, C3–H and C2–H), 3.71–3.59 (m, 1H, C5–H); ^{13}C NMR ($CDCl_3$): δ 154.8, 138.2, 138.1, 134.4, 130.1, 129.8, 128.6, 128.5, 128.3, 128.3, 128.3, 128.1, 128.0, 128.0, 127.8, 127.3, 126.7, 124.6, 119.0, 101.8, 90.4 (J = 182.4), 81.4 (J = 19.0), 80.1 (J = 9.4), 63.1 (J = 35.7). HRMS calcd for $C_{29}H_{27}FO_4Na^+$ [M+Na]: 481.1786; found: 481.1810.

4.1.28. 2-Naphthyl 2,3-di-O-benzyl-4-deoxy-α-L-threopyranoside (24)

This was prepared from **6b** as described for **19** (93%.). $[\alpha]_D^{20}$ 17° (c 1.1, $CHCl_3$); ^{1}H NMR (MeOH-d_4) δ 7.73–7.70 (m, 3H, ArH), 7.45–7.21 (m, 14H, ArH), 5.14 (d, 1H, J = 7.0, C1–H), 4.96 (d, 1H, J = 11.3, CH -Ph), 4.84 (d, 1H, J = 11.3, CH -Ph), 4.72 (d, 1H, J = 11.8, CH–Ph), 4.67 (d, 1H, J = 11.8, CH–Ph), 4.02 (ddd, 1H, J = 11.9, 4.7, 2.8, C5–H), 3.73 (ddd, 1H, J = 13.3, 8.4, 5.1, C3–H), 3.62 (td, 1H, J = 11.9, 2.5, C5–H), 3.56 (dd, 1H, J = 8.4, 7.0, C2–H), 2.16 (dp, 1H, J = 13.3, 2.5, C4–H), 1.74–1.64 (m, 1H, C4–H); ^{13}C NMR (MeOH-d_4) δ 155.1, 135.9, 131.3, 130.5, 128.9, 128.7, 128.6, 128.1, 127.4, 125.3, 119.9, 110.4, 101.4, 81.6, 77.9, 74.6, 71.5, 60.8, 27.8; HRMS calcd for $C_{29}H_{28}O_4Na^+$ [M+Na]: 463.1880; found: 463.1888.

4.2. NMR spectroscopy and molecular modeling

NMR experiments for conformational analysis were performed on three different spectrometers: a Bruker Avance III 600 MHz spectrometer equipped with a 5 mm PFG triple resonance probe, a Bruker AVANCE 500 MHz spectrometer and a Bruker AVANCE III 700 MHz spectrometer; the latter two equipped with 5 mm TCI Z-Gradient CryoProbes. Chemical shifts are reported in ppm using residual methanol (δ_H 3.31) and methanol-d_4 (δ_C 49.0) as references. NMR samples were prepared by dissolving 1–2 mg of the xylosides in 0.5 mL of methanol-d_4 (99.9%, Aldrich). ^{1}H and ^{13}C NMR chemical shift assignments were performed at 37 °C using 2D ^{1}H,^{1}H-TOCSY,[48] ^{1}H,^{13}C-HSQC,[49] ^{1}H,^{13}C-H2BC[50] and ^{1}H,^{13}C-HMBC[51] experiments. ^{1}H chemical shifts and $^nJ_{HH}$ coupling constant of compounds **1a**, **1a** (**Na**), **1b**, **2a**, **3a**, **3b**, **3d** and **4a** were determined with aid of the PERCH NMR spin simulation software (PERCH Solutions Ltd., Kuopio, Finland). Chemical shifts and coupling constants were altered iteratively until the simulated and experimental spectra appeared highly similar according to visual inspection and the total root-mean-square value was close to or below 0.1%. $^2J_{HH}$ and $^4J_{HH}$ were assumed to have negative signs whereas $^2J_{FH}$ have a positive sign.[52,53] 3D models of compounds **1a**, **1b**, **2a**, **3a**, **3b**, **3d** and **4a** were built using the VEGA ZZ software (release 2.3.1.2).[54] The molecular structures were energy minimized, using algorithms included in the program, in consecutive order (i) steepest descent; (ii) conjugate gradient and (iii) truncated Newton. Calculated $^nJ_{HH}$ coupling constants for the different ring conformations of compounds **1a**, **1b**, **2a**, **3a**, **3b**, **3d** and **4a** were based on the Karplus-type relationships proposed by Haasnot et al.[55] as implemented in the JANOCCHIO software.[56]

4.3. Biological testing

4.3.1. Materials

Breast cancer cells from patients with infiltrative ductal cancer (HCC70 cells) and normal fibroblasts from breast tissue of patients with infiltrative ductal cancer (CCD-1095Sk cells) were obtained from ATCC, LGC Promochem AB, Borås, Sweden. Regular cell culture media, L-glutamine, penicillin–streptomycin, trypsin, and donor calf serum were obtained from Life Technologies. Minimal essential Medium (MEM) and Ham's F-12 medium were purchased from Sigma. $Na_2{}^{35}SO_4$ (1310 Ci/mmol) was obtained from Perkin Elmer Sverige AB. D-[1-3H]galactose (20 Ci/mmol) was obtained from Bionuclear Scandinavia AB, Bromma, Sweden. Crystal violet was obtained from Merck, Germany. The prepacked Superose 6 HR 10/30m, Dextran T-500 and octyl-Sepharose CL-4B were from Pharmacia-LKB, Sweden, and DE-53 DEAE-cellulose was from Whatman. Water for HPLC-analysis was from a Millipore Milli-Q system.

4.3.2. Cell culture, radiolabeling and extraction procedures

Cells were cultured as monolayers according to manufacturer's instructions. Confluent cells were incubated in low-sulfate, $MgCl_2$-labeling medium supplemented with 2 mM glutamine, 20 mCi/mL of [^{35}S] sulfate and different xylosides. Dilutions were made from 20 mM stock solutions in Me_2SO/water (1:1, v/v). After the incubation period, culture medium was collected and pooled with two washings of ice-cold PBS (0.137 M NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.5). Cells were extracted with 0.1–0.2 mL/Cm^2 dish of 0.15 M NaCl, 10 mM EDTA, 2%(v/v) Triton X-100, 10 mM KH_2PO_4, pH 7.5, 5 μg/ml ovalbumin containg 10 mM N-ethylmaleimide, and 1 mM diisopropylphosphoro-fluoridate on a slow shaker at 4 °C for 10 min.

4.3.3. Isolation of xyloside-primed radiolabeled GAG

The procedures have been described in detail previously.[9] [^{35}S] Sulfate labeled polyanionic macromolecules were isolated from the culture medium by ion exchange chromatography on DEAE–cellulose at 4 °C. Samples were passed over a 0.2 mL-column of DEAE equilibrated with 6 M urea, 0.5 M NaOAc, pH 5.8, 5 μg/mL ovalbumin, 0.1% Triton X-100. After sample application, the columns were washed successively with (a) equilibration buffer, (5 mL), (b) 6 M urea, 10 mM Tris, pH 8.0, 5 μg/mL ovalbumin, 0.1% Triton X-100 (5 mL) and (c) 50 mM Tris, pH 7.5 (10 mL). Bound material was eluted with 2 x 0.5 mL of 4 M guanidine–HCl, 50 mM NaOAc, pH 5.8. The eluted free xyloside-primed GAG chains were separated from PG by hydrophobic interaction chromatography on Octyl-Sepharose column equilibrated with 4 M guanidine–HCl, 50 mM NaOAc, pH 5.8. After equilibration, BSA were added and columns washed again with 2.5 mL of 4 M guanidine–HCl, 50 mM NaOAc, pH 5.8 to avoid unspecific binding. After sample application the unbound xyloside-primed GAG were washed with 3 × 0.2 mL of 4 M guanidine–HCl, 50 mM NaOAc, pH 5.8. Radioactive samples were precipitated with 5 vol of 95% ethanol overnight at −20 °C using 100 μg of dextran as carrier. After centrifugation in a Beckman JS-7.5 at 4000 rpm for 30 min, material was dissolved in 4 M guanidine–HCl, 50 mM NaOAc, 0.2% Triton X-100, pH 5.8 followed by gel permeation FPLC on Superose 6. Radioactivity was determined in a β-counter.

4.3.4. In vitro growth assay using crystal violet method

Cells were harvested by trypsinization and seeded into 96-well microculture plates at 5000 cells/well in MEM supplemented with 10% fetal calf serum, 1% L-glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL) or RPMI-1640 supplemented with 10% fetal calf serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). After 24 h of plating the cells were allowed to proliferate in serum-free Ham's F-12 medium supplemented with insulin (10 μg/mL) and transferrin (25 μg/mL) in the presence of increasing concentrations of xyloside. Controls without xylosides were included. The total exposure-time was 96 h. Cells were then fixed in 1% glutaraldehyde dissolved in Hanks' balanced salt solution (NaCl 80 g/L, KCl 4 g/L, glucose 10 g/L, KH_2PO_4 600 mg/L, $NaHPO_4$ 475 mg/L) for 20 min, then the cells nuclei were stained with 0.1% crystal violet. After washing and cell lysis for 24 h in 1% Triton X-100, the amount of bound dye was measured at A_{600nm} in a microplate photometer (Titertek multiscan). The inhibitory effect of the compounds is expressed as the percentage of growth in the absence of drugs.

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Supplementary data

Supplementary data (experimental details, ^{1}H NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.007.

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