Efficient Synthesis of 4-Amino-4-deoxy-L-arabinose and Spacer-Equipped 4-Amino-4-deoxy-L-arabinopyranosides by Transglycosylation Reactions

Bernhard Müller,[a] Markus Blaukopf,[a] Andreas Hofinger,[a] Alla Zamyatina,[a] Helmut Brade,[b] Paul Kosma*[a]

[a] Department of Chemistry, University of Natural Resources and Applied Life Sciences-Vienna, Muthgasse 18, 1190 Vienna, Austria

[b] Research Center Borstel, Leibniz Center for Medicine and Biosciences, Parkallee 22, 23845 Borstel, Germany
Fax +43(1)476546059; E-mail: paul.kosma@boku.ac.at

Received 21 April 2010

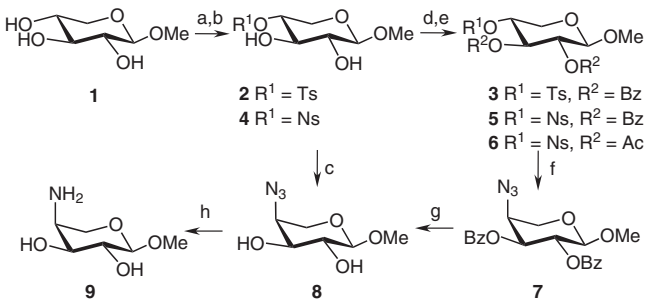
Abstract: Methyl 4-azido-4-deoxy-β-L-arabinopyranoside has been synthesized in five steps starting from methyl β-D-xylopyranoside in a multigram scale without chromatographic purification in 78% overall yield. The transformation relied on selective tosylation/nosylation at O-4 followed by acylation, S_N2 displacement with sodium azide, and subsequent deprotection. The methyl 4-azido-4-deoxy-arabinoside was then converted into allyl, propenyl, ω-bromohexyl, and chloroethoxyethyl spacer glycosides by transglycosylation with the respective alcohols in good yields and fair anomeric selectivity. Reduction of the azido group and further transformations of the aglycone afforded ω-thiol-containing spacer derivatives. Coupling to maleimide-activated BSA provided a potent immunogen, which was used to generate murine and rabbit polyclonal sera binding to LPS-core epitopes containing 4-amino-4-deoxy-arabinose residues.

Key words: aminoarabinose, neoglycoconjugate, transglycosylation, lipopolysaccharide, spacer

4-Amino-4-deoxy-L-arabinose (Ara4N) is an important sugar constituent of complex glycolipids constituting the outer leaflet of the cell wall of Gram-negative bacteria. Specifically, Ara4N has been detected in substoichiometric up to stoichiometric amounts ester-linked to the 1- and 4′-phosphate groups of the glucosamine disaccharide backbone of lipid A, which inserts the lipopolysaccharide (LPS) chain into the outer membrane.[1–3] In addition, Ara4N residues have been found glycosidically linked to position 8 of the higher carbon sugar 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) as in the core region of *Proteus* strains[4,5] and also linked to D-*glycero*-D-*talo*-oct-2-ulosonic acid (Ko) detected in the core region of *Burkholderia*,[6–9] as well as in a *Serratia marcescens* strain.[10] The incorporation of Ara4N has been implicated in the onset and maintenance of antibiotic resistance in bacteria by masking the anionic charges of the phosphate and carboxylic acid groups.[11] In order to study the antigenic properties of Ara4N-substituted LPS domains and develop monoclonal antibodies for diagnostic applications, a high-yielding approach to generate sufficient amounts of Ara4N building blocks was needed. Furthermore, to produce neoglycoconjugates carrying covalently linked Ara4N ligands, suitable spacer groups should be introduced allowing for a selective attachment onto the protein carrier. The conjugation chemistry has to be compatible with the presence of both amino and carboxylic acid groups in order to retain the antigenic properties of LPS core sugars. The selection of conjugation conditions would also have to take into account the labile phosphodiester linkages of Ara4N as well as the acid-labile glycosidic linkages of Kdo residues.

In this communication, we describe the preparation of Ara4N building blocks in multigram amounts as well as their conversion into a thiol-equipped spacer derivative, which was coupled to bovine serum albumin (BSA).

Previously, Ara4N has been prepared from methyl β-D-xylopyranoside via isopropylidene protection of O-2 and O-3 followed by triflation of O-4 and subsequent inversion.[12,13] Since in our hands the formation of the acetonide turned out to be unsuitable for large-scale preparation of the compound, we resorted to regioselective tosylation of O-4 via the intermediate stannylene acetal.[14–18] Thus, reaction of the commercially available methyl xyloside **1** with dibutyltin oxide and *p*-toluenesulfonyl chloride in 1,4-dioxane afforded the 4-*O*-tosylate **2** in 99% yield, followed by benzoylation with benzoyl chloride in pyridine, which gave the known dibenzoate **3** in 92% yield.[19] (Scheme 1). As an alternative option, the 4-*O*-nosylate **4** was prepared from **1** in 99% yield.[20] The subsequent conversion of nosylate **4** into the dibenzoate **5**, however, suffered from reduced yields, whereas the acetylation was smoothly effected in 93% yield to give the 2,3-di-*O*-



Scheme 1 *Reagents and conditions*: (a) Bu_2SnO, toluene, reflux, 5 h, then TsCl, 1,4-dioxane, r.t., 48 h, 99% yield for **2**, or (b) NsCl, dioxane, 20 h r.t., 99% yield for **4**; (c) from **4**: NaN_3, DMSO, 45 °C, 6 h, 85% yield for **8** (Method B); (d) BzCl, pyridine, r.t., 15 h, 92% yield for **3**, 47% yield for **5**; (e) from **4**: Ac_2O, pyridine, 0 °C, 2 h, 93% yield for **6**; (f) from **3**: NaN_3, DMSO, 110 °C, 15 h, 93%; (g) 0.1 M NaOMe, MeOH, r.t., 1 h, 93%; (h) 10% Pd/C, MeOH, H_2, r.t., 8 h, 99%.

SYNTHESIS 2010, No. 18, pp 3143–3151
Advanced online publication: 16.07.2010
DOI: 10.1055/s-0030-1258174; Art ID: P06510SS

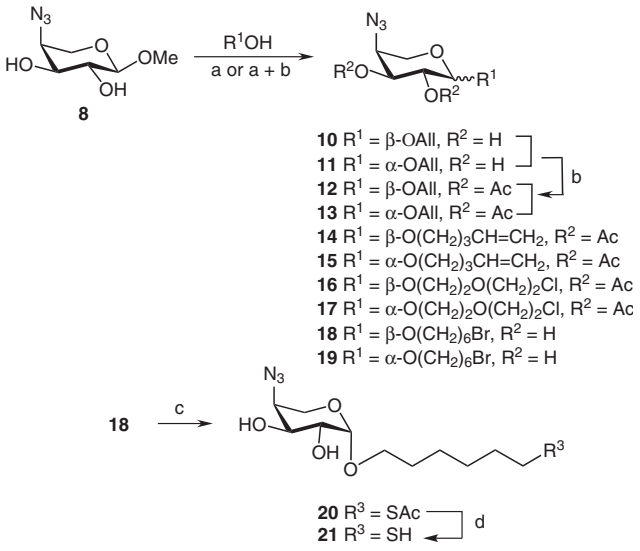
acetyl-4-*O*-nosyl derivative **6**. The introduction of the 4-azido group was achieved uneventfully by treatment of **3** with sodium azide in DMSO at 110 °C to furnish methyl α-L-*arabino*-pyranoside **7** in 93% yield. Transesterification of the benzoate groups under Zemplén conditions then afforded the 4-azido derivative **8** in 93% yield.

In addition, direct conversion of the unprotected nosylate **4** into compound **8** could be achieved in 85% yield. This three-step approach required a chromatography purification thereby limiting a large-scale application.

Reduction of the azide-group of **8** was accomplished by hydrogenation to furnish methyl 4-amino-4-deoxy-α-L-*arabino*-pyranoside **9** in 99% yield.

The synthesis of **8** could thus be achieved in multigram amounts via **7** without the need of chromatography and more than twice overall yield to previous reports.[12] The methyl glycoside **8** constitutes a suitable precursor for further transformations into glycosyl donors as well as spacer glycosides. Whereas acid hydrolysis of the methyl glycoside **8** under various conditions suffered from reduced yields and side reactions, transglycosylation has turned out as a versatile approach for direct conversion into glycosides.

Thus, an anomeric mixture of allyl arabinosides **10** and **11** was generated from the methyl glycoside precursor **8** by reaction with excess allyl alcohol and 1 M ethereal HCl at 60 °C (Table 1, entry 1, Scheme 2). The glycosides were obtained in a combined yield of 55% with a higher proportion of the axially oriented β-glycoside **10** (ratio of **10**:**11** ~2:1).



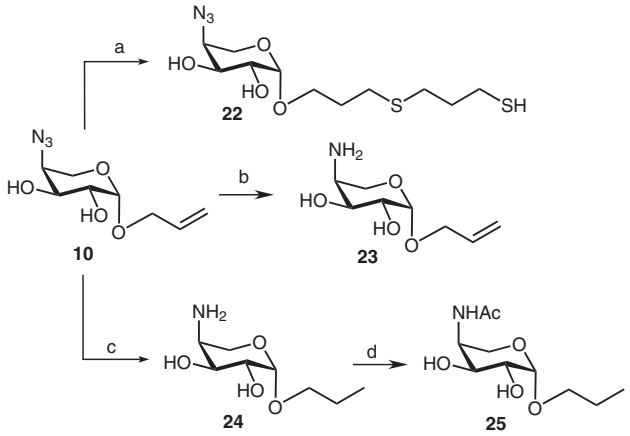
Scheme 2 *Reagents and conditions*: (a) see Table 1; (b) Ac_2O, pyridine, 0 °C, 2 h, 95% yield for **12/13**; (c) KSAc, DMF, r.t., 15 h, 83%; (d) 0.2 M NaOH, 1.5 h, r.t., 87%.

Reaction of the methyl glycoside **8** with acetyl chloride and excess of allyl alcohol proceeded smoothly at room temperature and gave a higher product yield (Table 1, entry 2) due to less by-product formation. Also, these conditions resulted in slightly higher enrichment of the respective β-anomers (entries 3 and 4). Similarly, the pent-4-enyl glycosides **14**, **15**, the (2-chloroethoxy)ethyl derivatives **16**, **17**, and the ω-bromohexyl glycosides **18** and **19** were obtained in satisfactory yields (Table 1, entries 3–6). The anomeric mixtures of **10/11** and **18/19** could be partially resolved by HPLC-separation, whereas the acetylated allyl glycosides **12** and **13** were amenable to separation on a standard silica gel phase. The pent-4-enyl derivatives **14/15** and the (2-chloroethoxy)ethyl derivatives **16/17** were also isolated as the 2,3-di-*O*-acetyl derivatives in order to allow for a facile separation of the excess alcohol components via their respective acetates. The assignment of the anomeric configuration of the β-glycosides **10**, **12**, **14**, **16**, and **18** was based on the low-field shifted ^1H NMR signals of the anomeric proton (in the range of δ = 5.19–4.85) and the small value of the homonuclear coupling constant ($J_{1,2}$ = ~3.6 Hz). In addition, the values of the optical rotation showed a higher dextrorotation for the axial glycosides **10**, **12**, **14**, and **18** in comparison to their equatorial counterparts.

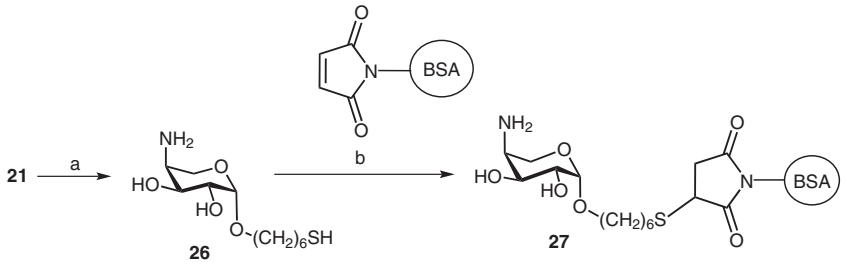
Table 1 Conditions and Yields for Transglycosylation Reactions

Entry	R^1 of R^1OH	Acid	Temp (°C)	Time (h)	Yield (%)	α/β
1	All[a]	HCl[b]	60/r.t.	4/8	55	1:2.0
2	All	AcCl	r.t.	18	76	1:2.3
3	$(CH_2)_3CH=CH_2$	AcCl	r.t.	24	70[c]	1:3.1
4	$(CH_2)_2O(CH_2)_2Cl$	HCl	80/r.t.	1/18	77	1:2.0
5	$(CH_2)_2O(CH_2)_2Cl$	AcCl	r.t.	22	72[c]	1:2.9
6	$(CH_2)_6Br$	HCl	70/r.t.	4/18	72	1:3.5
7	$(CH_2)_6Br$	AcCl	r.t.	18	65	1:3.2

[a] All = allyl.
[b] 1 M solution in Et_2O.
[c] Isolated as 2,3-di-*O*-acetate.



Scheme 3 *Reagents and conditions*: (a) $HS(CH_2)_3SH$, *hν*, MeOH, r.t., h, 65%; (b) $HS(CH_2)_3SH$, aq pyridine, r.t., 15 h, 91%; (c) 10% Pd/C, MeOH, H_2, r.t., 18 h, 97%; (d) Ac_2O, pyridine, r.t. 36 h, then 0.1 M NaOMe, MeOH, r.t., 15 h, 95%.



Scheme 4 *Reagents and conditions*: (a) $HS(CH_2)_3SH$, aq pyridine, r.t., 15 h, 80% yield; (b) aq buffer (pH 6.6), 4 °C, 16 h.

In order to introduce reactive end groups for subsequent conjugation, the terminal bromide group of the glycoside derivative **18** was exchanged for a thioacetyl group by reaction with KSAc in DMF to afford the ω-thioacetyl derivative **20** in 83% yield. Finally, the *S*-acetyl group was hydrolyzed under alkaline conditions and with exclusion of oxygen to furnish the ω-thiol compound **21** with minimal dimer formation (Scheme 2).

Notably, the allyl aglycone in compound **10** could also be diversified with respect to spacer elongation (Scheme 3).

Reaction of **10** with propane-1,3-dithiol under UV irradiation led to chain elongation and provided the thioether bridged ω-thiol derivative **22** in 65% yield (Scheme 3). A small portion of the respective dimer was also present in the product mixture. This strategy thus broadens the scope of using allyl glycosides as precursor of spacer glycosides, which have previously been obtained by reaction with cysteamine, cysteine, by epoxide formation, or via oxidative cleavage of the double bond. Alternatively, selective reduction of the azido group leaving the allyl group unaffected could be accomplished by reaction of **10** with propane-1,3-dithiol in the absence of UV irradiation in aqueous pyridine to give compound **23** in 91% yield.[21]

As additional haptenic ligands to be used in inhibition studies, the 4-amino-4-deoxy-derivative **24** and the N-acetylated derivative **25** were synthesized. Thus, the propyl 4-amino-4-deoxy-glycoside **24** was prepared by reduction of the 4-azido group with concomitant saturation of the allyl group by hydrogenation on palladium/charcoal in 97% yield. The ^{13}C NMR data of the glycoside **24** compare favorably with those reported for arabinoside units in LPS core and lipid A oligosaccharides, confirming the configurational assignments.[7] The *N*-acetyl derivative **25**

Table 2 Serum Antibody Titers by ELISA[a] in Rabbits and Mice after Immunization with Ara4N-BSA (**27**)

Animal	**28**	BSA	*E. coli*	*Proteus mirabilis*	*Burkh. cepacia*	*Salmon. enterica*
Rabbit						
K446[b]	<1000	<1000	<500	<500	<500	<500
K446[c]	1.024.000	<1000	<500	8.000	64.000	<500
K447[b]	1.000	<1000	<500	<500	<500	<500
K447[c]	256.000	<1000	<500	1000	32.000	<500
Mouse						
M1[d]	512.000	<1000	<100	100	<100	100
M2	128.000	<1000	<100	100	<100	<100
M3	256.000	<1000	100	100	<100	100
M4	<1000	<1000	<100	<100	<100	<100
M5	128.000	<1000	<100	<100	<100	<100
M6	512.000	<1000	100	800	800	<100
M7	256.000	<1000	100	100	100	<100
M8	1.024.000	<1000	100	3200	200	100
M9	64.000	<1000	<100	<100	100	<100
M10	256.000	<1000	100	100	100	100

[a] Final dilution yielding OD405 >0.2 with immobilized BSA, **27**, or LPS.
[b] Before immunization.
[c] 56 days after immunization.
[d] 35 days after immunization.

was produced in 95% yield via peracetylation of **24** followed by Zemplén de-O-acetylation.

For the synthesis of the neoglycoconjugate **27**, the 4-azido-4-deoxy spacer derivative **21** was converted into the corresponding 4-amino compound **26** by reaction with propane-1,3-dithiol in 81% yield (Scheme 4). The material contained ca. 15% of the corresponding disulfide and was directly used for the conjugation step. Coupling of the thiol-containing ligand to maleimide-activated BSA at pH 7.4 afforded the neoglycoprotein **27**. MALDI MS analysis of the Ara4N-BSA product indicated a high ligand density of ~34 mol ligand/mol BSA.

Immunization of rabbits and mice with Ara4N-BSA resulted in a specific response in all animals except one mouse against the immunizing antigen as measured by ELISA. The titers reached values of up to 1 million whereby no reactivity (<1000) was seen with BSA alone (Table 2). We also tested a panel of bacterial LPS known to contain Ara4N in a terminal position. No reactivity was observed with R*e*-mutant LPS of *E. coli*, which is not substituted with Ara4N, and LPS of *S. enterica* serovar Minnesota, which carries Ara4N on the 4′-phosphate of the lipid A backbone in nonstoichiometric amounts. However, both rabbits developed antibodies against the LPS of *P. mirabilis* and *B. cepacia* whereby the titers against the latter antigen were significantly higher. From 10 mice only two (animal M6 and M8) had developed antibodies against LPS of *P. mirabilis* and *B. cepacia*. Interestingly, animal M8 had a higher titer against *P. mirabilis* LPS than against *B. cepacia* LPS of 3200 and 800, respectively.

Maleimide-activated BSA was purchased from Sigma Aldrich. Known compounds were identified by comparison with reported melting points as well as ^{1}H and ^{13}C NMR data. Melting points were determined with a Kofler hot stage microscope and are uncorrected. Optical rotations were measured with a Perkin-Elmer 243 B polarimeter. $[\alpha]_D^{20}$ Values are given in units of 10^{-1} degcm^2g^{-1}. ^{1}H NMR spectra were recorded at 297 K with a Bruker DPX instrument operating at 300 MHz or 400 MHz for ^{1}H using $CDCl_3$ as solvent and TMS as standard, unless otherwise stated. ^{13}C NMR spectra were measured at 75.47 or 100.62 MHz and referenced to 1,4-dioxane (δ = 67.40). Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software. MALDI-TOF-MS ionization spectra were recorded in the positive ion mode, with sinapic acid as matrix. TLC was performed on Merck precoated plates (5 × 10 cm, layer thickness 0.25 mm, silica gel 60 F_{254}); spots were detected by spraying with anisaldehyde/H_2SO_4. For column chromatography, silica gel (0.040–0.063 mm) was used. Concentration of solutions was performed at reduced pressure at temperatures <40 °C. Elemental analyses were provided by Dr. J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien. LPS of R*e* mutants of *E. coli* (strain F576), *P. mirabilis* (strain R45) and *S. enterica* serovar Minnesota (strain R595) and LPS from *B. cepacia* (strain Ko2b) were obtained by extraction of bacteria with phenol–chloroform–light petroleum ether as described.[22]

Methyl 4-*O*-*p*-Toluenesulfonyl-β-D-xylopyranoside (2); Large-Scale Preparation

Commercially available methyl β-D-xylopyranoside (**1**; 25.00 g, 152.3 mmol) and Bu_2SnO (37.92 g, 152.3 mmol) were suspended in anhyd toluene (500 mL) and heated to reflux with a Dean–Stark trap. Full conversion was accomplished when 2.74 mL of H_2O had been collected after 5 h. The reaction mixture was cooled to 40 °C and concentrated. The remaining, slightly yellow solid was dried under reduced pressure overnight and used without further purification. A solution of TsCl (28.95 g, 151.85 mmol) in anhyd 1,4-dioxane (110 mL) was added dropwise with cooling to a suspension of the stannylidene derivative in anhyd 1,4-dioxane (400 mL). The reaction mixture was stirred for 48 h at r.t. SiO_2 (25 g) was added and the solvent was removed. The remaining, slightly yellowish solid was purified by flash chromatography first with toluene (1.2 L) followed by elution with EtOAc (1.2 L). The EtOAc fraction was concentrated until a slightly yellow oil remained, which crystallized overnight. Crystallization from *n*-hexane–EtOAc gave **2** (48.16 g, 99%) as colorless needles; mp 128–130 °C (Lit.[18] mp 135–136 °C).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.82 (d, *J* = 12.0 Hz, 2 H_{arom}), 7.36 (d, *J* = 12.0 Hz, 2 H_{arom}), 4.36 (dt, *J* = 4.5, 8.0 Hz, 1 H, H-4), 4.23 (d, *J* = 6.5 Hz, 1 H, H-1), 4.05 (dd, *J* = 4.8, 12.2 Hz, 1 H, H-5$_{eq}$), 3.68 (dt, *J* = 4.5, 8.0 Hz, 1 H, H-3), 3.49 (s, 3 H, OCH_3), 3.35–3.44 (m, 2 H, H-2, H-5$_{ax}$), 3.04–3.12 (br s, 1 H, 3-OH), 2.81–2.92 (br s, 1 H, 2-OH), 2.45 (s, 3 H, ArCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 145.44 (C1-Ar), 132.84 (C4-Ar), 130.01 (C3-, C5-Ar), 128.05 (C2-, C6-Ar), 103.27 (C-1), 77.58 (C-4), 72.39 (C-2, C-3), 62.05 (C-5), 56.97 (OCH_3), 21.70 (ArCH_3).

Methyl 2,3-Di-*O*-benzoyl-4-*O*-*p*-toluenesulfonyl-β-D-xylopyranoside (3)

Benzoyl chloride (2.30 mL, 9.71 mmol) was added dropwise to a stirred solution of **2** (3.00 g, 9.4 mmol) in anhyd pyridine (15 mL) at 0 °C. The colorless solution turned pink and a colorless solid precipitated. The ice-bath was removed and the reaction mixture was stirred overnight at r.t. The mixture was dissolved in $CHCl_3$ (50 mL) and washed with H_2O (50 mL). The aqueous layer was extracted with $CHCl_3$ (40 mL), the organic were layers combined, washed with aq 0.1 M HCl (50 mL) and sat. aq $NaHCO_3$ (50 mL), and dried ($MgSO_4$). The solution was concentrated and the solid residue was crystallized from *n*-hexane–EtOAc to give **3**; yield: 4.34 g (92%); colorless solid; mp 138–140 °C (Lit.[19] mp 139–141 °C); $[\alpha]_D^{20}$ +39.5 (*c* 0.31, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.93–7.87 (m, 2 H_{arom}), 7.68 (d, *J* = 8.3 Hz, 2 H_{arom}), 7.61 (d, *J* = 8.3 Hz, 2 H_{arom}), 7.53–7.45 (m, 2 H_{arom}), 7.37–7.28 (m, 4 H_{arom}), 6.95 (d, *J* = 8.3 Hz, 2 H_{arom}), 5.57 (t, *J* = 8.7 Hz, 1 H, H-3), 5.24 (dd, *J* = 6.8, 8.8 Hz, 1 H, H-2), 4.70–4.60 (m, 1 H, H-4), 4.58 (d, *J* = 6.8 Hz, 1 H, H-1), 4.38 (dd, *J* = 5.1, *J* = 12.0 Hz, 1 H, H-5$_{eq}$), 3.69 (dd, *J* = 12.0, 8.8 Hz, 1 H, H-5$_{ax}$), 3.47 (s, 3 H, OCH_3), 2.17 (s, 3 H, ArCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.00, 164.74 (C=O), 144.92, 133.23, 133.17, 132.45, 129.73, 129.71, 128.91, 128.60, 128.26, 128.11, 127.57 (C-Ar), 101.59 (C-1), 74.93 (C-4), 70.73 (C-2, C-3), 62.86 (C-5), 56.91 (OCH_3), 21.50 (ArCH_3).

Methyl 4-*O*-4-Nitrobenzenesulfonyl-β-D-xylopyranoside (4)

Compound **4** was prepared according to the procedure described for **2** from the stannylidene intermediate (4.00 g, 10.1 mmol) and nosyl chloride (2.20 g, 9.9 mmol) in dioxane (30 mL) with stirring for 20 h at r.t. and worked up as described; yield: 3.46 g (99%); colorless solid; mp 64–67 °C (Lit.[20] mp 62–65 °C).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (d, *J* = 9.1 Hz, 2 H_{arom}), 8.17 (d, *J* = 9.1 Hz, 2 H_{arom}), 4.39 (dt, *J* = 5.4, 9.3 Hz, 1 H, H-4), 4.14 (dd, *J* = 5.2, 11.5 Hz, 1 H, H-5$_{eq}$), 4.13 (d, *J* = 7.4 Hz, 1 H, H-1), 3.57 (app. t, *J* = 8.9 Hz, 1 H, H-3), 3.51 (s, 3 H, OCH_3), 3.43 (dd, *J* = 9.8, 11.8 Hz, 1 H, H-5$_{ax}$), 3.36 (br s, 2 H, 2-OH, 3-OH), 3.22 (dd, *J* = 7.4, 9.0 Hz, 1 H, H-2).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.65 (C4-Ar), 141.71 (C1-Ar), 129.40 (C3-, C5-Ar), 124.09 (C2-, C6-Ar), 103.88 (C-1), 79.11 (C-4), 73.24 (C-2), 72.90 (C-3), 62.88 (C-5), 56.95 (OCH_3).

Methyl 2,3-Di-*O*-benzoyl-4-*O*-4-nitrobenzenesulfonyl-β-D-xylopyranoside (5)

Compound **5** was prepared from **4** (1.71 g, 3.89 mmol) and benzoyl chloride (1.16 mL, 9.71 mmol) in anhyd pyridine (12 mL) with stirring for 4 h at r.t. according to the procedure described for **3**. Workup as described for **3** gave compound **5**; yield: 1.29 g (47%); colorless crystals; mp 151–152 °C (*n*-hexane); $[\alpha]_D^{20}$ +6.7 (*c* 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20–7.23 (m, 14 H_{arom}), 5.57 (t, *J* = 8.6 Hz, 1 H, H-3), 5.26 (dd, *J* = 6.8, 8.8 Hz, 1 H, H-2), 4.79 (dt, *J* = 5.1 Hz, 1 H, H-4), 4.61 (d, *J* = 6.8 Hz, 1 H, H-1), 4.17 (dd, *J* = 5.1, 12.1 Hz, 1 H, H-5$_{eq}$), 3.75 (dd, *J* = 12.1, 8.8 Hz, 1 H, H-5$_{ax}$), 3.50 (s, 3 H, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.48, 164.92 (C=O), 150.17, 141.30, 133.88, 133.37, 129.71, 129.44, 128.73, 128.33, 128.28, 128.03, 124.21 (C2-, C6-Ar), 101.67 (C-1), 76.57 (C-4), 70.72 (C-3), 70.60 (C-2), 62.90 (C-5), 57.00 (OCH_3).

Anal. Calcd for $C_{26}H_{23}NO_{11}S$: C, 55.75; H, 3.95; N, 2.51. Found: C, 56.01; H, 4.16; N, 2.51.

Methyl 2,3-Di-*O*-acetyl 4-*O*-4-nitrobenzenesulfonyl-β-D-xylopyranoside (6)

Compound **6** was prepared from **4** (3.00 g, 8.6 mmol) and Ac_2O (15 mL, 132 mmol) in anhyd pyridine (15 mL) with stirring for 2 h at r.t. The formed precipitate was filtered off and the filtrate was concentrated to 1/3 volume, which led to additional precipitation. Solids were combined and dried at 40 °C under reduced pressure to afford **6**; yield: 3.4 g (93%); colorless crystals; mp 157 °C (*n*-hexane); $[\alpha]_D^{20}$ –65.7 (*c* 0.4, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, *J* = 8.8 Hz, 2 H_{arom}), 8.11 (d, *J* = 8.8 Hz, 2 H_{arom}), 5.15 (app. t, *J* = 8.5 Hz, 1 H, H-3), 4.84 (dd, *J* = 7.0, 8.5 Hz, 1 H, H-2), 4.67 (dt, *J* = 5.1, 8.6 Hz, 1 H, H-4), 4.38 (d, *J* = 6.8 Hz, 1 H, H-1), 4.17 (dd, *J* = 5.1, 11.9 Hz, 1 H, H-5$_{eq}$), 3.51 (dd, *J* = 12.1, 9.1 Hz, 1 H, H-5$_{ax}$), 3.46 (s, 3 H, OCH_3), 2.03 (s, 3 H, 2-OAc), 1.85 (s, 3 H, 3-OAc).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.50, 169.38 (C=O), 150.95 ($ArNO_2$), 141.75 (C1-Ar), 129.24 (C3-, C5-Ar), 124.52 (C2-, C6-Ar), 101.47 (C-1), 75.68 (C-4), 70.82 (C-3), 70.64 (C-2), 62.36 (C-5), 56.90 (OCH_3), 20.61 ($COCH_3$), 20.46 ($COCH_3$).

Anal. Calcd for $C_{16}H_{19}NO_{11}S$: C, 44.34; H, 4.42; N, 3.23. Found: C, 44.00; H, 4.23; N, 3.22.

Methyl 4-Azido-2,3-di-*O*-benzoyl-4-deoxy-α-L-arabinopyranoside (7)

Compound **3** (69.15 g, 131 mmol) and NaN_3 (13.7 g, 210.6 mmol) were suspended in anhyd DMSO (280 mL) and stirred at 90 °C overnight. The reaction mixture was dissolved in $CHCl_3$ (0.8 L) and washed with H_2O (3 × 400 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ (400 mL) and brine (400 mL), dried (Na_2SO_4), and concentrated. The residual oil was dissolved in EtOAc (20 mL) and *n*-hexane was added until a colorless solid precipitated. The crystals were collected and the filtrate was evaporated to dryness and to afford an additional crop. Crystallization from 2:1 *n*-hexane–EtOAc afforded **7**; yield: 48.64 g (93%); mp 104–105 °C (EtOH); $[\alpha]_D^{20}$ +18.6 (*c* 0.3, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.06–7.97 (m, 4 H_{arom}), 7.58–7.50 (m, 2 H_{arom}), 7.45–7.38 (m, 4 H_{arom}), 5.58 (dd, *J* = 5.4, 7.9 Hz, 1 H, H-2), 5.51 (dd, *J* = 3.2, 7.9 Hz, 1 H, H-3), 4.60 (d, *J* = 5.6 Hz, 1 H, H-1), 4.22–4.14 (m, 2 H, H-4, H-5$_{eq}$), 3.84–3.75 (m, *J* = 4.3, 13.8 Hz, 1 H, H-5$_{ax}$), 3.50 (s, 3 H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.63, 165.03 (C=O), 133.57, 133.32, 130.01, 129.80, 129.30, 128.78, 128.48, 128.39, (C-Ar), 101.05 (C-1), 71.47 (C-3), 69.18 (C-2), 61.66 (C-5), 57.56 (C-4), 56.45 (OCH_3).

Anal. Calcd for $C_{20}H_{19}N_3O_6$: C, 60.45; H, 4.82; N, 10.57. Found: C, 60.58; H, 4.78; N, 10.36.

*Synthesis of **7** under Microwave Irradiation*: Compound **3** (3.70 g, 7.03 mmol) and NaN_3 (0.69 g, 10.6 mmol) were suspended in anhyd DMSO (10 mL) and heated in a MSL Microwave to 110 °C (max. 250 W). After 30 min, a second portion of NaN_3 (200 mg) was added and heating was continued for 15 h. Cooling to r.t and workup as described above afforded **7** (2.26 g, 81%) as colorless needles.

Methyl 4-Azido-4-deoxy-α-L-arabinopyranoside (8)

*Method A, from **7***: Azide **7** (47.42 g, 119.3 mmol) was suspended in anhyd MeOH (300 mL). A 1 M solution of methanolic NaOMe (12 mL) was added and the mixture was stirred for 1 h at r.t. The solution was made neutral by adding DOWEX 50 H^+ resin. The resin was removed by filtration and the filtrate was concentrated. *n*-Hexane (200 mL) was added, the suspension was stirred overnight and the solid product **8** was collected by filtration and dried; yield: 20.97 g (93%); colorless solid; mp 94–95 °C {Lit.[12] mp 94–95 °C); $[\alpha]_D^{20}$ –22.6 (*c* 0.3, $CHCl_3$); Lit.[8] –26.1 (*c* 0.5, $CHCl_3$)}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.48–5.43 (m, 1 H, 3-OH), 5.25–5.20 (m, 1 H, 2-OH), 4.04 (d, *J* = 7.2 Hz, 1 H, H-1), 3.83–3.71 (m, 2 H, H-4, H-5$_{eq}$), 3.70–3.60 (m, 1 H, H-3), 3.56–3.48 (m, 1 H, H-5$_{ax}$), 3.38 (s, 3 H, OCH_3), 3.34–3.24 (m, 1 H, H-2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 104.33 (C-1), 72.54 (C-3), 70.66 (C-2), 63.03 (C-5), 61.13 (C-4), 55.84 (OCH_3).

*Method B, from **4***: Nosylate **4** (0.200 g, 0.57 mmol) and NaN_3 (0.40 g, 6.15 mmol) were suspended in anhyd DMSO (1 mL) and stirred at 45 °C for 5 h. The reaction mixture was dissolved in $CHCl_3$ (30 mL) and washed with H_2O (20 mL), sat. aq $NaHCO_3$ (2 × 20 mL), and brine (15 mL). The organic layer was dried ($MgSO_4$) and concentrated. Purification of the residue by column chromatography (*n*-hexane–EtOAc, 3:1) afforded **8**; yield: 0.092 g (85%); colorless solid.

Methyl 4-Amino-4-deoxy-α-L-arabinopyranoside (9)

Pd/C (10 mol%) was added to a solution of azide **8** (308 mg, 1.63 mmol) in MeOH (25 mL) and the suspension was stirred at r.t. overnight under H_2 at atmospheric pressure. The catalyst was removed by filtration over Celite and the filtrate was concentrated and dried to give **9**; yield: 264 mg (99%); colorless syrup; $[\alpha]_D^{20}$ –36.4 (*c* 0.31, MeOH).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.33 (d, *J* = 6.9 Hz, 1 H, H-1), 3.86 (dd, *J* = 3.2, 12.4 Hz, 1 H, H-5$_{eq}$), 3.76 (dd, *J* = 4.2, 8.8 Hz, 1 H, H-3), 3.71 (dd, *J* = 2.4, 12.4 Hz, 1 H, H-5$_{ax}$), 3.56 (s, 3 H, OCH_3), 3.52–3.59 (m, 1 H, H-2), 3.08–3.14 (m, 1 H, H-4).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 103.84 (C-1), 72.07 (C-3), 70.19 (C-2), 65.29 (C-5), 56.88 (OCH_3), 49.72 (C-4).

Allyl 4-Azido-4-deoxy-β-L-arabinopyranoside (10) and Allyl 4-Azido-4-deoxy-α-L-arabinopyranoside (11)

Method A: Azide **8** (1.03 g, 5.44 mmol) was dissolved in anhyd allyl alcohol (3 mL) and a 1 M solution of HCl in Et_2O (2.0 mL) was added. The solution was stirred for 4 h at 60 °C and for further 18 h at r.t. The brownish reaction mixture was diluted with EtOAc (30 mL), neutralized with sat. aq $NaHCO_3$ (30 mL), and washed with brine (20 mL). The solvent was removed and the remaining yellowish oil was purified by column chromatography (100 g SiO_2, 10 μm, toluene–EtOAc, 3.5:1). The product-containing fractions were pooled and concentrated to afford **10**.

10

Yield: 320 mg (27%); R_f = 0.66 (EtOAc); mp 32–33 °C; $[\alpha]_D^{20}$ +192.4 (*c* 0.4, $CHCl_3$).

^{1}H NMR (400 MHz, CD_3OD): δ = 5.93–6.04 (m, 1 H, =CH), 5.35 (qd, *J* = 1.7, 17.2 Hz, 1 H, CH=CH_{2trans}), 5.21 (qd, *J* = 1.5, 10.4 Hz,

1 H, CH=CH_{2cis}), 4.85 (d, J = 3.6 Hz, 1 H, H-1), 4.20 (tdd, J = 1.5, 5.2, 13.0 Hz, 1 H, OCH_2), 4.04 (dd, J = 3.6, 9.6 Hz, 1 H, H-3), 4.01–4.08 (m, 1 H, OCH_2), 3.86–3.93 (m, 2 H, H-5$_{eq}$, H-4), 3.75 (dd, J = 3.6, 9.7 Hz, 1 H, H-2), 3.62 (dd, J = 2.6, 12.8 Hz, 1 H, H-5$_{ax}$).

^{13}C NMR (100 MHz, CD_3OD): δ = 133.37 (=CH), 118.02 (=CH_2), 97.70 (C-1), 70.41 (C-3), 69.75 (C-2), 68.74 (OCH_2), 61.42 (C-4), 60.85 (C-5).

Anal. Calcd for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53. Found: C, 44.68; H, 5.79; N, 18.79.

Further elution provided a mixture of **10** and **11**; yield: 233 mg (20%), followed by **11**.

11

Yield: 98 mg (8%); R_f = 0.55 (EtOAc); mp 39–42 °C; $[\alpha]_D^{20}$ +61.8 (c 0.51, $CHCl_3$).

^{1}H NMR (400 MHz, CD_3OD): δ = 5.89–6.00 (m, 1 H, =CH), 5.32 (qd, J = 1.7, 17.3 Hz, 1 H, CH=CH_{2trans}), 5.16 (qd, J = 1.5, 10.4 Hz, 1 H, CH=CH_{2cis}), 4.29 (tdd, J = 1.5, 5.2, 12.9 Hz, 1 H, OCH_2), 4.22 (d, J = 7.2 Hz, 1 H, H-1), 4.09 (tq, J = 1.4, 6.3 Hz, 1 H, OCH_2), 3.89 (dd, J = 2.7, 12.7 Hz, 1 H, H-5$_{eq}$), 3.74 (dd, $J_{3,4}$ = 3.9 Hz, J = 8.8 Hz, 1 H, H-3), 3.76–3.80 (m, 1 H, H-4), 3.58 (dd, J = 1.8, 12.7 Hz, 1 H, H-5$_{ax}$), 3.50 (dd, 1 H, J = 7.2, 8.9 Hz, H-2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 133.48 (=CH), 118.21 (=CH_2), 101.70 (C-1), 72.60 (C-3), 71.23 (C-2), 69.95 (OCH_2), 62.98 (C-5), 60.06 (C-4).

Anal. Calcd for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53. Found: C, 44.51; H, 6.03; N, 19.22.

Method B: Freshly distilled AcCl (0.5 mL) was added at r.t. to a solution of **8** (400 mg; 2.11 mmol) in anhyd allyl alcohol (2.5 mL). Upon addition a strong exothermic reaction occurred. After cooling to r.t., the solution was stirred for 18 h. The slightly yellowish solution was diluted with EtOAc (30 mL), treated with sat. aq $NaHCO_3$ (2 × 20 mL), washed with brine (20 mL), and dried ($MgSO_4$). The solvent was removed and the remaining oil was purified by column chromatography (60 g, 63 μm SiO_2, *n*-hexane–EtOAc, 4:1). The product-containing fractions were pooled and concentrated to afford 522 mg (76%) **10** and **11** as a 2.3:1 anomeric mixture.

Allyl 2,3-Di-*O*-acetyl-4-azido-4-deoxy-β-L-arabinopyranoside (12) and Allyl 2,3-Di-*O*-acetyl-4-azido-4-deoxy-α-L-arabinopyranoside (13)

A solution of the anomeric mixture **10**,**11** (100 mg, 0.46 mmol) in anhyd pyridine (5 mL) and Ac_2O (500 μL, 3.9 mmol) was stirred at r.t. overnight. The solution was co-evaporated with toluene (2 × 20 mL) and concentrated. Purification of the residue on silica gel (toluene–EtOAc, 8:1) afforded first **12** as a colorless oil.

12

Yield: 25 mg (18%); colorless syrup; R_f = 0.55 (*n*-hexane–EtOAc, 3:1); $[\alpha]_D^{20}$ +187.8 (c 0.4, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.92–5.79 (m, 1 H, =CH), 5.40 (dd, J = 3.8, 10.5 Hz, 1 H, H-3), 5.30 (dq, 1 H, J = 1.6, 17.2 Hz, CH=CH_{2trans}), 5.21 (dq, 1 H, J = 1.3, 10.5 Hz, CH=CH_{2cis}), 5.20 (dd, J = 3.6, 10.5 Hz, 1 H, H-2), 5.09 (d, J = 3.6 Hz, 1 H, H-1), 4.19 (ddt, 1 H, J = 1.5, 5.1, 13.1 Hz, OCH_2), 4.11 (m, 1 H, H-4), 4.03–3.94 (m, 2 H, OCH_2, H-5$_{eq}$), 3.67 (dd, J = 2.0, 12.6 Hz, 1 H, H-5$_{ax}$), 2.12 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.22 and 170.17 (C=O), 133.23 (=CH), 117.84 (=CH_2), 95.51 (C-1), 69.28 (C-3), 68.56 (OCH_2), 68.20 (C-2), 60.01 (C-5), 59.86 (C-4), 20.78 and 20.59 (CH_3C=O).

Further elution of the column afforded **13**.

13

Yield: 10 mg (8%); R_f = 0.45 (*n*-hexane–EtOAc, 3:1); $[\alpha]_D^{20}$ +26.3 (c 0.2 $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.92–5.78 (m, 1 H, =CH), 5.28 (dq, J = 1.7, 17.2 Hz, 1 H, CH=CH_{2trans}), 5.19 (dq, J = 1.4, 10.4 Hz, 1 H, CH=CH_{2cis}), 5.17 (dd, J = 5.4, 7.9 Hz, 1 H, H-2), 5.07 (dd, J = 3.6, 7.7 Hz, 1 H, H-3), 4.49 (d, J = 5.4 Hz, 1 H, H-1), 4.28 (ddt, J = 1.6, 4.8, 13.2 Hz, 1 H, OCH_2), 4.05 (dd, J = 5.2, 12.0 Hz, 1 H, H-5$_{eq}$), 4.04 (ddt, J = 5.2, 12.0 Hz, 1 H, OCH_2), 3.96–3.90 (m, 1 H, H-4), 3.61 (dd, J = 2.6, 12.1 Hz, 1 H, H-5$_{ax}$), 2.12 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.59 and 169.50 (C=O), 133.43 (=CH), 117.23 (CH=CH_2), 98.65 (C-1), 71.06 (C-3), 69.11 (OCH_2), 68.63 (C-2), 61.36 (C-5), 56.98 (C-4), 20.78 and 20.63 ($COCH_3$).

ESI-TOF: m/z calcd for $C_{12}H_{17}O_6N_3$ + Na $[M + Na]^+$: 322.1010; found: 322.1024.

Pent-4-enyl 2,3-Di-*O*-acetyl-4-azido-4-deoxy-β-L-arabinopyranoside (14) and Pent-4-enyl 2,3-Di-*O*-acetyl-4-azido-4-deoxy-α-L-arabinopyranoside (15)

Compound **8** (400 mg, 2.11 mmol) and anhyd pent-4-en-1-ol (2.5 mL) was treated with freshly distilled AcCl (0.50 mL) and processed as described for the allyl derivatives **10** and **11** (Method B). Purification of the crude material by column chromatography (*n*-hexane–EtOAc, 4:1) afforded first a pure fraction of **14**.

14

Yield: 250 mg (34%); colorless oil; $[\alpha]_D^{20}$ +171.9 (c 0.36, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.87–5.72 (m, 1 H, =CH), 5.37 (dd, J = 3.8, 10.5 Hz, 1 H, H-3), 5.16 (dd, J = 3.6, 10.5 Hz, 1 H, H-2), 5.04 (d, J = 3.6 Hz, 1 H, H-1), 5.07–4.95 (m, 2 H, CH=CH_2), 4.12–4.07 (m, 1 H, H-4), 3.94 (dd, J = 1.9, 12.6 Hz, 1 H, H-5$_{eq}$), 3.62–3.73 (m, 2 H, CH_2O and H-5$_{ax}$), 3.45–3.35 (m, 1 H, CH_2O), 2.18–2.06 (m, 2 H, CH_2), 2.12 (s, 3 H, $COCH_3$), 2.07 (s, 3 H, $COCH_3$), 1.74–1.63 (m, 2 H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.18 and 170.28 (C=O), 137.76 (=CH), 115.21 (CH=CH_2), 96.41 (C-1), 69.39 (C-3), 68.42 (C-2), 67.75 (OCH_2), 59.92 (C-5), 59.86 (C-4), 30.09 and 28.49 (CH_2), 20.73 ($COCH_3$), 20.59 ($COCH_3$).

ESI-TOF: m/z calcd for $C_{14}H_{21}N_3O_6$ $[M + Na]^+$: 350.1323; found: 350.1328.

Further elution gave a fraction containing **14** and **15** (208 mg, 28%) and pure **15**.

15

Yield: 70 mg (9%); colorless oil; $[\alpha]_D^{20}$ –14.3 (c 0.21, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.87–5.72 (m, 1 H, CH=), 5.10–4.93 (m, 3 H, CH=CH_2, H-3), 5.37 (dd, J = 3.8, 10.5 Hz, 1 H, H-3), 5.14 (dd, J = 5.4, 7.7 Hz, 1 H, H-2), 4.44 (d, J = 5.3 Hz, 1 H, H-1), 4.03 (dd, J = 5.3, 12.1 Hz, 1 H, H-5$_{eq}$), 3.95–3.89 (m, 1 H, H-4), 3.81 (dt, J = 6.3, 9.5 Hz, 1 H, CH_2O), 3.62 (dd, J = 2.6, 12.1 Hz, 1 H, H-5$_{ax}$), 3.44 (dt, J = 6.4, 9.5 Hz, 1 H, CH_2O), 2.17–2.06 (m, 2 H, CH_2), 2.12 (s, 3 H, $COCH_3$), 2.07 (s, 3 H, $COCH_3$), 1.72–1.62 (m, 2 H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.10 and 169.15 (C=O), 137.84 (=CH), 114.98 (CH=CH_2), 99.78 (C-1), 71.11 (C-3), 68.70 (C-2), 68.33 (OCH_2), 61.30 (C-5), 57.02 (C-4), 29.94 and 28.61 (CH_2), 20.71 ($COCH_3$), 20.56 ($COCH_3$).

ESI-TOF: m/z calcd for $C_{14}H_{21}N_3O_6$ $[M + Na]^+$: 350.1323; found: 350.1329.

2-(2-Chloroethoxy)ethyl 2,3-Di-*O*-acetyl-4-azido-4-deoxy-β-L-arabinopyranoside (16) and 2-(2-Chloroethoxy)ethyl 2,3-Di-*O*-acetyl-4-azido-4-deoxy-α-L-arabinopyranoside (17)

Compound **8** (400 mg, 2.11 mmol) and anhyd (2-chloroethoxy)ethanol (2.5 mL) were treated with freshly distilled AcCl (0.50 mL) and processed as described for the allyl derivatives **10** and **11** (Method B). The product-containing fractions were pooled, and concentrated to afford 430 mg (72%) **16** and **17** as a ~2.9:1 anomeric mixture of colorless oil.

β-Anomer **16**

NMR-data were extracted from anomeric mixture.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.41 (dd, *J* = 3.7, 10.5 Hz, 1 H, H-3), 5.19 (dd, *J* = 3.6, 10.5 Hz, 1 H, H-2), 5.11 (d, *J* = 3.6 Hz, 1 H, H-1), 4.12–4.09 (m, 1 H, H-4), 4.05 (ddd, *J* = 1.8, 12.6 Hz, 1 H, H-5$_{eq}$), 3.85–3.79 (m, 1 H, CH_2O), 3.78–3.74 (m, 2 H, CH_2O), 3.71–3.60 (m, 6 H, CH_2, H-5$_{ax}$), 2.12 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$).

^{13}C NMR (100 MHz, $CHCl_3$): δ = 170.11 (C=O), 96.48 (C-1), 71.36, 71.34, 70.12, 69.25 (C-3), 68.25 (C-2), 67.37, 59.97 (C-5), 59.89 (C-4), 42.81 (CH_2Cl), 20.59 ($CO\mathit{C}H_3$), 20.79 ($CO\mathit{C}H_3$).

α-Anomer **17**

NMR-data were extracted from anomeric mixture.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.16 (dd, *J* = 5.5, 8.0 Hz, 1 H, H-2), 5.07 (dd, *J* = 3.6, 8 Hz, 1 H, H-3), 4.53 (d, *J* = 5.6 Hz, 1 H, H-1), 4.09–4.12 (m, 1 H, H-4), 4.05 (dd, *J* = 5.1, 12.2 Hz, 1 H, H-5$_{eq}$), 3.91–3.95 (m, 1 H, H-4), 3.88–3.91 (m, 1 H, CH_2O), 3.58–3.77 (m, 6 H), 2.12 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.22 (C=O), 99.90 (C-1), 71.37, 71.20, 70.33, 68.61 (C-3), 68.25 (C-2), 68.15, 61.60 (C-5), 57.13 (C-4), 42.78 (CH_2Cl), 20.74 ($CO\mathit{C}H_3$), 20.56 ($CO\mathit{C}H_3$).

ESI-TOF: *m*/*z* calcd for $C_{13}H_{20}ClN_3O_7$ [M + Na]$^+$: 388.0882; found: 388.0887.

6-Bromohexyl 4-Azido-4-deoxy-β-L-arabinopyranoside (18) and 6-Bromohexyl 4-azido-4-deoxy-α-L-arabinopyranoside (19)

Method A: 6-Bromohexanol (1.6 mL) was added to a suspension of compound **8** (700 mg, 3.7 mmol) in 1 M ethereal HCl (1.0 mL) and heated for 4 h at 70 °C in a 25 mL two-necked flask. The resulting solution was then stirred overnight at r.t. EtOAc (80 mL) and solid Na_2CO_3 (2.5 g) were added and the suspension was filtered. The filtrate was washed with brine (100 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (120 g SiO_2, *n*-hexane–EtOAc, 7:3 → 0:1) yielding the pure β-anomer **18**.

18

Yield: 542 mg (44%); colorless crystals; mp 33–34 °C; $[\alpha]_D^{20}$ +132.8 (*c* 1.3, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.87 (d, *J* = 3.7 Hz, 1 H, H-1), 3.97 (dd, *J* = 3.8, 9.2 Hz, 1 H, H-3), 3.96–3.92 (m, 1 H, H-4), 3.83 (dd, *J* = 1.7, 12.5 Hz, 1 H, H-5$_{eq}$), 3.79 (dd, *J* = 3.7, 9.2 Hz, 1 H, H-2), 3.72 (dt, *J* = 6.8, 9.7 Hz, 1 H, OCH_2), 3.69 (dd, *J* = 2.2, 12.5 Hz, 1 H, H-5$_{ax}$), 3.46 (dt, *J* = 6.3, 9.5 Hz, 1 H, OCH_2), 3.35 (t, *J* = 6.7 Hz, 2 H, $BrCH_2$), 2.34 (br s, 2 H, OH), 1.84–1.76 (m, 2 H, $C\mathit{H}_2CH_2Br$), 1.61–1.51 (m, 2 H, CH_2), 1.45–1.26 (m, 4 H, CH_2).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 98.44 (C-1), 70.74 (C-2), 69.93 (C-3), 68.39 (OCH_2), 61.19 (C-4), 60.53 (C-5), 33.75 ($BrCH_2$), 32.54, 29.24, 27.83, 25.30 (CH_2).

Anal. Calcd for $C_{11}H_{20}BrN_3O_4$: C, 39.07; H, 5.96; N, 12.42. Found: C, 39.44; H, 5.71; N, 12.32.

Further elution gave a fraction containing **18** and **19** (240 mg, 19%) and pure **19**.

19

Yield: 108 mg (9%); colorless crystals; mp 47–49 °C; $[\alpha]_D^{20}$ –11 (*c* 0.8, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.26 (d, *J* = 6.4 Hz, 1 H, H-1), 3.90–3.81 (m, 3 H, H-3, H-4, OCH_2), 3.77 (dd, *J* = 3.8, 12.6 Hz, 1 H, H-5$_{eq}$), 3.70 (dd, *J* = 6.4, 7.9 Hz, 1 H, H-2), 3.59 (dd, *J* = 2.5, 12.6 Hz, 1 H, H-5$_{ax}$), 3.49 (td, *J* = 6.6, 9.6 Hz, 1 H, OCH_2), 3.41 (t, *J* = 6.7 Hz, 2 H, $BrCH_2$), 2.94 (br s, 1 H, OH), 2.54 (br s, 1 H, OH), 1.84–1.76 (m, 2 H, $C\mathit{H}_2CH_2Br$), 1.61–1.51 (m, 2 H), 1.45–1.26 (m, 4 H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 102.45 (C-1), 72.61 (C-2), 71.38 (C-3), 69.55 (OCH_2), 62.54 (C-4), 59.33 (C-5), 33.78 ($BrCH_2$), 32.55, 29.30, 27.82, 25.15 (CH_2).

Anal. Calcd for $C_{11}H_{20}BrN_3O_4$: C, 39.07; H, 5.96; N, 12.42. Found: C, 39.36; H, 5.78; N, 12.28.

Method B: Freshly distilled AcCl (0.25 mL) was added at r.t. to a solution of **8** (200 mg; 1.6 mmol) in 6-bromohexanol (1 mL, 6.6 mmol). Upon addition a strong exothermic reaction occurred. After cooling to r.t., the solution was stirred for 18 h. The slightly yellowish solution was diluted with EtOAc (50 mL), treated with sat. aq $NaHCO_3$ (60 mL), washed with brine (40 mL), and dried (Na_2SO_4). The solvent was removed and the remaining oil was purified by column chromatography (50 g, 63 μm SiO_2, *n*-hexane–EtOAc, 4:1). The product-containing fractions were pooled and concentrated to afford 238 mg (65%) **18** and **19** as a 3:1 anomeric mixture.

6-*S*-Thioacetylhexyl 4-Azido-4-deoxy-β-L-arabinopyranoside (20)

A solution of KSAc (17 mg, 0.15 mmol) in anhyd DMF (1mL) was added to a solution of bromide **18** (98 mg, 0.29 mmol) in anhyd DMF (2 mL) and the solution was stirred at r.t. overnight whereupon a colorless solid precipitated.The reaction mixture was diluted with EtOAc (20 mL) and poured onto H_2O (20 mL). The aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with sat. $NaHCO_3$ (40 mL), brine (30 mL), and dried ($MgSO_4$). Concentration of the solution afforded a slightly yellow oil, which was further purified by column chromatography (15.0 g SiO_2; *n*-hexane–EtOAc, 3:2) to furnish **20**; yield: 82 mg (83%); colorless syrup; $[\alpha]_D^{20}$ +133 (*c* 0.5 $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.86 (d, *J* = 3.7 Hz, 1 H, H-1), 4.00–3.91 (m, 2 H, H-4, H-5$_{eq}$), 3.88–3.65 (m, 4 H, H-2, H-3, H-5$_{ax}$, OCH_2), 3.49–3.39 (m, 1 H, OCH_2), 2.86 (t, *J* = 7.2 Hz, 2 H, SCH_2), 2.32 (s, 3 H, CH_3COS), 2.17 (br s, 2 H, OH), 1.68–1.51 (m, 4 H, CH_2), 1.46–1.29 (m, 4 H, CH_2).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.11 (C=O), 98.46 (C-1), 70.82 (C-2), 69.97 (C-3), 68.46 (OCH_2), 61.17 (C-4), 60.57 (C-5), 30.66 (CH_3), 29.37, 29.23, 28.88, 28.35, 25.60 (CH_2).

Anal. Calcd for $C_{11}H_{20}BrN_3O_4$: C, 46.83; H, 6.95; N, 12.60. Found: C, 46.94; H, 6.96; N, 12.35.

6-Mercaptohexyl 4-Azido-4-deoxy-β-L-arabinopyranoside (21)

Thioacetate **20** (210 mg, 0.629 mmol) was stirred with aq 0.2 M NaOH (50 mL) under argon at r.t. for 1.5 h. The reaction mixture was extracted with EtOAc (3 × 40 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated to give **21** as a colorless oil, which solidified at r.t.; yield: 169 mg (87%); R_f = 0.14 (*n*-hexane–EtOAc, 1:1); $[\alpha]_D^{20}$ +146 (*c* 0.5, MeOH).

^{1}H NMR (300 MHz, CD_3OD): δ = 4.77 (d, *J* = 3.7 Hz, 1 H, H-1), 4.00 (dd, *J* = 3.8, 9.7 Hz, 1 H, H-3), 3.91–3.84 (m, 2 H, H-4, H-5$_{eq}$), 3.74 (dd, *J* = 3.7 Hz, 1 H, H-2), 3.69 (dt, *J* = 7.2, 9.7 Hz, 1 H, OCH_2), 3.61 (dd, *J* = 2.6, 12.8 Hz, 1 H, H-5$_{ax}$), 3.46 (dt, *J* = 6.3, 9.7 Hz, 1 H, OCH_2), 2.52 (t, *J* = 7.1 Hz, 2 H, SCH_2), 1.72–1.55 (m, 4 H, CH_2), 1.50–1.36 (m, 4 H, CH_2).

^{13}C NMR (75 MHz, CD_3OD): δ = 100.72 (C-1), 70.90 (C-3), 70.55 (C-2), 69.41 (OCH_2), 64.07 (C-4), 61.63 (C-5), 35.11 (SCH_2), 30.46, 29.16, 26.76, 24.88 (CH_2).

ESI-TOF: *m*/*z* calcd as the disulfide $C_{22}H_{40}O_8N_6S_2$ $[M - H]^-$: 579.2276; found: 579.2275.

3-Mercaptopropyl-3-thiopropyl 4-Azido-4-deoxy-β-L-arabinopyranoside (22)

Propane-1,3-dithiol (0.2 mL) was added to a solution of azide **10** (42 mg, 0.19 mmol) in MeOH (1.0 mL) in a quartz flask. The reaction mixture was stirred under UV radiation (254 nm) for 4 h at r.t. to give full conversion. The solvent was removed and the residue was purified by chromatography (*n*-hexane–EtOAc–MeOH, 1:1:0.1) to afford **22** as a colorless oil; yield: 40 mg (65%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.88 (d, *J* = 3.7 Hz, 1 H, H-1), 4.02–3.75 (m, 5 H, H-2, H-3, H-4, H-5$_{eq}$, OCH_2), 3.69 (dd, *J* = 2.2, 12.6 Hz, 1 H, H-5$_{ax}$), 3.53 (dt, *J* = 6.1, 9.9 Hz, 1 H, OCH_2), 2.70–2.57 (m, 6 H, CH_2), 1.95–1.81 (m, 4 H, CH_2), 1.38 (t, *J* = 8.0 Hz, 1 H, SH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 98.65 (C-1), 70.68 (C-3), 69.89 (C-2), 67.24 (OCH_2), 61.17 (C-4), 60.63 (C-5), 33.16, 30.51, 29.09, 29.03, 23.34 (CH_2).

ESI-TOF: *m*/*z* calcd for $C_{11}H_{20}N_3O_4S_2$ $[M - H]^-$: 322.0901; found: 322.0905.

Allyl 4-Amino-4-deoxy-β-L-arabinopyranoside (23)

A solution of azide **10** (50 mg, 0.23 mmol) in pyridine–H_2O (10:1, 2.2 mL) was purged with argon. Et_3N (0.48 mL) and propane-1,3-dithiol (0.49 mL, 4.65 mmol) were added at r.t. and the mixture was stirred overnight. The mixture was co-evaporated with toluene (2 × 10 mL) and the residue was purified on an Isolute Si column (500 mg) using EtOAc as eluent. The first eluate (15 mL) contained residual propane-1,3-dithiol. Further elution with MeOH and pooling of the fractions afforded product **23**; yield: 40 mg (91%); colorless syrup; R_f = 0.18 ($CHCl_3$–MeOH–AcOH, 4:1:0.1); $[\alpha]_D^{20}$ +183.6 (*c* 0.4, MeOH).

^{1}H NMR (300 MHz, CD_3OD): δ = 6.03–5.88 (CH=), 5.32 (dq, *J* = 1.7, 17.3 Hz, 1 H, CH=C*H*$_{2trans}$), 5.16 (dq, *J* = 1.5, 10.4 Hz, 1 H, CH=C*H*$_{2cis}$), 4.75 (d, *J* = 3.5 Hz, 1 H, H-1), 4.19 (m, 1 H, OCH_2), 4.02 (m, 1 H, OCH_2), 3.87 (dd, *J* = 2.5, 11.9 Hz, 1 H, H-5$_{eq}$), 3.82 (dd, *J* = 4.1, 9.2 Hz, 1 H, H-3), 3.68 (dd, *J* = 3.4, 9.2 Hz, 1 H, H-2), 3.48 (dd, *J* = 3.1, 11.9 Hz, 1 H, H-5$_{ax}$), 3.06–3.01 (m, 1 H, H-4).

^{13}C NMR (75 MHz, CD_3OD): δ = 134.23 (=CH), 116.07 (CH=*C*H_2), 98.33 (C-1), 69.32 (C-3), 68.84 (C-2), 68.27 (OCH_2), 62.29 (C-5), 50.96 (C-4).

ESI-TOF: *m*/*z* calcd for $C_8H_{15}NO_4$ $[M + Na]^+$: 212.0893; found: 212.0898.

Propyl 4-Amino-4-deoxy-β-L-arabinopyranoside (24)

A suspension of azide **10** (37.0 mg, 0.53 mmol) and 10% Pd/C (15 mg) in anhyd MeOH (4 mL) was stirred at r.t. under H_2 at atmospheric pressure for 18 h. The suspension was filtered over a bed of Celite and the filtrate was concentrated. The remaining oil was further dried under high vacuum yielding the pure product **24**; yield: 33 mg (97%); colorless oil; R_f = 0.17 ($CHCl_3$–MeOH–AcOH, 2:1:0.1); $[\alpha]_D^{20}$ +197.5 (*c* 0.28, MeOH).

^{1}H NMR (300 MHz, CD_3OD): δ = 4.75 (d, *J* = 3.6 Hz, 1 H, H-1), 3.87 (dd, *J* = 2.6, 11.9 Hz, 1 H, H-5$_{eq}$), 3.81 (dd, *J* = 4.2, 9.1 Hz, 1 H, H-3), 3.66 (dd, *J* = 3.6, 9.0 Hz, 1 H, H-2), 3.69–3.60 (m, 1 H, OCH_2), 3.47 (dd, *J* = 3.1, 11.9 Hz, 1 H, H-5$_{ax}$), 3.45–3.36 (m, 1 H, OCH_2), 3.07–3.01 (m, 1 H, H-4), 1.66–1.61 (m, 2 H, CH_2), 0.95 (t, *J* = 7.4 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, CD_3OD): δ = 100.5 (C-1), 71.1 (OCH_2), 70.6 (C-3), 70.2 (C-2), 66.99 (C-3), 63.4 (C-5), 52.3 (C-4), 23.7 (CH_2), 10.9 (CH_3).

MS: ESI-TOF: *m*/*z* calcd for $C_8H_{17}NO_4$ $[M + H]^+$: 192.123; found: 192.1231.

Propyl 4-Acetamido-4-deoxy-β-L-arabinopyranoside (25)

A solution of amine **24** (40 mg, 0.209 mmol) in anhyd pyridine (2 mL) and Ac_2O (0.5 mL) was stirred for 36 h at r.t. The reaction mixture was diluted with EtOAc (15 mL) and stirred for 15 min with EtOH (0.5 mL). The organic phase was washed with aq 2 M HCl (8 mL), H_2O (10 mL), and sat. aq $NaHCO_3$ (10 mL), and dried ($MgSO_4$). Concentration afforded a colorless residue (63 mg, 95%). A portion of the residue (56 mg, 0.176 mmol) was dissolved in anhyd MeOH (2 mL) and treated with 0.1 M methanolic NaOMe (1.4 mL) for 15 h at r.t. The solution was neutralized with Dowex 50 H^+ resin. The resin was filtered off, washed with MeOH (10 mL), and the filtrate was concentrated to give **25**; yield: 30 mg (95%); colorless oil; R_f = 0.48 ($CHCl_3$–MeOH, 4:1); $[\alpha]_D^{20}$ +138 (*c* 0.3, MeOH).

^{1}H NMR (300 MHz, CD_3OD): δ = 5.97 (d, *J* = 8.7 Hz, 1 H, $NHCOCH_3$), 4.78 (d, *J* = 3.3 Hz, 1 H, H-1), 4.20–4.26 (m, 1 H, H-4), 3.92 (dd, *J* = 4.4, 9.0 Hz, 1 H, H-3), 3.83 (dd, *J* = 2.7, 11.8 Hz, 1 H, H-5$_{eq}$), 3.68 (dd, *J* = 3.3, 9.2 Hz, 1 H, H-2), 3.59–3.69 (m, 1 H, OCH_2), 3.50 (dd, *J* = 3.6 Hz, 11.8 Hz, 1 H, H-5$_{ax}$), 3.37–3.46 (m, 1 H, OCH_2), 1.99 (s, 3 H, $COCH_3$), 1.63 (m, *J* = 7.2 Hz, 2 H, CH_2), 0.96 (t, *J* = 7.4 Hz, 3 H, CH_3).

^{13}C NMR (75 MHz, CD_3OD): δ = 100.43 (C-1), 71.28 (C-1′), 70.82 (C-3), 69.71 (C-2), 62.51 (C-5), 51.21 (C-4), 23.79 ($NHCOCH_3$), 22.62 (C-2′), 10.96 (C-3′).

ESI-TOF: *m*/*z* calcd for $C_{10}H_{19}NO_5$ + Na $[M + Na]^+$: 256.1155; found: 256.1151.

6-Mercaptohexyl 4-Amino-4-deoxy-β-L-arabinopyranoside (26)

Azide **21** (150 mg, 0.51 mmol) was dissolved in pyridine (5 mL), deionized H_2O (0.6 mL), and Et_3N (0.6 mL). The solution was purged with argon for 5 min and propane-1,3-dithiol (0.6 mL) was added. Stirring at r.t. overnight gave full conversion. The reaction mixture was co-evaporated with toluene (2 × 20 mL), and purified by flash chromatography over SiO_2 (6 g) using EtOAc and MeOH as eluents. The methanolic product fractions were pooled and evaporated to dryness to give **26** as a syrup, which was stored under argon; yield: 110 mg (80%, containing 15% disulfide according to NMR spectroscopy).

^{1}H NMR (300 MHz, CD_3OD): δ = 4.76 (d, *J* = 3.7 Hz, 1 H, H-1), 3.86 (dd, *J* = 2.7, 11.8 Hz, 1 H, H-5$_{eq}$), 3.81 (dd, *J* = 4.4, 9.5 Hz, 1 H, H-3), 3.74–3.66 (m, 1 H, OCH_2), 3.67 (dd, *J* = 3.3, 9.5 Hz, 1 H, H-2), 3.52–3.39 (m, 2 H, H-5$_{ax}$, OCH_2), 3.06–3.01 (m, 1 H, H-4), 2.52 (t, 2 H, SCH_2), 1.78–1.54 (m, 4 H, CH_2), 1.53–1.33 (m, 4 H, CH_2).

^{13}C NMR (75 MHz, CD_3OD): δ = 100.56 (C1), 70.69 (C3), 70.24 (C2), 69.34 (OCH_2), 63.56 (C5), 52.32 (C4), 35.08 (CH_2S), 30.46 (CH_2), 29.14 (CH_2), 26.73 (CH_2), 24.92 (CH_2).

BSA-Conjugate 27

According to the general procedure of the commercially available 'Sigma-Aldrich Maleimide Activated BSA, KLH Conjugatio Kit-Stock No. MBK-1': Thiol **26** (5 mg) was dissolved in buffer (0.5 mL; 20 mM sodium phosphate buffer, 100 mM EDTA, and 80 mM sucrose, pH 6.6) and immediately added to a solution of 5 mg/mL maleimide-activated BSA (20 mM sodium phosphate buffer, 230 mM NaCl, 2 mM EDTA, and 80 mM sucrose, pH 6.6). The reaction mixture was purged with argon for 2 min, and stirred at 4 °C overnight. The reaction mixture was purified over a Sephadex G-25M column (part of the BSA-Kit), and as eluent 0.01 M PBS-buffer in double distilled H_2O was used. The protein containing fractions

were pooled and lyophilized to afford 1.5 mg of **27** as colorless solid.

Immunization of Mice and Rabbits with Ara4N-BSA (27)

Ten mice (Balb/c) were injected on day 0 subcutaneously into four sites of the back skin with Ara4N-BSA conjugate (50 μg in 200 μL of PBS) emulsified with an equal volume of Freund's complete adjuvant. The animals received a booster injection intraperitoneally on day 28 (50 μg in 200 μL of PBS emulsified with an equal volume of Freund's incomplete adjuvant) and bled on day 35. From two rabbits (chinchilla bastards) preimmune sera were collected on day –2. On day 0 they were injected with Ara4N-BSA conjugate (100 μg in 1 mL of PBS) emulsified with an equal volume of Freund's complete adjuvant into the popliteal and elbow lymph nodes. The animals received a booster injection subcutaneously on day 47 (150 μg in 1 mL of PBS emulsified with an equal volume of Freund's incomplete adjuvant) into 6 sites of the back skin and were bled on day 56.

ELISA Using Neoglycoconjugate and LPS Antigens

i) Neoglycoconjugates in carbonate buffer (50 mM, pH 9.2) were coated onto MaxiSorp microtiter plates (96-well, U-bottom, NUNC) at 4 C overnight. Antigen solutions were adjusted to equimolar concentrations based on the amount of ligand present in the respective glycoconjugate. If not stated otherwise, a volume of 50 μL was used. Plates were washed twice in PBS supplemented with Tween 20 (0.05%, Bio-Rad) and thimerosal (0.01%, PBS-T) and were then blocked with PBS-T supplemented with casein (2.5%, PBS-TC) for 1 h at 37 °C on a rocker platform followed by two washings. Appropriate antibody dilutions in PBS-TC supplemented with 5% BSA (PBS-TCB) were added and incubated for 1 h at 37 °C. After two washings, peroxidase-conjugated goat anti-mouse IgG or goat-antirabbit IgG (both heavy and light chain specific; Dianova; diluted 1:1.000 in PBS-TCB) was added and incubation was continued for 1 h at 37 °C. The plates were washed three times with BPS-T. Substrate solution was freshly prepared and was composed of 2,2′-azinobis(3-ethylbenzthiazolinsulfonic acid) diammonium salt (1 mg) dissolved in substrate buffer (0.1 M sodium citrate, pH 4.5; 1 mL) followed by the addition of H_2O_2 (25 μL of a 0.1% solution). After 30 min at 37 °C, the reaction was stopped by the addition of aq oxalic acid (2%) and the plates were read by a microplate reader (Tecan Sunrise) at 405 nm. Tests were run twice in quadruplicates with confidence values not exceeding 10%.

ii) When LPS was used as an antigen in ELISA, microtiter polyvinyl plates (96-well, Falcon 3911; Becton Dickinson) were coated with LPS of varying concentration (2 to 250 ng/well) diluted in PBS (pH 7.2) and were incubated overnight at 4 °C. PBS and PBS-containing solutions were supplemented with thimerosal (0.01%). Further incubation steps were performed at 37 °C under gentle agitation. The coated plates were washed four times with PBS and were blocked for 1 h with PBS supplemented with casein (2.5%, Sigma; PBS-C; 200 μL per well). Serial serum dilutions in PBS-C were subsequently added, and the mixture was incubated for 1 h at 37 °C. After washing as described above, secondary antibodies diluted in PBS-C (same source and dilution as above) were added. After four washings in PBS, the following steps were done as described for ELISA using neoglycoconjugate antigens.

Acknowledgment

The authors are grateful to Buko Lindner (Research Center Borstel) for recording the MALDI MS and Stephan Hann and Madeleine Dell'mour (BOKU Vienna) for recording the ESI-TOF MS. Financial support by Fonds zur Förderung der wissenschaftlichen Forschung (FWF grant P 19295) is gratefully acknowledged.

References

(1) For reviews, see: (a) Raetz, C.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635. (b) Holst, O.; Molinaro, A. In: *Microbial Glycobiology*; Moran, A. P.; Holst, O.; Brennan, P. J.; von Itzstein, M., Eds.; Elsevier: Amsterdam, **2009**, 29–55; and references cited therein.
(2) Gatzeva-Topalova, P. Z.; May, A. P.; Sousa, M. C. *Biochem.* **2005**, *44*, 5328.
(3) Breazeale, S. D.; Ribeiro, A. A.; Raetz, C. R. H. *J. Biol. Chem.* **2003**, *278*, 24731.
(4) Sidorczyk, Z.; Kaca, W.; Brade, H.; Rieschel, E. T.; Sinnwell, V.; Zähringer, U. *Eur. J. Biochem.* **1987**, *168*, 269.
(5) Vinogradov, E. V.; Sidorczyk, Z.; Knirel, Y. *Aust. J. Chem.* **2002**, *55*, 61.
(6) For reviews, see: (a) Vinion-Dubiel, A. D.; Goldberg, J. B. *J. Endotoxin Res.* **2003**, *9*, 201. (b) De Soyza, A.; Silipo, A.; Lanzetta, R.; Govan, J. R.; Molinaro, A. *Innate Immun.* **2008**, *14*, 127.
(7) Isshiki, A.; Kawahara, K.; Zähringer, U. *Carbohydr. Res.* **1998**, *313*, 21.
(8) Gronow, S.; Xia, G.; Brade, H. *Eur. J. Cell Biol.* **2010**, *89*, 3.
(9) Gronow, S.; Noah, C.; Blumenthal, A.; Lindner, B.; Brade, H. *J. Biol. Chem.* **2003**, *278*, 1647.
(10) Vinogradov, E.; Lindner, B.; Seltmann, G.; Radziejewska-Lebrecht, J.; Holst, O. *Chem. Eur. J.* **2006**, *12*, 6692.
(11) Moskowitz, S. M.; Ernst, R. K.; Miller, S. I. *J. Bacteriol.* **2004**, *186*, 575.
(12) Naleway, J. J.; Raetz, C. H. R.; Anderson, L. *Carbohydr. Res.* **1988**, *179*, 199.
(13) Kline, T.; Trent, S. M.; Stead, C. M.; Lee, M. S.; Sousa, M. C.; Felise, H. B.; Nguyen, H. V.; Miller, S. I. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1507.
(14) Helm, R. F.; Ralph, J.; Anderson, L. *J. Org. Chem.* **1991**, *56*, 7015.
(15) Nilsson, M.; Westman, J.; Svahn, C.-M. *J. Carbohydr. Chem.* **1993**, *12*, 23.
(16) Tadeo, K.; Ohguchi, Y.; Hasegawa, R.; Kitamura, S. *Carbohydr. Res.* **1995**, *278*, 301.
(17) Ziser, L.; Withers, S. G. *Carbohydr. Res.* **1994**, *265*, 9.
(18) Tsuda, Y.; Nishimura, M.; Kobayashi, T.; Sato, Y.; Kanemitsu, K. *Chem. Pharm. Bull.* **1991**, *39*, 2883.
(19) Kondo, Y. *Carbohydr. Res.* **1982**, *110*, 339.
(20) Buchanan, J. G.; Edgar, A. R.; Large, D. G. *J. Chem. Soc., Chem. Commun.* **1969**, *10*, 558.
(21) Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, *19*, 3633.
(22) Westphal, O.; Jann, K. *Meth. Carbohydr. Chem.* **1965**, *5*, 83.