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Synthesis of lipid A and inner-core lipopolysaccharide (LPS) ligands containing 4-amino-4-deoxy-L-arabinose units*

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Abstract: Attachment of 4-amino-4-deoxy-L-arabinose (Ara4N) to phosphates or sugar hydroxyl groups of lipopolysaccharide (LPS) contributes to bacterial resistance against common antibiotics. For a detailed study of antigenic properties and binding interactions, Ara4N-containing inner-core ligands related to *Burkholderia* and *Proteus* LPS have been synthesized in good yields. Glycosylation at position 8 of allyl glycosides of oct-2-ulosonic acids (Ko, Kdo) has been accomplished using an *N*-phenyltrifluoroacetimidate 4-azido-4-deoxy-L-arabinosyl glycosyl donor followed by azide reduction and global deprotection. The β-L-Ara4N-(1 → 8)-α-Kdo disaccharide was further extended into the branched β-L-Ara4N-(1 → 8)[α-Kdo-(2 → 4)]-α-Kdo trisaccharide via a regioselective glycosylation of a protected triol intermediate. Synthesis of Ara4N-modified lipid A part structure occurring in the LPS of *Burkholderia*, *Pseudomonas*, and *Klebsiellla* strains was accomplished using the *H*-phosphonate approach. The stereocontrolled assembly of the phosphodiester linkage connecting glycosidic centers of two aminosugars was elaborated employing an anomeric *H*-phosphonate of cyclic silyl-ether protected 4-azido-4-deoxy-β-L-arabinose, which was coupled to the hemiacetal of the lipid A GlcN-disaccharide backbone. Conditions for global deprotection, which warrant the integrity of "double anomeric" phosphodiester linkage, were successfully developed. Introduction of thiol-terminated spacer at the synthetic ligands allows both coupling to bovine serum albumin (BSA) and immobilization on gold nanoparticles as well as generation of glycoarrays.

Keywords: antimicrobial activity; carbohydrates; endotoxins; Kdo; lipid A; lipopolysaccharide; organic synthesis; phosphodiesters.

INTRODUCTION

Within the last two decades, infections caused by Gram-negative bacteria have developed into major threats in clinical practice owing to a steady increase of antibiotic-resistant strains [1]. On the other hand, the number of FDA-approved novel antimicrobial drugs has dramatically decreased [2], restricting therapeutic measures to a single antibiotic in several cases. Major challenges have been encountered in infections caused by nosocomial pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Burkholderia cepacia*, or *Acinetobacter baumannii* [3,4]. In the search for novel strategies in antibacterial therapies, lipopolysaccharide (LPS), located in the outer leaflet of the Gram-negative bacterial cell wall, has been identified as a promising target [5]. LPS consists of the amphiphilic lipid A

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part—which anchors the polysaccharide in the outer membrane—a core region containing the higher carbon sugars 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptose and the *O*-antigenic polysaccharide [6]. Antibiotic resistance of several bacterial species is associated with the presence of 4-amino-4-deoxy-L-arabinose (Ara4N), which has been frequently detected phosphate-ester-linked to the 4'-position as well as to the reducing end of the β-(1 → 6)-linked glucosamine disaccharide unit of lipid A [7,8]. Ara4N modification of the lipid A phosphates is believed to have a profound impact on the interaction of lipid A with TLR4-MD-2 complex and initiation of innate immune response [9–11]. In addition, Ara4N has been found as a glycosyl moiety linked to position 8 of Kdo [12] as well as to the Kdo-isosteric D-*glycero*-D-*talo*-oct-2-ulosonic acid (Ko), respectively, as shown in Fig. 1 [13]. The antibiotic properties of these Ara4N-substitution motifs have been linked to the inhibition of antimicrobial cationic peptides—components of the innate immune defense—by masking the charged carboxylate and phosphate groups in the inner core and lipid A region, respectively [14]. In particular, resistance mechanisms against polymyxin B as one of the last resorts for the treatment of multidrug-resistant bacteria, has been referred to the presence of Ara4N in *P. aeruginosa*, *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, *B. cenocepacia*, *Helicobacter pylori*, and *Yersinia pestis* [15,16]. The molecular details of binding interactions to antimicrobial peptides as well as the antigenic properties of the domains harboring Ara4N have not been investigated in full depth. Thus, we

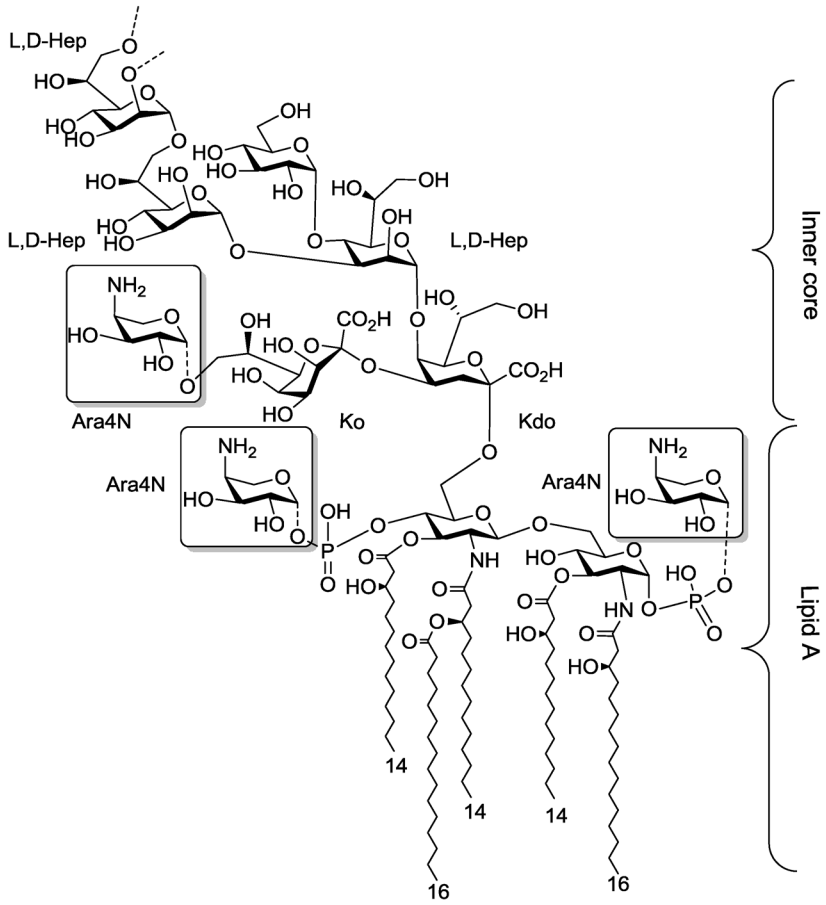
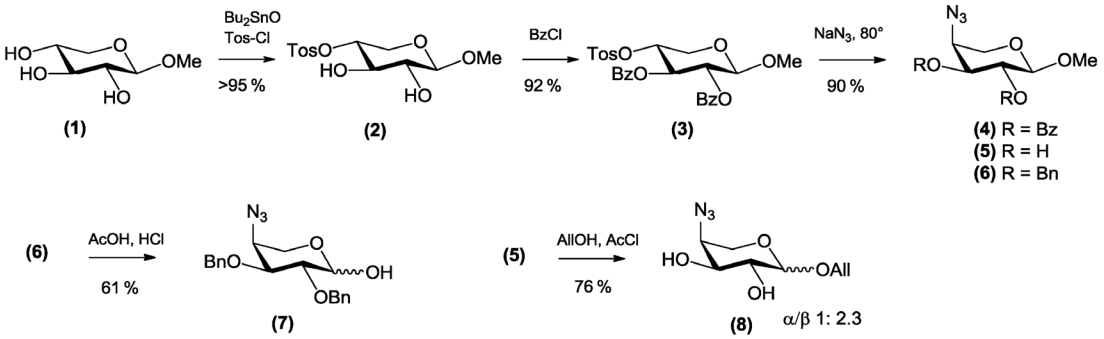


Fig. 1 Structure of the lipid A and inner-core region of LPS from *Burkholderia pyrrocinia* [13].

have set out to synthesize these LPS determinants as monovalent ligands as well as linked to proteins for ongoing immunochemical and conformational studies.

SYNTHESIS OF 4-AZIDO-4-DEOXY-L-ARABINOSE

For the assembly of the inner-core and lipid A-related saccharides, multigram amounts of Ara4N were needed. The previously reported preparation of Ara4N via introduction of a 2,3-*O*-isopropylidene group onto methyl β-D-xylopyranoside **1** followed by suitable conversion into the corresponding 4-azido-4-deoxy derivative could not be elaborated into a high-yielding procedure [17]. Alternatively, a short step sequence capitalizing on an intermediate tin acetal followed by benzoylation and tosylate displacement by sodium azide furnished the corresponding glycoside **4** comprising a single chromatographic separation in 78 % overall yield [18]. After removal of the benzoates, benzyl groups were introduced—as non-participating groups for the glycosylation steps—to afford glycoside **6** in high yield (Scheme 1).



Scheme 1 Synthesis of methyl 4-azido-4-deoxy-α-L-arabinosides (**4–6**), hemiacetal (**7**), and allyl glycoside (**8**).

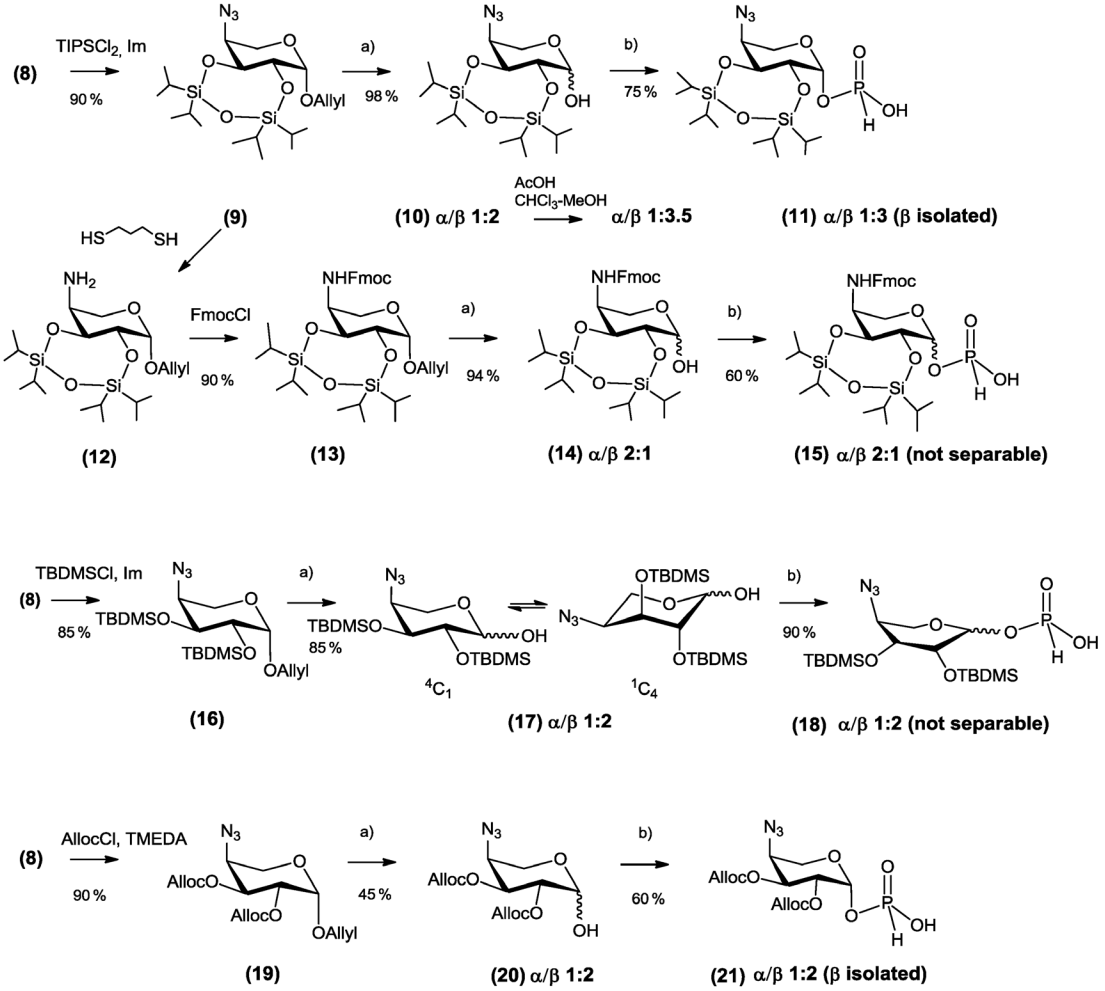
Removal of the anomeric methyl group of **6** could then be accomplished either by acid hydrolysis or by conversion into allyl glycosides via transglycosylation [18]. Treatment of **6** with 2 M HCl in acetic acid at 65 °C furnished hemiacetal **7** in 61 % yield, whereas reaction of **5** with acetyl chloride in allyl alcohol at room temperature afforded a 2.3:1 anomeric mixture of the allyl β- and α-L-glycosides **8** in 76 % yield. The allyl glycoside allowed for subsequent separation of the anomers and exchange of protecting groups.

SYNTHESIS OF ANOMERIC *H*-PHOSPHONATES OF 4-AZIDO-4-DEOXY-L-ARABINOSE

In a series of experiments directed to the stereocontrolled formation of the phosphodiester linkage connecting glycosidic centers of the diglucosamine backbone of lipid A and the anomeric position of the second aminosugar, the phosphoramidite and *H*-phosphonate methodologies were exploited. The *H*-phosphonate approach was shown to be the method of choice, allowing for better yields and higher stereocontrol of the arising "double anomeric" phosphodiester linkage. The most straightforward way to the anomeric *H*-phosphonate is the acylation of the corresponding hemiacetal with chlorophosphites followed by hydrolysis, and thus the anomeric configuration of the formed glycosidic *H*-phosphonate depends on the configuration of the mother hemiacetal.

To investigate the anomeric preferences of the hemiacetals of Ara4N, a series of divergently 2,3-*O*-protected 4-azido-4-deoxy-L-arabinose derivatives has been synthesized. Thus, cyclic 2,3-*O*-tetraisopropyldisiloxane-1,3-diyl (TIPS) as well as *tert*butyldimethylsilyl (TBDMS) and allyloxycarbonyl (Alloc) protecting groups were installed in β-anomeric compounds **9**, **16**, and **19** in excel-

lent yields (Scheme 2). After cleavage of the allyl group employing a two-step procedure (isomerization and hydrolysis with aq I_2 at 0 °C) and chromatography on silica gel, the α/β-anomeric ratio in the resulting hemiacetals having 2,3-*O*-TIPS (**10**), 2,3-di-*O*-TBDMS (**17**), and 2,3-di-*O*-Alloc (**20**) protecting groups was compared. Conformationally constrained TIPS-protected compound **10** showed, after anomerization by treatment with acetic acid in $CHCl_3$:MeOH, the highest proportion of β-(*axial*) configured hemiacetal (α:β = 1:3, $J_{1,2}$ = 7.2 and 3.7 Hz, respectively). In an attempt to improve the sterical outcome in favor of the β-anomer, the azido group in position 4 in compound **9** was substituted, after reduction with 1,3-propane dithiol, by an Fmoc group to furnish **13**. Contrary to the expectations that a bulky Fmoc group would exert conformational influence on the anomeric center and shield the α-face, the anomeric ratio in the hemiacetal **14** shifted toward equatorial α-anomer (α:β = 2:1). Noteworthy, the TBDMS-protected hemiacetal **17** underwent $^4C_1 \leftrightarrow {}^1C_4$ conformational rearrangement with formation of, most probably, distorted half-chair forms as deduced from the coupling constants ($J_{1,2}$ = 2.1 and 4.6 Hz for two anomers), indicating the inherent tendency of TBDMS groups to adopt axial arrangements. The 2,3-di-*O*-Alloc-protected hemiacetal **20** (α:β = 1:2, $J_{1\beta,2}$ = 3.4 Hz) was prepared in the same way, albeit with lower yields, owing to partial cleavage of Alloc groups upon



Scheme 2 Synthesis of divergently protected anomeric *H*-phosphonates of Ara4N (**11**, **15**, **18**, and **21**). Reagents and conditions: (a) [Ir(I)(MePPh$_2$)$_2$-(cod)]PF$_6$, H_2, then aq. I_2, 0 °C; (b) SalPCl, pyr, then H_2O.

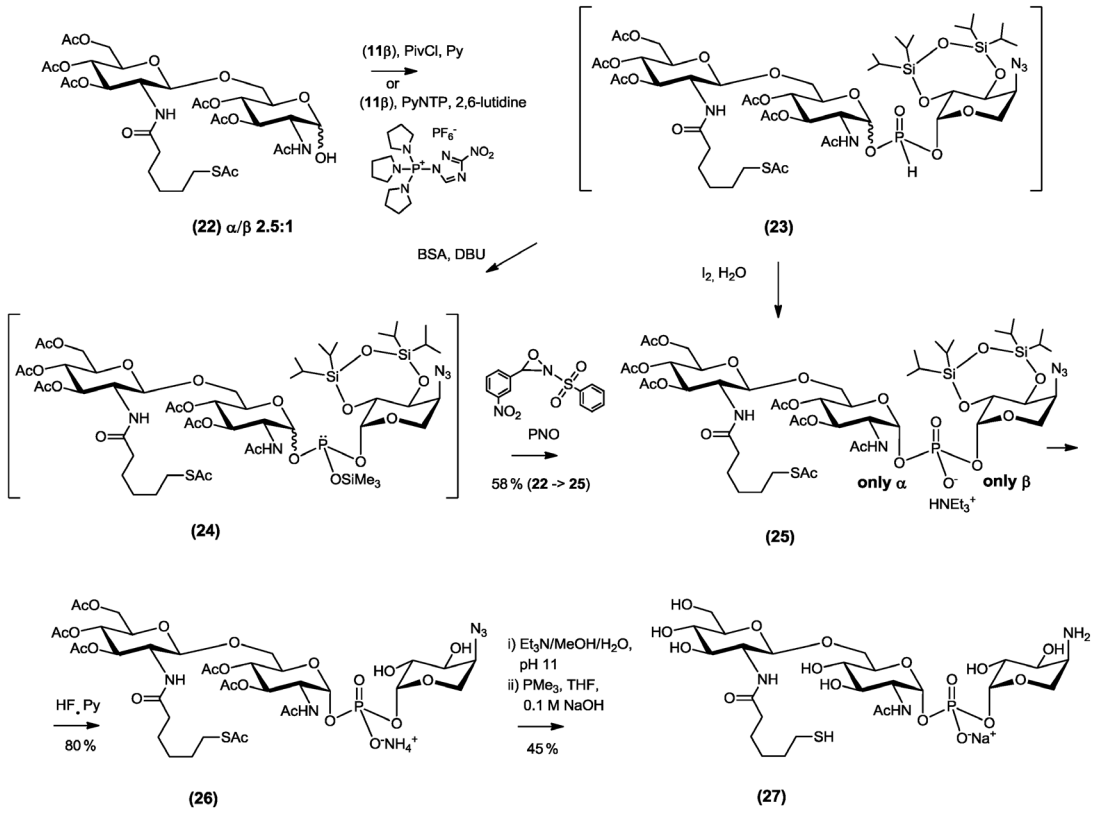
anomeric deprotection. Phosphitylation of the hemiacetals **10**, **14**, **17**, and **20** was performed with salicyl chlorophosphite (SalPCl, Py) [19], followed by aqueous work-up to provide *H*-phosphonates **11**, **15**, **18**, and **21** as anomeric mixtures in 70–90 % yields. 2,3-*O*-TIPS-4-azido-protected β-*H*-phosphonate **11** ($J_{P,H}$ = 652 Hz, $J_{1,2}$ = 3.5 Hz, $J_{1,P}$ = 9.6 Hz) was successfully isolated (36 %) and applied for further phosphodiester coupling. 2,3-*O*-TIPS-4-*N*-Fmoc-protected *H*-phosphonate **15** was obtained as an unseparable anomeric mixture with considerable preference for the equatorial anomer (α:β = 2:1). The axial *H*-phosphonate of TBDMS-protected derivative **18** could neither be isolated in pure form. Moreover, the assignment of the anomeric configurations in **18** was complicated by conformational flexibility of pyranose ring in 2,3-di-*O*-TBDMS-1-*O*-phosphite-substituted Ara4N, which adopted distorted half-boat conformation ($J_{1,2}$ = 2.7 and 3.9 Hz and heteronuclear coupling constants $J_{C1,H1}$ = 169.0 and 170.4 Hz, respectively). The anomeric mixture of Alloc-protected *H*-phosphonates **21** (α:β = 1:2, β-anomer: $J_{1,2}$ = 3.3 Hz, $J_{1,P}$ = 8.6 Hz; α-anomer: $J_{1,2}$ = 6.8 Hz, $J_{1,P}$ = 9.0 Hz) was successfully separated to furnish β-*H*-phosphonate in 40 % yield.

SYNTHESIS OF LIPID A SUBUNITS MODIFIED BY 4-AMINO-4-DEOXY-L-ARABINOSE

Penta- to hexaacylated diglucosamine backbone of lipid A was replaced with β-D-GlcN-(1 → 6)-α-D-GlcNAc **22** in which the amino group of the nonreducing moiety was acylated with thiol-terminated C6 spacer to enable subsequent attachment to bovine serum albumin (BSA) or gold nanoparticles. For the assembly of "double anomeric" phosphodiester β-D-GlcN-(1 → 6)-α-D-GlcNAc-(1 → P ← 1)-β-L-Ara4N **27**, the cyclic silyl ether protected *H*-phosphonate **11** was coupled to hemiacetal **22** under standard conditions (pivaloyl chloride, PivCl, Py). The resulting intrinsically labile *H*-phosphonate phosphodiester **23** ($J_{P,H}$ = 752 and 730 Hz) was oxidized in situ with I_2/H_2O to give fully protected phosphodiester **25** (Scheme 3). This protocol provided highly variable yields, apparently, owing to the propensity of PivCl to over-activate *H*-phosphonates with formation of bisacylphosphites [20] and to esterify free hydroxyl groups, leading to 1-pivaloylation of GlcNAc hemiacetal **22** and subsequent oxazoline formation. The following oxidation of the *H*-phosphonate to phosphate with aqueous I_2 is considered to be the most problematic step in the contemporary *H*-phosphonate approach, particularly when glycosidic *H*-phosphonates are involved, resulting often in a high degree of hydrolysis of phosphodiester linkage.

The tendency to side-product formation observed with PivCl as coupling agent and aqueous I_2 as oxidizing reagent prompted us to examine the properties of phosphonium-type condensing reagents, which were originally developed for peptide synthesis, in conjunction with milder oxidation procedure. Thus, the scope of coupling reaction employing one of the most powerful reagents for the activation of *H*-phosphonate monoesters 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP) [21] was explored.

The coupling efficiency and reproducibility in the preparation of *H*-phosphonate phosphodiester **23** employing PyNTP-activated **11β** and **22** or divergently protected β-D-GlcN(thiohexanoyl)-(1 → 6)-α-D-GlcNAc hemiacetals (not shown) outperformed the efficacy of using PivCl. Next, anhydrous neutral conditions for the oxidation of **23** with 2-(phenylsulfenyl)-3-(3-nitrophenyl)oxaziridine (PNO) were exploited [22]. After the phosphite **23** (^{31}P NMR: δ 4.5 and 6.5 ppm) was stabilized in a highly reactive three-coordinate form as silyl ester **24** (^{31}P NMR: δ 128 and 132 ppm) by treatment with excess of *N,O*-bis-(trimethylsilyl)acetamide in the presence of DBU, the addition of PNO resulted in oxidation to phosphate **25** (δ –4.0 ppm) within 5 min. Phosphodiester **25** was isolated by chromatography on silica gel as anomerically pure compound in 58 % yield. The β-configured phosphodiester at the GlcNAc site (arising from approx 30 % of β-hemiacetal in **22**) was apparently destroyed during chromatography. Given that glycosyl phosphates have proven to be excellent glycosylating agents, we were doubtful regarding the stability of the glycosidic phosphodiester **25** upon conditions of global deprotection. Hence, numerous experiments were performed to work out the deprotection protocols amenable to conditions compatible with anomeric phosphodiester linkage. The cleavage of 2,3-*O*-TIPS group from



Scheme 3 Synthesis of the part structure of *Burkholderia* lipid A substituted with Ara4N at reducing phosphate.

Ara4N moiety was performed by treatment with HF·Py within 3 days to furnish **26** in good yield (80 %). The sluggishness of the reaction turned out to be beneficial since neither hydrolysis nor decomposition products were detected.

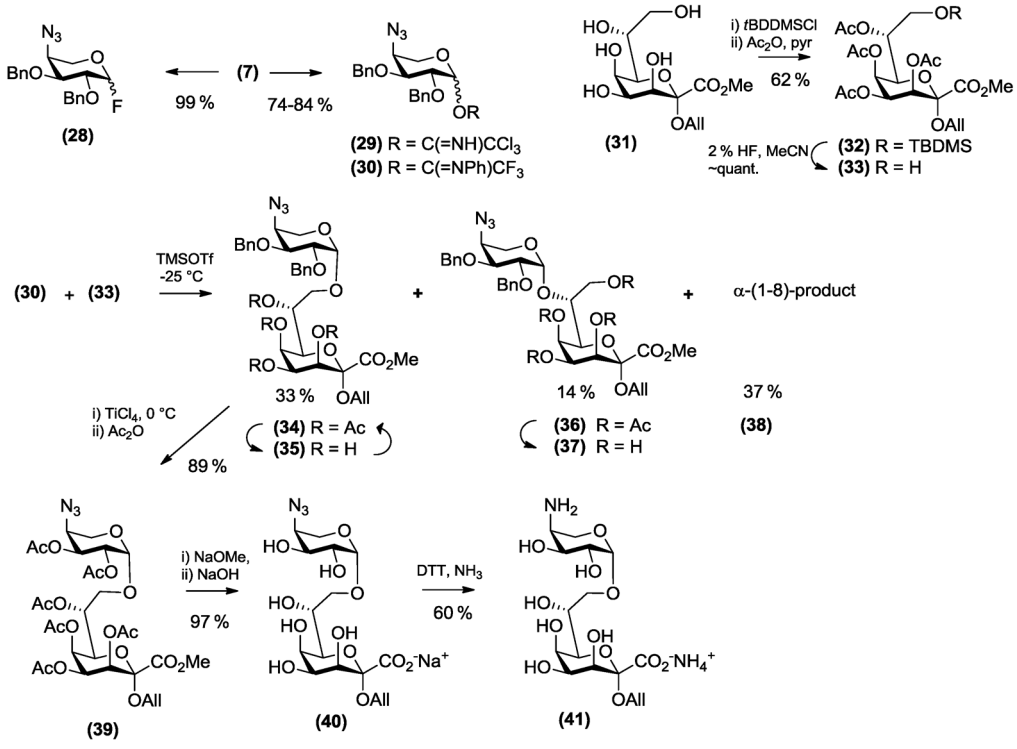
Subsequent hydrolysis of the acetate groups with $Et_3N/MeOH/H_2O$ (pH 10.5–11) and reduction of 4-azido group by application of Staudinger conditions (PMe_3, 0.1 M NaOH) provided amphipathic phosphate **27**. Following chromatography on LH-20, the product was purified by high-performance liquid chromatography (HPLC) on RP-18 in acetonitrile-water.

In conclusion, we have developed an efficient stereospecific synthetic route to the part structure of lipid A, which is substituted by anomerically pure β-L-Ara4N at the glycosidic phosphate, that can be applied to the preparation of other lipid A-related glycosidically linked phosphodiesters where two aminosugars are involved.

SYNTHESIS OF Ara4N-(1 → 8)-Ko RELATED TO *BURKHOLDERIA* LPS

Ara4N residues attached to position 8 of Ko have been found in several *Burkholderia* strains [13,23,24] as well as in a *Serratia marcescens* strain [25]. The trisaccharide β-Ara4N-(1 → 8)-α-Ko-(2 → 4)-α-Kdo-(2 → lipid A) is a unique structural motif in *B. cepacia*. The glycosyl donors, fluoride **28**, trichloroacetimidate **29**, and *N*-phenyltrifluoroacetimidate **30** were derived from hemiacetal **7** in good yields. As glycosyl acceptor, the previously prepared methyl ester allyl glycoside derivative **31** [26] was selectively protected as 8-*O*-TBDMS derivative followed by *O*-acetylation to give compound **32** in 62 % yield (Scheme 4). Coupling of **32** with fluoride donor **28** promoted by a large excess of boron tri-

fluoride etherate produced the anomeric disaccharide in varying (but up to 80 %) yields and with low anomeric selectivity. Cleavage of the 8-*O*-silyl ether with 2 % HF furnished acceptor **33** which was glycosylated with donor **30** in 84 % yield and a 1.6:1 β to α ratio, albeit with formation of a (1 → 7)-linked disaccharide byproduct **36** arising via acetyl migration of the adjacent 7-*O*-acetate. Following removal of the α-(1 → 8) isomer **38** by column chromatography, the latter byproduct was separated after Zemplén deacetylation of the β-(1 → 8)- and β-(1 → 7)-linked product mixture leading to substantial differences in chromatographic mobility of the tetraol derivatives **35** and **37**, respectively. Reacetylation afforded the target disaccharide **34** in isolated 33 % yield. Since the allyl group had to be preserved for later incorporation of a spacer moiety, the benzyl groups had to be removed by other alternative routes than hydrogenolysis. Gratifyingly, treatment of peracetate **34** with $TiCl_4$ led to smooth cleavage of the benzylic protecting groups [27]. For ease of purification, the resulting diol was acetylated to eventually give the hexa-*O*-acetyl compound **39** in 89 % combined yield. Global deprotection was achieved by transesterification of the acetyl groups followed by saponification of the methyl ester with aqueous NaOH to afford the sodium salt **40** in high yield. Reduction of the 4'-azido group was effected by treatment with dithiothreitol followed by purification of the product by ion-exchange chromatography and conversion into the ammonium salt **41** in good yield.

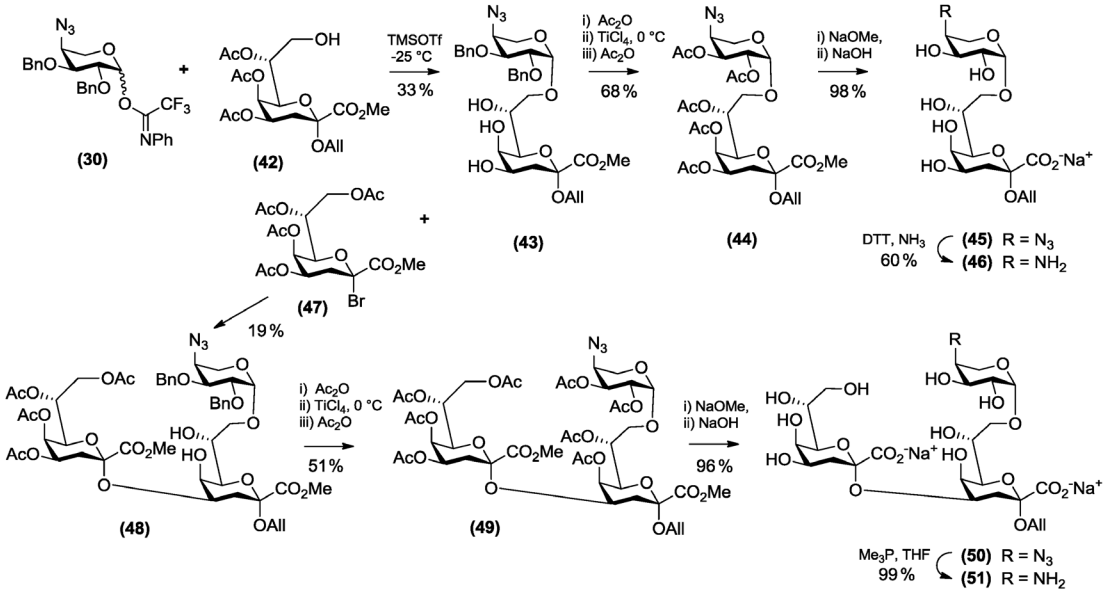


Scheme 4 Synthesis of Ara4N-(1 → 8)-Ko disaccharide **41**.

SYNTHESIS OF Ara4N-(1 → 8)-Kdo LIGANDS RELATED TO *PROTEUS* LPS

In many *Proteus mirabilis* and *Proteus penneri* strains, Ara4N is attached to position 8 of Kdo, which, however, is directly linked to lipid A and contains an α-(2 → 4)-linked side-chain Kdo unit [28]. The synthesis of the corresponding di- and trisaccharide ligands could be elaborated similar to the *Burkholderia* specific disaccharide **41**. Whereas coupling of the 4,5,7-tri-*O*-acetyl Kdo acceptor deriv-

ative **42** with the arabinosyl trichloroacetimidate donor **29** gave only a 38 % yield of disaccharide products, the NPTFA donor **30** again furnished a good yield (77 %) of products that were separated as described for disaccharide **34** to eventually provide disaccharide **43** in 33 % yield (Scheme 5). Exchange of the 2',3'-di-*O*-benzyl groups for acetates by the action of $TiCl_4$ also worked smoothly despite the presence of the acid-sensitive ketosidic linkage of Kdo to give the pentaacetate derivative **44** in 68 % yield. Zemplén de-*O*-acetylation followed by methyl ester saponification gave the 4'-azido derivative **45** in 98 % yield. Final reduction of the azido group by treatment with dithiothreitol afforded the target β-Ara4N-(1 → 8)-α-Kdo allyl glycoside **46** in 60 % yield [29].



Scheme 5 Synthesis of *P. mirabilis* and *P. penneri* di- and trisaccharide ligands.

For the synthesis of the branched *P. mirabilis* inner core subunit, the 4,5,7-triol derivative **43** was subjected to a regioselective glycosylation using the Kdo bromide methyl ester derivative **47** under Helferich conditions in nitromethane. The α-(2 → 4)-linked trisaccharide was separated from minor amounts of the α-(2 → 7)-linked regioisomer as well as from the β-(2 → 4)-linked anomer by HPLC to give the branched trisaccharide **48** in 19 % isolated yield. Again, exchange of benzyl groups for acetates was accomplished in fair yield to give **49**. Removal of the ester groups was achieved as described above giving **50**, whereas attempted reduction of the azide group with dithiothreitol led to concomitant formation of byproducts which could not be removed by chromatography. Alternatively, Staudinger reduction of **50** with trimethyl phosphine was successful and gave the target trisaccharide **51** in 99 % yield [29].

NMR spectroscopic characterization

^{13}C NMR data for saccharides **41**, **46**, and **51** were obtained at 100 or 150 MHz, respectively, and were assigned on the basis of HSQC, APT, and HMBC data. The NMR assignments (Table 1) compare favorably with published values of allyl β-L-arabinopyranoside [18], allyl D-*glycero*-D-*talo*-oct-2-ulopyranoside [26] and the data of β-L-Ara4N-(1 → 8)-α-Ko-(2 → 4)-α-Kdo-(2 → OMe) obtained from *B. cepacia* [23] and a heptosylated oligosaccharide fragment from *P. penneri* [28], respectively. Glycosylation at C-8 by an Ara4N residue resulted in significant downfield shifts of the C-8 signals in

Ara4N-Ko (**41**) and Ara4N-Kdo glycosides (**46**, **51**) accompanied by shifts of the neighboring C-7 signal to higher field. Glycosylation at position 4 of Kdo (**51**) was identified by a low-field shifted signal of C-4 and small high-field shifts observed for the neighboring C-3 and C-5 carbons.

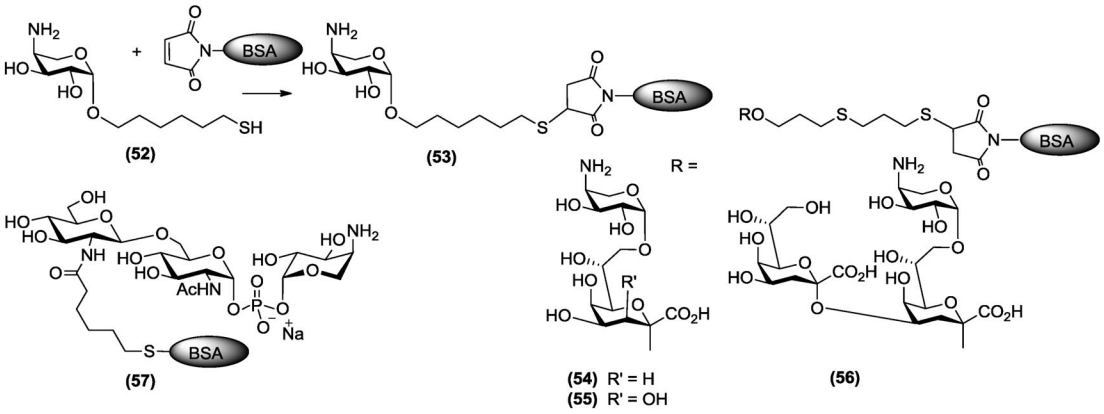
Table 1 ^{13}C NMR data[a] (ppm) of compounds **41**, **46**, and **51**.

Carbon atom	Ara4N[b]	Ko	**41**	Ref. [23]	Kdo	**46**	**51**	Ref. [28]
Ara4N								
1	98.33		99.88	99.24		99.91	99.15	99.0
2	68.84		68.80[c]	68.79		68.53	68.17	68.5
3	69.32		67.59	66.40		68.90	65.92	66.1
4	50.96		51.97	52.84		52.23	51.66	52.2
5	62.29		60.63	58.91		60.07	57.97	58.7
K(d)o								
1		174.41	174.01	174.14	176.1	176.00	n.d.[e]	n.d.
2		102.83	102.54	102.74	101.1	100.99	99.32	n.d.
3		72.56	72.20[d]	72.75	35.1	34.92	33.21	34.7
4		67.32	67.02	66.77	66.9	67.03[c]	68.50	70.6
5		69.10	68.78[c]	69.14	67.2	66.79	64.20	69.4
6		72.45	72.34[d]	73.49	72.5	72.34	71.41	72.9
7		70.38	68.41	69.29	70.4	67.23[c]	67.86	68.2
8		63.92	70.64	70.87	64.1	70.85	70.00	71.0
Kdo								
1							n.d.	n.d.
2							100.20	n.d.
3							34.51	34.9
4							66.13	66.6
5							66.27	66.8
6							72.40	72.4
7							69.91	70.7
8							63.18	63.4
Allyl								
1	68.27	65.44	64.32		65.4	65.19	64.03	
2	134.23	134.41	133.49		134.8	134.60	134.07	
3	116.07	118.77	117.51		118.8	118.50	117.13	

[a]In D_2O at 297 K.
[b]In CD_3OD.
[c,d]Assignments within a column may be reversed.
[e]n.d.: not determined

SYNTHESIS OF NEOGLYCOCONJUGATES CONTAINING 4-AMINO-4-DEOXY-L-ARABINOSE EPITOPES

The 6-mercaptohexyl Ara4N glycoside **52** was conjugated to maleimide-activated BSA and the neoglycoprotein **53** was used for generation of polyclonal antibodies [18]. Thus, the allyl glycosides **41**, **46**, and **51** were extended with a 3-thiopropylthio spacer by radical addition of 1,3-propanedithiol to the allylic aglycon and were then coupled to activated BSA to give neoglycoconjugates **53**–**56** (Scheme 6). Similarly, the lipid A derivative **27** was converted into conjugate **57**. Immunization of rabbits with the Ara4N-neoglycoconjugate **53** resulted in high titer polyclonal sera, which were tested with LPS from *E. coli*, *S. enterica*, *P. mirabilis*, and *B. cepacia* (Table 2).



Scheme 6 Synthesis of neoglycoproteins **53–57**.

Table 2 Serum antibody titers by enzyme-linked immunoabsorbent assay (ELISA) in rabbits.

Serum dilution yielding OD_{405} >0.2 with LPS		Ara4N units in LPS
E. coli (R*e*)	<500	–
S. enterica sv. Minnesota R595	<500	Ara4N-(1 → 4')-P-lipid A
B. cepacia	64 000	Ara4N-(1 → 8)-Ko-(2 → 4)-Kdo
P. mirabilis R45	8000	Ara4N-(1 → 8)[Kdo-(2 → 4)]-Kdo

No reactivity was observed with *E. coli* LPS owing to the absence of Ara4N in its LPS; also, the Ara4N unit linked in a phosphodiester linkage to the 4'-position of lipid A in *S. enterica* was not reactive with the Ara4N-specific polyclonal sera. High titers were obtained for antibodies against the Ara4N-(1 → 8)-Ko epitope of *B. cepacia* with slightly lower reactivity observed for the branched epitope present in *P. mirabilis*, which might probably be due to higher steric congestion owing to the presence of the side-chain Kdo. Ongoing studies with the novel neoglycoconjugates **54–57** are in progress and will be published in due course.

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