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# Stereoselective Synthesis of α- and β-L-Ara4N Glycosyl H-Phosphonates and a Neoglycoconjugate Comprising Glycosyl Phosphodiester Linked β-L-Ara4N

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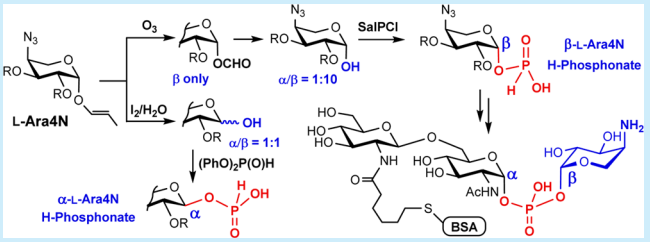
Supporting Information

**ABSTRACT:** Stereoselective synthesis of variably protected α- and β-L-Ara4N glycosyl H-phosphonates as key intermediates in the syntheses of β-L-Ara4N-modified LPS structures and α-L-Ara4N-containing biosynthetic precursors is reported. A facile one-pot approach toward β-L-Ara4N glycosyl H-phosphonates includes anomeric deallylation of protected 4-azido β-L-Ara4N via terminal olefin isomerization followed by ozonolysis and methanolysis of formyl groups to furnish exclusively β-configured lactols that are phosphitylated with retention of configuration. The carbohydrate epitope of β-L-Ara4N-modified Lipid A, βGlcN(1→6)αGlcN(1→P←1)β-L-Ara4N, was stereoselectively synthesized and linked to maleimide-activated bovine serum albumin.




Modification of the terminal portion of lipopolysaccharide (LPS), a glycophospholipid Lipid A, with 4-amino-4-deoxy-β-L-arabinose (L-Ara4N) is an adaptive mechanism that enables Gram-negative bacteria to resist recognition by the components of the host immune system.[1,2] Inducible addition of L-Ara4N to at least one of the phosphate groups of Lipid A (at the 1 and/or 4′ positions) in *Escherichia coli*, *Salmonella enterica* serovar *Typhimurium*, *Yersinia pestis*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* complex is required for bacterial viability and largely contributes to antibiotic resistance and bacterial virulence (Figure 1).[3−9] Inducible addition of cationic

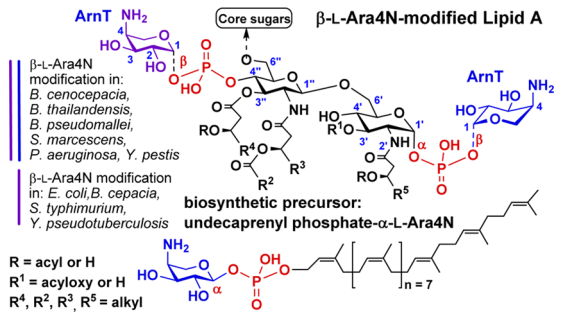



**Figure 1.** Structure of β-L-Ara4N-modified Lipid A and a biosynthetic precursor undecaprenyl phosphate-α-L-Ara4N.

sugar L-Ara4N reduces the net negative charge of the bacterial membrane, which protects it from recognition by the cationic antimicrobial peptides (CAMPs) that comprise the foremost component of the innate immune response at epithelial surfaces. Occurrence of profound resistance to exogenous CAMP polymyxin B, a "last line of defence" antibiotic for the treatment of multi-drug-resistant Gram-negative infections, is also attributed to incorporation of L-Ara4N into the Lipid A moiety of LPS.[3,4,10] L-Ara4N biosynthesis occurs in the cytoplasm, whereas in the final step, L-Ara4N is delivered by the long-chain isoprene lipid carrier undecaprenyl phosphate (UndP) to the periplasmic face of the inner bacterial membrane.[11] There, a membrane lipid-to-lipid glycosyltransferase ArnT catalyzes transfer of L-Ara4N from undecaprenylphosphate-α-L-Ara4N to the phosphate groups of Lipid A.[11,12] ArnT family is the last enzyme in the Ara4N biosynthesis pathway in Gram-negative bacteria and is thus an attractive target for development of antibacterial agents affecting LPS biosynthesis, which necessitates a synthetic access to α-L-Ara4N-containing UndP derivatives.[13]

Ara4N-modified LPS structures can hardly be obtained in pure form by isolation from bacterial sources, due to the inherent lability of the anomeric phosphodiester functionality. To clarify immuno-modulating and immunogenic potential of the Ara4N modification, a reliable synthetic approach toward β-L-Ara4N glycosyl phosphate-containing LPS partial structures is highly desirable. The Lipid A-based neoglycoconjugate, containing conserved epitope βGlcN(1→6)αGlcN(1→P←1)β-L-Ara4N of highly virulent Gram-negative human pathogens, is an important antigen that could be applied to help generate specific monoclonal antibodies. Such mAbs could be used in diagnostic immunoaffinity assays for rapid antigen determination in clinical samples and applied to screen not-yet-identified Ara4N-producing mutants in, for example, *Y. pestis* flea infection models.[6]

In contrast to the abundantly prevailing phosphodiester bonds connecting one anomeric and one non-anomeric sugar hydroxyl

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group, the L-Ara4N-modified Lipid A involves a glycosyl phosphodiester linkage connecting anomeric centers of amino-sugars Ara4N and GlcN (Figure 1). Assembly of such a binary glycosyl phosphodiester requires both rigorous anomeric stereocontrol and very mild reaction conditions that allow for preservation of the labile glycosyl phosphoester intermediates. We have recently shown that application of the H-phosphonate approach is advantageous for such a purpose, compared to other phosphitylation methodologies.[14,15] Preparation of anomerically pure $\alpha$- and $\beta$- H-phosphonate monoesters of orthogonally protected L-Ara4N in a stereoselective manner comprises a major synthetic challenge in the synthesis of Ara4N-containing phosphodiesters (Figure 2).

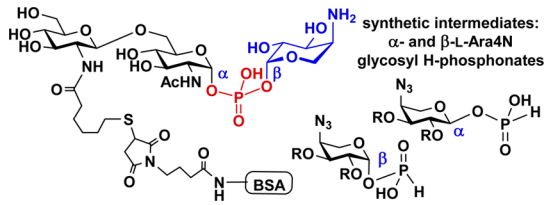
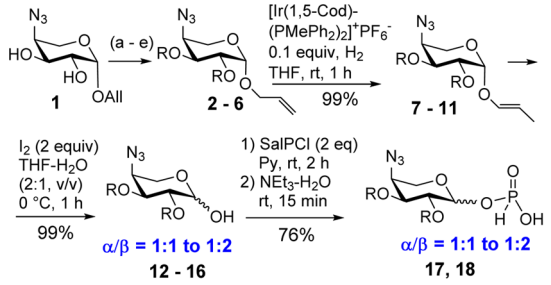



**Figure 2.** Synthetic neoglycoconjugate based on the diglucosamine backbone of Lipid A modified with $\beta$-L-Ara4N at the anomeric phosphate group.

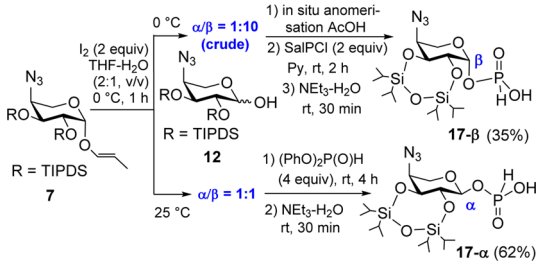
Our synthesis of L-Ara4N glycosyl H-phosphonates relied on the initial protection of hydroxyl groups in positions 2 and 3 in azide **1**[16] to furnish 2,3-*O*-tetraisopropyldisiloxane-1,3-diyl (TIPDS), **2**, 2,3-di-*O*-*tert*-butyldimethylsilyl (TBDMS), **3**, 2,3-di-*O*-benzyl, **4**, PMB, **5**, and 2,3-di-*O*-acetyl (Ac), **6**, protected $\beta$-allyl glycosides (Scheme 1). Stereoselectivity of phosphitylation

**Scheme 1. Synthesis of L-Ara4N Glycosyl H-Phosphonates via Conventional Approach**




at the anomeric center generally relies on the ultimate anomeric ratio in the lactol precursors[17,18] and demands the preparation of anomerically enriched hemiacetals which should be straightforwardly converted into the H-phosphonates. Anomeric deallylation of **2** and **3** was carried out by sequential double bond isomerization with $[Ir(1,5\text{-Cod})(PMePh_2)_2]^+PF_6^-$ followed by $I_2$-assisted prop-1-enyl cleavage, to furnish anomeric mixtures **12** and **13**, respectively ($\alpha/\beta$ = 1:1). Lactols **12** and **13** could be enriched with the $\beta$-anomer ($\alpha/\beta$ = 1:3) by treatment with diluted AcOH. Subsequent phosphitylation by reaction with 2-chloro-1,3,2-benzodioxaphosphorin-4-one (SalPCl)[19] in pyridine yielded anomeric H-phosphonates **17** and **18** ($\alpha/\beta$ = 1:3). 2,3-*O*-TIPDS-protected **17-*β*** was smoothly isolated in pure form, though in a moderate 35% yield (Scheme 2). In contrast, separation of the 2,3-di-*O*-TBDMS-protected $\alpha/\beta$ mixture **18** was challenging and ineffective.

**Scheme 2. Stereocontrolled Synthesis of *α*-L-Ara4N and *β*-L-Ara4N Glycosyl H-Phosphonates**




A predominant formation of the $\beta$-configured H-phosphonate was achieved by application of fairly reactive SalPCl, which quickly trapped the excess of axial $\beta$-lactol in **12**, such that the initial $\alpha/\beta$ ratio was preserved. To guide in situ anomerization in favor of the $\alpha$-lactol, a less reactive phosphitylation agent that would primarily react with the more nucleophilic equatorial 1-OH group, to shift the $\alpha/\beta$ ratio in favor of $\alpha$-anomer, could be of use. Indeed, slow addition of diphenylphosphite to a solution of **12** ($\alpha/\beta$ = 1:1) in pyridine resulted in preponderant formation of the kinetic product, an equatorial glycosyl H-phosphonate ($\alpha/\beta$ = 2:1) **17-*α*** readily isolated in 62% yield (Scheme 2, Table 1).

Monitoring the progress of $I_2$-mediated hydrolysis of the prop-1-enyl group in **7** by $^1$H NMR indicated that exclusively the $\beta$-lactol was formed when the reaction was performed at 0 °C ($\alpha/\beta$ ratio varied from 0:10 to 1:10), while the proportion of the $\alpha$-configured lactol increased at a higher reaction temperature (25 °C, $\alpha/\beta$ = 1:1). Insight into the mechanistic pathway of $I_2$-assisted prop-1-enyl glycoside cleavage in **7** suggested formation of the intermediate halohydrin (at 0 °C), which is cleaved without affecting the anomeric configuration, to form exclusively the $\beta$-configured lactol (Figures S1 and S2).[20,21] At 25 °C, and upon chromatography on silica gel, a rapid anomerization toward $\alpha$-lactol sets in. Attempts to avoid the purification step and to conduct the phosphitylation of the crude reaction mixture **12** enriched with the $\beta$-lactol ($\alpha/\beta$ = 1:10) failed due to formation of numerous byproducts.

To elaborate an efficient approach toward anomerically pure $\beta$-L-Ara4N H-phosphonates, we scrutinized the options for traceless hydrolysis of $\beta$-allyl glycosides without affecting the axial anomeric configuration at C-1. To this end, after allyl group isomerization, the anomeric prop-1-enyl ether could be oxidized by ozonolysis to give a stable formyl intermediate under mild conditions (Scheme 3).[22−24] The formate group could be selectively hydrolyzed to give a $\beta$-lactol and volatile methyl formate, so that the crude $\beta$-lactol could be directly subjected to phosphitylation without needing chromatographic purification.

Accordingly, prop-1-enyl intermediates **7** and **9**−**11** were treated with ozone at −78 °C followed by addition of thiourea. Formyl glycosides **19**−**22** were isolated in pure form by chromatography on deactivated silica gel. Finally, methanolysis ($NEt_3$/MeOH) of the formate esters at −40 °C furnished solely $\beta$-configured lactols **12**−**16**, which were used without further purification. Subsequent phosphitylation with SalPCl provided anomerically pure $\beta$-glycosyl H-phosphonates **17-*β***, **23-*β***, **24-*β***,



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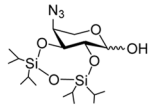
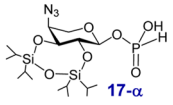
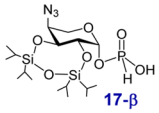
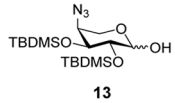
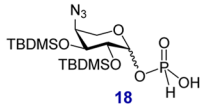
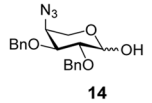
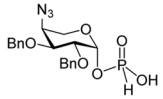
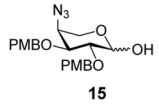
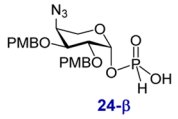
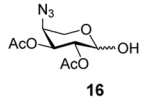
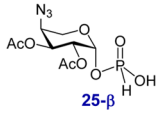
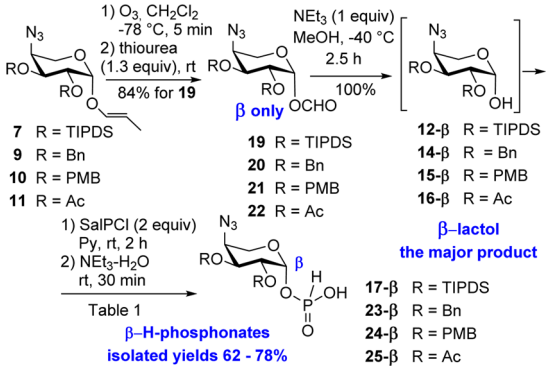
**Table 1. Stereoselective Synthesis of $\alpha$- and $\beta$-Glycosyl H-Phosphonates of L-Ara4N**

| lactol<br>method[a]<br>α/β ratio | H-phosphonate | α/β ratio<br>yield<br>isolated yield[b] |
|---|---|---|
|  <br>**12**<br>A: α/β = 1:1<br>B: α/β = 1:3<br>C: α/β = 1:10 |  <br>**17-α** | A: α/β = 2:1<br>pure α: 62%<br>B: α/β = 1:3<br>(84%)<br>pure β: 35% |
| |  <br>**17-β** | C: α/β = 1:6<br>(90%)<br>pure β: 78% |
|  <br>**13**<br>A: α/β = 1:1<br>B: α/β = 1:2 |  <br>**18** | A: α/β = 2:1<br>(76%)<br>inseparable<br>B: α/β = 1:2<br>(90%)<br>inseparable |
|  <br>**14**<br>C: α/β = 1:4 |  <br>**23-β** | α/β = 1:4 (78%)<br>pure β: 62% |
|  <br>**15**<br>C: α/β = 1:6 |  <br>**24-β** | α/β = 1:6 (84%)<br>pure β: 77% |
|  <br>**16**<br>C: α/β= 1:10 |  <br>**25-β** | α/β = 1:8<br>pure β: 63% |

[a]Method for removal of anomeric prop-1-enyl ether and phosphitylation: (A) (1) $I_2$ (2 equiv), THF/$H_2O$ (2:1, v/v), rt; (2) slow addition (4 h) of $(PhO)_2P(O)H$ (4 equiv), Py; (3) $NEt_3/H_2O$, rt, 30 min; (B) (1) $I_2$ (2 equiv), THF/$H_2O$ (2:1, v/v), 0 °C; (2) in situ anomerization with AcOH; (3) SalPCl (2 equiv), Py; (4) $NEt_3/H_2O$, rt, 30 min; (C) (1) $O_3$, −78 °C, 5 min, thiourea (1.3 equiv); (2) MeOH, $NEt_3$, −40 °C; (3) SalPCl (2 equiv), Py; (4) $NEt_3/H_2O$, rt, 30 min. [b]Yield of glycosyl $\beta$-L- or $\alpha$-L-Ara4N H-phosphonate after conventional column chromatography on silica gel (not HPLC).

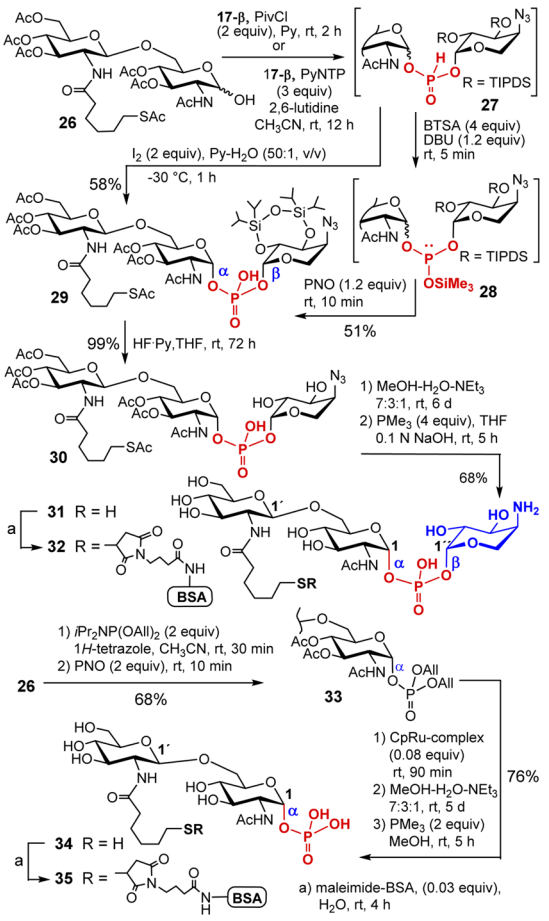
**Scheme 3. Stereoselective Synthesis of $\beta$-L-Ara4N Glycosyl H-Phosphonates**




and **25-$\beta$** in 62−78% yield (Table 1). Exchange of the 4-azido group in **2** for the Fmoc-protected amino group, followed by anomeric deallylation and phosphitylation with SalPCl, furnished an inseparable mixture of $\alpha$- and $\beta$-L-Ara4N glycosyl H-phosphonates in 84% yield, in which the $\alpha$-configured product prevailed ($\alpha/\beta$ = 2:1, Scheme S1).

Glycosyl H-phosphonate **17-$\beta$** was coupled to the $\beta(1\rightarrow6)$-linked diglucosamine lactol **26**[14] ($\alpha/\beta$ = 2:1) in pyridine using pivaloyl chloride (PivCl) as activating agent to furnish H-phosphonate glycosyl phosphodiester **27** as an anomeric mixture at GlcN moiety ($\alpha/\beta$ = 2:1, according to $^1$H and $^{31}$P NMR data) (Scheme 4). Oxidation of **27** by treatment with aq $I_2$ at −40 °C

**Scheme 4. Synthesis of $\beta$GlcN(1→6)-$\alpha$GlcN(1→P←1)-$\beta$-L-Ara4N Epitope and BSA Conjugate**




followed by chromatographic purification afforded anomerically pure binary glycosyl phosphodiester **29** in 58% yield, whereas an undesired $\beta$-anomeric product was expectedly destroyed upon aqueous $I_2$-mediated oxidation and isolation by chromatography on silica gel.[25] Despite the apparent simplicity, this procedure requires strict control of reaction conditions since minor alterations can result in hydrolysis of the binary glycosyl phosphodiester or the appearance of byproducts. PivCl-mediated coupling can instigate formation of P-acyl byproducts[26] resulting from an over-reaction of **17** or **27** with PivCl and the formation of GlcNAc-derived oxazolines.[27] Oxidation of **27** into the P(V) counterpart **29** by treatment with aq $I_2$ could result in rapid hydrolysis, with the loss of Ara4N, if the optimized reaction conditions were not rigorously followed. To circumvent these possible drawbacks, we exchanged the activating agent to 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)-phosphonium hexafluorophosphate (PyNTP), which selectively reacted with the electrophilic phosphorus atom of **17** to form a P−N-activated intermediate.[15,28] Formation of the binary glycosyl H-phospho-



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nate diester **27** was confirmed by appearance of the P−H-coupled signals ($^{31}$P NMR, $\delta$: 7.3 ppm, $J_{PH}$ = 730 Hz and 5.6 ppm, $J_{PH}$ = 754 Hz) corresponding to the $\beta,\beta$- and $\alpha,\beta$-linked H-phosphonate diesters (Figure S3).

Anhydrous oxidation of tetra-coordinated H-phosphonate **27** was performed in two steps: by treatment with *N,O*-bis-(trimethylsilyl)acetamide (BTSA) in the presence of DBU which transformed **27** into the three-coordinated silylphosphite **28**, and by oxidation[29] of **28** with 2-(phenylsulfonyl)-3-(3-nitrophenyl)-oxaziridine (PNO) to furnish 1,1′-glycosyl phosphodiester **29**. Despite the somewhat lower yield, the mildness of reaction conditions renders this approach an expedient alternative to the PivCl-mediated coupling. Stepwise deprotection of **29** included treatment with HF·Py to remove the TIPDS protecting group, a deacetylation of **30** with MeOH/$H_2O$/$NEt_3$ and final reduction of the 4-azido group by reaction with trimethylphosphine[30] in aq NaOH/THF to provide **31**. Direct coupling of **31** to a maleimide-activated BSA gave the neoglycoconjugate **32** comprising up to an average of 19 pseudotrisaccharide units per BSA molecule. An Ara4N-free counterpart of **32**, a neoglycoconjugate **35**, was synthesized as a negative control. To this end, **26** was phosphitylated by applying the phosphoramidite procedure. The intermediate phosphite triester was oxidized with PNO to give phosphate triester **33**, and the allyl protecting groups at phosphorus were removed by treatment with [CpRu(IV)($\pi$-$C_3H_5$)-(2-quinolinecarboxylato)]-$PF_6$ complex.[15] Deacetylation followed by reduction of the eventually formed disulfide bond with $PMe_3$ and ensuing coupling to the maleimide activated BSA furnished **35**.

A novel efficient approach for anomeric deallylation with retention of configuration has allowed for the stereoselective synthesis of anomerically pure $\beta$-L-Ara4N glycosyl H-phosphonates and could be of general use for the anomeric deprotection of other allyl pyranosides. A facile access to both anomers of L-Ara4N glycosyl H-phosphonates now creates opportunities for the synthesis of $\alpha$-L-Ara4N-containing phosphodiesters as prospective antibiotics to combat infections by multi-drug-resistant Ara4N-producing Gram-negative strains and for synthetic preparation of $\beta$-L-Ara4N-containing antigenic LPS epitopes as potential diagnostics.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03358.

Additional figures, experimental procedures, and NMR data (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Raetz, C. R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635.
(2) Needham, B. D.; Trent, M. S. *Nat. Rev. Microbiol.* **2013**, *11*, 467.
(3) Hamad, M. A.; Di Lorenzo, F.; Molinaro, A.; Valvano, M. A. *Mol. Microbiol.* **2012**, *85*, 962.
(4) Moskowitz, S. M.; Ernst, R. K.; Miller, S. I. *J. Bacteriol.* **2004**, *186*, 575.
(5) Winfield, M. D.; Latifi, T.; Groisman, E. A. *J. Biol. Chem.* **2005**, *280*, 14765.
(6) Aoyagi, K. L.; Brooks, B. D.; Bearden, S. W.; Montenieri, J. A.; Gage, K. L.; Fisher, M. A. *Microbiology* **2015**, *161*, 628.
(7) Zhou, Z.; Ribeiro, A. A.; Lin, S.; Cotter, R. J.; Miller, S. I.; Raetz, C. R. H. *J. Biol. Chem.* **2001**, *276*, 43111.
(8) Breazeale, S. D.; Ribeiro, A. A.; Raetz, C. R. H. *J. Biol. Chem.* **2003**, *278*, 24731.
(9) De Soyza, A.; Silipo, A.; Lanzetta, R.; Govan, J. R.; Molinaro, A. *Innate Immun.* **2008**, *14*, 127.
(10) Rubin, E. J.; Herrera, C. M.; Crofts, A. A.; Trent, M. S. *Antimicrob. Agents Chemother.* **2015**, *59*, 2051.
(11) Raetz, C. R. H.; Reynolds, C. M.; Trent, M. S.; Bishop, R. E. *Annu. Rev. Biochem.* **2007**, *76*, 295.
(12) Tavares-Carreón, F.; Patel, K. B.; Valvano, M. A. *Sci. Rep.* **2015**, *5*, 10773.
(13) Petrou, V. I.; Herrera, C. M.; Schultz, K. M.; Clarke, O. B.; Vendome, J.; Tomasek, D.; Banerjee, S.; Rajashankar, K. R.; Belcher Dufrisne, M.; Kloss, B.; Kloppmann, E.; Rost, B.; Klug, C. S.; Trent, M. S.; Shapiro, L.; Mancia, F. *Science* **2016**, *351*, 608.
(14) Baum, D.; Kosma, P.; Zamyatina, A. *Org. Lett.* **2014**, *16*, 3772.
(15) Hollaus, R.; Ittig, S.; Hofinger, A.; Haegman, M.; Beyaert, R.; Kosma, P.; Zamyatina, A. *Chem. - Eur. J.* **2015**, *21*, 4102.
(16) Muller, B.; Blaukopf, M.; Hofinger, A.; Zamyatina, A.; Brade, H.; Kosma, P. *Synthesis* **2010**, *2010*, 3143.
(17) Oscarson, S.; Sehgelmeble, F. W. *Tetrahedron: Asymmetry* **2005**, *16*, 121.
(18) Morelli, L.; Cancogni, D.; Tontini, M.; Nilo, A.; Filippini, S.; Costantino, P.; Romano, M. R.; Berti, F.; Adamo, R.; Lay, L. *Beilstein J. Org. Chem.* **2014**, *10*, 2367.
(19) Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661.
(20) Yang, H.; Wang, P. *J. Org. Chem.* **2013**, *78*, 1858.
(21) Barbier, M.; Grand, E.; Kovensky, J. *Carbohydr. Res.* **2007**, *342*, 2635.
(22) Kajihara, K.; Arisawa, M.; Shuto, S. *J. Org. Chem.* **2008**, *73*, 9494.
(23) Smith, A. B.; Rivero, R. A.; Hale, K. J.; Vaccaro, H. A. *J. Am. Chem. Soc.* **1991**, *113*, 2092.
(24) Marshall, J. A.; Garofalo, A. W. *J. Org. Chem.* **1993**, *58*, 3675.
(25) Zamyatina, A.; Kosma, P. In *Carbohydrate Chemistry. Chemical and Biological Approaches*; Rauter, A. P., Lindhorst, T. K., Eds.; RSC Publishing: Cambridge, UK, 2009; Vol. 35, p 71.
(26) Froehler, B. C.; Matteucci, M. D. *Tetrahedron Lett.* **1986**, *27*, 469.
(27) Westerduin, P.; Veeneman, G. H.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 6271.
(28) Saito, K.; Wada, T. *Tetrahedron Lett.* **2014**, *55*, 1991.
(29) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710.
(30) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Chou, T. C.; Dong, H.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 10913.




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