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Synthesis of and evaluation of lipid A modification by 4-substituted 4-deoxy arabinose analogs as potential inhibitors of bacterial polymyxin resistance

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Abstract—Three sets of novel 4-deoxy-L-arabinose analogs were synthesized and evaluated as potential inhibitors of the bacterial resistance mechanism in which lipid A, on the outer membrane, is modified with 4-amino-4-deoxy-L-arabinose (L-Ara4N). One compound diminished the transfer of L-Ara4N onto lipid A. These results suggest that small molecules might be designed that would effect the same reversal of bacterial resistance observed in genetic knockouts.

Polymyxin is a currently prescribed antibiotic whose use is predominately topical as a result of its systemic toxicity. With the advent of devices that include as 'topical' the aerosol route of administration, polymyxin is now widely used for chronic airway infections, notably in cystic fibrosis patients for whom this is a significant cause of morbidity and mortality.[1] Polymyxin, like all cationic antimicrobial peptides (CAPs), binds to negative charges on phosphates of the lipopolysaccharide component lipid A that makes up the outer leaflet of the Gram-negative bacterial outer membrane. Lipid A binding by CAPs results in critical disorganization of the outer membrane.[2] Bacteria can resist the action of polymyxin by modification of lipid A phosphates, which decreases surface negative charge and presumably reduces polymyxin binding to the altered electrostatic topography of the remodeled membrane.[3–5]

The pathway that results in aminoarabinose incorporation into lipid A has been elucidated in some detail. Five enzymes catalyze seven reactions that produce an undecaprenylated aminosugar at the cytoplasmic surface of the inner membrane. This glycolipid is then translocated to the periplasmic side of the inner membrane where ArnT/PmrK catalyzes the displacement of the prenyl group by the 4′- and/or 1′-phosphate(s) of lipid A.[6]

If the mechanisms of lipid A modification could be disabled, CAP-resistant bacteria might be returned to a susceptible state. An agent capable of this restoration could also promote the activity of host antimicrobial peptides in controlling infection, and indeed, mutants defective for the addition of aminoarabinose are attenuated for virulence in mouse models of infection. A re-sensitizing agent could also expand the repertoire of utility for polymyxin and enable the durable therapeutic utility of the newer CAPs under investigation.[7] While the ultimate goal of the project is to develop small molecule inhibitors of the L-Ara4N-lipid A that could exogenously reproduce the effects of genetic knockouts, we have initially focused on the synthesis of compounds with inhibitory activity in vitro. We now report on the synthesis and evaluation of a series of 4-modified arabinose analogs.

Keywords: Lipid A remodeling; Aminoarabinose; 4-Deoxy-4-substituted arabinose analogs; Polymyxin resistance.
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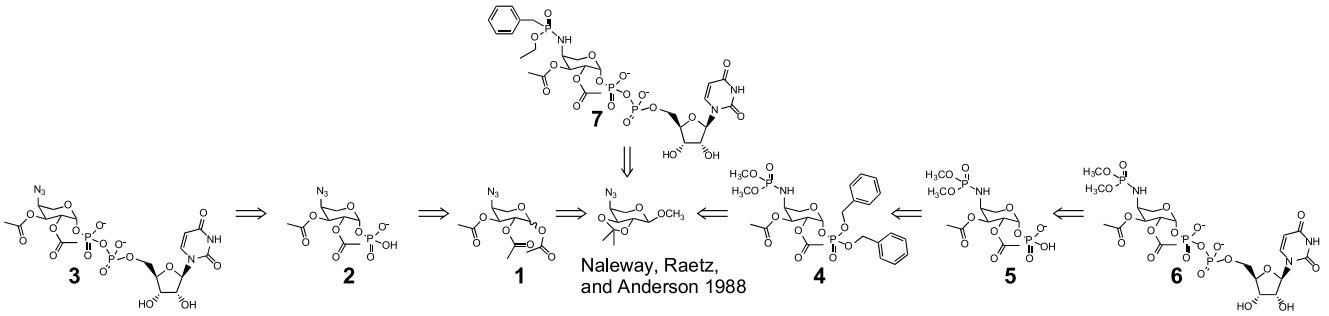
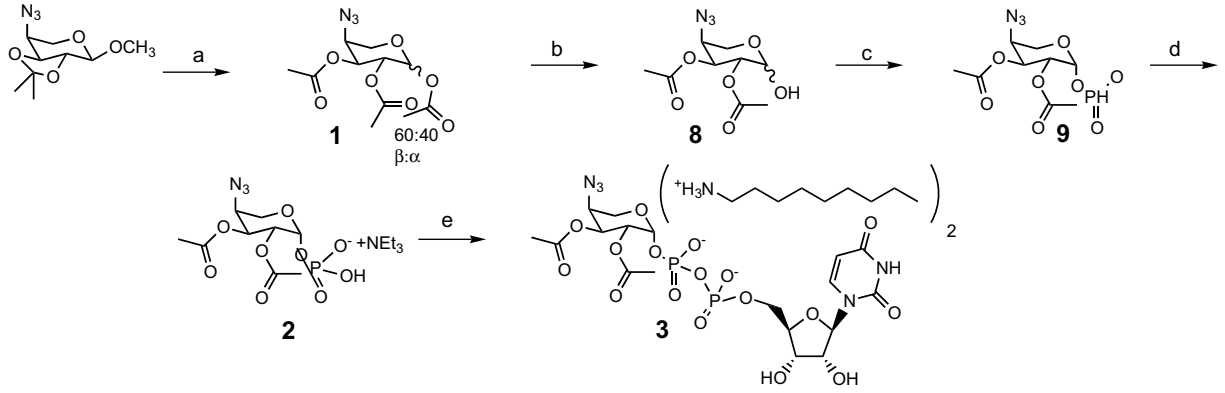


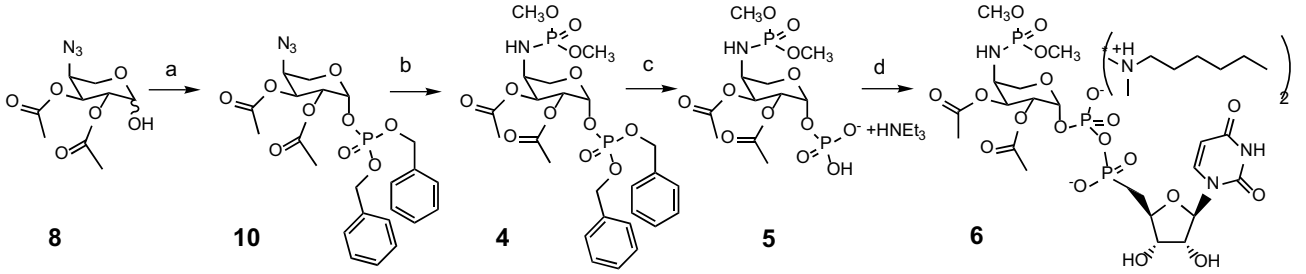
Figure 1.

The 4-amino function of aminoarabinose is formylated for several of the cytoplasmic biosynthetic steps. N-formylation drives the otherwise-unfavorable transamination reaction (ArnB/PmrH) forward,[8] and the formyl group is subsequently removed by ArnD.[9] We constructed analogs (Fig. 1) that might mimic the N-formylation transition state or serve as surrogates for the formylated amine. Three isosteres for the N–C(OH)–N framework were selected, phosphonamidate, phosphoramidate, and azide. For the azide and phosphoramidate, several substituents at the anomeric carbon were explored: *O*-phosphoryl, *O*-acetyl, *O*-benzyl, and, the most substrate-mimetic, *O*-uridyldiphosphoryl. Although the initial biological characterization was to be in cell-free enzyme preparations, we designed in some structural features that anticipated our longer term plans for compounds having good stability in the human host and the ability to penetrate bacterial outer membranes. Since five of the L-Ara4N biosynthetic enzymes, both the soluble and membrane bound, lie beyond the inner membrane, while the lipid A modifying enzyme, ArnT/PmrK, is on the inner membrane, access to these enzymes was expected to be a significant determinant of our compounds' ultimate capabilities against intact bacteria. With this in mind, the 2- and 3-hydroxyls were acetylated, and the phosphoramidates/phosphonamidates maintained as esters. We anticipated that these esters would encounter nonselective esterases and be hydrolyzed in vivo. The selection of the compounds benefited from an economy in design, since many candidates were also intermediates en route to the more complex structures. The 4-azido-2,3-isopropylidene-1-methyl acetal[10] served as the single common precursor.

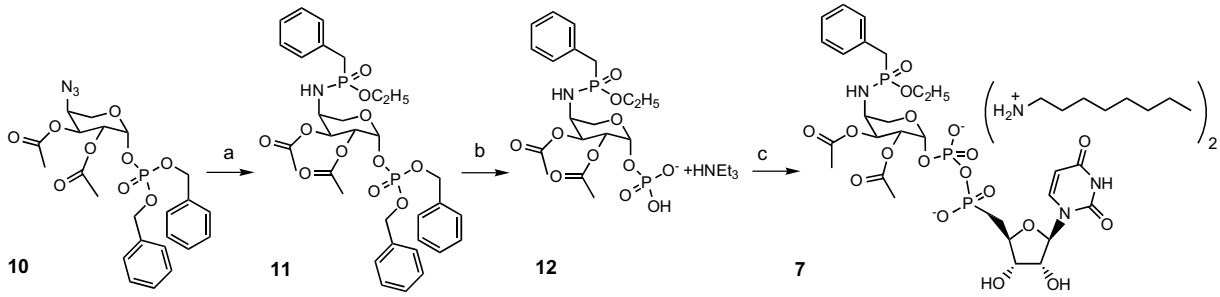
The 4-azido analogs **1**, **2**, and **3** were synthesized as shown in Scheme 1. Conversion of the isopropylidene



Scheme 1. Reagents and conditions: (a) i—1 M HCl/acetone, then $NaHCO_3$, ii—1 M HCl, 90 °C, iii—Li_2CO_3, iv—Ac_2O, NaOAc, reflux; (b) $(CH_3)_2NH$, CH_3CN, −20 °C; (c) i—2-chloro-4[*H*]-benzodioxaphosphorin-4-one, NEt_3, THF–dioxane, 0 °C, ii—H_2O; (d) i—Dowex 50W × 8, H^+ form, THF–dioxane, ii—*t*-BuOOH/decane, cat. I_2, NEt_3; (e) i—tri-*n*-octylamine/pyridine, ii—UMP morpholidate, DCCM salt, tetrazole, pyridine.



Scheme 2. Reagents: (a) i—*n*-BuLi, THF, ii—tetrabenzylpyrophosphate, THF; (b) $(CH_3)_3P$, pyridine, CH_2Cl_2; (c) i—H_2, Pd–C, CH_3OH, ii—NEt_3; (d) UMP morpholidate, tetrazole, pyridine.



Scheme 3. Reagents: (a) i—ethyl benzyl H-phosphinate, BTSTFA, pyridine, ii—1:3 CH_3OH–$CHCl_3$, Δ; (b) i—H_2, Pd–C, CH_3OH, ii—NEt_3; (c) i—tri-*n*-octylamine, pyridine, ii—UMP morpholidate, tetrazole, pyridine.

methyl acetal directly to the triacetate was accomplished in a three-step sequence using Takeo's procedure.[11] Selective 1-*O*-deacetylation was achieved by treatment of the triacetate with a saturated solution of dimethylamine in acetonitrile at −20 °C.[12] Installation of the phosphate via the two-step method[13] gave the β-L-arabinose phosphate **2** in modest yield but good stereochemical purity.[14] Reaction of the 1-phosphate, as its tri-*n*-octylamine salt, with uridine monophosphoryl morpholidate[15] in the presence of tetrazole, completed the synthesis of **3**. For the synthesis of **4**, **5**, and **6** the azide was converted via a modified Staudinger reaction to the phosphoramidate (Scheme 2).[16] Without the synthetic incompatibility presented by the azide function, dibenzylphosphate could be directly introduced (**8**–**10**) and readily removed (**4**–**5**).

The efficiency of the Staudinger reaction that retains the N–P bond for the preparation of phosphoramidates encouraged us to develop a similar strategy for the synthesis of phosphonamidate **7**. Intermediate **10** was reacted with ethyl benzyl H-phosphinate[17,18] in the presence of 6 equivalents of BTSTFA, followed by thermal rearrangement to give phosphonamidate **11** (Scheme 3).[16,19] Hydrogenolysis of the benzyl esters gave the free phosphate **12**. Solubility mandated prior formation of the bis-*n*-octylammonium salt for the reaction with UMP morpholodate, giving **7** as the corresponding bis-*n*-octylammonium salt.

Compounds **1**–**7** were evaluated in vitro in a single global assay for the decarboxylase and transformylase activity encoded in ArnA/PmrI as well as the transaminase activity of ArnB/PmrH as previously described.[20,21] These enzymes were unaffected at compound concentrations up to 100 μM. Crystal structures of ArnA and ArnB[20–22] suggest that the 2- and 3-acetates, as well as excessive functionality at the 4-position, may be impeding rather than promoting activity against these two aminoarabinose biosynthetic enzymes.

Considering that the 4-azido group might be a neutral surrogate for the 4-amino group, compounds **1**, **2**, and **3** were selected for evaluation in an assay against PmrK/ArnT.[6] Compound **7** was included as a structural (and presumed negative) control of the most substrate-like of the azide trio, compound **3**. To monitor PmrK/ArnT activity, whole membranes from the Gram-negative bacterium *Salmonella typhimurium* strain JSG435[23] were assayed for the addition of aminoarabinose to ^{32}P labeled precursors of lipid A.[6] *Salmonella* JSG435 overproduces the enzymatic machinery required for the modification of lipid A with aminoarabinose, including the membrane-bound glycosyl transferase PmrK/ArnT.[6] As determined by densiometry[24] of the ^{32}P TLC (Fig. 2), compound **1** effected a threefold diminution of aminoarabinose addition to the phosphate groups of the lipid A precursor lipid IV_A, demonstrating inhibition of PmrK/ArnT.

Acting against the transferase PmrK/ArnT, azido compound **1** represents the first reported small molecule to

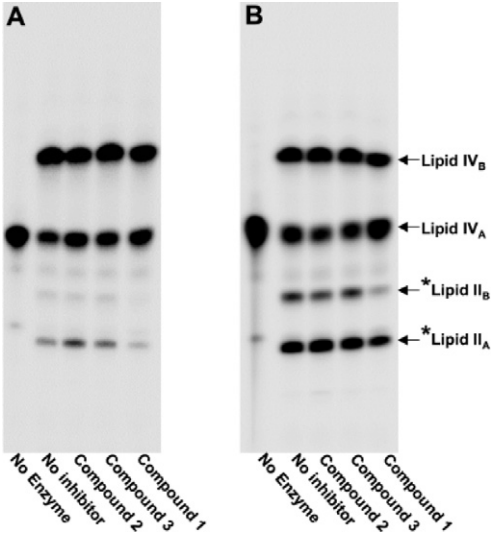


Figure 2. Assay of PmrK/ArnT aminoarabinose transferase activity. Membranes from *S. typhimurium* strain JSG435 were assayed for the transfer of L-Ara4N from endogenous sources to the tetra-acylated lipid A precursor 4′-^{32}P-lipid IV_A. The protein concentration was 1 mg/ml and the concentration of the inhibitor when present was 1 mM. Assays were carried out as previously described[6] for 0.5 h (A) or for 6 h (B) at 30 C using 5 μM 4′-^{32}P-lipid IV_A (20,000 cpm/nmol). The reaction products were separated by thin layer chromatography in the solvent chloroform, pyridine, 88% formic acid, water (50:50:16:5, v/v) and visualized by Phosphorimaging. Lipid II_A arises from the addition of L-Ara4N to the starting substrate lipid IV_A. Lipids II_B and IV_B arise from the PagP-dependent addition of palmitate to lipid II_A and lipid IV_A, respectively. Reaction products modified with L-Ara4N are indicated by an asterisk.

interfere with lipid A modification. With the synthetic methods developed, the structural biology of the enzymes well characterized, and with compound **1** as a potential lead, additional analogs may now be prepared with increased activity against the lipid A modifying enzymes and against resistant bacteria.[25]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.061.

References and notes

1. Beringer, P. *Curr. Opin. Pulm. Med.* **2001**, *7*, 434.
2. Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238.
3. Peschel, A. *Trends Microbiol.* **2002**, *10*, 179.
4. McPhee, J. B.; Lewenza, S.; Hancock, R. E. *Mol. Microbiol.* **2003**, *50*, 205.
5. Bader, M. W.; Navarre, W. W.; Shiau, W.; Nikaido, H.; Frye, J. G.; McClelland, M.; Fang, F. C.; Miller, S. I. *Mol. Microbiol.* **2003**, *50*, 219.
6. Trent, M. S.; Ribeiro, A. A.; Lin, S.; Cotter, R. J.; Raetz, C. R. *J. Biol. Chem.* **2001**, *276*, 43122.
7. Baron, C.; Coombes, B. *Infect. Disord. Drug Targets* **2007**, *7*, 19.
8. Breazeale, S. D.; Ribeiro, A. A.; McClerren, A. L.; Raetz, C. R. *J. Biol. Chem.* **2005**, *280*, 14154.
9. Williams, G. J.; Breazeale, S. D.; Raetz, C. R.; Naismith, J. H. *J. Biol. Chem.* **2005**, *280*, 23000.
10. Naleway, J. J.; Raetz, C. R.; Anderson, L. *Carbohydr. Res.* **1988**, *179*, 199.
11. Takeo, K.; Murata, Y.; Kitamura, S. *Carbohydr. Res.* **1992**, *224*, 311.
12. Upreti, M. R. Dipali; Vishwakarma, Ram A. *Tetrahedron* **2000**, *56*, 6577.
13. Takaya, K.; Nagahori, N.; Kurogochi, M.; Furuike, T.; Miura, N.; Monde, K.; Lee, Y. C.; Nishimura, S. *J. Med. Chem.* **2005**, *48*, 6054.
14. For the synthesis of compound **2** and analytical data, please see Supplementary information.
15. Wittmann, V.; Wong, C. H. *J. Org. Chem.* **1997**, *62*, 2144.
16. For the synthesis of compound **4** and analytical data, please see Supplementary information.
17. Abrunhosa-Thomas, I.; Ribiere, Patrice; Adcock, Alicia C. *Synthesis* **2006**, 325.
18. Deprele, S. M.; Jean-Luc, A. *J. Organiomet. Chem.* **2002**, *634–644*, 154.
19. For the synthesis of compound **11** and analytical data, please see Supplementary information. Compound **11**.
20. Gatzeva-Topalova, P. Z.; May, A. P.; Sousa, M. C. *Biochemistry* **2005**, *44*, 5328.
21. Gatzeva-Topalova, P. Z.; May, A. P.; Sousa, M. C. *Structure* **2005**, *13*, 929.
22. Gatzeva-Topalova, P. Z.; May, A. P.; Sousa, M. C. *Biochemistry* **2004**, *43*, 13370.
23. Gunn, J. S.; Ryan, S. S.; Van Velkinburgh, J. C.; Ernst, R. K.; Miller, S. I. *Infect. Immun.* **2000**, *68*, 6139.
24. TLC plates were exposed overnight to a PhosphorImager Screen and product formation detected using a Bio-Rad Molecular Imager PhosphorImager. Enzyme activity was calculated by determining the percentage of the substrate converted to product by densitometry using Quantity One Software.
25. A preliminary assay against whole bacteria showed that none of the compounds, at highest concentrations ranging from 90 mM (compound **1**) to 500 mM (compound **7**), were able to restore susceptibility to the CAP polymyxin in *Salmonella* strain JSG435 that constitutively synthesizes a lipid A modified with aminoarabinose. The lack of effect against intact bacteria could result from insufficient activity against the target, poor permeability/retention, metabolic inactivation, or a combination of these factors.