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Synthesis of Lipid-Linked Arabinofuranose Donors for Glycosyltransferases

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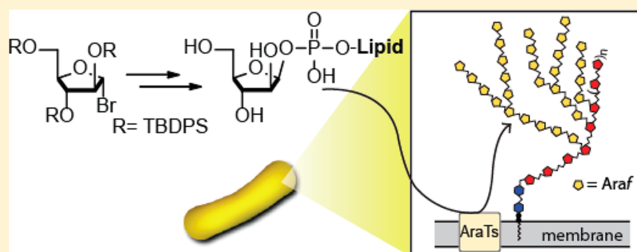
Supporting Information

ABSTRACT: Mycobacteria and corynebacteria use decaprenylphosphoryl-*β*-D-arabinofuranose (DPA) as a critical cell wall building block. Arabinofuranosyltransferases that process this substrate to mediate cell wall assembly have served as drug targets, but little is known about the substrate specificity of any of these enzymes. To probe substrate recognition of DPA, we developed a general and efficient synthetic route to *β*-D-arabinofuranosyl phosphodiesters. In this approach, the key glycosyl phosphodiester bond-forming reaction proceeds with high *β*-selectivity. In addition to its stereoselectivity, our route provides the means to readily access a variety of different lipid analogues, including aliphatic and polyprenyl substrates.



INTRODUCTION

Mycobacteria and corynebacteria have a robust cell wall that is crucial for their survival and pathogenesis. In the case of *Mycobacterium tuberculosis,* the enzymes responsible for cell wall biosynthesis have been identified as promising targets for the development of new drugs to treat tuberculosis.[1] A significant proportion of the mycobacterial cell envelope is composed of arabinofuranose (Ara*f*), which is found in the form of a large branched heteropolysaccharide known as the arabinogalactan (AG, Figure 1a) as well as the lipoarabinomannan (LAM).[1−3] The assembly of the arabinan polysaccharide is catalyzed by a series of arabinofuranosyltransferases, which utilize the lipid-linked donor substrate, decaprenylphosphoryl-*β*-D-arabinofuranose (DPA, Figure 1b), to add single Ara*f* residues to a growing arabinan polysaccharide.[4] Some of these arabinofuranosyltransferases are targets of the first line antitubercular agent ethambutol.[5] Despite their known potential value as therapeutic targets, little is known about the mechanism of substrate preferences of any of these enzymes.

Paramount to the study of glycan biosynthesis is the ability to readily obtain renewable quantities of glycosyl donor and acceptor substrates or functional analogues thereof.[6,7] DPA can be accessed by total synthesis; however, the required decaprenol precursor is costly and only available in small quantities.[5,8] Additionally, the long decaprenyl lipid of DPA confers poor aqueous solubility upon the glycosyl donor, thereby complicating the study of arabinofuranosyltransferases. A more readily available, shorter lipid analogue of DPA could facilitate a wide range of mechanistic studies. A recent report provides impetus to generate DPA analogues, as proteoliposomes containing the mycobacterial arabinofuranosyltransferase AftC could use a (*Z*,*Z*)-farnesyl lipid analogue to transfer a single Ara*f* residue to a synthetic acceptor.[9] To explore

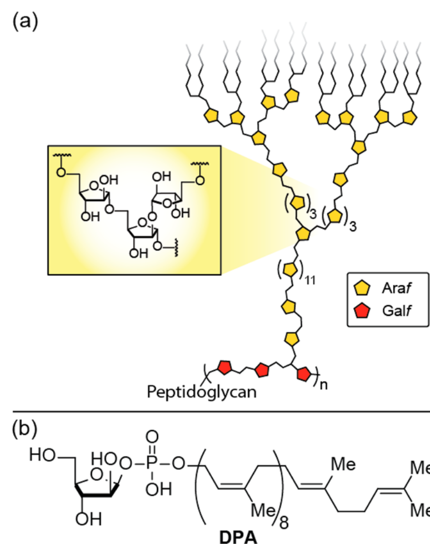


Figure 1. (a) Schematic depiction of a portion of the mycobacterial cell wall showing the galactofuranose (red) and arabinofuranose (yellow) polysaccharide components and (b) decaprenylphosphoryl-*β*-D-arabinofuranose (DPA) is the building block used to incorporate arabinofuranose residues.

arabinose incorporation into the cell wall, we sought to prepare various analogues of DPA with both saturated and unsaturated lipid substituents. Using the known synthetic methods, we were unable to generate a wide range of *β*-D-arabinofuranosyl

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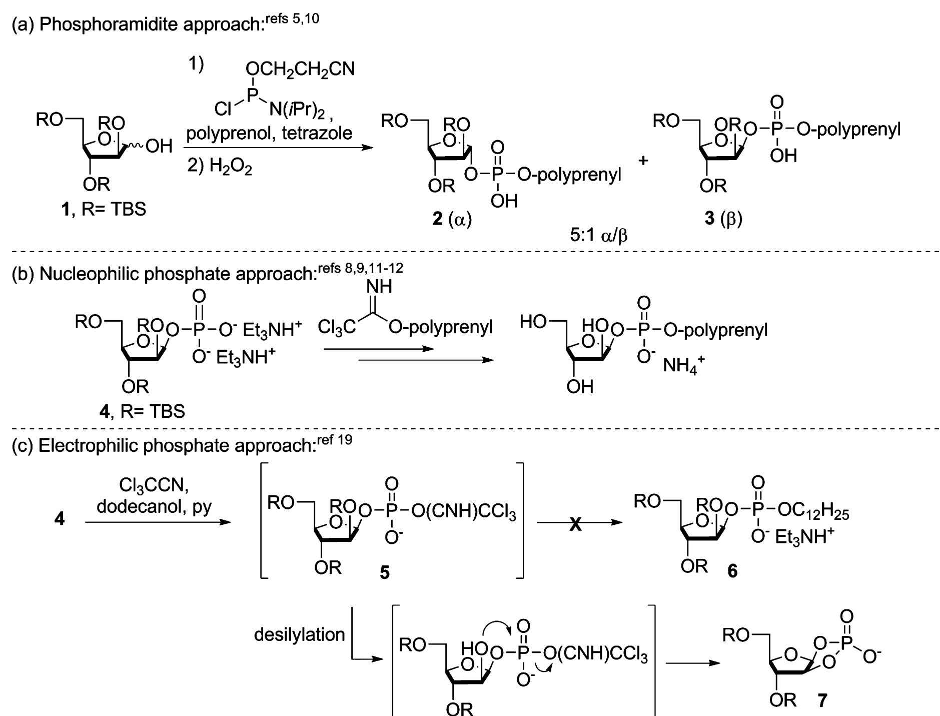


Figure 2. Synthetic approaches to DPA and analogues.

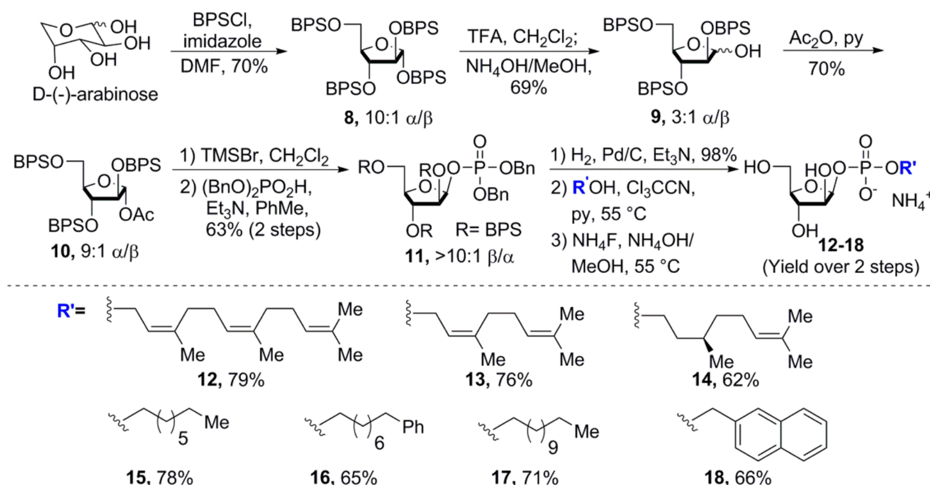
phosphodiesters. Some methods have restrictions on the type of lipid that can be appended, and many approaches give rise to mixtures of glycosylphosphate derivatives that are difficult to separate. Because only β-glycosyl phosphodiesters are candidate substrates for the arabinofuranosyltransferases, the most valuable synthetic method would be one that generates the β-anomer selectively. Here we describe a route to β-D-arabinofuranosyl phosphodiesters that provides both saturated and unsaturated products with high stereoselectivity for the β-anomer.

■ RESULTS AND DISCUSSION

To date, there have been two different approaches to the synthesis of DPA and shorter polyprenyl analogues. One approach developed by Lee and co-workers uses a phosphoramidite coupling method to join 2,3,5-silyl protected Ara*f* (**1**) with a corresponding polyprenol (Figure 2a).[5,10] After oxidation of the intermediate phosphite, the desired phosphodiester is formed as a 5:1 mixture of α- and β-anomers (**2** and **3**). This approach is advantageous in that any number of DPA analogues, both saturated and unsaturated, can be prepared by simply changing the alcohol coupling partner. A disadvantage, however, is that the desired β-anomer is the minor component of the product mixture. Another approach developed by Liav and Brennan relies on first installing a phosphoryl group at the C_1 position of arabinose. In a subsequent step, the resulting Ara*f* phosphate (**4**) is coupled to a lipid-linked trichloroacetimidate intermediate (Figure 2b).[8,9,11,12] This approach favors formation of the desired β-anomer (2:1−4:1 β/α), but it is applicable only to activated lipid acetimidates (i.e., allylic or benzylic) and not aliphatic substrates.[13] Furthermore, this approach requires an extra synthetic step to convert the lipid moiety into the corresponding trichloroacetimidate derivative.

To access both saturated and unsaturated substrates, we envisioned using an alternative strategy in which the phosphodiester moiety is generated directly from monophosphate salt **4** and a lipid alcohol (Figure 1c). Of the known coupling agents for linking alcohols and phosphoryl groups to form phosphodiesters (e.g., carbodiimides, sulfonyl chlorides, Mitsunobu conditions, etc.),[13] we were attracted to the method of Cramer and co-workers, which uses trichloroacetonitrile to activate the phosphoryl group.[14−17] Applying this approach to our targets involves generating iminophosphate intermediate **5**, which can undergo nucleophilic attack by an alcohol. When we attempted to carry out this reaction on a TBS-protected substrate, we found the phosphorylated Ara*f* derivative **4** to be quite unstable. We traced this instability to the lability of its silyl groups, which underwent cleavage even during storage at −20 °C. When phosphodiester coupling was attempted using freshly prepared **4** and dodecanol, only the minor α-anomer underwent coupling whereas the desired β-anomer afforded the corresponding C_1,C_2 cyclic phosphate **7**.[18] Although it was not noted, others appear to have encountered this problem previously.[19] The production of this undesired product is a consequence of the deleterious removal of the C_2 *tert*-butyldimethylsilyl (TBS) ether and subsequent intramolecular cyclization via the electrophilic iminophosphate intermediate. Thus, the unwanted desilylation not only decreased the yield of our target compound but also precluded the production of the requisite β-anomer.

To obtain our desired DPA analogues, a protecting group strategy for Ara*f* was needed that circumvented the propensity of the TBS ether at the 2-position to undergo cleavage. It has previously been shown that nonparticipating protecting groups such as benzyl groups provide high selectivity for the β-anomer during phosphorylation of the corresponding protected α-glycosyl bromide intermediate.[20] Still, the conditions needed to remove the benzyl protecting groups would interfere with accessing substrates that possess alkene-containing lipids. Alternatively, ester protecting groups are compatible with installing a wide variety of lipids but offer poor selectivity in the glycosylation reaction due to competing neighboring group



Scheme 1. Synthesis of β-D-Arabinofuranosyl Phosphodiesters 12−18

participation.[20−22] For the synthesis of DPA derivatives, silyl groups offer an ideal combination of selectivity and functional group tolerance. We therefore reasoned that a potential solution to the observed instability of the TBS protecting group would be to use a more hindered *tert*-butyldiphenylsilyl (BPS) protecting group.

On the surface, the use of the BPS rather than TBS group seemed to be a straightforward perturbation, but there were uncertainties. The first was whether this exchange would indeed allow us to generate the β-linked Ara*f* derivatives (vide infra). Perhaps the most pressing unknown, however, was whether the steric demands of this protecting group would prevent generating the target glycosyl donor. For example, a recent attempt by Dureau and co-workers to persilylate galactose with BPSCl led only to a mixture of partially silylated products.[23] Fortunately, when D-arabinose was treated with BPSCl and imidazole, the desired tetra-BPS-protected Ara*f* intermediate **8** was obtained in 70% yield. This product was obtained as a 10:1 mixture of anomers favoring the α-configuration (Scheme 1). Selective removal of the anomeric BPS group was accomplished by treating **7** with excess trifluoroacetic acid for 5 min, followed by carefully pouring the reaction mixture into a solution of ammonium hydroxide in methanol at −15 °C. The desired product, 2,3,5-(BPS)-Ara*f* (**9**), was obtained as a 3:1 mixture of α- and β-anomers. Acetylation of **9** resulted in glycosyl acetate **10**, which was formed as a 9:1 ratio of anomers, again favoring the α-anomer.

The C_1 phosphate moiety was installed in two steps: first, conversion of acetate **10** into the corresponding glycosyl bromide intermediate was effected using bromotrimethylsilane, and second, displacement of the anomeric bromide substituent was carried out with dibenzyl phosphate serving as a nucleophile to afford **11**. This two-step sequence was highly selective for the desired β-anomer (>10:1 β/α).[24] This selectivity is notable. When the same reaction sequence was performed on the analogous TBS-protected intermediate, the process was much less selective (a 2:1−4:1 mixture of β- and α-anomers). When BPS protection was employed, therefore, not only did the desired β-linked phosphotriester predominate, but it was obtained in high yield.

We considered the origin of the higher selectivity obtained with the substrate bearing BPS relative to that possessing TBS protecting groups. Since the glycosylation reaction of the phosphodiester is anticipated to proceed through an S_N2 mechanism, one might anticipate that the ratio of glycosyl bromide anomers would be reflected in the ratio of phosphorylated products.[20] NMR analysis of the crude glycosyl bromides, however, revealed that the identity of the silyl group has little influence on the ratio of glycosyl bromides; both substrates had a preference for the α-configuration (92:8 α/β for TBS and >95:5 α/β for BPS).[25,26] More flexibility in the TBS-protected substrate may allow the reaction to proceed through not only the S_N2 reaction but also a competing S_N1-like pathway involving an oxocarbenium intermediate.[27] The increased steric demands in the BPS-protected substrate may make it difficult for this species to adopt a conformation that can accommodate an oxocarbenium intermediate. Whatever the origins of the selectivity, this phosphorylation reaction is a remarkable case in which the identity of the silyl group has a large effect on selectivity in a furanose ring system. It should be noted that bulky silicon protecting groups have been exploited in pyranose systems to control ring conformation and anomeric selectivity in glycosylation reactions.[28−30] Moreover, Bols and co-workers demonstrated that conformational preferences in pyranose glycosyl donors enforced by bulky silicon protecting groups can dramatically enhance their reactivity in glycosylation reactions.[31−34] No such effects had been reported in furanose sugars. We postulate that the dramatic influence of the sterically demanding silyl substituents that we observed may be exploited to produce other furanosides with high stereoselectivity.

With the C_1 phosphoryl group installed in the desired β-configuration, we focused on forming the requisite phosphodiester. First, the benzyl substituents on the phosphotriester were removed by hydrogenolysis to provide the intermediate monophosphate. As expected, this entity was stable; we found no evidence of the silyl group lability that plagued our reactions when TBS was used as a protecting group. We could promote phosphodiester formation using a variety of alcohols (polyprenyl, saturated *n*-alkanes, and naphthyl) by heating the alcohol and protected Ara*f* monophosphate in pyridine at 55 °C in the presence of trichloroacetonitrile. The crude phosphodiesters were then treated with ammonium fluoride in a 15% solution of aqueous ammonium hydroxide in methanol to afford the corresponding β-D-arabinofuranosyl phosphodiesters **12−18** in 62−79% yield over two steps.

■ CONCLUSION

The synthetic sequence described here can provide β-D-arabinofuranosyl phosphodiesters in 8 synthetic steps with an overall yield of 13−16%. This approach is not only highly selective for the β-anomer, but it also offers flexibility. Essentially any derivative can be prepared from the corresponding alcohol and Ara*f* monophosphate salt. We envision that this route will facilitate the study of the mycobacterial arabinofuranosyltransferases as it efficiently and reliably provides access to shorter and more aqueous soluble analogues of the endogenous Ara*f* donor, DPA.

■ EXPERIMENTAL SECTION

General Experimental Procedures, Materials, and Instrumentation. Solvents were purified according to the guidelines in *Purification of Common Laboratory Chemicals*.[35] All reactions were run under nitrogen atmosphere unless otherwise stated. Analytical thin-layer chromatography (TLC) was carried out on E. Merck (Darmstadt) TLC plates precoated with silica gel 60 F254 (250 μm layer thickness). Analyte visualization was accomplished using a UV lamp and by charring with *p*-anisaldehyde solution. Flash column chromatography was performed with Silicycle flash silica gel (40−63 μm, 60 Å pore size) using reagent-grade hexanes and ACS grade ethyl acetate (EtOAc) or methanol and CH_2Cl_2. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a 300 MHz spectrometer (acquired at 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR), a 400 MHz spectrometer (acquired at 400 MHz for ^1H NMR and 101 MHz for ^{13}C NMR), or a 500 MHz spectrometer (acquired at 500 MHz for ^1H NMR and 126 MHz for ^{13}C NMR). Chemical shifts are reported relative to tetramethylsilane or residual solvent peaks in parts per million ($CHCl_3$: ^1H, 7.27, ^{13}C, 77.23; MeOH: ^1H, 3.31, ^{13}C, 49.15). Peak multiplicity is reported as singlet (s), doublet (d), doublet of doublets (dd), doublets of doublets of doublets (ddd), triplet (t), doublet of triplets (dt), etc. High-resolution mass spectra (HRMS) were obtained on an electrospray ionization-time of flight (ESI-TOF) mass spectrometer.

1,2,3,5-Tetra-*O*-*tert*-butyldiphenylsilyl-D-arabinofuranose (8). A mixture of D-(−)-arabinose (200 mg, 1.33 mmol) and imidazole (633 mg, 9.31 mmol) were azeotropically dried by evaporation with toluene (3 × 3 mL). The mixture was taken up in DMF (9 mL), and *tert*-butyldiphenylsilyl chloride (2.1 mL, 8.0 mmol) was added. The reaction mixture was stirred for 48 h at 70 °C and was then quenched by the addition of diethanolamine (0.38 mL, 4.0 mmol). Stirring was continued for 2 h, and the reaction mixture was then poured into a stirring mixture of 15% EtOAc/hexanes (25 mL) and water (25 mL). The phases were separated, and the organic phase was washed with water (3 × 25 mL) and brine (30 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on silica gel, eluting with 4% EtOAc/hexanes. The product containing fractions were combined and concentrated under reduced pressure to provide **8** (1.03g, 70%) as a white foam: R_f = 0.53 (10% EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 7.71−7.49 (m, 16H), 7.41−7.07 (m, 34H), 5.23 (s, 1H), 4.51 (ddd, *J* = 7.4, 5.2, 2.3 Hz, 1H), 4.29 (s, 1H), 4.10 (d, *J* = 2.3 Hz, 1H), 3.53 (dd, *J* = 10.4, 7.2 Hz, 1H), 3.41 (dd, *J* = 10.5, 5.2 Hz, 1H), 1.00 (s, 9H) 0.97−0.95 (m, 18H), 0.70 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.1, 136.1, 136.1, 136.0, 135.8, 135.8, 135.7, 134.2, 133.8, 133.7, 133.5, 133.4, 132.9, 129.8, 129.8, 129.7, 129.7, 129.6, 129.5, 129.2, 128.4, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 125.5, 103.9, 88.9, 84.8, 81.2, 65.5, 27.1, 27.1, 27.0, 26.8, 19.4, 19.4, 19.4, 19.1; HRMS (ESI-TOF$^+$) calcd for $C_{69}H_{86}NO_5Si_4$ (M + NH_4^+) 1120.5578, found 1120.5588.

2,3,5-Tri-*O*-*tert*-butyldiphenylsilyl-D-arabinofuranose (9). To a stirred solution of **8** (1.27g, 1.15 mmol) in CH_2Cl_2 (11.5 mL) was added trifluoroacetic acid (2.90 mL, 37.9 mmol). The reaction mixture was stirred at rt for 5 min and was then slowly poured into a 15% solution of concentrated ammonium hydroxide in MeOH (38 mL) at −15 °C. The mixture was stirred at −15 °C for 30 min and was then warmed to rt. The mixture was partitioned between 1:1 CH_2Cl_2/water (50 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on silica gel, eluting with 4% EtOAc/hexanes. The product-containing fractions were combined and concentrated under reduced pressure to provide **9** (681 mg, 69%) as a clear, highly viscous oil: R_f = 0.31 (10% EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 7.63−7.12 (m, 30H), 5.28 (dd, *J* = 12.6, 3.4 Hz, 0.25H, H-1β), 5.10 (d, *J* = 12.1, 0.75H, H-1α), 4.56 (dd, *J* = 6.9, 6.9 Hz, 0.75H, H-4α), 4.26 (dd, *J* = 1.7, 1.7 Hz, 0.25H, H-2β), 4.22 (s, 0.75H, H-2α), 4.05 (s, 1H, H-3α,β), 3.97 (dd, *J* = 5.8, 5.8 Hz, 0.25H, H-4β), 3.72 (dd, *J* = 10.2, 6.7 Hz, 0.75H, H-5α), 3.68 (d, *J* = 12.1 Hz, 0.75H, OHα), 3.57−3.49 (m, 0.75H, H-5′α), 3.39 (dd, *J* = 10.5, 6.1 Hz, 0.25H, H-5β), 3.29 (dd, *J* = 10.5, 5.7 Hz, 0.25H, H-5′β), 0.98 (s, 2.3H, BPS-β), 0.96 (s, 6.7H, BPS-α), 0.95 (s, 6.7H, BPS-α), 0.92 (s, 2.3H, BPS-β), 0.88 (s, 2.3H, BPS-β), 0.80 (s, 6.7H, BPS-α); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.0, 135.9, 135.9, 135.8, 135.8, 135.7, 135.7, 135.0, 133.8, 133.5, 133.5, 133.4, 133.2, 133.0, 132.9, 132.58, 132.5, 132.5, 132.4, 132.2, 130.2, 130.2, 130.1, 130.0, 129.9, 129.9, 129.9, 129.8, 129.8, 129.7, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 104.0, 98.7, 88.8, 85.5, 81.2, 79.4, 78.7, 78.1, 65.2, 65.0, 27.1, 27.0, 27.0, 26.9, 26.8, 19.3, 19.3, 19.3, 19.2, 19.1; HRMS (ESI-TOF$^+$) calcd for $C_{53}H_{68}NO_5Si_3$ (M + NH_4^+) 882.4400, found 882.4431.

1-*O*-Acetyl-2,3,5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranose (10). To a stirred solution of **9** (0.64 g, 0.74 mmol) in pyridine (7.4 mL) were added 4-(dimethylamino)pyridine (9 mg, 0.07 mmol) and acetic anhydride (0.21 mL, 2.2 mmol). The reaction mixture was stirred at rt for 3.5 h and was then quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL). The mixture was partitioned between CH_2Cl_2 (15 mL) and water (15 mL), the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by flash column chromatography on a 2.5 × 19 cm silica gel column, eluting with 4% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (19−29) were combined and concentrated under reduced pressure to provide **10** (473 mg, 70%) as a clear, highly viscous oil: R_f = 0.30 (6% EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 7.62−7.13 (m, 30H), 5.97 (s, 1H), 4.49 (ddd, *J* = 7.6, 6.2, 1.3 Hz, 1H), 4.33 (s, 1H), 4.16 (d, *J* = 0.9 Hz, 1H), 3.66 (dd, *J* = 10.3, 6.4 Hz, 1H), 3.49 (dd, *J* = 10.3, 7.3 Hz, 1H), 1.94 (s, 3H), 0.97 (s, 9H), 0.96 (s, 9H), 0.83 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 135.9, 135.9, 135.9, 135.8, 135.7, 133.7, 133.5, 133.4, 133.3, 132.7, 132.6, 130.1, 130.0, 129.9, 129.9, 129.8, 129.7, 127.9, 127.9, 127.8, 127.8, 102.6, 90.5, 82.4, 79.1, 64.8, 27.0, 26.9, 21.3, 19.4, 19.4, 19.1; HRMS (ESI-TOF$^+$) calcd for $C_{55}H_{70}NO_6Si_3$ (M + NH_4^+) 924.4506, found 924.4528.

Dibenzyl (2,3,5-*O*-*tert*-Butyldiphenylsilyl-β-D-arabinofuranosyl)-1-phosphate (11). To a stirred solution of **10** (0.46 g, 0.50 mmol) in CH_2Cl_2 (5 mL) was added trimethylsilyl bromide (133 μL, 1.01 mmol). The reaction mixture was stirred at rt for 6 h and was then concentrated under reduced pressure. The crude glycosyl bromide was azeotropically dried with toluene (3 × 2 mL) and then used immediately.

To a stirred solution of azeotropically dried dibenzyl phosphate (281 mg, 1.01 mmol) in toluene (3 mL) were added powdered 4 Å molecular sieves (400 mg) and triethylamine (182 μL, 1.31 mmol). The mixture was cooled to 0 °C, and a solution of the aforementioned glycosyl bromide in toluene (1 mL) was added slowly via cannula. The transfer was completed by rinsing twice with toluene (2 × 0.5 mL). Stirring was continued at 0 °C for 1 h and then at rt overnight. The reaction mixture was filtered through a pad of sand and Celite, rinsing with EtOAc, and the solvent was removed under reduced pressure. Purification was accomplished by flash column chromatography on a 2.5 × 19 cm silica gel column, eluting with 15% EtOAc/hexanes, collecting 13 × 100 mm test tube fractions. The product containing fractions (10−29) were combined and concentrated under reduced pressure to provide **11** (356 mg, 63% over two steps) as a clear viscous

oil: R_f = 0.26 (15% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.41 (m, 13H), 7.40−7.10 (m, 27H), 5.78 (dd, *J* = 5.0, 3.0 Hz, 1H), 4.98 (dd, *J* = 11.9, 6.9 Hz, 1H), 4.87 (dd, *J* = 11.9, 7.7 Hz, 1H), 4.77 (d, *J* = 6.5 Hz, 2H), 4.32 (s, 1H), 4.24 (td, *J* = 7.0, 1.5 Hz, 1H), 4.12 (dd, *J* = 2.4, 2.4 Hz, 1H), 3.72 (dd, *J* = 10.4, 7.2 Hz, 1H), 3.61 (dd, *J* = 10.4, 6.8 Hz, 1H), 0.92 (s, 9H), 0.92 (s, 9H), 0.87 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.2, 136.1, 136.1, 136.0, 136.0, 135.9, 135.9, 135.7, 133.7, 133.5, 133.2, 132.8, 132.8, 130.0, 129.9, 129.9, 129.8, 129.7, 128.5, 128.5, 128.4, 128.3, 128.0, 127.9, 127.9, 127.9, 127.9, 127.8, 127.7, 101.1(d), 87.0, 78.3, 78.2(d), 69.1(d), 69.0(d), 64.7, 27.1, 27.0, 26.9, 19.5, 19.3; HRMS (ESI-TOF$^+$) calcd for $C_{67}H_{81}NO_8Si_3$ (M + NH_4^+) 1142.5003, found 1142.5027.

Bis-triethylammonium (2,3,5-*O*-*tert*-Butyldiphenylsilyl-*β*-D-arabinofuranosyl)-1-phosphate (S3). To a stirred solution of **11** (259 mg, 0.230 mmol) in 10% EtOH/EtOAc (8.2 mL) were added triethylamine (0.8 mL, 5.75 mmol) and 10% palladium on carbon (17 mg, 0.016 mmol). The reaction vessel was purged with N_2 and then equipped with a H_2-filled balloon. The reaction mixture was stirred at rt overnight and was then filtered through a plug of sand and Celite with 10% EtOH/EtOAc. The solvent was removed under reduced pressure to provide monophosphoryl salt **S3** (259 mg, 98%) as a white powder: R_f = 0.5 (15% MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75−7.67 (m, 2H), 7.56 (m, 4H), 7.53−7.48 (m, 2H), 7.46−7.40 (m, 2H), 7.40−7.21 (m, 14H), 7.15 (m, 4H), 7.07 (t, *J* = 7.5 Hz, 2H), 5.86 (dd, *J* = 7.1, 3.0 Hz, 1H), 4.22 (s, 1H), 4.10 (brt, *J* = 2.4 Hz, 1H), 4.05 (t, *J* = 7.2 Hz, 1H), 3.70 (dd, *J* = 10.4, 7.6 Hz, 1H), 3.47 (m, 1H), 2.97 (q, *J* = 7.3 Hz, 12H), 1.25 (t, *J* = 7.3 Hz, 18H), 0.93 (s, 9H), 0.92 (s, 9H), 0.85 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.7, 136.0, 135.8, 135.2, 135.7, 134.1, 133.7, 133.7, 133.6, 133.0, 132.7, 129.7, 129.7, 129.6, 129.4, 127.8, 127.7, 127.7, 127.6, 99.8(d), 85.3, 78.9, 78.5(d), 65.2, 45.6, 27.2, 27.0, 27.0, 19.6, 19.3, 19.2, 8.7. HRMS (ESI-TOF$^+$) calcd for $C_{53}H_{69}NO_8PSi_3$ (M + NH_4^+) 962.4064, found 962.4080.

(*Z*,*Z*)-Farnesylphosphoryl-*β*-D-arabinofuranose (12).[9] A mixture of monophosphate salt **S3** (86 mg, 0.075 mmol) and (*Z*,*Z*)-farnesol[36] (67 mg, 0.30 mmol) were azeotropically dried with anhydrous pyridine (3 × 0.5 mL). The mixture was taken up in pyridine (1 mL), and trichloroacetonitrile (75 μL, 0.75 mmol) was added. The reaction mixture was stirred at 55 °C for 12 h. The solvent was removed under reduced pressure, and the crude phosphodiester was carried onto deprotection without purification.

To a solution of the aforementioned BPS-protected phosphodiester in a 15% solution of concd ammonium hydroxide in methanol (1.5 mL) was added ammonium fluoride (83 mg, 2.2 mmol). The reaction mixture was stirred at 55 °C for 10 h. After the mixture was cooled to rt, CH_2Cl_2 (2 mL) was added, and the precipitate that had formed was removed by filtration through Celite. The solvent was removed under reduced pressure, and purification was accomplished by flash column chromatography on a 1 × 6 cm silica gel column. The column was eluted with 10% MeOH/CH_2Cl_2 (fractions 1−10) then 1% H_2O/20% MeOH/CH_2Cl_2 (fractions 11−60) while collecting 1.5 mL test tube fractions. The product-containing fractions (14−65) were combined and concentrated under reduced pressure to provide **12** (27 mg, 79%) as a clear colorless oil: R_f = 0.27 (4% H_2O/25% MeOH/71% CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 5.48 (t, *J* = 4.5 Hz, 1H), 5.45−5.38 (m, 1H), 5.19−5.07 (m, 2H), 4.43 (t, *J* = 6.3 Hz, 2H), 4.13−4.04 (m, 1H), 3.90−3.93 (m, 1H), 3.82−3.69 (m, 2H), 3.63 (dd, *J* = 12.2, 5.7 Hz, 1H), 2.17−1.98 (m, 8H), 1.74 (s, 3H), 1.68 (s, 6H), 1.61 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 140.9, 136.7, 132.5, 125.9, 125.5, 123.5(d), 99.2(d), 85.1, 79.5(d), 75.3, 64.2, 63.4(d), 33.4, 33.1, 27.8, 27.7, 26.1, 23.9, 23.8, 17.9; HRMS (ESI-TOF$^-$) calcd for $C_{20}H_{34}O_8P$ (M^-) 433.1996, found 433.1991.

(*Z*)-Nerylphosphoryl-*β*-D-arabinofuranose (13). Prepared in the same manner as described for compound **12** from intermediate monophosphate salt **S3** (30 mg, 0.026 mmol) and nerol (20 μL, 0.11 mmol). Compound **13** (7.6 mg, 76%) was generated as a clear colorless oil: R_f = 0.26 (4% H_2O/25% MeOH/71% CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 5.48 (t, *J* = 4.6 Hz, 1H), 5.41 (t, *J* = 6.9 Hz, 1H), 5.15−5.09 (m, 1H), 4.49−4.38 (m, 2H), 4.08 (dd, *J* = 8.0, 7.0 Hz, 1H), 3.96 (ddd, *J* = 8.1, 4.3, 2.1 Hz, 1H), 3.80−3.69 (m, 2H), 3.63 (dd, *J* = 12.2, 5.8 Hz, 1H), 2.17−2.01 (m, 4H), 1.74 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 141.0, 132.9, 125.2, 123.5(d), 99.2(d), 85.1, 79.5(d), 75.3, 64.1, 63.5(d), 33.2, 27.9, 26.1, 23.8, 17.9; HRMS (ESI-TOF$^-$) calcd for $C_{15}H_{26}O_8P$ (M^-) 365.1370, found 365.1373.

((*R*)-Citronellyl)phosphoryl-*β*-D-arabinofuranose (14). Prepared in the same manner as described for compound **12** from intermediate monophosphate salt **S3** (30 mg, 0.026 mmol) and (*R*)-citronellol (21 μL, 0.11 mmol). Compound **14** (6.2 mg, 62%) was generated as a clear colorless oil: R_f = 0.24 (4% H_2O/25% MeOH/71% CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 5.47 (t, *J* = 4.6 Hz, 1H), 5.14−5.08 (m, 1H), 4.08 (dd, *J* = 8.1, 6.9 Hz, 1H), 4.00−3.89 (m, 3H), 3.81−3.69 (m, 2H), 3.62 (dd, *J* = 12.1, 5.8 Hz, 1H), 2.09−1.91 (m, 2H), 1.75−1.63 (m, 1H), 1.67 (s, 3H), 1.61(s, 3H), 1.48−1.33 (m, 3H), 1.22−1.11 (m, 1H), 0.92 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 132.0, 126.1, 99.2(d), 85.1, 79.5(d), 75.3, 65.3(d), 64.2, 39.0(d), 38.5, 30.5, 26.6, 26.0, 19.9, 17.9, 17.8. HRMS (ESI-TOF$^-$) calcd for $C_{15}H_{28}O_8P$ (M^-) 367.1527, found 367.1534.

***n*-Octylphosphoryl-*β*-D-arabinofuranose (15).** Prepared in the same manner as described for compound **12** from intermediate monophosphate salt **S3** (30 mg, 0.026 mmol) and *n*-octanol (18 μL, 0.11 mmol). Compound **15** (7.3 mg, 78%) was generated as a clear colorless oil: R_f = 0.27 (4% H_2O/25% MeOH/71% CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 5.47 (t, *J* = 4.5 Hz, 1H), 4.07 (dd, *J* = 8.3, 6.9 Hz, 1H), 3.99−3.93 (m, 1H), 3.89 (q, *J* = 6.4 Hz, 2H), 3.82−3.69 (m, 2H), 3.62 (dd, *J* = 12.1, 5.7 Hz, 1H), 1.63 (p, *J* = 6.8 Hz, 2H), 1.47−1.21 (m, 10H), 0.90 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 99.2(d), 85.1, 79.5(d), 75.3, 67.0(d), 64.2, 33.2, 32.0(d), 30.6, 27.1, 27.0, 23.9, 14.6; HRMS (ESI-TOF$^-$) calcd for $C_{13}H_{26}O_8P$ (M^-) 341.1370, found 341.1365.

(8-Phenyloctyl)phosphoryl-*β*-D-arabinofuranose (16). Prepared in the same manner as described for compound **12** from intermediate monophosphate salt **S3** (30 mg, 0.026 mmol) and 8-phenyl-1-octanol (24 mg, 0.11 mmol). Compound **16** (7.4 mg, 65%) was generated as a clear colorless oil: R_f = 0.30 (4% H_2O/25% MeOH/71% CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (t, *J* = 7.6 Hz, 2H), 7.19−7.08 (m, 3H), 5.47 (t, *J* = 4.6 Hz, 1H), 4.15−4.03 (m, 1H), 3.99−3.92 (m, 1H), 3.92−3.81 (m, 2H), 3.80−3.70 (m, 2H), 3.62 (dd, *J* = 12.1, 5.8 Hz, 1H), 2.59 (t, *J* = 7.7 Hz, 2H), 1.67−1.56 (m, 4H), 1.49−1.20 (m, 8H); ^{13}C NMR (101 MHz, CD_3OD) δ 144.1, 129.5, 129.4, 126.7, 99.2(d), 85.1, 78.9, 75.3, 67.0(d), 64.2, 37.1, 32.9, 31.9(d), 30.75, 30.6, 30.4, 27.0; HRMS (ESI-TOF$^-$) calcd for $C_{19}H_{30}O_8P$ (M^-) 417.1683, found 417.1693.

***n*-Dodecylphosphoryl-*β*-D-arabinofuranose (17).** Prepared in the same manner as described for **12** from intermediate monophosphate salt **S3** (89 mg, 0.078 mmol) and *n*-dodecanol (70 μL, 0.31 mmol). Compound **17** (23 mg, 71%) was generated as a colorless wax: R_f = 0.30 (4% H_2O/30% MeOH/66% CH_2Cl_2); ^{1}H NMR (300 MHz, CD_3OD) δ 5.47 (t, *J* = 4.6 Hz, 1H), 4.12−4.03 (m, 1H), 3.97 (ddd, *J* = 8.1, 4.3, 2.2 Hz, 1H), 3.90 (q, *J* = 6.5 Hz, 2H), 3.82−3.69 (m, 2H), 3.68−3.58 (m, 1H), 1.69−1.55(m, 2H), 1.48−1.17 (m, 18H), 0.90 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 99.2(d), 85.08, 79.4(d), 75.33, 67.0(d), 64.2, 33.2, 32.0, 31.9, 30.9, 30.9, 30.9, 30.7, 30.6, 27.0, 23.9, 14.6, 9.3; HRMS (ESI-TOF$^-$) calcd for $C_{17}H_{34}O_8P$ (M^-) 397.1996, found 397.1995.

(2-Naphthalenemethyl)phosphoryl-*β*-D-arabinofuranose (18). Prepared in the same manner as described for compound **12** from intermediate monophosphate salt **S3** (30 mg, 0.026 mmol) and 2-naphthalenemethanol (18 mg, 0.11 mmol). Compound **18** (6.6 mg, 65%) was generated as a clear colorless oil: R_f = 0.24 (4% H_2O/25% MeOH/71% CH_2Cl_2); ^{1}H NMR (400 MHz, CD_3OD) δ 7.90 (s, 1H), 7.88−7.79 (m, 3H), 7.55 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.50−7.41 (m, 2H), 5.58 (t, *J* = 4.7 Hz, 1H), 5.13 (d, *J* = 5.9 Hz, 2H), 4.11 (t, *J* = 7.6 Hz, 1H), 4.03−3.97 (m, 1H), 3.85−3.69 (m, 2H), 3.64 (dd, *J* = 12.1, 5.8 Hz, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 137.4, 137.3, 134.9, 134.6, 129.1, 129.1, 128.8, 127.18, 127.0, 126.8, 99.4(d), 85.2, 79.5, 75.3, 68.7(d), 64.3; HRMS (ESI-TOF$^-$) calcd for $C_{16}H_{18}O_8P$ (M^-) 369.0744, found 369.0743.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This paper is dedicated to the memory of our colleague Howard Zimmerman, who inspired us with his relentless pursuit of mechanistic understanding.

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