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Note

Synthesis of 2-deoxy-2-fluoro analogs of polyprenyl β-D-arabinofuranosyl phosphates

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Abstract—Described is the synthesis of polyprenyl 2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate derivatives, including an analog of decaprenyl β-D-arabinofuranosyl phosphate, the donor species used by the arabinosyltransferases involved in mycobacterial cell-wall biosynthesis. The targets were synthesized via a route involving the synthesis of a protected β-D-arabinofuranosyl phosphate derivative, its coupling with a polyprenyl trichloroacetimidate, and then deprotection of the resulting product. The use of arabinofuranosyl phosphates with the monosaccharide hydroxyl groups protected as either silyl ethers or benzoate esters was explored. Although the coupling yields between the phosphate and polyprenyl trichloroacetimidates were comparable with either type of protecting group, access to the benzoyl-protected derivative was more efficient and therefore gave the products in higher overall yield.

Keywords: Glycosyl phosphate; Polyprenol; Mycobacteria; Arabinosyltransferase

The two major polysaccharide components of the mycobacterial cell wall are an arabinogalactan (AG) and a lipoarabinomannan (LAM), both of which contain an arabinan domain composed entirely of arabinose residues in the furanose ring form.[1] This arabinan is assembled by a family of arabinosyltransferases (AraT's) that use decaprenyl β-D-arabinofuranosyl phosphate, more commonly known as decaprenol phosphoarabinose (DPA, **1**), as the donor species.[2,3] The development of AraT assays[3,4] requires efficient access to **1**, and two methods have been reported to date. One method involves the coupling of sugar and polyprenol via a phosphoramidite coupling followed by oxidation to the phosphate.[3,5] In the second approach, a β-D-arabinofuranosyl phosphate moiety is synthesized, which is then coupled to decaprenyl trichloroacetimidate.[6]

As part of a larger program on the synthesis of inhibitors of mycobacterial AraT's,[7] we needed to synthesize a DPA analog in which the C-2 hydroxyl group had been replaced with fluorine (**2**). In evaluating both of the approaches described above, we selected the latter method as the most suitable for our purposes and report here its application to the synthesis of **2**, as well as a panel of shorter chain polyprenyl 2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphates that contain neryl (**3**), geranyl (**4**), or farnesyl (**5**) moieties.

We envisioned an approach for the synthesis of **2**–**5** as illustrated in Figure 1. A protected 2-deoxy-2-fluoro-arabinofuranosyl bromide (**6** or **7**) could be converted into the corresponding glycosyl phosphate (**8** or **9**), which could in turn be coupled with the appropriate polyprenyl trichloroacetimidate (**10**). The product obtained, **11**, could then be deprotected yielding **2**–**5**. The use of silyl ether protecting groups was in direct analogy to earlier work on the synthesis of **1**.[3,5,6] We also chose to explore the feasibility of an alternate approach, involving protection of the carbohydrate hydroxyl groups as benzoate esters, as the required glycosyl bromide (**7**) could be obtained in fewer steps overall than the corresponding silyl-protected bromide **6**.

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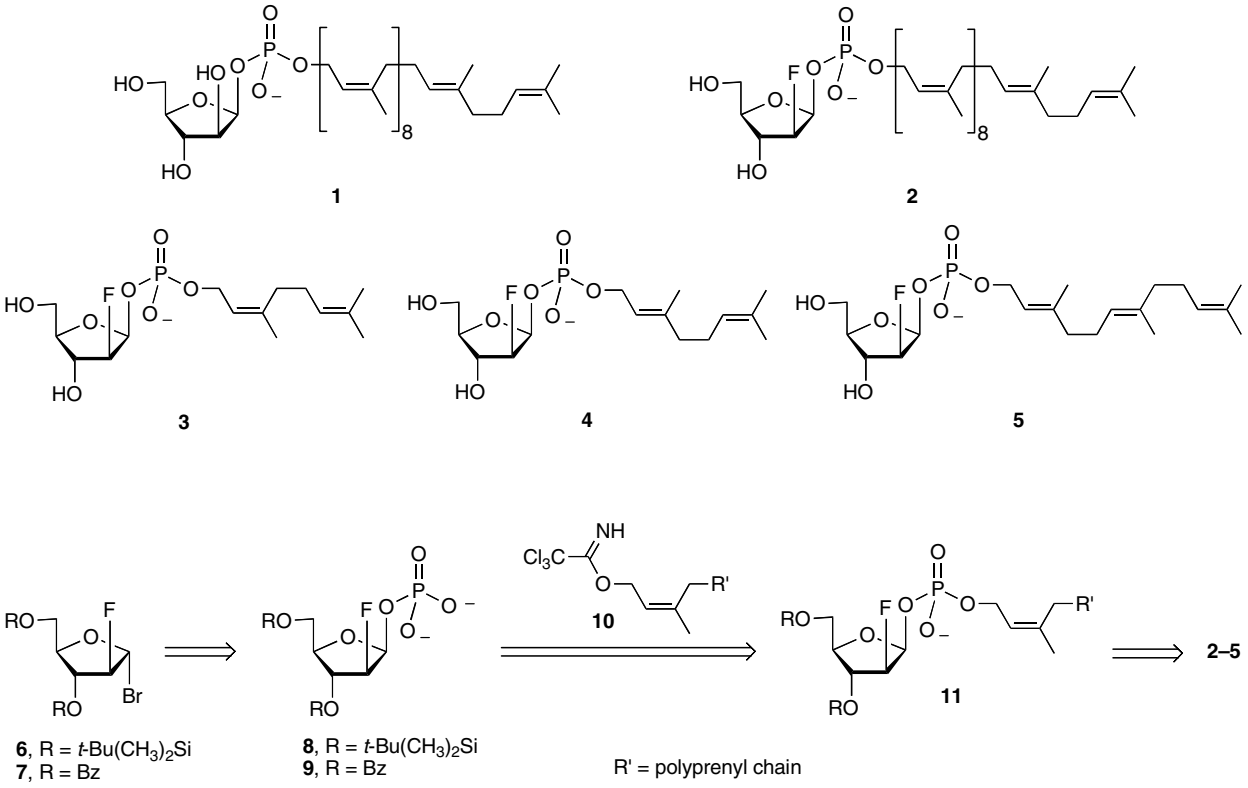
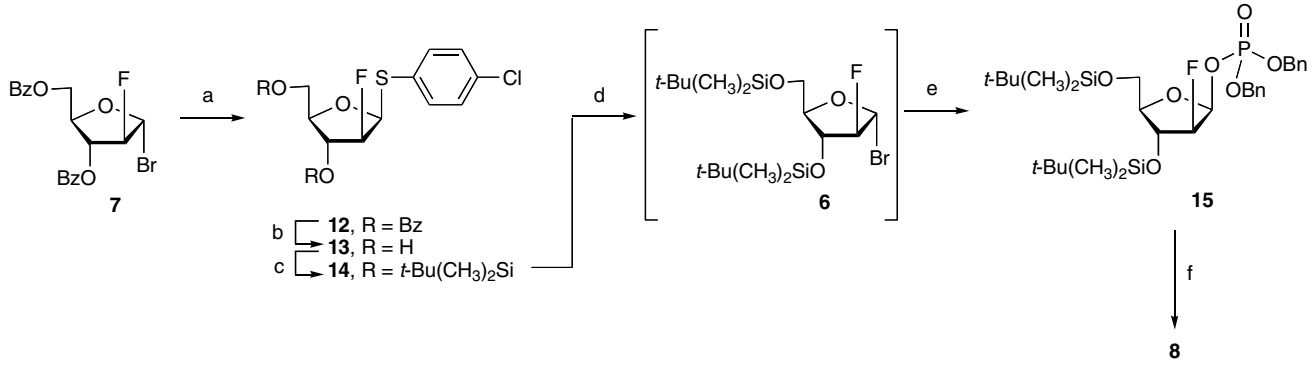


Figure 1. Retrosynthesis of **2**–**5**.

The synthesis of the targets using silyl protection began (Scheme 1) from the known[8] glycosyl bromide **7**, which was reacted with *p*-chlorothiophenol under phase-transfer conditions[9] to provide an 83% yield of the corresponding thioglycoside **12**. Subsequent treatment with sodium methoxide in methanol afforded diol **13**, which was then silylated under conventional conditions (*tert*-butylchlorodimethylsilane, imidazole, *N*,*N*-dimethylformamide) providing **14** in 81% yield over the two steps. The reaction of **14** with bromine in carbon tetrachloride yielded the corresponding glycosyl bromide **6**. This labile intermediate was not isolated but instead immediately treated with dibenzyl phosphate and triethylamine, affording a 67% yield of glycosyl phosphate **15** as a 1:5 α:β mixture. The diastereomers were separated by column chromatography along with 8% of unreacted **14** and 10% of its hydrolysis product. In the ^{1}H NMR spectrum of the major product, the anomeric proton signal appeared at 5.89 ppm as a doublet of doublets with coupling constants of 4.3 and 5.5 Hz. Selective ^{31}P decoupling transformed this signal into a doublet with a coupling constant of 4.3 Hz; clearly indicating a $^{3}J_{H1,P}$ of 5.5 Hz. The ^{19}F NMR spectrum of the major isomer showed a $^{3}J_{H1,F}$ of 0 Hz. Therefore, the

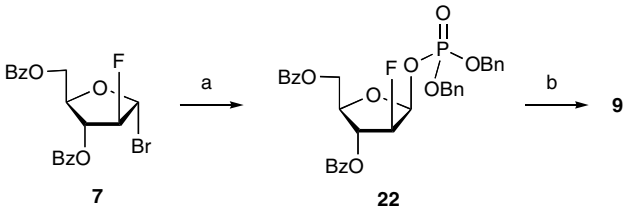


Scheme 1. Reagents and conditions: (a) *p*-ClC_6H_4SH, KOH, H_2O, CH_2Cl_2, *n*-Bu_4NBr, rt, 83%; (b) $NaOCH_3$, CH_3OH, CH_2Cl_2, rt, 87%; (c) *t*-$Bu(CH_3)_2SiCl$, imidazole, DMF, rt, 83%; (d) Br_2, CCl_4, rt; (e) dibenzyl phosphate, Et_3N, CCl_4, 67% from **14**; (f) H_2, Pd/C, EtOAc, Et_3N, rt.

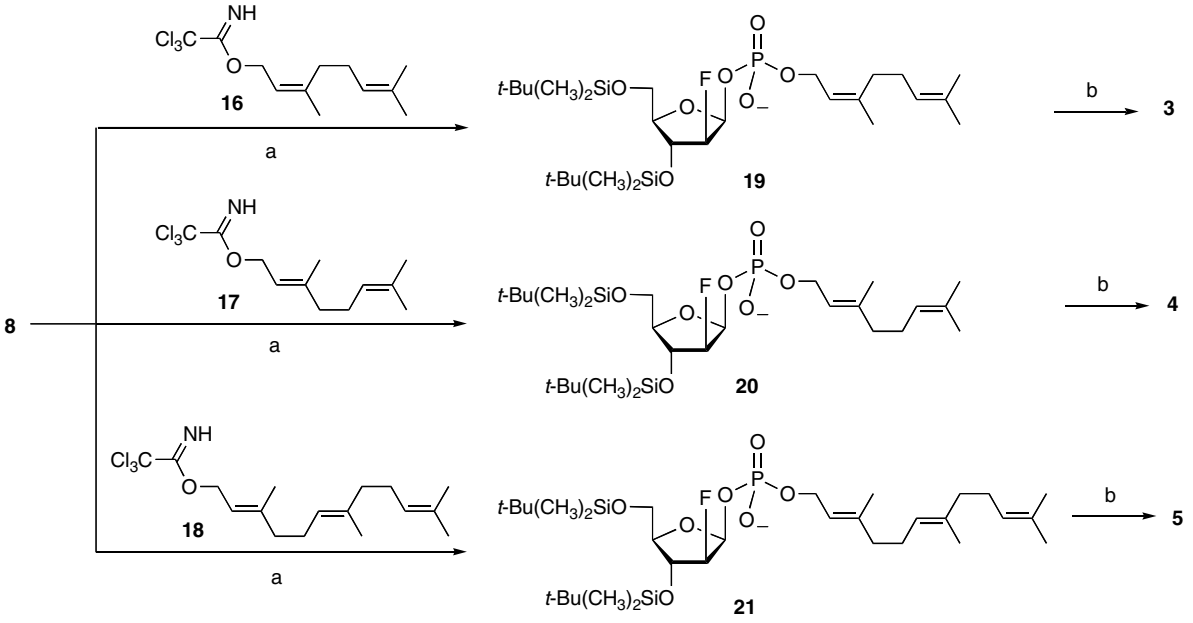
4.3 Hz coupling was assigned to $^{3}J_{H1,H2}$, indicating the β stereochemistry for the phosphate group at the anomeric centre.[10] Hydrogenation of **15** (H_2, Pd/C) provided **8** together with 15–20% of partially desilylated byproducts. Attempts to find conditions under which cleavage of the silyl ethers did not occur were unsuccessful, and hence the mixture of compounds obtained after the reaction was used directly in the next step.

With glycosyl phosphate **8** in hand, we investigated its coupling to activated derivatives of nerol, geraniol, and *trans*,*trans*-farnesol (Scheme 2). Thus, each of the three prenols was treated with DBU and trichloroacetonitrile to afford the corresponding trichloroacetimidate derivatives (**16**–**18**), which were of limited stability. Purification could be achieved by rapid filtration through silica gel using 12:1 hexanes–ethyl acetate as the eluant. We also found that simple filtration of the reaction mixture through cotton and sodium sulfate provided trichloroacetimidates that could be used in the subsequent couplings in comparable yield to the reactions with the compounds purified by chromatography. Upon their formation, each prenyl trichloroacetimidate was reacted with **8** in a solution of 5:1 toluene–DMF at 65 °C, which provided each of the coupled products (**19**–**21**) in modest (42–44%) yields. Subsequent treatment with ammonium fluoride in methanol at 65 °C led to cleavage of the silyl ethers, affording the deprotected targets **3**–**5** in 54–58% yields.

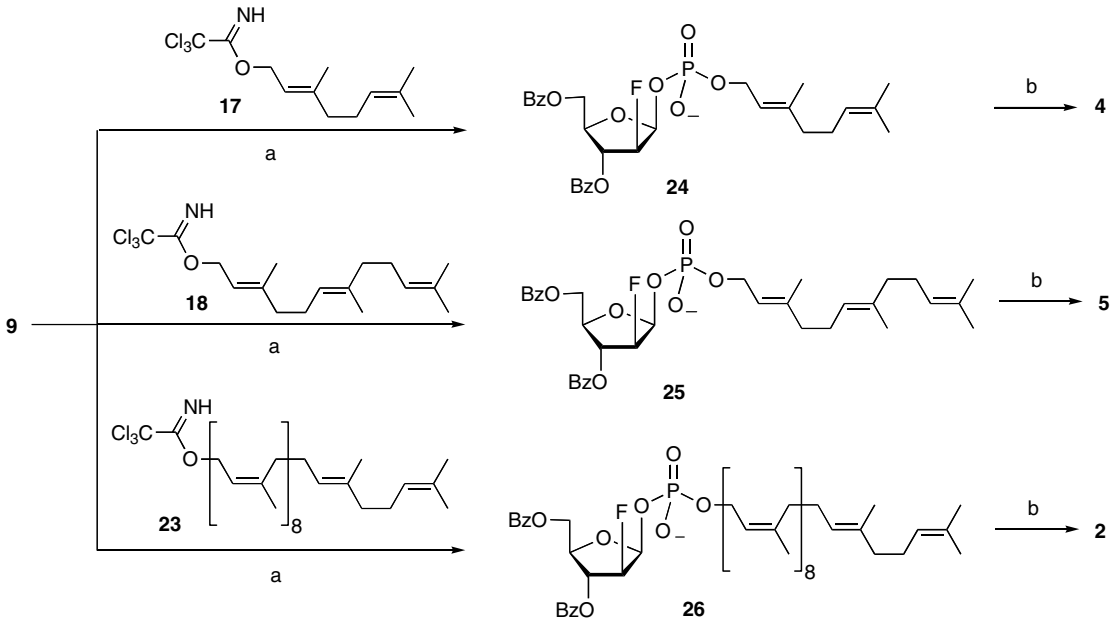
The modest overall yield and the number of steps required to synthesize **8** prompted us to consider the possibility of using benzoate ester protecting groups in place of the silyl ethers. The required β-arabinofuranosyl phosphate derivative, **9** (Fig. 1), could be synthesized very efficiently as outlined in Scheme 3. The reaction of glycosyl bromide **7** with dibenzyl phosphate and triethylamine yielded the expected phosphate derivative **22** in 82% yield as a 1:6.4 α:β mixture, which could be separated by chromatography. In the ^{1}H NMR spectrum of the major product, the anomeric proton signal appeared at 6.11 ppm as a doublet of doublets with coupling constants 4.3 and 5.8 Hz. The same series of experiments described above for **15** were used to establish that $^{3}J_{H1,H2}$ in **22** is 4.3 Hz, thus supporting the β stereochemistry of this arabinofuranosyl phosphate derivative. Similar analysis of the ^{13}C, ^{1}H, and ^{19}F spectra for the minor isomer yielded couplings for the anomeric hydrogen resonance (doublet of doublets at 6.04 ppm) of 4.8, 8.1, and 0.0 Hz for the $^{3}J_{H1,P}$, $^{3}J_{H1,F}$, and $^{3}J_{H1,H2}$ couplings, respectively, thus indicating the α stereochemistry.[10] Hydrogenation of the benzyl ethers gave **9** in 91% yield. Compared to the synthesis of the silyl ether-protected phosphate **8**, benzoyl-protected **9** can be prepared not only in fewer steps but also in better



Scheme 3. Reagents and conditions: (a) dibenzyl phosphate, Et_3N, $ClCH_2CH_2Cl$, CH_2Cl_2, 82%; (b) H_2, Pd/C, EtOAc, Et_3N, rt, 91%.



Scheme 2. Reagents and conditions: (a) toluene, DMF, 65 °C, 44% for **16**, 43% for **17**, 42 for **18**; (b) NH_4F, NH_3, CH_3OH, 65 °C, 58% for **19**, 54% for **20**, 54% for **21**.



Scheme 4. Reagents and conditions: (a) toluene, DMF, 65 °C, 45% for **17**, 46% for **18**, 40% for **23**; (b) CH_3OH, H_2O, Et_3N, rt, 64% for **24**, 60% for **25**, 63% for **26**.

yield and with higher stereoselectivity from the glycosyl bromide precursor **7**.

The coupling of **9** to the polyprenyl imidate derivatives was next explored by its reaction with **17** or **18** as was done for the silyl-protected intermediates (Scheme 4). The yields of the coupled products, **24** and **25**, were comparable with those obtained from **8**, and therefore changing the protecting groups from silyl to benzoyl does not substantially improve the yield of the coupling step. Cleavage of the benzoate esters in **24** and **25** could be achieved by stirring each compound in a mixture of methanol, triethylamine, and water, which provided **4** and **5** in 64% and 60% yield, respectively.

Based on the results described above, we chose to synthesize **2** from the benzoyl-protected phosphate substrate **9** (Scheme 4). Thus, decaprenyl trichloroacetimidate derivative **23** was prepared from decaprenol and reacted with **9**, affording a 40% yield of **26**. Subsequent debenzoylation under the conditions described previously gave **2** in 63% yield.

In summary, we describe here the synthesis of a 2-deoxy-2-fluoro analog of DPA, the donor substrate used by mycobacterial arabinosyltransferases. The key step in this sequence was the coupling of a benzoyl-protected 2-deoxy-2-fluoro-β-arabinofuranosyl phosphate derivative with decaprenyl trichloroacetimidate. Product **2** was obtained in 19% overall yield in four steps from the known and readily prepared 2-deoxy-2-fluoro-arabinofuranosyl bromide **7**. Using the same route, a series of shorter chain polyprenyl β-arabinofuranosyl phosphates were also synthesized.

1. Experimental

1.1. General methods

The reactions were carried out in an oven-dried glassware. The reaction solvents were distilled from appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on Silica Gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by charring with acidified *p*-anisaldehyde solution in EtOH. Unless otherwise indicated, all column chromatography was performed on silica gel (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 22 ± 2 °C. ^{1}H NMR spectra were recorded at 500 MHz, and chemical shifts were referenced to either TMS (0.0, $CDCl_3$) or CD_3OD (3.30, CD_3OD). ^{1}H data are reported as though they were of first order. ^{13}C NMR spectra were recorded at 125 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.23, $CDCl_3$), or CD_3OD (48.9, CD_3OD). ^{19}F spectra were recorded at 376 or 468 MHz, and chemical shifts were referenced to external $CFCl_3$.[11] ^{31}P spectra were recorded with proton decoupling at 162 MHz, and chemical shifts were referenced using external 85% H_3PO_4.[11] In the processing of the reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. The organic solutions were concentrated under vacuum at <40 °C. Electrospray-ionization mass spectra were recorded on samples suspended in

mixtures of THF with CH_3OH and added NaCl. Decaprenol was purchased from Larodan Fine Chemicals, Sweden, as a solution in hexane, and was used as such.

1.2. General procedure for the preparation of polyprenyl trichloroacetimidates

To a solution of the alcohol (1 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C was added DBU (15 μL, 0.1 mmol). The mixture was stirred at 0 °C for 30 min and then warmed to rt over 30 min. The solvent was then removed and dry hexane (3 mL) was added. After stirring for 5 min, this solution was passed through a plug of cotton and Na_2SO_4. The resulting solution was then concentrated to yield the trichloroacetimidate derivative, which could be used without further purification. Alternatively, the residue obtained after the initial solvent evaporation following the reaction could be quickly filtered through silica gel using 12:1 hexanes–EtOAc as the eluant. The fractions containing the trichloroacetimidate derivative were concentrated and used immediately.

1.3. General procedure for coupling of 8 with polyprenyl trichloroacetimidates and subsequent deprotection

To a solution of **8** (0.1 mmol) in dry toluene (1.5 mL) was added the corresponding trichloroacetimidate derivative (0.15 mmol) followed by DMF (0.3 mL). The reaction mixture was heated at 65 °C for 8 h and then cooled to rt. The mixture was concentrated to a syrupy residue that was purified by column chromatography (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH) to yield the corresponding product (**19**–**21**) in 42–44% yield. The coupled products (0.05 g) were immediately dissolved in a mixture of methanolic aq NH_3 (4 mL; 0.6 mL 33% NH_4OH in 3.4 mL CH_3OH), and solid NH_4F (0.15 g) was added. The reaction mixture was heated at 65 °C for 14 h and then cooled to rt and diluted with CH_2Cl_2 (2 mL). The precipitated salts were filtered, and the filtrate was concentrated to a syrupy residue that was purified by column chromatography (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH) to yield **3**–**5** in 54–58% yield.

1.4. General procedure for coupling of 9 with polyprenyl trichloroacetimidates 17 and 18 and subsequent deprotection

To a solution of **9** (0.1 mmol) in dry toluene (1.5 mL) was added the corresponding trichloroacetimidate derivative (0.15 mmol) followed by DMF (0.3 mL). The reaction mixture was heated at 65 °C for 16 h and then cooled to rt before being concentrated to a syrupy residue that was purified by column chromatography (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH) to yield the corresponding coupled products (**24** or **25**) in 45–46% yield. The coupled product (0.05 g) was dissolved in a solution of 5:2:1 CH_3OH–H_2O–Et_3N (2 mL), and the solution was stirred for 48–60 h. The reaction mixture was directly concentrated and the syrupy residue was purified by column chromatography (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH) to yield **2**, **4**, or **5** in 60–64% yield.

1.5. Decaprenyl 2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate (2)

To a solution of sugar 1-phosphate **9** (0.05 mg, 0.1 mmol) in dry toluene (1.5 mL) was added decaprenyl trichloroacetimidate (0.1 mmol) followed by DMF (0.3 mL). The reaction mixture was heated at 65 °C for 16 h and then cooled to rt before being concentrated to a syrupy residue that was purified by column chromatography (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH) to yield the coupled product **26** (5 mg, 40%): ESIMS: m/z calcd for $[C_{69}H_{97}FO_9P]^-$: 1119.6859. Found: 1119.6864. Compound **26** (4 mg) was dissolved in a solution of 5:2:1 CH_3OH–H_2O–Et_3N (0.4 mL) and stirred for 42 h before the mixture was concentrated to a syrupy residue that was purified by column chromatography (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH) to yield **2** (2.3 mg, 63%): R_f 0.2 (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH); $[\alpha]_D$ −8.6 (*c* 0.2, CH_3OH); ^1H NMR (600 MHz, CD_3OD, δ_H) 5.62 (dd, 1H, *J* 4.5, 4.5 Hz, H-1), 5.40 (dd, 1H, *J* 7.2, 7.2 Hz, CH=), 5.05–5.16 (m, 9H, CH=), 4.80 (dddd, 1H, *J* 2.0, 4.5, 6.3, 53.0 Hz, H-2), 4.44–4.36 (m, 3H, H-3, OCH_2CH=), 3.80 (ddd, 1H, *J* 3.1, 5.6, 6.7 Hz, H-4), 3.73 (dddd, 1H, *J* 1.1, 3.1, 3.9, 12.8 Hz, H-5), 3.64 (dd, 1H, *J* 5.6, 12.8 Hz, H-5′), 2.20–1.92 (m, 36H, $18\times CH_2CH$=), 1.72–1.50 (m, 21H, $7\times CH_3$), 1.36–1.25 (m, 6H, $2\times CH_3$), 0.92–0.86 (m, 6H, $2\times CH_3$); ^{31}P NMR (162 MHz, CD_3OD, δ_P) 3.85; ^{19}F NMR (376 MHz, CD_3OD/CD_2Cl_2, δ_F) −208.19 (dd, *J* 17.2, 53.0 Hz); ESIMS: m/z calcd for $[C_{55}H_{89}FO_7P]^-$: 911.6335. Found: 911.6333.

1.6. Neryl 2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate (3)

R_f 0.13 (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH); $[\alpha]_D$ −39.8 (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 5.62 (dd, 1H, *J* 4.3, 9.1 Hz, H-1), 5.38 (ddd, 1H, *J* 0.6, 6.7, 6.7 Hz, CH=), 5.14–5.07 (m, 1H, CH=), 4.82 (dddd, 1H, *J* 1.8, 4.3, 6.1, 52.7 Hz, H-2), 4.45–4.34 (m, 3H, OCH_2CH=, H-3), 3.81 (ddd, 1H, *J* 3.2, 5.6, 6.9 Hz, H-4), 3.74 (dd, 1H, *J* 3.2, 12.3 Hz, H-5), 3.64 (dd, 1H, *J* 5.6, 12.3 Hz, H-5′), 2.14–2.04 (m, 4H, $2\times CH_2CH$=), 1.73 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.59 (s, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ_C) 141.10 (=CCH_3), 132.83 (=CCH_3), 124.97 (CH=), 123.11 (d, *J* 8.7 Hz, CH=), 96.76 (dd, *J* 5.7, 18.1 Hz,

C-1), 96.50 (dd, *J* 7.2, 199.8 Hz, C-2), 84.27 (d, *J* 9.3 Hz, C-4), 73.08 (d, *J* 21.2 Hz, C-3), 63.74 (O*C*H_2CH=), 63.38 (d, *J* 5.2 Hz, C-5), 33.08 (*C*H_2CH=), 27.76 (*C*H_2CH=), 25.92 (CH_3), 23.67 (CH_3), 17.79 (CH_3); ^{31}P NMR (162 MHz, CD_3OD, δ_P) 3.28; ^{19}F NMR (376 MHz, D_2O, δ_F) −206.73 (dd, *J* 17.6, 52.7 Hz). ESIMS: *m*/*z* calcd for $[C_{15}H_{25}FO_7P]^-$: 367.1316. Found: 367.1319.

1.7. Geranyl 2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate (4)

R_f 0.12 (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH); $[\alpha]_D$ −34.6 (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 5.63 (dd, 1H, *J* 4.2, 4.2 Hz, H-1), 5.39 (ddd, 1H, *J* 1.2, 6.9, 6.9 Hz, CH=), 5.13–5.07 (m, 1H, CH=), 4.82 (dddd, 1H, *J* 1.8, 4.2, 6.1, 53.4 Hz, H-2), 4.42–4.36 (m, 3H, OCH_2CH=, H-3), 3.80 (ddd, 1H, *J* 3.2, 5.6, 6.9 Hz, H-4), 3.74 (dd, 1H, *J* 3.2, 12.3 Hz, H-5), 3.65 (dd, 1H, *J* 5.6, 12.3 Hz, H-5′), 2.14–2.06 (m, 2H, CH_2CH=), 2.05–2.00 (m, 2H, CH_2CH=), 1.67 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.59 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 140.97 (=CCH_3), 132.49 (=CCH_3), 125.07 (CH=), 122.24 (d, *J* 8.2 Hz, CH=), 96.71 (dd, *J* 5.7, 18.1 Hz, C-1), 96.53 (dd, *J* 7.7, 199.8 Hz, C-2), 84.25 (d, *J* 9.8 Hz, C-4), 73.03 (d, *J* 20.6 Hz, C-3), 63.70 (O*C*H_2CH=), 63.56 (d, *J* 5.7 Hz, C-5), 40.61 (*C*H_2CH=), 27.47 (*C*H_2CH=), 25.87 (CH_3), 17.75 (CH_3), 16.46 (CH_3); ^{31}P NMR (162 MHz, CD_3OD, δ_P) 3.50; ^{19}F NMR (376 MHz, CD_3OD, δ_F) −208.50 (dd, *J* 17.3, 53.4 Hz); ESIMS: *m*/*z* calcd for $[C_{15}H_{25}FO_7P]^-$: 367.1316. Found: 367.1317.

1.8. *trans*,*trans*-Farnesyl 2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate (5)

R_f 0.15 (7:1:0.1 CH_2Cl_2–CH_3OH–33% NH_4OH); $[\alpha]_D$ −44.0 (*c* 1.1, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 5.62 (dd, 1H, *J* 4.2, 4.2 Hz, H-1), 5.40 (ddd, 1H, *J* 1.2, 6.8, 8.0 Hz, CH=), 5.14–5.05 (m, 2H, CH=), 4.81 (dddd, 1H, *J* = 1.8, 4.2, 6.1, 53.4 Hz, H-2), 4.41–4.35 (m, 3H, OCH_2CH=, H-3), 3.80 (ddd, 1H, *J* 3.2, 5.8, 6.9 Hz, H-4), 3.74 (dd, 1H, *J* 3.1, 12.3 Hz, H-5), 3.65 (dd, 1H, *J* 5.8, 12.3 Hz, H-5′), 2.30–1.92 (m, 8H, 4 × CH_2CH=), 1.67 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.59 (s, 6H, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 140.90 (=CCH_3), 136.23 (=CCH_3), 132.10 (=CCH_3), 125.43 (CH=), 125.17 (CH=), 122.28 (CH=), 96.69 (dd, *J* 5.2, 17.6 Hz, C–1), 96.48 (dd, *J* 7.2, 199.8 Hz, C-2), 84.27 (d, *J* 9.8 Hz, C-4), 73.05 (d, *J* 21.2 Hz, C-3), 63.81 (O*C*H_2CH=), 63.54 (d, *J* 5.7 Hz, C-5), 40.85 (*C*H_2CH=), 40.63 (*C*H_2CH=), 27.81 (*C*H_2CH=), 27.40 (*C*H_2CH=), 25.89 (CH_3), 17.76 (CH_3), 16.48 (CH_3), 16.07 (CH_3); ^{31}P NMR (162 MHz, CD_3OD, δ_P) 3.87; ^{19}F NMR (468 MHz, CD_3OD, δ_F) −208.60 (dd, *J* 17.0, 53.4 Hz); ESIMS: *m*/*z* calcd for $[C_{20}H_{33}FO_7P]^-$: 435.1942. Found: 435.1941.

1.9. 3,5-Di-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate (8)

Dibenzylphosphate **15** (50 mg, 0.08 mmol) was dissolved in 10:1 EtOAc–Et_3N (6 mL), and 10% Pd/C was added (10 mg). The reaction mixture was hydrogenated at 1 atm pressure for 14 h, before the catalyst was filtered and washed with EtOAc (2 mL). The combined filtrate was concentrated and dried under vacuum to provide debenzylated product **8**, which was used immediately in the next step. The product was contaminated with 15–20% of desilylated byproducts. Data for the major product: ^{1}H NMR (400 MHz, CD_3OD, δ_H) 5.72–5.64 (m, 1H, H-1), 4.88–4.82 (m, 1H, H-2), 4.38–4.32 (m, H-3), 3.85–3.57 (m, 3H, H-4, 5, 5′), 1.00–0.85 (m, 18H, C(CH_3)$_3$), 0.15–0.05 (m, 4 × $SiCH_3$); ^{31}P NMR (162 MHz, CD_3OD, δ_P) 3.40.

1.10. 3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl phosphate (9)

Compound **22** (0.2 g, 0.32 mmol) was dissolved in 10:1 EtOAc–Et_3N (10 mL) and 10% Pd/C was added (30 mg). The reaction mixture was stirred under hydrogen at 1 atm pressure for 14 h before the catalyst was filtered and washed with 3:7 EtOAc–CH_3OH (6 mL). The combined filtrate was concentrated and dried under vacuum to provide **9** (as its triethylammonium salt, 0.16 g, 91%), sufficiently pure for use in the next step: ^{1}H NMR (500 MHz, CD_3OD, δ_H) 8.05–7.95 (m, 4H, ArH), 7.65–7.60 (m, 1H, ArH), 7.55–7.45 (m, 3H, ArH), 7.40–7.32 (m, 2H, ArH), 5.88 (ddd, 1H, *J* 1.6, 4.0, 5.6 Hz, H-1), 5.81 (ddd, 1H, *J* 5.4, 5.4, 16.6 Hz, H-3), 5.31 (dddd, 1H, *J* 1.2, 4.0, 5.4, 52.5 Hz, H-2), 4.68 (dd, 1H, *J* 6.0, 11.4 Hz, H-5), 4.62 (ddd, 1H, *J* 0.7, 6.1, 11.4 Hz, H-5′), 4.42 (ddd, 1H, *J* 5.4, 6.0, 6.1 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 167.62 (C=O), 167.07 (C=O), 134.80 (Ar), 134.27 (Ar), 131.02 (Ar), 130.80 (2C, Ar), 130.73 (2C, Ar), 130.41 (Ar), 129.72 (2C, Ar), 129.47 (2C, Ar), 97.41 (dd, *J* 4.6, 18.1 Hz, C-1), 94.08 (dd, *J* 6.7, 200.1 Hz, C-2), 79.45 (d, *J* 7.7 Hz, C-3), 77.86 (d, *J* 24.3 Hz, C-4), 67.15 (C-5); ^{31}P NMR (162 MHz, CD_3OD, δ_P) 3.52; ^{19}F NMR (376 MHz, CD_3OD, δ_F) −207.08 (dd, *J* 16.6, 52.5 Hz); ESIMS: *m*/*z* calcd for $[C_{19}H_{17}FO_9P]^-$: 439.0588. Found 439.0589.

1.11. *p*-Chlorophenyl 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-1-thio-β-D-arabinofuranoside (12)

To a solution of **7** (0.5 g, 1.2 mmol) in CH_2Cl_2 (12 mL) was added *p*-chlorothiocresol (0.26 g, 1.8 mmol) followed by an aq solution of KOH (0.14 g in 1.8 mL water) and *n*-Bu_4NBr (0.08 g in 1.8 mL water). The reac-

tion mixture was stirred for 16 h and then diluted with CH_2Cl_2 (20 mL). The CH_2Cl_2 layer was separated, dried (Na_2SO_4) and concentrated to a syrup that was purified by column chromatography (9:1 hexanes–EtOAc) to yield **12** (0.48 g, 83%) as a syrup: R_f 0.4 (85:15 hexanes–EtOAc); $[\alpha]_D$ −72.4 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.13–8.08 (m, 2H, ArH), 8.04–7.99 (m, 2H, ArH), 7.63–7.50 (m, 4H, ArH), 7.42–7.49 (m, 4H, ArH), 7.32–7.26 (m, 2H, ArH), 5.66 (ddd, 1H, *J* 1.2, 2.7, 16.2 Hz, H-3), 5.55 (dd, 1H, *J* 3.4, 27.5 Hz, H-1), 5.36 (ddd, 1H, *J* 1.2, 3.4, 50.0 Hz, H-2), 4.72 (dd, 1H, *J* 5.7, 11.8 Hz, H-5), 4.65 (dd, 1H, *J* 5.3, 11.8 Hz, H-5′), 4.49 (ddd, 1H, *J* 2.7, 5.3, 5.7 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.18 (C=O), 165.10 (C=O), 134.12 (Ar), 133.86 (Ar), 133.30 (2C, Ar), 133.15 (Ar), 132.11 (Ar), 132.10 (Ar), 129.83 (4C, Ar), 129.69 (Ar), 129.24 (2C, Ar), 128.60 (2C, Ar), 128.38 (2C, Ar), 95.78 (d, *J* 189.6 Hz, C-2), 89.29 (d, *J* 18.9 Hz, C-1), 82.14 (C-4), 77.71 (d, *J* 30.8 Hz, C-3), 63.71 (d, *J* 2.5 Hz, C-5); ^{19}F NMR (376 MHz, $CDCl_3$, δ_F) −192.70 (ddd, *J* 16.2, 27.5, 50.0 Hz). ESIMS: *m/z* calcd for $[C_{25}H_{20}ClFO_5S]Na^+$: 509.0596. Found: 509.0600.

1.12. *p*-Chlorophenyl 2-deoxy-2-fluoro-1-thio-β-D-arabinofuranoside (13)

To a solution of **12** (0.4 g, 0.8 mmol) in 1:1 CH_2Cl_2–CH_3OH (12 mL) was added $NaOCH_3$ (0.03 g, 0.5 mmol). After stirring for 3 h, the solution was neutralized with a few drops of glacial HOAc and concentrated to a syrupy residue that was purified by column chromatography (1:1 hexanes–EtOAc) to yield **13** (0.2 g, 87%) as a syrup: R_f 0.3 (1:1 hexanes–EtOAc); $[\alpha]_D$ −147.3 (*c* 1.0, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.37–7.44 (m, 2H, ArH), 7.26–7.21 (m, 2H, ArH), 5.42 (dd, 1H, *J* 3.6, 25.3 Hz, H-1), 4.99 (ddd, 1H, *J* 2.0, 3.0, 51.7 Hz, H-2), 4.28 (ddd, 1H, *J* 2.0, 3.4, 18.1 Hz, H-3), 3.90–3.85 (m, 1H, H-4), 3.76–3.68 (m, 2H, H-5, 5′); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 133.58 (Ar), 132.57 (Ar), 132.28 (2C, Ar), 129.17 (2C, Ar), 98.2 (d, *J* 188.4 Hz, C-2), 88.43 (d, *J* 18.6 Hz, C-1), 86.28 (d, *J* 2.1 Hz, C-4), 74.91 (d, *J* 26.3 Hz, C-3), 61.88 (d, *J* 1.6 Hz, C-5); ^{19}F NMR (376 MHz, $CDCl_3$, δ_F) −192.31 (ddd, *J* 18.1, 25.3, 51.7 Hz); ESIMS: *m/z* calcd for $[C_{11}H_{12}ClFO_3S]Na^+$: 301.0071. Found 301.0068.

1.13. *p*-Chlorophenyl 3,5-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-fluoro-1-thio-β-D-arabinofuranoside (14)

To a solution of **13** (0.2 g, 0.7 mmol) in dry DMF (3 mL) was added imidazole (0.3 g, 4.4 mmol), followed by *t*-Bu$(CH_3)_2SiCl$ (0.27 g, 1.8 mmol). The reaction mixture was stirred for 14 h and then poured into ice water (50 mL) and extracted with toluene (2 × 15 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated to a syrupy residue that was purified by column chromatography (30:1 hexanes–EtOAc) to yield the title compound **14** (0.34 g, 93%) as a syrup: R_f 0.46 (25:1 hexanes–EtOAc); $[\alpha]_D$ −88.8 (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.46–7.42 (m, 2H, ArH), 7.25–7.22 (m, 2H, ArH), 5.46 (dd, 1H, *J* 3.4, 27.7 Hz, H-1), 4.92 (ddd, 1H, *J* 1.8, 3.4, 51.6 Hz, H-2), 4.43 (ddd, 1H, *J* 1.8, 1.8, 17.3 Hz, H-3), 3.94 (ddd, 1H, *J* 1.8, 5.2, 7.8 Hz, H-4), 3.79 (ddd, 1H, *J* 2.0, 5.2, 10.2 Hz, H-5), 3.67 (ddd, 1H, *J* 1.8, 7.8, 10.2 Hz, H-5′), 0.92 (s, 9H, $C(CH_3)_3$), 0.89 (s, 9H, $C(CH_3)_3$), 0.13 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$), 0.09 (s, 3H, $SiCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 133.39 (Ar), 133.22 (Ar), 132.43 (2C, Ar), 129.08 (2C, Ar), 98.43 (d, *J* 188.4 Hz, C-2), 88.55 (d, *J* 18.1 Hz, C-1), 87.39 (C-4), 75.95 (d, *J* 25.8 Hz, C-3), 62.55 (d, *J* 3.6 Hz, C-5), 25.92 (3C, $C(CH_3)_3$), 25.67 (3C, $C(CH_3)_3$), 18.37 ($C(CH_3)_3$), 17.93 ($C(CH_3)_3$), −4.86 (2C, $SiCH_3$), −5.36 (2C, $SiCH_3$); ^{19}F NMR (376 MHz, $CDCl_3$, δ_F) −190.71 (ddd, *J* 17.3, 27.7, 51.6 Hz); ESIMS: *m/z* calcd for $[C_{23}H_{40}ClFO_3SSi_2]Na^+$: 529.1801. Found 529.1801.

1.14. Dibenzyl (3,5-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl) phosphate (15)

A solution of **14** (0.2 g, 0.4 mmol) in dry CCl_4 (12 mL) containing powdered 4 Å molecular sieves (0.2 g) was stirred for 30 min before a solution of Br_2 in dry CCl_4 (44 mg in 0.7 mL CCl_4) was added. This solution was stirred for 1.5 h. Simultaneously, a solution of dibenzyl phosphate (0.14 g, 0.52 mmol) in CH_2Cl_2 (8 mL) was stirred together with powdered 4 Å molecular sieves (~0.2 g) for 20 min before Et_3N (94 μL, 0.67 mmol) was added. After stirring for 3 min, this solution was added to the mixture prepared from **14**. The resulting mixture was stirred for 16 h, neutralized with a few drops of Et_3N, and then filtered. The filtrate was concentrated to a syrupy residue that was purified by column chromatography (85:15 hexanes–EtOAc) to yield **15** (0.168 g, 67%) as a separable α:β mixture (α:β 1:5) in addition to 8% of unreacted **14** and 10% of hydrolyzed **14**. Data for the major isomer: R_f 0.22 (85:15 hexanes–EtOAc); $[\alpha]_D$ −22.9 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.25 (m, 10H, ArH), 5.89 (dd, 1H, *J* 4.3, 5.5 Hz, H-1), 5.16–5.02 (m, 4H, PhCH_2), 4.85 (dddd, 1H, *J* 1.7, 4.3, 6.5, 52.6 Hz, H-2), 4.44 (ddd, 1H, *J* 6.5, 6.5, 16.5 Hz, H-3), 3.89 (ddd, 1H, *J* 4.3, 5.7, 6.5 Hz, H-4), 3.77 (dd, 1H, *J* 4.3, 11.3 Hz, H-5), 3.72 (dd, 1H, *J* 5.7, 11.3 Hz, H-5′), 0.90 (s, 9H, $C(CH_3)_3$), 0.87 (s, 9H, $C(CH_3)_3$), 0.13 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$), 0.03 (s, 6H, 2 × $SiCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 135.80 (Ar), 135.73 (Ar), 128.47 (Ar), 128.41 (2C, Ar), 128.38 (3C, Ar), 127.92 (2C, Ar), 127.85 (2C, Ar), 97.50 (dd, *J* 4.6, 18.6 Hz,

C-1), 95.24 (dd, *J* 5.7, 201.3 Hz, C-2), 84.28 (d, *J* 9.3 Hz, C-4), 73.21 (d, *J* 21.2 Hz, C–3), 69.28 (d, *J* 5.2 Hz, Ph*C*H_2), 69.17 (d, *J* 5.2 Hz, Ph*C*H_2), 63.54 (C-5), 25.90 (3C, C(*C*H_3)$_3$), 25.59 (3C, C(*C*H_3)$_3$), 18.35 (*C*(CH$_3$)$_3$), 17.85 (*C*(CH$_3$)$_3$), −4.66 (SiCH$_3$), −4.98 (SiCH$_3$), −5.39 (SiCH$_3$), −5.42 (SiCH$_3$); ^{31}P NMR (162 MHz, $CDCl_3$, δ_P) −1.78; ^{19}F NMR (468 MHz, $CDCl_3$, δ_F) −204.49 (dd, *J* 16.5, 52.6 Hz); ESIMS: *m*/*z* calcd for $[C_{31}H_{50}FO_7PSi_2]Na^+$: 663.2709. Found, 663.2706.

1.15. Dibenzyl (3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl) phosphate (22)

A solution of **7** (0.55 g, 1.3 mmol) in 1,2-dichloroethane (6 mL) containing powdered 4 Å molecular sieves (0.2 g) was stirred for 20 min. In a separate flask, a solution of dibenzyl phosphate (0.47 g, 1.69 mmol) in CH_2Cl_2 (6 mL) was stirred together with powdered 4 Å molecular sieves (0.2 g) for 20 min and then Et_3N (0.28 mL, 2.2 mmol) was added. After stirring for another 3 min, this solution was added to the mixture containing **7**. The resulting reaction mixture was heated at 60 °C for 2 h and then cooled to rt before being filtered. The filtrate was concentrated to a syrupy residue that was purified by column chromatography (7:3 hexanes–EtOAc) to yield the title compound **22** (0.66 g, 82%) as a separable mixture (α:β 1:6.4). Data for the major isomer: R_f 0.1 (4:1 hexanes–EtOAc); $[\alpha]_D$ −23.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–7.98 (m, 4H, ArH), 7.64–7.60 (m, 1H, ArH), 7.53–7.44 (m, 3H, ArH), 7.40–7.22 (m, 12H, ArH), 6.11 (dd, 1H, *J* 4.3, 5.8 Hz, H-1), 5.84 (ddd, 1H, *J* 6.1, 6.1, 16.6 Hz, H-3), 5.34 (dddd, 1H, *J* 1.7, 4.3, 6.1, 52.0 Hz, H-2), 5.14–5.00 (m, 4H, 2 × PhCH_2), 4.72 (dd, 1H, *J* 4.0, 11.9 Hz, H-5), 4.57 (dd, 1H, *J* 6.4, 11.9 Hz, H-5′), 4.49 (ddd, 1H, *J* 4.0, 6.1, 6.4 Hz, H-4); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.96 (C=O), 165.56 (C=O), 135.47 (Ar), 135.43 (Ar), 135.42 (Ar), 135.37 (Ar), 133.82 (Ar), 133.11 (Ar), 129.89 (2C, Ar), 129.77 (2C, Ar), 129.50 (Ar), 128.61 (Ar), 128.59 (2C, Ar), 128.56 (Ar), 128.49 (2C, Ar), 128.45 (Ar), 128.32 (2C, Ar), 127.99 (2C, Ar), 127.78 (2C, Ar), 97.31 (dd, *J* 5.2, 18.6 Hz, C-1), 92.56 (dd, *J* 6.7, 204.4 Hz, C-2), 80.07 (d, *J* 7.7 Hz, C-4), 75.04 (d, *J* 24.3 Hz, C-3), 69.61 (d, *J* 5.2 Hz, OCH$_2$), 69.41 (d, *J* 5.7 Hz, OCH$_2$), 64.93 (C-5); ^{31}P NMR (162 MHz, $CDCl_3$, δ_P) −1.66; ^{19}F NMR (376 MHz, $CDCl_3$, δ_F) −203.82 (dd, *J* 16.6, 52.0 Hz); ESIMS: *m*/*z* calcd for $[C_{33}H_{30}FO_9P]Na^+$: 643.1503. Found, 643.1500.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.08.020.

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