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## Investigation and Conformational Analysis of Fluorinated Nucleoside Antibiotics Targeting Siderophore Biosynthesis

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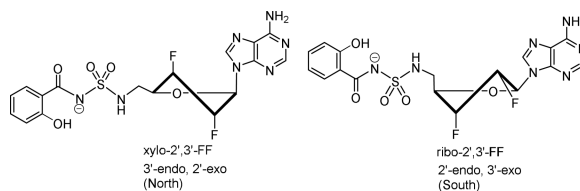
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### Abstract

Antibiotic resistance represents one of the greatest threats to public health. The adenylation inhibitor 5'-O-[N-(salicyl)sulfamoyl]adenosine (SAL-AMS) is the archetype for a new class of nucleoside antibiotics that target iron acquisition in pathogenic microorganisms and is especially effective against *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Strategic incorporation of fluorine at the 2' and 3' positions of the nucleoside was performed by direct fluorination to enhance activity and improve drug disposition properties. The resulting SAL-AMS analogs were comprehensively assessed for biochemical potency, whole-cell antitubercular activity, and *in vivo* pharmacokinetic parameters. Conformational analysis suggested a strong preference of fluorinated sugar rings for either a 2'-endo, 3'-exo (South) or a 3'-endo, 2'-exo (North) conformation. The structure–activity relationships revealed a strong conformational bias for the C3'-endo conformation to maintain potent biochemical and whole-cell activity whereas improved pharmacokinetic properties were associated with the C2'-endo conformation.

### Graphical Abstract



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**Supporting Information:** <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F NMR spectra for all compounds, HPLC traces and conformational analysis of **12–17**, and a complete description of the rat pharmacokinetic studies, MbtA inhibition assay, and *M. tuberculosis* MIC assay. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

## INTRODUCTION

Iron is the most abundant element in the universe and essential for all domains of life where it serves as a redox cofactor for enzymes involved in diverse metabolic pathways. However, in many environments, iron is severely depleted, which is particularly challenging for microorganisms that can only assimilate nutrients from their immediate surroundings. To prevent bacterial colonization and growth, mammals use iron binding proteins such as transferrin to withhold iron. The concentration of unbound  $\text{Fe}^{3+}$  in human serum and body fluids is  $\sim 10^{-24}$  M, an astonishingly low concentration close to the reciprocal of Avogadro's number.<sup>1</sup> To survive under these iron-restricted conditions, many pathogenic bacteria synthesize, secrete, and reimport small-molecule iron-chelators known generically as siderophores.<sup>2</sup> *Mycobacterium tuberculosis*, the leading cause of bacterial infectious disease mortality, and the focus of the present report, relies on mycobactins **1** (Figure 1), which are aryl-capped siderophores, for iron acquisition *in vivo*.<sup>3</sup> Given the vital role that siderophores play in bacterial iron acquisition, disruption of siderophore biosynthesis or trafficking could provide a new strategy to combat drug resistant bacteria. This strategy is particularly effective for *M. tuberculosis* since it relies exclusively on the mycobactins for siderophore iron mobilization, whereas other bacteria such as *Escherichia coli* and *Acinetobacter baumannii* synthesize multiple siderophores.

The nucleoside antibiotic 5'-O-[N-(salicyl)sulfamoyl]adenosine (SAL-AMS, **10**, Figure 2B) is a prototype for a new class of antibiotics that targets iron acquisition through inhibition of aryl acid adenylating enzymes (AAAEs) in several pathogenic bacteria including MbtA from *M. tuberculosis*, EntE from *Escherichia coli* and *Klebsiella pneumoniae*, BasE from *Acinetobacter baumannii*, YbtE from *Yersinia pestis*, VibE from *Vibrio cholerae*, and PchA from *Pseudomonas aeruginosa*.<sup>4</sup> AAAEs catalyze the first committed step in the synthesis of the respective aryl-capped siderophores **2–6** produced by each bacteria (Figure 1). Adenylation, the ligation of a carboxylic acid substrate with AMP to form an acyl-AMP intermediate, is a ubiquitous process and adenylation inhibitors like SAL-AMS (**10**) mimic the acyl-AMP intermediate, through replacement of the labile acyl-phosphate moiety with an acyl-sulfamate bioisostere (Figure 2).<sup>5</sup> The specificity of SAL-AMS is derived from the salicyl moiety, which allows it to bind to adenylating enzymes that utilize salicylic acid or related carboxyl acid substrates. Affinity-based protein profiling of SAL-AMS in *M. tuberculosis* showed it binds only to MbtA from more than 60 functionally related adenylating enzymes in mycobacteria.<sup>6</sup> Isosteric substitution of the phenol of SAL-AMS with an amino group, completely obliterated biological activity, due to a H-bond repulsion in the enzyme active site, illustrating the high level of selectivity that one can obtain with such adenylation inhibitors.<sup>7</sup> SAL-AMS has demonstrated proof-of-concept *in vitro*<sup>4a,c</sup> and *in vivo*,<sup>8</sup> but like other adenylation inhibitors that mimic their native acyl-adenylates, it is marred by poor physicochemical properties. To further advance this new class of antibiotics, key improvements in the molecular properties of SAL-AMS will be required.

One of the most common means to modulate chemical properties of small molecules is to introduce fluorine, often considered an isostere of hydrogen; although fluorine's van der Waal radius (1.47 Å) is closer to oxygen (1.52 Å) than hydrogen (1.20 Å). Fluorination also has a long tradition in nucleoside chemistry and the synthetic provenance can be traced to a

report by Fox and co-workers.<sup>9</sup> The replacement of the 2' or 3' hydroxyl groups of a nucleoside with a fluorine atom causes only a minor change in the overall structure, but profoundly affects the stereoelectronic properties of the sugar moiety.<sup>10</sup> Such dominating effects can control the conformational equilibria and lock the sugar ring into either a North (C3'-*endo* pucker) or a South (C2'-*endo* pucker) conformation,<sup>11</sup> can stabilize the glycosidic bond towards hydrolysis,<sup>12</sup> and can also enhance the lipophilicity.<sup>13</sup>

Based on the beneficial effects of fluorination, we postulated that strategic introduction of fluorine at the 2' and/or 3' position in both  $\alpha$  and  $\beta$ - configuration of SAL-AMS may enhance the biological activity and pharmacokinetic behavior. We predicted compounds that mimic the C3'-*endo* pucker of the native acyl-adenylate intermediate<sup>14</sup> would exhibit greater biological activity than compounds that adopt the C2'-*endo* pucker. However, we could not anticipate the impact of ring pucker or extent of fluorination on *in vivo* pharmacokinetic parameters. Herein we describe synthesis and conformational analysis of a systematic series of fluorinated nucleoside derivatives of SAL-AMS **12–17** (Figure 2C), along with their enzyme inhibition, antibacterial activity, and complete *in vivo* pharmacokinetic characterization.

## RESULTS AND DISCUSSION

### Synthesis

The synthesis of **12–17** was accomplished in two stages: synthesis of the requisite fluorinated nucleoside followed by installation of the *N*-(salicyl)sulfamide moiety at C-5'. For the second stage, we developed a new methodology while for the first stage, we used an amalgamation of methods for the direct introduction of fluorine into the targeted adenine nucleosides.<sup>15</sup> The main synthetic challenge was to install fluorine at the desired position of the sugar moiety and with the desired stereochemistry. There are two general synthetic approaches towards fluorinated nucleosides: (1) transformation of natural nucleosides using various fluorinating reagents and (2) glycosylation of fluorinated sugar with an appropriate heterocyclic base. With the first approach, one can start with a pre-defined  $\alpha$  or  $\beta$  configuration at C-1', but the main limitation is the sluggish reactivity of certain pentofuranose hydroxyls towards fluorinating reagents. In the second approach, a wide variety of nucleosides can be prepared, but the formation of regioisomers and anomers ( $\alpha$  and  $\beta$  isomers) are the major drawbacks. We selected the first approach since it usually involves fewer synthetic steps when suitable protecting groups are available.

The direct introduction of fluorine into each nucleoside building block is illustrated in Schemes 1–4. For the preparation of 3'-deoxy-3'-F-*xylo* **23** and 2'-deoxy-2'-F-*arabino* **24**, a unified synthetic approach was employed (Scheme 1). Regioselective PMB protection<sup>16</sup> of the secondary alcohols of adenosine **18** provided a 3:1 mixture of 2'-OPMB adenosine **19** and 3'-OPMB adenosine **20** in 75% yield. The ratio of **19** and **20** in the mixture was determined by <sup>1</sup>H NMR integrations of the anomeric protons. Without further separation of regioisomers, subsequent tritylation of the 5'-OH and 6-NH<sub>2</sub> afforded a 3:1 mixture of **21** and **22** that was fluorinated with attendant inversion of configuration using DAST<sup>17</sup> to obtain **23** (55% from **21**) and **24** (42% from **22**), which were easily separated by chromatography. The assignment of fluorine configuration was based on proton and fluorine

spectra. In the case of 3'-deoxy-3'-F-xylo **23**, the vicinal coupling of fluorine with both 2'-H and 4'-H ( $^3J(2',F3') = 13.9$ ,  $^3J(4',F3') = 31.4$  Hz), and germinal coupling with 3'-H ( $^2J(3',F3') = 50.6$  Hz) confirmed the position of fluorine at 3'. The coupling constants also suggest 3'-F has an *anti* relationship with 4'-H and a *syn* relationship with 2'-H, which indicates a *xylo* configuration. The absence of detectable  $^3J(1',2')$  is consistent with a 3'-*endo* conformation.<sup>18</sup> The configuration at C2' of 2'-deoxy-2'-F-*arabino* **24** was unequivocally assigned by the presence of a five bond coupling between 2'-F and C8-H of the adenine base ( $^5J(8,F2') = 3.1$  Hz), which is only observed in *arabino*-2'-F compounds.<sup>17</sup>

The key intermediate **21** also served as an entry point for synthesis of 3'-deoxy-3'-F-*ribo* **27** through a two-step oxidation–reduction sequence<sup>19</sup> that inter-converted the *ribo* sugar to a *xylo* sugar (Scheme 2). This was accomplished by Dess-Martin periodinane (DMP) mediated oxidation of **21** to 3'-ketoadenosine derivative **25**, which was stereoselectively reduced with sodium triacetoxyborohydride to furnish alcohol **26**. The bulky reducing agent NaBH(OAc)<sub>3</sub> delivers hydride from the more accessible  $\alpha$ -face of the nucleoside. Fluorination of **26** with DAST gave **27** in 61% yield. Once again, the coupling constants of fluorine with 2'-H and 4'-H ( $^3J(2',F3') = 21.6$ ,  $^3J(4',F3') = 26.4$  Hz) compared to those of 3'-deoxy-3'-F-*xylo* **23** indicate a *ribo*-configuration. A significantly large coupling between 1'-H and 2'-H ( $^3J(1',2') = 7.5$ ) and the absence of  $^3J(3',4')$  further confirms the 2'-*endo* conformation of 3'-deoxy-3'-F-*ribo* compounds.

The novel difluorinated nucleoside 2',3'-dideoxy-2',3'-FF-*xylo* **31** was synthesized from 2'-fluoroadenosine **28**<sup>20</sup> by trityl protection to provide **29** and subsequent treatment with DAST furnished **31** in 62% overall yield (Scheme 3). The 2'-deoxy-2'-F-*ribo* **30** building block was prepared by PMB protection of **29**. As the configuration of fluorine at C2' is known, a strong vicinal *trans* coupling between two the fluorines ( $^3J(F2',F3') = 15.0$ ) in **31** indicate the 2',3'-*xylo* difluoro configuration. The absence of  $^3J(1',2')$  is consistent with the 3'-*endo* conformation.

The difluorinated 2',3'-dideoxy-2',3'-FF-*ribo* **35** was prepared from the known *lyxo*-epoxide<sup>21</sup> **32** (Scheme 4) following the Watanabe two-step process initiated by epoxide opening with a fluoride ion exclusively from the  $\alpha$ -face of the sugar to afford a 3:1 ratio of regioisomers 3'-fluoro-*arabino* **33** and 2'-fluoro-*xylo* **34** in a 65% combined yield. Treatment of this mixture with DAST furnished **35** in a 62% yield.<sup>22</sup> The regioisomeric ratio was determined by integration of the relevant peaks in <sup>19</sup>F NMR spectrum of the crude products. There was no need for the separation of **33** and **34** because both provide the same difluoro product **35** after treatment with DAST. The presence of rather weak *cis* vicinal coupling between two fluorines ( $^3J(F2',F3') = 3.8$ ) and a strong coupling between 1'-H and 2'-H ( $^3J(1',2') = 5.0$ ) indicates the 2',3'-*ribo* difluoro configuration and C2'-*endo* conformation.

Each of the fully protected fluorinated nucleosides was globally deprotected with 80% TFA in chloroform to obtain fluorinated nucleosides **36–40** (Scheme 5). This allowed unequivocal structural assignment for **36**,<sup>23</sup> **37**,<sup>24</sup> **38**,<sup>17, 25</sup> and **40**<sup>26</sup> by comparison to the literature data of these nucleosides synthesized through alternate synthetic routes, but not **39** since this has not been previously reported. Illustrating the efficiency of direct fluorination,

our synthesis of **40** proceeded in 6 steps and 8.5% overall yield from adenosine, whereas, the only described synthesis that utilized glycosylation of adenine with a fluorinated carbohydrate required 13 steps in 0.4% overall yield.<sup>26</sup>

The fluorinated nucleoside building blocks were elaborated to the corresponding SAL-AMS derivatives **12–17** (Scheme 6). The primary motivation for using the trityl protecting group among the plethora of common nucleoside protecting groups was based upon prior reports that had shown *O*-Tr groups could be chemoselectively removed in the presence of a *N*-Tr group<sup>27</sup> as well as the improved lipophilicity engendered by this nonpolar group that facilitated chromatographic purification. De-*O*-tritylation of **30**, **24**, **23**, **27**, **31**, and **35** was successively achieved with 0.4 M HCl in 1,4-dioxane to afford **41a–f**. Mono-deprotection was complete within 1 h and longer reaction times led to undesired PMB deprotection. The resulting C5' alcohols in **41a–f** were converted to the C5' azides using the methodology of Liu and Austin<sup>28</sup> to provide **42a–f**. This two-step one-pot method converts the 5'-OH to a phosphate leaving group in the first-step, which is displaced by azide in the second step. We observed that the presence of fluorine in the  $\beta$  ('up') configuration found in **41b**, **41c**, and **41e** significantly suppressed the reactivity of 5' phosphates towards nucleophilic azides. For comparison, **42c** was obtained in 57% yield and conversion was still incomplete after 24 h of reflux, whereas **42d** was obtained in 81% yield and complete conversion was observed within 3 h. This can be explained in terms of electrostatic repulsion between electron rich fluorine and the incoming azide anion. The azides **42a–f** were reduced to amino derivatives in quantitative yields by catalytic hydrogenation or with zinc in AcOH. The crude 5'-aminonucleosides were then transformed to the 5'-sulfamides **43a–f** in 84–94% yield by refluxing with sulfamide (NH<sub>2</sub>SO<sub>2</sub>NH<sub>2</sub>) in 1,4-dioxane for 2 h. This new method of sulfamide synthesis is superior to previous examples in terms of yield and experimental simplicity.<sup>4c, 29</sup> The synthesis of the target molecules was completed by salicylation of sulfamides **43a–f** with NHS-ester **44** to obtain fully protected coupled products **45a–f** and global deprotection furnished **7–12** in 39–50% yield.<sup>30</sup>

### Conformational Analysis

The solution phase conformations adopted by the sugar rings of nucleosides **12–17** were studied by <sup>1</sup>H NMR <sup>3</sup>*J*(H,H) scalar coupling constants of ring protons and electronegativities of ring substituents through the use of pseudorotation analysis (program: Matlab Pseudorotation GUI).<sup>31</sup> <sup>1</sup>H NMR were obtained in DMSO-*d*<sub>6</sub> at 23 °C and the multiplets were analyzed (Table 1). Detailed conformation analysis including coupling constants and optimized pseudorotation parameters are provided in Supporting Information (Table S2). The percent North or C3'-*endo* conformation (%*N*) adopted by **12–17** agree closely with the reported values for the free nucleosides (Table 1). Most compounds displayed a substantial conformational preference for either the C2'-*endo* or C3'-*endo* pucker except for 2'-deoxy-2'-F-ribo **12** and 2'-deoxy-2'-F-arabino **13**. The 3'-deoxy-3'-F-xylo **14** primarily adopted the C3'-*endo* conformation (~90% *N*) whereas, 3'-deoxy-3'-F-ribo **15** was almost exclusively present in the C2'-*endo* conformation. Similar conformational rigidity was also observed with 2',3'-dideoxy-2',3'-FF-xylo **16** and 2',3'-dideoxy-2',3'-FF-ribo **17**; the former nearly exclusively in the C3'-*endo* (96% *N*) whereas the latter was largely in the C2'-*endo* (20% *N*) conformation. Marquez and co-workers previously

observed similar extreme inclination of 2',3'-dideoxy-2',3'-FF-*xylo* uridine nucleosides towards C3'-*endo* conformation (~100% N).<sup>32</sup>

### Biochemical and Antitubercular Evaluation

The nucleoside derivatives were evaluated for enzyme inhibition against MbtA, the molecular target of SAL-AMS in *M. tuberculosis*.<sup>4a,c</sup> The effect of configuration at C2' on enzyme inhibition was inconsequential since both 2'- $\alpha$ -fluoro **12** and 2'- $\beta$ -fluoro **13** were equipotent with  $\text{app}K_i$ 's of 1.7–1.8 nM (Table 1). By contrast, the enzyme inhibition was more sensitive to the stereochemical configuration at C3' and 3'- $\beta$ -fluoro **14** was approximately 10-fold more potent than 3'- $\alpha$ -fluoro **15**. The impact of configuration at C3' was amplified in difluorinated analogs **16** and **17** (both contain a 2'- $\alpha$  fluoro group), 3'- $\beta$ -fluoro **16** ( $\text{app}K_i = 1.4$  nM) was nearly 100-fold more potent than 3'- $\alpha$ -fluoro **17**. All compounds were then evaluated for antibacterial activity against virulent *M. tuberculosis* strain H37Rv and the concentration of inhibitor that resulted in complete inhibition of observable growth was defined as the minimum inhibitory concentration (MIC). The relative trend in antibacterial activities mirrored the biochemical results (Table 1). Thus, the C2'-fluoro diastereomers **12** and **13** had nearly identical MICs while the C3'-fluoro analog **14** was fourfold more potent than **15**. The activities of the difluorinated analogs also significantly diverged and **16** (MIC = 0.78  $\mu\text{M}$ ) was ca. 50-fold more potent than **17**. Comparison of the conformational disposition of each inhibitor with the observed biochemical and antibacterial activities shows an excellent qualitative positive correlation between the C3'-*endo* conformation and biological activity.

### In Vivo Pharmacokinetics

All compounds were then subjected to single dose pharmacokinetic (PK) studies in a crossover experiment using cannulated rats and administered orally (*p.o.*) at 25 mg/kg then intravenously (*i.v.*) at 2.5 mg/kg after a 3-day washout period. The serum concentration versus time curves (Figure 2) were employed for the calculation of pharmacokinetic parameters by non-compartmental analysis. Each of the fluorinated analogs exhibited improved oral bioavailability (F%), half-life ( $t_{1/2}$ ), maximum serum concentration ( $C_{\text{max}}$ ), and area under the concentration-time curve (AUC) relative to SAL-AMS **10** as well as decreased clearance (CL) (Table 2). Difluorinated analog **17** achieved the most improved pharmacokinetic profile compared to SAL-AMS (**10**) with about a fourfold increase in F%, 15-fold higher  $C_{\text{max}}$  and ca. 75-fold increased oral exposure as defined by its oral AUC, which is largely attributed to its remarkable ca. 25-fold increase in half-life to 267 min. This is also clearly noticeable on concentration versus time plots. Pairwise comparison (i.e. **12** vs. **13**, **14** vs. **15**, and **16** vs. **17**) of the oral exposure levels indicated a strong correlation between sugar conformation and relative oral AUC levels. In all cases, analogs favoring the C2'-*endo* conformation possessed ca. twofold enhanced %F and 3–13-fold greater oral AUCs relative to the diastereomer favoring the C3'-*endo* conformation.

## CONCLUSION

We have reported the efficient synthesis and conformational analysis of a systematic series of fluorinated analogues of SAL-AMS featuring direct fluorination of the nucleoside. The



structure–activity relationships (SAR) revealed a strong conformational bias for the C3'-*endo* conformation to maintain potent biochemical and whole-cell activity. Fluorination of SAL-AMS was also shown to have a dramatic impact on pharmacokinetic properties increasing half-life up to ca. 25-fold, oral exposure 75-fold, and oral bioavailability 10-fold. Further investigations of these fluorinated SAL-AMS derivatives and the application of this strategy to other adenylation inhibitors are ongoing. The results of these studies may have more widespread utility since adenylate-forming enzymes are involved in a myriad of biochemical pathways in DNA, RNA, protein, amino acid, and cofactor biosynthesis as well as post-translational protein modifications.<sup>5, 35</sup>

## EXPERIMENTAL SECTION

### General Chemistry Methods

All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system using packed columns of neutral alumina was used for drying THF and CH<sub>2</sub>Cl<sub>2</sub>, while packed columns of 4 Å molecular sieves were used to dry DMF, and the solvents were dispensed under nitrogen. All reactions were performed under an inert atmosphere of argon in oven dried (130-150 °C) glassware. Thin-layer chromatography was performed on a pre-coated silica gel 60 F<sub>254</sub> plates. The detection of compounds was carried out with UV light. Purification by flash chromatography was performed using a medium-pressure flash chromatography system and flash column silica cartridges with the indicated solvent system. HPLC purifications were performed on instrument equipped with reverse-phase Phenomenex Gemini 10 μm C18 110Å (250 × 21.2 mm) column. All NMR spectra were recorded on a 400 MHz spectrometer at 400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C, and 376 MHz for <sup>19</sup>F. <sup>1</sup>H NMR spectra were referenced to residual CDCl<sub>3</sub> (7.27 ppm), DMSO-d<sub>6</sub> (2.50 ppm), or CD<sub>3</sub>OD (3.31 ppm); <sup>13</sup>C NMR spectra were referenced to CDCl<sub>3</sub> (77.23 ppm) DMSO-D<sub>6</sub> (39.51 ppm), or CD<sub>3</sub>OD (49.15 ppm); and <sup>19</sup>F NMR spectra were referenced to hexafluorobenzene (-162.9 ppm)<sup>36</sup> or trifluoroacetic acid (-76.5 ppm). NMR chemical shift data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, ABq = AB quartet, dm = doublet of multiplets), coupling constant, integration. Coupling constants are given in Hertz (Hz). <sup>1</sup>H and <sup>13</sup>C NMR peak assignments were based on gCOSY and gHMQC NMR spectra, respectively. <sup>19</sup>F NMR peaks were assigned using proton-fluorine coupling constants. High-resolution mass spectra (HRMS) were obtained on a TOF instrument.

### General Procedure 1: 5'-O,N<sup>6</sup>-Ditritylation

The nucleoside of choice was dried prior use by coevaporation with anhydrous pyridine. To a stirred solution of the nucleoside (1 mmol, 1 equiv) in pyridine (13 mL) was added DMAP (0.8 equiv) and trityl chloride (2.3 equiv) and the reaction mixture was heated at 80 °C for 4 h. The reaction mixture was cooled down to rt, quenched with EtOH (7 mL), concentrated under reduced pressure and coevaporated with toluene (2 × 10 mL) to obtain a crude residue. Purification by flash chromatography (dry loading, SiO<sub>2</sub>, EtOAc/hexanes gradient) afforded the 5'-O,N<sup>6</sup>-ditritylated product.

***N*<sup>6</sup>-Trityl-9-[2-*O*-(4-methoxybenzyl)-5-*O*-trityl- $\beta$ -*D*-ribofuranosyl]adenine (21) and *N*<sup>6</sup>-Trityl-9-[3-*O*-(4-methoxybenzyl)-5-*O*-trityl- $\beta$ -*D*-ribofuranosyl]adenine (22)**

An isomeric mixture of 2'-OPMB adenosine **19** and 3'-OPMB adenosine **20** was prepared from adenosine **18** employing a published procedure.<sup>16</sup> A mixture of **21** and **22** was prepared from a mixture of **19** and **20** (5.3 g, 13.7 mmol) using the general procedure 1. Flash chromatography (SiO<sub>2</sub>, gradient: hexanes to 50% EtOAc in hexanes) afforded a mixture of **21** and **22** (5.0 g, 42%) as a white solid which was used in the next step without further separation. The ratio of **21** and **22** in the mixture was determined to be ~3:1 by <sup>1</sup>H NMR integrations of anomeric protons.

Major isomer **21**: *R*<sub>f</sub> = 0.53 (1:1 EtOAc/hexanes); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.99, 7.85 (2s, 2H; H-2, H-8), 7.42–7.21 (m, 30H; 6 $\times$ C<sub>6</sub>H<sub>5</sub>), 7.14 (d, *J* = 8.6 Hz, 2H; –PMB), 6.93 (s, 1H; *N*<sup>6</sup>-H), 6.77 (d, *J* = 8.6 Hz, 2H; –PMB), 6.09 (d, <sup>3</sup>*J*(1',2') = 4.4 Hz, 1H; H-1'), 4.68–4.66 (m, 1 H; H-2'), 4.64 (ABq,  $\delta$  = 42.8 Hz, *J* = 11.6 Hz, 2H; –PMB), 4.31–4.27 (m, 1H; H-3'), 4.19 (td, <sup>3</sup>*J*(5',4') = 3.3, <sup>3</sup>*J*(3',4')  $\approx$  <sup>3</sup>*J*(5'',4')  $\approx$  4.6 Hz, 1H; H-4'), 3.77 (s, 3H; –PMB), 3.47 (dd, <sup>3</sup>*J*(5',4') = 3.3, <sup>2</sup>*J*(5'',5') = 10.6 Hz, 1H; H-5'), 3.34 (dd, <sup>3</sup>*J*(5'',4') = 4.5, <sup>2</sup>*J*(5'',5') = 10.6 Hz, 1H; H-5''), 2.66 (d, <sup>3</sup>*J*(OH,3') = 5.6 Hz, 1H; 3'-OH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  160.0, 154.3, 152.5, 148.8, 145.2, 143.8, 138.7, 130.0, 129.2, 128.9, 128.8, 128.1, 128.0, 127.4, 127.1, 121.6, 114.2, 87.3, 87.2, 84.2, 80.5, 73.0, 71.6, 70.3, 63.6, 55.5; HRMS (ESI +): *m/z* calcd for C<sub>56</sub>H<sub>49</sub>N<sub>5</sub>O<sub>5</sub>Na [(M + Na)<sup>+</sup>] 894.3626, found 894.3658 (– 3.5 ppm, acquired as a mixture of **21** and **22**).

***N*<sup>6</sup>-trityl-9-[2-*O*-(4-methoxybenzyl)-3-deoxy-3-fluoro-5-*O*-trityl- $\beta$ -*D*-xylofuranosyl]adenine (23) and *N*<sup>6</sup>-Trityl-9-[3-*O*-(4-methoxybenzyl)-2-deoxy-2-fluoro-5-*O*-trityl- $\beta$ -*D*-arabinofuranosyl]adenine (24)**

To a solution of a mixture of **21** and **22** (4.70 g, 5.39 mmol, 1.0 equiv, 73% **21**) in CH<sub>2</sub>Cl<sub>2</sub> (45 mL) and pyridine (4.3 mL, 43.27 mmol, 10 equiv) in a polypropylene tube was added DAST (3.5 mL, 26.49 mmol, 5.0 equiv) dropwise and the mixture was stirred at rt for 24 h. CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added to the reaction mixture and the organic layer was washed with sat. NaHCO<sub>3</sub> and water. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, gradient: hexanes to 30% EtOAc in hexanes) afforded **23** (1.86 g, 55% from **21**) and **24** (0.55 g, 42% from **22**) as white solids.

**23**: *R*<sub>f</sub> = 0.35 (1:3 EtOAc/hexanes); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.07, 7.77 (2s, 2H; H-2, H-8), 7.49–7.24 (m, 32H; 6 $\times$ C<sub>6</sub>H<sub>5</sub> and –PMB), 6.96 (s, 1H; *N*<sup>6</sup>-H), 6.86 (d, *J* = 8.8 Hz, 2H; –PMB), 6.31 (br s, 1H; H-1'), 5.07 (dd, <sup>3</sup>*J*(3',4') = 2.5, <sup>2</sup>*J*(3',F3') = 50.6 Hz, 1H; H-3'), 4.76 (ABq,  $\delta$  = 62.8 Hz, *J* = 11.6 Hz, 2H; –PMB), 4.56 (dtd, <sup>3</sup>*J*(3',4') = 2.3, <sup>3</sup>*J*(5',4') = 6.1, <sup>3</sup>*J*(5'',4') = 6.2, <sup>3</sup>*J*(4',F3') = 31.3 Hz, 1H; H-4'), 4.38 (d, <sup>3</sup>*J*(2',F3') = 13.9 Hz, 1H; H-2'), 3.79 (s, 3H; –PMB), 3.62 (dd, <sup>3</sup>*J*(5'',4') = 6.8, <sup>2</sup>*J*(5',5'') = 9.4 Hz, 1H; H-5''), 3.48 (dd, <sup>3</sup>*J*(5',4') = 5.9, <sup>2</sup>*J*(5',5'') = 9.7 Hz, 1H; H-5'); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  159.9, 154.2, 152.5, 148.5, 145.2, 143.7, 137.8 (d, <sup>5</sup>*J*(C8,F3') = 5.8 Hz), 129.9, 129.2, 128.8, 128.8, 128.1, 128.1, 127.4, 127.1, 121.2, 114.2, 94.1 (d, <sup>1</sup>*J*(C3',F3') = 184.9 Hz), 88.4, 87.3, 85.3 (d, <sup>2</sup>*J*(C2',F3') = 27.3 Hz), 82.1 (d, <sup>2</sup>*J*(C4',F3') = 19.0 Hz), 72.5, 71.6, 60.8 (d, <sup>3</sup>*J*(C5',F3') = 9.4 Hz), 55.5; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>):  $\delta$  –202.3 (ddd, <sup>3</sup>*J*(2',F3') = 14.0, <sup>3</sup>*J*(4',F3') =



31.4,  $^2J(3',F3') = 50.6$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{56}H_{48}FN_5O_4Na [(M + Na)^+]$  896.3583, found 896.3604 (  $-2.3$  ppm).

**24:**  $R_f = 0.27$  (1:3 EtOAc/hexanes);  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  8.04 (s, 1H; H-2), 7.97 (d,  $^5J(8,F2') = 3.1$  Hz, 1H; H-8), 7.45–7.19 (m, 30H;  $6 \times C_6H_6$ ), 7.18 (d,  $J = 8.4$  Hz, 2H; –PMB), 6.95 (s, 1H;  $N^6$ -H), 6.82 (d,  $J = 8.4$  Hz, 2H; –PMB), 6.42 (dd,  $^3J(1',2') = 3.0$ ,  $^3J(1',F2') = 21.6$  Hz, 1H; H-1'), 5.05 (dd,  $^3J(1',2') = 3.2$ ,  $^2J(2',F2') = 51.2$  Hz, 1H; H-2'), 4.53 (ABq,  $\delta = 24.8$  Hz,  $J = 11.5$  Hz, 2H; –PMB), 4.26 (dd,  $^3J(3',4') = 3.7$ ,  $^3J(3',F2') = 17.9$  Hz, 1H; H-3'), 4.17–4.16 (m, 1H; H-4'), 3.77 (s, 3H; –PMB), 3.40 (dd,  $^3J(4',5') = 5.4$ ,  $^2J(5',5'') = 10.4$  Hz, 1H; H-5'), 3.33 (dd,  $^3J(4',5'') = 5.0$ ,  $^2J(5',5'') = 10.2$  Hz, 1H; H-5'');  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  159.8, 154.3, 152.6, 148.8, 145.2, 143.8, 139.6 (d,  $^4J(C8,F2') = 6.3$  Hz), 129.8, 129.2, 128.8, 128.7, 128.1, 128.0, 127.4, 127.1, 120.4, 114.2, 93.3 (d,  $^1J(C2',F2') = 192.7$  Hz), 87.1, 83.2 (d,  $^2J(C1',F2') = 16.8$  Hz), 81.8, 81.6 (d,  $^2J(C3',F2') = 25.7$  Hz), 72.3, 71.6, 63.3, 55.5;  $^{19}F$  NMR (376 MHz,  $CDCl_3$ ):  $\delta$  –197.3 (dddd,  $^5J(8,F2') = 3.0$ ,  $^3J(3',F2') = 17.9$ ,  $^3J(1',F2') = 21.3$ ,  $^2J(2',F2') = 51.3$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{56}H_{48}FN_5O_4Na [(M + Na)^+]$  896.3583, found 896.3624 (  $-4.5$  ppm).

#### ***N*<sup>6</sup>-Trityl-3'-deoxy-2'-O-(4-methoxybenzyl)-3'-oxo-5'-O-trityladenosine (25)**

To a solution of Dess-Martin periodinane (2.74 g, 6.468 mmol 1.2 equiv) in  $CH_2Cl_2$  (50 mL) and *t*-BuOH (0.67 mL) was added a solution of alcohol **21** (4.7 g, 5.39 mmol, 1 equiv) in  $CH_2Cl_2$  (50 mL). Anhydrous  $Na_2CO_3$  powder (0.1 g) was added and the reaction mixture was stirred at rt for 3 h. The reaction was quenched by adding 1 M  $Na_2S_2O_3$  (5 mL), sat.  $NaHCO_3$  (5 mL), brine (5 mL), and EtOAc (15 mL). The layers were separated and the aqueous layer was extracted with EtOAc ( $2 \times 15$  mL). The combined organic layers were dried ( $MgSO_4$ ), filtered, and concentrated under reduced pressure. Purification by flash chromatography ( $SiO_2$ , 40:60 EtOAc/hexanes) afforded the product (3.9 g, 83%) as a white solid.  $R_f = 0.45$  (1:1 EtOAc/hexanes);  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  7.92, 7.75 (2s, 2H; H-2, H-8), 7.39–7.17 (m, 30H;  $6 \times C_6H_5$ ), 7.02 (d,  $J = 8.5$  Hz, 2H; –PMB), 6.96 (s, 1H;  $N^6$ -H), 6.69 (d,  $J = 8.5$  Hz, 2H; –PMB), 6.10 (d,  $^3J(1',2') = 7.8$  Hz, 1H; H-1'), 5.21 (d,  $^3J(1',2') = 7.8$  Hz, 1H; H-2'), 4.73 (ABq,  $\delta = 34.4$  Hz,  $J = 11.7$  Hz, 2H; –PMB), 4.32–4.31 (m, 1H; H-4'), 3.72 (s, 3H; –PMB), 3.49 (dd,  $^3J(5'',4') = 4.0$ ,  $^2J(5',5'') = 10.5$  Hz, 1H; H-5'), 3.40 (dd,  $^3J(5',4') = 2.4$ ,  $^2J(5',5'') = 10.5$  Hz, 1H; H-5'');  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  209.1, 159.9, 154.3, 152.7, 149.2, 145.1, 143.4, 138.7, 130.0, 129.2, 128.8, 128.2, 128.1, 128.0, 127.4, 127.2, 121.4, 114.1, 87.4, 84.6, 81.3, 78.3, 72.8, 71.7, 63.3, 55.4; **HRMS** (ESI+):  $m/z$  calcd for  $C_{56}H_{47}N_5O_5Na [(M + Na)^+]$  892.3469, found 892.3425 (  $-4.9$  ppm).

#### ***N*<sup>6</sup>-Trityl-9-[2-O-(4-methoxybenzyl)-5-O-trityl- $\beta$ -D-xylofuranosyl]adenine (26)**

To AcOH (57 mL) cooled to 4 °C was added  $NaBH_4$  (1.30 g, 34.37 mmol, 6.5 mmol) and the mixture was stirred for 10 min. Ketone **25** (4.60 g, 5.29 mmol, 1 equiv) was added as a dry powder and the reaction mixture was stirred for 2 h at 4 °C during which time the reaction solidified. The resulting frozen reaction mixture was allowed to melt by warming to rt, immediately evaporated, and coevaporated with EtOH ( $2 \times 50$  mL) under reduced pressure. The residue was partitioned between  $CHCl_3$  (100 mL) and  $H_2O$  (100 mL), and the organic layer was washed with sat.  $NaHCO_3$ , brine, dried ( $Na_2SO_4$ ), and evaporated under reduced pressure. Purification by flash chromatography ( $SiO_2$ , 50:50 EtOAc/hexanes)

afforded the product (3.37 g, 73%) as a white solid.  $R_f = 0.58$  (1:1 EtOAc/hexanes);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.89, 7.72 (2s, 2H; H-2, H-8), 7.44–7.16 (m, 32H;  $6\times\text{C}_6\text{H}_5$  and –PMB), 7.10 (d,  $^3J(\text{OH},3') = 11.0$  Hz, 1H; 3'-OH), 7.04 (s, 1H;  $N^6\text{-H}$ ), 6.88 (d,  $J = 8.5$  Hz, 2H; –PMB), 5.77 (d,  $^3J(1',2') = 1.7$  Hz, 1H; H-1'), 4.57 (ABq,  $\delta = 73.2$  Hz,  $J = 11.3$  Hz, 2H; –PMB), 4.35 (d,  $^3J(1',2') = 1.7$  Hz, 1H; H-2'), 4.28 (ddd,  $^3J(3',4') = 3.2$ ,  $^3J(5',4') = 5.7$ ,  $^3J(5'',4') = 6.4$ , 1H; H-4'), 4.23 (dd,  $^3J(3',4') = 3.2$ ,  $^3J(\text{OH},3') = 11.0$  Hz, 1H; H-3'), 3.81 (s, 3H; –PMB), 3.58–3.51 (m, 2H; H-5'/5'');  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.8, 154.6, 151.6, 146.9, 144.9, 144.0, 140.3, 129.7, 129.3, 129.1, 128.9, 128.2, 127.9, 127.2, 127.1, 122.1, 114.2, 91.3, 90.1, 87.2, 82.8, 74.6, 72.6, 71.7, 62.3, 55.5; **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{56}\text{H}_{49}\text{N}_5\text{O}_5\text{Na}$  [(M + Na) $^+$ ] 894.3626, found 894.3673 (–5.2 ppm).

### ***N*<sup>6</sup>-Trityl-9-[3-deoxy-3-fluoro-2-*O*-(4-methoxybenzyl)-5-*O*-trityl- $\beta$ -D-ribofuranosyl]adenine (27)**

To a solution of alcohol **26** (2.3 g, 2.64 mmol, 1.0 equiv) in  $\text{CH}_2\text{Cl}_2$  (50 mL) and pyridine (2.1 mL, 26.4 mmol, 10 equiv) was added DAST (2.1 mL, 15.84 mmol, 6.0 equiv) dropwise and the mixture was stirred at rt for 24 h.  $\text{CH}_2\text{Cl}_2$  (50 mL) was added to the reaction mixture and the organic layer was washed with sat.  $\text{NaHCO}_3$ , water, and brine. The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated under reduced pressure. Purification by flash chromatography ( $\text{SiO}_2$ , 40:60 EtOAc/hexanes) afforded the product (1.4 g, 61%) as a white solid.  $R_f = 0.35$  (1:2 EtOAc/hexanes);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.91, 7.79 (2s, 2H; H-2, H-8), 7.38–7.22 (m, 30H;  $6\times\text{C}_6\text{H}_5$ ), 7.09 (d,  $J = 8.5$  Hz, 2H; –PMB), 6.93 (s, 1H;  $N^6\text{-H}$ ), 6.74 (d,  $J = 8.6$  Hz, 2H; –PMB), 6.06 (d,  $^3J(1',2') = 7.5$  Hz, 1H; H-1'), 5.11 (ddd,  $^3J(2',3') = 4.3$ ,  $^3J(1',2') = 7.6$ ,  $^3J(2',\text{F}3') = 21.6$  Hz, 1H; H-2'), 4.99 (ddd,  $^3J(3',4') = 1.4$ ,  $^3J(2',3') = 4.4$ ,  $^3J(3',\text{F}3') = 54.4$  Hz, 1H; H-3'), 4.54 (ABq,  $\delta = 23.2$  Hz,  $J = 11.7$  Hz, 2H; –PMB), 4.42 (dm,  $^3J(4',\text{F}3') = 26.4$  Hz, 1H; H-4'), 3.75 (s, 3H; –PMB), 3.47 (dd,  $^3J(5',4') = 4.8$ ,  $^2J(5',5'') = 10.5$  Hz, 1H; H-5'), 3.30 (dd,  $^3J(5'',4') = 4.1$ ,  $^2J(5',5'') = 10.5$  Hz, 1H; H-5'');  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.8, 154.3, 152.5, 149.1, 145.2, 143.6, 139.5, 129.8, 129.2, 129.0, 128.8, 128.1 ( $\times 2$ ), 127.4, 127.1, 121.8, 114.1, 90.4 (d,  $^1J(\text{C}3',\text{F}3') = 186.6$  Hz), 87.5, 86.5, 82.7 (d,  $^2J(\text{C}2',\text{F}3') = 23.5$  Hz), 77.6 (d,  $^2J(\text{C}4',\text{F}3') = 15.8$  Hz), 72.7, 71.6, 63.0 (d,  $^3J(\text{C}5',\text{F}3') = 9.9$  Hz), 55.5;  $^{19}\text{F NMR}$  (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  –199.3 (ddd,  $^3J(2',\text{F}3') = 21.6$ ,  $^3J(4',\text{F}3') = 26.4$ ,  $^2J(3',\text{F}3') = 54.3$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{56}\text{H}_{48}\text{FN}_5\text{O}_4\text{Na}$  [(M + Na) $^+$ ] 896.3583, found 896.3546 (–4.1 ppm).

### ***N*<sup>6</sup>-Trityl-9-(2-deoxy-2-fluoro-5-*O*-trityl- $\beta$ -D-ribofuranosyl)adenine (29)**

This was prepared from 2'-deoxy-2'-fluoroadenosine **28** (0.50 g, 1.86 mmol) using the general procedure 1. Purification by flash chromatography ( $\text{SiO}_2$ , 50:50 EtOAc/hexanes) afforded the product (1.10 g, 79%) as a white solid.  $R_f = 0.40$  (1:1 EtOAc/hexanes);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.98, 7.92 (2s, 2H; H-2, H-8), 7.42–7.18 (m, 30 H;  $6\times\text{C}_6\text{H}_5$ ), 7.10 (s, 1H;  $N^6\text{-H}$ ), 6.17 (dd,  $^3J(1',2') = 2.4$ ,  $^3J(1',\text{F}2') = 17.6$  Hz, 1H; H-1'), 5.54 (ddd,  $^3J(2',1') = 2.4$ ,  $^3J(2',3') = 4.5$ ,  $^2J(2',\text{F}2') = 53.1$  Hz, 1H; H-2'), 4.73 (ddd,  $^3J(2',3') = 4.5$ ,  $^3J(3',4') = 6.9$ ,  $^3J(3',\text{F}2') = 17.4$  Hz, 1H; H-3'), 3.55–3.52 (m, 1H; H-4'), 3.53 (dd,  $^3J(4',5') = 3.1$ ,  $^2J(5',5'') = 10.7$  Hz, 1H; H-5'), 3.41 (dd,  $^3J(4',5'') = 4.6$ ,  $^2J(5',5'') = 10.7$  Hz, 1H; H-5'');  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  154.3, 152.6, 148.4, 145.0, 143.6, 138.8, 129.2, 128.8, 128.1 ( $\times 2$ ), 127.4, 127.1, 121.4, 93.4 (d,  $^1J(\text{C}2',\text{F}2') = 186.8$  Hz), 87.3, 87.0 (d,  $^2J(\text{C}1',\text{F}2') = 33.1$

(Hz), 82.6, 71.6, 70.3 (d,  $^2J(\text{C}3',\text{F}2') = 16.5$  Hz), 63.0;  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  -205.7 (dt,  $^3J(3',\text{F}2') = 17.4$ ,  $^3J(1',\text{F}2') = 17.4$ ,  $^2J(2',\text{F}2') = 53.1$  Hz); HRMS (ESI+):  $m/z$  calcd for  $\text{C}_{48}\text{H}_{41}\text{FN}_5\text{O}_3$  [(M + H) $^+$ ] 754.3188, found 754.3195 ( -0.9 ppm).

### ***N*<sup>6</sup>-Trityl-9-[2-deoxy-2-fluoro-3-*O*-(4-methoxybenzyl)-5-*O*-trityl- $\beta$ -D-ribofuranosyl]adenine (30)**

To a solution of **29** (0.35 g, 0.464 mmol, 1 equiv) in THF (7 mL) at 0 °C was added 60% NaH (23 mg, 0.575 mmol, 1.2 equiv) and the mixture was stirred for 10 min. TBAI (23 mg, 0.062 mmol, 0.12 equiv) and PMBCl (77  $\mu\text{L}$ , 0.568 mmol, 1.2 equiv) were sequentially added and the mixture was stirred at that temperature for further 30 min. The reaction was slowly warmed to rt and stirred for 15 h. The reaction was quenched with MeOH (7 mL), evaporated, partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ . The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  and the combined organics were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. Purification by flash chromatography ( $\text{SiO}_2$ , gradient: hexanes to 50:50 EtOAc/hexanes) afforded the product (0.32 g, 78%) as a white solid.  $R_f = 0.21$  (1:2 EtOAc/hexanes);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.98, 7.91 (2s, 2H; H-2, H-8), 7.34–7.17 (m, 32H;  $6 \times \text{C}_6\text{H}_6$ , -PMB), 7.01 (s, 1H;  $N^6$ -H), 6.88 (d,  $J = 8.6$  Hz, 2H; -PMB), 6.15 (d,  $^3J(1',\text{F}2') = 18.0$  Hz, 1H; H-1'), 5.64 (dd,  $^3J(3',2') = 4.0$ ,  $^2J(2',\text{F}2') = 53.1$  Hz, 1H; H-2'), 4.68–4.66 (m, 1H; H-3'), 4.53 (ABq,  $\delta = 48.0$  Hz,  $J = 11.4$  Hz, 2H; -PMB), 4.27 (dt,  $^3J(4',5') = 3.5$ ,  $^3J(4',5'') = 3.5$ ,  $^3J(3',4') = 7.4$  Hz, 1H; H-4'), 3.80 (s, 3H; -PMB), 3.48 (dd,  $^3J(4',5') = 3.1$ ,  $^2J(5',5'') = 10.9$  Hz, 1H; H-5'), 3.24 (dd,  $^3J(4',5'') = 4.2$ ,  $^2J(5',5'') = 10.3$  Hz, 1H; H-5'');  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -204.6 (dt,  $^3J(1',\text{F}2') = 18.5$ ,  $^3J(3',\text{F}2') = 18.5$ ,  $^2J(2',\text{F}2') = 52.8$  Hz); HRMS (ESI+):  $m/z$  calcd for  $\text{C}_{56}\text{H}_{49}\text{FN}_5\text{O}_4$  [(M + H) $^+$ ] 874.3763, found 874.3756 ( -0.8 ppm).

### ***N*<sup>6</sup>-Trityl-9-(2,3-dideoxy-2,3-difluoro-5-*O*-trityl- $\beta$ -D-xylofuranosyl)adenine (31)**

To a solution of alcohol **29** (2.30 g, 3.05 mmol, 1.0 equiv) in  $\text{CH}_2\text{Cl}_2$  (50 mL) and pyridine (2.40 mL, 29.8 mmol, 10 equiv) was added DAST (2.0 mL, 15.14 mmol, 5.0 equiv) dropwise and the mixture was stirred at rt for 25 h.  $\text{CH}_2\text{Cl}_2$  (50 mL) was added to the reaction mixture and the organic layer was washed with sat.  $\text{NaHCO}_3$  and water. The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated under reduced pressure. Purification by flash chromatography ( $\text{SiO}_2$ , 30:70 EtOAc/hexanes) afforded the product (1.79 g, 78%) as a white solid.  $R_f = 0.45$  (1:2 EtOAc/hexanes);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.08, 7.77 (2s, 2H; H-2, H-8), 7.51–7.24 (m, 30H;  $6 \times \text{C}_6\text{H}_5$ ), 6.97 (s, 1H;  $N^6$ -H), 6.40 (d,  $^3J(1',\text{F}2') = 20.2$  Hz, 1H; H-1'), 5.42 (dd,  $^3J(2',\text{F}3') = 9.8$ ,  $^2J(2',\text{F}2') = 48.0$  Hz, 1H; H-2'), 5.27 (ddd,  $^3J(3',4') = 3.0$ ,  $^3J(3',\text{F}2') = 7.7$ ,  $^2J(3',\text{F}3') = 50.0$  Hz, 1H; H-3'), 4.56 (dm,  $^3J(4',\text{F}3') = 31.2$  Hz, 1H; H-4'), 3.68 (dd,  $^3J(5',4') = 6.5$ ,  $^2J(5',5'') = 9.8$  Hz, 1H; H-5'), 3.54 (dd,  $^3J(5'',4') = 6.0$ ,  $^2J(5',5'') = 9.8$  Hz, 1H; H-5'');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  154.2, 152.8, 148.6, 145.1, 143.5, 137.5 (d,  $J = 6.2$  Hz), 129.2, 128.8, 128.2, 128.1, 127.5, 127.1, 121.1, 96.1 (dd,  $^2J(\text{C}2',\text{F}3') = 31.7$ ,  $^1J(\text{C}2',\text{F}2') = 186.1$  Hz), 92.4 (dd,  $^2J(\text{C}3',\text{F}2') = 30.1$ ,  $^1J(\text{C}3',\text{F}3') = 184.9$  Hz), 87.7 (d,  $^2J(\text{C}1',\text{F}2') = 36.6$  Hz), 87.5, 81.8 (d,  $^2J(\text{C}4',\text{F}3') = 19.4$  Hz), 71.6, 60.4 (d,  $^3J(\text{C}5',\text{F}3') = 9.0$  Hz);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -191.9 (dddd,  $^4J(4',\text{F}2') = 3.4$ ,  $^3J(3',\text{F}2') = 7.9$ ,  $^3J(\text{F}2',\text{F}3') = 15.0$ ,  $^3J(1',\text{F}2') = 20.3$ ,  $^2J(2',\text{F}2') = 47.7$  Hz; F-2'), -208.4 (dddd,  $^3J(2',\text{F}3') = 9.6$ ,  $^3J(\text{F}2',\text{F}3') = 14.9$ ,  $^3J(4',\text{F}3') =$

31.1,  $^2J(3',F3') = 49.5$  Hz; F-3'); **HRMS** (ESI+):  $m/z$  calcd for  $C_{48}H_{40}F_2N_5O_2 [(M + H)^+]$  756.3145, found 756.3138 ( -0.9 ppm).

***N*<sup>6</sup>-Trityl-9-(3-deoxy-3-fluoro-5-*O*-trityl- $\beta$ -*D*-arabinofuranosyl)adenine (33) and *N*<sup>6</sup>-Trityl-9-(2-deoxy-2-fluoro-5-*O*-trityl- $\beta$ -*D*-xylofuranosyl)adenine (34)**

To a solution of epoxide **32**<sup>21</sup> 3.20 g, 4.36 mmol, 1.0 equiv) in 2-ethoxyethanol (40 mL) was added  $KHF_2$  (1.50 g, 19.2 mmol, 4.4 equiv) and NaF (2.20 g, 52.39 mmol, 12.0 equiv) and the mixture was heated under reflux at 145 °C for 15 h. The reaction mixture was allowed to cool to rt, solvent was removed under reduced pressure and the residue was diluted with  $CHCl_3$  (40 ml) and washed with water. The organic layer was dried ( $MgSO_4$ ), filtered, and concentrated under reduced pressure. The ratio of **33** and **34** was determined to be 3.1:1 by  $^1H$  NMR of the crude product. Purification by flash chromatography ( $SiO_2$ , 35:65 EtOAc/hexanes) afforded the product as a mixture of **33** and **34** (2.12 g, 65% combined) as a white solid. A few pure fractions of each regioisomer were separately collected and characterized.

**33:**  $R_f = 0.44$  (1:1 EtOAc/hexanes);  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  8.05, 7.96 (2s, 2H; H-2, H-8), 7.44–7.18 (m, 30H;  $6 \times C_6H_5$ ), 7.07 (s, 1H; *N*<sup>6</sup>-H), 6.18–6.17 (m, 1H; H-1'), 5.52 (d,  $^3J(2',OH) = 7.6$  Hz, 1H; -OH), 5.04 (dm,  $^2J(3',F3') = 51.6$  Hz, 1H; H-3'), 4.53–4.48 (m, 1H; H-4'), 4.30 (dm,  $^3J(2',F3') = 28.8$  Hz, 1H; H-2'), 3.57–3.44 (m, 2H; H-5', H-5'');  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  154.4, 152.1, 148.3, 144.9, 143.4, 140.5, 129.1, 128.8, 128.2, 128.1, 127.5, 127.1, 120.8, 96.9 (d,  $^1J(C3',F3') = 183.0$  Hz), 87.8, 86.0, 81.7 (d,  $^2J(C2',F3') = 25.4$  Hz), 74.4 (d,  $^2J(C4',F3') = 25.2$  Hz), 71.6, 63.4 (d,  $^3J(C5',F3') = 9.6$  Hz);  $^{19}F$  NMR ( $CDCl_3$ ):  $\delta$  -182.9 ppm (dddd,  $^4J(5',F3') = 2.5$ ,  $^3J(4',F3') = 12.2$ ,  $^3J(2',F3') = 28.9$ ,  $^2J(3',F3') = 51.7$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{48}H_{40}FN_5O_3Na [(M + Na)^+]$  776.3007, found 776.3028 ( 2.7 ppm).

**34:**  $R_f = 0.51$  (1:1 EtOAc/hexanes);  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  7.86, 7.84 (2s, 2H; H-2, H-8), 7.43–7.13 (m, 31H;  $6 \times C_6H_5$  and -OH), 7.12 (s, 1H; *N*<sup>6</sup>-H), 5.93 (d,  $^3J(1',F2') = 25.6$  Hz, 1H; H-1'), 5.29 (dm,  $^2J(2',F2') = 49.6$  Hz, 1H; H-2'), 4.34–4.28 (m, 2H; H-3', H-4'), 3.59–3.52 (m, 2H; H-5'/5'');  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  154.7, 151.7, 146.7, 144.8, 143.9, 140.1, 129.1, 128.9, 128.2, 127.9, 127.3, 127.2, 121.9, 101.0 (d,  $^1J(C2',F2') = 186.1$  Hz), 90.1 (d,  $^2J(C1',F2') = 39.6$  Hz), 87.3, 82.9, 74.6 (d,  $^2J(C3',F2') = 25.8$  Hz), 71.8, 62.0;  $^{19}F$  NMR ( $CDCl_3$ ):  $\delta$  -177.8 (ddd,  $^3J(3',F2') = 15.6$ ,  $^3J(1',F2') = 25.5$ ,  $^2J(2',F2') = 49.5$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{48}H_{40}FN_5O_3Na [(M + Na)^+]$  776.3007, found 776.3021 ( 1.8 ppm).

***N*<sup>6</sup>-Trityl-9-(2,3-dideoxy-2,3-difluoro-5-*O*-trityl- $\beta$ -*D*-ribofuranosyl)adenine (35)**

To a solution of a mixture of **33** and **34** (1.94 g, 2.57 mmol, 1.0 equiv) in toluene (30 mL) and pyridine (0.57 mL, 4.72 mmol, 1.8 equiv) was added DAST (2.04 ml, 15.44 mmol, 6.0 equiv) dropwise and the mixture was stirred at rt for 1 h before heating the mixture at 80 °C for an additional 3 h. EtOAc (100 mL) was added to the reaction mixture and the organic layer was washed with sat.  $NaHCO_3$  and water. The organic layer was dried ( $MgSO_4$ ), filtered, and concentrated under reduced pressure. Purification by flash chromatography ( $SiO_2$ , 20:80 EtOAc/hexanes) afforded the product (1.20 g, 62%) as a white solid.  $R_f = 0.33$  (1:2 EtOAc/hexanes);  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  7.93, 7.88 (2s, 2H; H-2, H-8), 7.39–

7.19 (m, 30H;  $6 \times C_6H_5$ ), 6.79 (s, 1H;  $N^6$ -H), 6.18 (ddd,  $^4J(1',F3') = 1.7$ ,  $^3J(1',2') = 5.0$ ,  $^3J(1',F2') = 14.2$  Hz, 1H; H-1'), 6.02 (ddt,  $^3J(2',3') \approx ^3J(1',2') \approx 4.8$ ,  $^3J(2',F3') = 13.2$ ,  $^2J(2',F2') = 50.9$  Hz, 1H; H-2'), 5.45 (ddt,  $^3J(3',2') \approx ^3J(3',4') \approx 4.0$ ,  $^3J(3',F2') = 7.6$ ,  $^2J(3',F3') = 53.0$  Hz, 1H; H-3'), 4.46 (dm,  $^3J(4',F3') = 21.8$  Hz, 1H; H-4'), 3.54 (dd,  $^3J(5',4') = 4.2$ ,  $^2J(5',5'') = 10.8$  Hz, 1H; H-5'), 3.39 (dd,  $^3J(5'',4') = 4.3$ ,  $^2J(5',5'') = 10.8$  Hz, 1H; H-5'');  **$^{13}C$  NMR** (100 MHz,  $CDCl_3$ ):  $\delta$  154.4, 152.7, 148.7, 145.1, 143.5, 139.3, 129.2, 128.8, 128.1 ( $\times 2$ ), 127.5, 127.2, 121.7, 89.7 (dd,  $^2J(C2',F3') = 14.4$ ,  $^1J(C2',F2') = 196.3$  Hz), 88.9 (dd,  $^2J(C3',F2') = 13.9$ ,  $^1J(C2',F2') = 190.4$  Hz), 87.5, 86.1 (dd,  $^3J(C1',F3') = 2.5$ ,  $^2J(C1',F2') = 31.1$  Hz), 81.3 (dd,  $^3J(C4',F2') = 2.5$ ,  $^2J(C4',F3') = 22.7$  Hz), 71.6, 62.5 (d,  $^3J(C5',F3') = 6.4$  Hz);  **$^{19}F$  NMR** (376 MHz,  $CDCl_3$ ):  $\delta$  -206.9 (dddd,  $^3J(F2',F3') = 3.8$ ,  $^3J(2',F3') = 13.5$ ,  $^3J(4',F3') = 21.8$ ,  $^2J(3',F3') = 53.3$  Hz; F-3'), -213.0 (dm,  $^2J(2',F2') = 51.1$  Hz; F-2'); **HRMS** (ESI+):  $m/z$  calcd for  $C_{48}H_{39}F_2N_5O_2Na$  [(M + Na) $^+$ ] 778.2964, found 778.2970 ( 0.8 ppm).

### 9-(3-Deoxy-3-fluoro- $\beta$ -D-xylofuranosyl)adenine (36)

To a flask containing compound **23** (0.075 g, 0.086 mmol) was added 80% aqueous TFA in  $CHCl_3$  (5 mL). After 1 h MeOH (5 mL) was added and the reaction mixture was then concentrated under reduced pressure. The residue was dissolved in MeOH (5 mL), residual TFA was neutralized by adding  $Et_3N$  (10 drops), and the mixture was concentrated under reduced pressure. To the residue was added  $H_2O$  (5 mL) and  $Et_2O$  (5 mL) and the mixture was stirred vigorously. The phases were separated and MeCN (2 mL) was added to the aqueous layer. The aqueous layer was filtered through a syringe filter and purification was performed by preparative reverse-phase HPLC using a Phenomenex Gemini 10  $\mu m$  C18 110 $\text{\AA}$  (250  $\times$  21.2 mm) column at a flow rate of 21 mL/min with a gradient from 5% to 30% MeCN in  $H_2O$  over 12 min. The appropriate fractions containing the product were pooled and lyophilized to afford the product (0.020 g, 87%) as a white solid.  **$^1H$  NMR** (400 MHz,  $CD_3OD$ ):  $\delta$  8.22, 8.15 (2s, 2H; H-2, H-8), 6.09 (d,  $^3J(1',2') = 2.0$  Hz, 1H; H-1'), 5.10 (ddd,  $^3J(2',3') = 1.8$ ,  $^3J(3',4') = 3.3$ ,  $^2J(3',F3') = 51.7$  Hz, 1H; H-3'), 4.72 (dt,  $^3J(2',3') = 1.8$ ,  $^3J(1',2') = 1.9$ ,  $^3J(2',F3') = 13.9$  Hz, 1H; H-2'), 4.47 (dtd,  $^3J(3',4') = 3.2$ ,  $^3J(5',4') \approx ^3J(5'',4') = 5.8$ ,  $^3J(4',F3') = 27.9$  Hz, 1H; H-4'), 3.98 (dd,  $^3J(5',4') = 5.7$ ,  $^3J(5'',5') = 11.8$  Hz, 1H; H-5'), 3.93 (ddd,  $^4J(5'',F3') = 1.6$ ,  $^3J(5'',4') = 6.1$ ,  $^3J(5'',5') = 11.9$  Hz, 1H; H-5'');  **$^{13}C$  NMR** (100 MHz,  $CD_3OD$ ):  $\delta$  157.5, 154.1, 150.4, 140.4 (d,  $^5J(C8,F3') = 6.4$  Hz), 120.4, 96.9 (d,  $^1J(C3',F3') = 184.0$  Hz), 91.6 (d,  $^3J(C1',F3') = 1.5$  Hz), 84.1 (d,  $^2J(C4',F3') = 19.3$  Hz), 79.9 (d,  $^2J(C2',F3') = 26.6$  Hz), 60.2 ppm (d,  $^3J(C5',F3') = 10.2$  Hz);  **$^{19}F$  NMR** (376 MHz,  $CD_3OD$ ):  $\delta$  -203.2 (ddd,  $^3J(2',F3') = 13.9$ ,  $^3J(4',F3') = 27.9$ ,  $^2J(3',F3') = 51.7$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{10}H_{12}FN_5O_3Na$  [(M + Na) $^+$ ] 292.0816, found 292.0823 ( 2.4 ppm).

### 9-[2-Deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl]adenine (37)

To a flask containing compound **24** (0.060 g, 0.069 mmol) was added 80% aqueous TFA in  $CHCl_3$  (5 mL) to form a brown solution. After 1 h MeOH (5 mL) was added and the reaction mixture was then concentrated under reduced pressure. The residue was dissolved in MeOH (5 mL), residual TFA was neutralized by adding  $Et_3N$  (10 drops), and the mixture was concentrated under reduced pressure. To the residue was added  $H_2O$  (5 mL) and  $Et_2O$



(5 mL) and the mixture was stirred vigorously. The phases were separated and MeCN (2 mL) was added to the aqueous layer. The aqueous layer was filtered through a syringe filter and purification was performed by preparative reverse-phase HPLC using a Phenomenex Gemini 10  $\mu\text{m}$  C18 110Å (250  $\times$  21.2 mm) column at a flow rate of 21 mL/min with a gradient from 5% to 30% MeCN in H<sub>2</sub>O over 12 min. The appropriate fractions containing the product were pooled and lyophilized to afford the product (0.017 g, 89%) as a white solid. **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD):  $\delta$  8.33 (d, <sup>3</sup>*J*(8,F2') = 2.1 Hz, 1H; H-8), 8.21 (s, 1H; H-2), 6.48 (dd, <sup>3</sup>*J*(1',2') = 4.1, <sup>3</sup>*J*(1',F2') = 16.1 Hz, 1H; H-1'), 5.14 (ddd, <sup>3</sup>*J*(2',3') = 3.0, <sup>3</sup>*J*(1',2') = 4.2, <sup>2</sup>*J*(2',F2') = 52.2 Hz, 1H; H-2'), 4.53 (ddd, <sup>3</sup>*J*(2',3') = 3.0, <sup>3</sup>*J*(3',4') = 4.8, <sup>3</sup>*J*(3',F2') = 18.2 Hz, 1H; H-3'), 4.01 (q, <sup>3</sup>*J*(4',5')  $\approx$  <sup>3</sup>*J*(4',5'')  $\approx$  <sup>3</sup>*J*(4',3') = 4.6 Hz, 1H; H-4'), 3.87 (ddd, <sup>5</sup>*J*(F2',5') = 1.4, <sup>3</sup>*J*(4',5') = 4.0, <sup>2</sup>*J*(5',5'') = 12.2 Hz, 1H; H-5'), 3.81 (dd, <sup>3</sup>*J*(4',5'') = 5.2, <sup>2</sup>*J*(5',5'') = 12.2 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>):  $\delta$  157.5, 154.2, 150.5, 141.8 (d, <sup>4</sup>*J*(C8,F2') = 4.3 Hz), 119.8, 97.1 (d, <sup>1</sup>*J*(C2',F2') = 192.9 Hz), 85.9 (d, <sup>3</sup>*J*(C4',F2') = 4.0 Hz), 84.5 (d, <sup>2</sup>*J*(C1',F2') = 17.2 Hz), 74.9 (d, <sup>2</sup>*J*(C3',F2') = 24.5 Hz), 62.3 (d, <sup>4</sup>*J*(C3',F2') = 1.5 Hz); **<sup>19</sup>F NMR** (376 MHz, CD<sub>3</sub>OD):  $\delta$  -199.1 (dt, <sup>3</sup>*J*(1',F2') = 17.2, <sup>3</sup>*J*(3',F2') = 17.2, <sup>2</sup>*J*(2',F2') = 52.2 Hz); **HRMS** (ESI+): *m/z* calcd for C<sub>10</sub>H<sub>12</sub>FN<sub>5</sub>O<sub>3</sub>Na [(M + Na)<sup>+</sup>] 292.0816, found 292.0803 ( -4.4 ppm).

### 9-(3-Deoxy-3-fluoro- $\beta$ -D-ribofuranosyl)adenine (38)

To a flask containing compound **27** (0.055 g, 0.063 mmol) was added 80% aqueous TFA in CHCl<sub>3</sub> (5 mL). After 1 h MeOH (5 mL) was added and the reaction mixture was then concentrated under reduced pressure. The residue was dissolved in MeOH (5 mL), residual TFA was neutralized by adding Et<sub>3</sub>N (10 drops), and the mixture was concentrated under reduced pressure. To the residue was added H<sub>2</sub>O (5 mL) and Et<sub>2</sub>O (5 mL) and the mixture was stirred vigorously. The phases were separated and MeCN (2 mL) was added to the aqueous layer. The aqueous layer was filtered through a syringe filter and purification was performed by preparative reverse-phase HPLC using a Phenomenex Gemini 10  $\mu\text{m}$  C18 110Å (250  $\times$  21.2 mm) column at a flow rate of 21 mL/min with a gradient from 5% to 30% MeCN in H<sub>2</sub>O over 12 min. The appropriate fractions containing the product were pooled and lyophilized to afford the product (0.017 g, 97%) as a white solid. **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD):  $\delta$  8.29, 8.18 (2s, 2H; H-2, H-8), 6.01 (d, <sup>3</sup>*J*(1',2') = 8.0 Hz, 1H; H-1'), 5.12 (dd, <sup>3</sup>*J*(2',3') = 4.3, <sup>2</sup>*J*(3',F3') = 54.5 Hz, 1H; H-3'), 4.98 (ddd, <sup>3</sup>*J*(2',3') = 4.3, <sup>3</sup>*J*(1',2') = 8.0, <sup>3</sup>*J*(2',F3') = 25.2 Hz, 1H; H-2'), 4.44 (dt, <sup>3</sup>*J*(4',5') = 2.5, <sup>3</sup>*J*(4',5'') = 2.5, <sup>3</sup>*J*(4',F3') = 27.6 Hz, 1H; H-4'), 3.87 (dt, <sup>4</sup>*J*(4',F3') = 2.4, <sup>3</sup>*J*(4',5') = 2.4, <sup>2</sup>*J*(5',5'') = 12.6 Hz, 1H; H-5'), 3.79 (dd, <sup>3</sup>*J*(4',5'') = 2.4, <sup>2</sup>*J*(5',5'') = 12.7 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD):  $\delta$  157.9, 153.7, 150.2, 142.3, 121.3, 94.7 (d, <sup>1</sup>*J*(C3',F3') = 182.0 Hz), 90.5, 86.6 (d, <sup>2</sup>*J*(C4',F3') = 22.1 Hz), 74.7 (d, <sup>2</sup>*J*(C2',F3') = 16.5 Hz), 63.2 (d, <sup>3</sup>*J*(C5',F3') = 11.6 Hz); **<sup>19</sup>F NMR** (376 MHz, CD<sub>3</sub>OD):  $\delta$  -199.6 (dt, <sup>3</sup>*J*(2',F3')  $\approx$  <sup>3</sup>*J*(4',F3') = 26.5, <sup>2</sup>*J*(3',F3') = 53.8 Hz); **HRMS** (ESI+): *m/z* calcd for C<sub>10</sub>H<sub>12</sub>FN<sub>5</sub>O<sub>3</sub>Na [(M + Na)<sup>+</sup>] 292.0816, found 292.0816 ( <1 ppm).

### 9-(2,3-Dideoxy-2,3-difluoro- $\beta$ -D-xylofuranosyl)adenine (39)

To a flask containing compound **31** (0.089 g, 0.118 mmol) was added 80% aqueous TFA in CHCl<sub>3</sub> (5 mL). After 1 h MeOH (5 mL) was added and the reaction mixture was then concentrated under reduced pressure. The residue was treated with sat. NaHCO<sub>3</sub> (20 ml) and



extracted with EtOAc (3 × 20 ml). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, 98:2 EtOAc/MeOH) afforded the product (0.023 g, 80%) as a white solid. *R<sub>f</sub>* = 0.28 (5:95 MeOH/EtOAc); **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD): δ 8.23, 8.17 (2s, 2H; H-2, H-8), 6.39 (dd, <sup>3</sup>*J*(1',2') = 2.4, <sup>3</sup>*J*(1',F2') = 18.9 Hz, 1H; H-1'), 5.73 (ddt, <sup>3</sup>*J*(1',2') ≈ <sup>3</sup>*J*(2',3') ≈ 1.9, <sup>3</sup>*J*(2',F3') = 11.6, <sup>2</sup>*J*(2',F2') = 48.5 Hz, 1H; H-2'), 5.47 (dddd, <sup>3</sup>*J*(2',3') = 1.8, <sup>3</sup>*J*(3',4') = 3.6, <sup>3</sup>*J*(3',F2') = 10.1, <sup>2</sup>*J*(3',F3') = 50.1 Hz, 1H; H-3'), 4.50 (dm, <sup>3</sup>*J*(4',F3') = 27.2 Hz, 1H; H-4'), 4.00 (dd, <sup>3</sup>*J*(4',5') = 5.5, <sup>2</sup>*J*(5',5'') = 11.9 Hz, 1H; H-5'), 3.97–3.92 (m, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CD<sub>3</sub>OD): δ 157.6, 154.3, 150.5, 140.3 (d, *J* = 5.7 Hz), 120.3, 97.5 (dd, <sup>2</sup>*J*(C2',F3') = 31.1, <sup>1</sup>*J*(C2',F2') = 184.9 Hz), 93.9 (dd, <sup>2</sup>*J*(C3',F2') = 29.2, <sup>1</sup>*J*(C2',F2') = 183.9 Hz), 88.5 (dd, <sup>3</sup>*J*(C1',F3') = 1.9, <sup>2</sup>*J*(C1',F2') = 36.2 Hz), 84.0 (d, <sup>2</sup>*J*(C4',F3') = 19.7 Hz), 59.8 (d, <sup>3</sup>*J*(C5',F3') = 9.8 Hz); **<sup>19</sup>F NMR** (376 MHz, CD<sub>3</sub>OD): δ -192.3 (ddm, <sup>3</sup>*J*(1',F2') = 19.0, <sup>2</sup>*J*(2',F2') = 48.5 Hz; F-2'), -207.3 (ddt, <sup>3</sup>*J*(F2',F3') = 11.8, <sup>3</sup>*J*(2',F3') = 11.8, <sup>3</sup>*J*(4',F3') = 27.0, <sup>2</sup>*J*(3',F3') = 50.5 Hz; F-3'); **HRMS** (ESI+): *m/z* calcd for C<sub>10</sub>H<sub>11</sub>F<sub>2</sub>N<sub>5</sub>O<sub>2</sub>Na [(M + Na)<sup>+</sup>] 294.0773, found 294.0768 ( -1.7 ppm).

### 9-(2,3-Dideoxy-2,3-difluoro-β-D-ribofuranosyl)adenine (40)

To a flask containing compound **35** (0.138 g, 0.182 mmol) was added 80% aqueous TFA in CHCl<sub>3</sub> (3 mL). After 1 h MeOH (5 mL) was added and the reaction mixture was then concentrated under reduced pressure. The residue was treated with sat. NaHCO<sub>3</sub> (7 mL) and extracted with EtOAc (3 × 7 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, 98:2 EtOAc/MeOH) afforded the product (0.039 g, 80%) as a white solid. *R<sub>f</sub>* = 0.40 (5:95 MeOH/EtOAc); **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]DMSO): δ 8.57 (s, 1H), 8.52 (br s, 2H; -NH<sub>2</sub>), 8.38 (s, 1H), 6.36 (ddd, <sup>4</sup>*J*(1',F3') = 1.6, <sup>3</sup>*J*(1',2') = 5.8, <sup>3</sup>*J*(1',F2') = 13.6 Hz, 1H; H-1'), 5.90 (dddd, <sup>3</sup>*J*(2',3') = 4.3, <sup>3</sup>*J*(1',2') = 5.8, <sup>3</sup>*J*(2',F3') = 16.6, <sup>2</sup>*J*(2',F2') = 50.3 Hz, 1H; H-2'), 5.53 (dm, <sup>2</sup>*J*(3',F3') = 52.9 Hz, 1H; H-3'), 4.42 (dm, <sup>3</sup>*J*(4',F3') = 24.0 Hz, 1H; H-4'), 3.70–3.67 (m, 2H; H-5'/5''); **<sup>13</sup>C NMR** ([D<sub>6</sub>]DMSO): δ 153.6, 149.4, 148.6, 140.8, 119.1, 90.6 (dd, <sup>2</sup>*J*(C2',F3') = 14.3, <sup>1</sup>*J*(C2',F2') = 193.8 Hz), 89.8 (dd, <sup>2</sup>*J*(C3',F2') = 13.3, <sup>1</sup>*J*(C2',F2') = 184.8 Hz), 84.7 (dd, <sup>3</sup>*J*(C1',F3') = 2.0, <sup>2</sup>*J*(C1',F2') = 31.5 Hz), 83.4 (dd, <sup>3</sup>*J*(C4',F2') = 2.4, <sup>2</sup>*J*(C4',F3') = 21.4 Hz), 60.2 (d, <sup>3</sup>*J*(C5',F3') = 8.4 Hz); **<sup>19</sup>F NMR** (376 MHz, [D<sub>6</sub>]DMSO): δ -206.2 (dddd, <sup>3</sup>*J*(F2',F3') = 4.9, <sup>3</sup>*J*(2',F3') = 16.6, <sup>3</sup>*J*(4',F3') = 22.6, <sup>2</sup>*J*(3',F3') = 53.4 Hz; F-3'), -214.4 (dm, <sup>2</sup>*J*(2',F2') = 50.7 Hz; F-2'); **HRMS** (ESI+): *m/z* calcd for C<sub>10</sub>H<sub>12</sub>F<sub>2</sub>N<sub>5</sub>O<sub>2</sub> [(M + H)<sup>+</sup>] 272.0954, found 272.0963 ( 3.3 ppm).

### General Procedure 2: Selective Deprotection of 5'-O-Trityl

A 0.4 M HCl solution in 1,4-dioxane was prepared by diluting commercially available 4 M HCl in 1,4-dioxane. A solution of 5'-O,N<sup>6</sup>-ditritylated compound (1 mmol) in 0.4 M HCl in 1,4-dioxane (38 mL) was stirred at rt for 1 h. MeOH (25 mL) was added and the reaction mixture was concentrated under reduced pressure. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with sat. NaHCO<sub>3</sub>, water, and brine. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, EtOAc/hexanes gradient) afforded the product.

**N<sup>6</sup>-Trityl-9-[2-deoxy-2-fluoro-3-O-(4-methoxybenzyl)-β-D-ribofuranosyl]adenine (41a)**

This was prepared from ditritylated compound **30** (0.10 g, 0.114 mmol) using general procedure 2. Purification by flash chromatography (SiO<sub>2</sub>, 70:30 EtOAc/hexanes) afforded the product (0.06 g, 86%) as a white solid. *R<sub>f</sub>* = 0.22 (1:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 7.98, 7.81 (2s, 2H; H-2, H-8), 7.35–7.21 (m, 17H; 3×C<sub>6</sub>H<sub>6</sub>, –PMB), 7.10 (s, 1H; N<sup>6</sup>-H), 6.90 (d, *J* = 8.6 Hz, 2H; –PMB), 6.47 (br s, 1H; –OH), 6.10 (dd, <sup>3</sup>*J*(1',2') = 6.8, <sup>3</sup>*J*(1',F2') = 12.0 Hz, 1H; H-1'), 5.79 (ddd, <sup>3</sup>*J*(2',3') = 4.8, <sup>3</sup>*J*(1',2') = 6.9, <sup>2</sup>*J*(2',F2') = 51.6 Hz, 1H; H-2'), 4.69 (ABq, δ = 72.8 Hz, *J* = 11.2 Hz, 2H; –PMB), 4.40 (dt, <sup>3</sup>*J*(3',F2') = 1.4, <sup>3</sup>*J*(3',4') = 1.4, <sup>3</sup>*J*(2',3') = 4.6 Hz, 1H; H-3'), 4.34–4.33 (m, 1H; H-4'), 3.86 (dm, <sup>2</sup>*J*(5',5'') = 13.1 Hz, 1H; H-5'), 3.80 (s, 3H; –PMB), 3.70 (dd, <sup>3</sup>*J*(4',5'') = 1.8, <sup>2</sup>*J*(5',5'') = 13.2 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): δ 159.7, 154.8, 152.0, 147.5, 144.8, 140.1, 129.8, 129.6, 129.1, 128.1, 127.2, 122.6, 114.2, 91.8 (d, <sup>1</sup>*J*(C2',F2') = 197.3 Hz), 88.8 (d, <sup>2</sup>*J*(C1',F2') = 31.0 Hz), 86.8 (d, <sup>3</sup>*J*(C4',F2') = 3.2 Hz), 77.1 (d, <sup>2</sup>*J*(C3',F2') = 13.0 Hz), 73.2 (d, <sup>4</sup>*J*(PMB-CH<sub>2</sub>-,F2') = 3.2 Hz), 71.7, 63.0, 55.5; **<sup>19</sup>F NMR** (376 MHz, CDCl<sub>3</sub>): δ –213.9 (dd, <sup>3</sup>*J*(1',F2') = 11.9, <sup>2</sup>*J*(2',F2') = 51.6 Hz); **HRMS** (ESI+): *m/z* calcd for C<sub>37</sub>H<sub>34</sub>FN<sub>5</sub>O<sub>4</sub>Na [(M + Na)<sup>+</sup>] 654.2487, found 654.2538 (–7.8 ppm).

**N<sup>6</sup>-Trityl-9-[2-deoxy-2-fluoro-3-O-(4-methoxybenzyl)-β-D-arabinofuranosyl]adenine (41b)**

This was prepared from ditritylated compound **24** (0.45 g, 0.515 mmol) using general procedure 2. Purification by flash chromatography (SiO<sub>2</sub>, 70:30 EtOAc/hexanes) afforded the product (0.25 g, 78%) as a white solid. *R<sub>f</sub>* = 0.29 (1:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.02 (s, 1H; H-2), 7.92 (d, <sup>5</sup>*J*(8,F2') = 1.9 Hz, 1H; H-8), 7.36–7.22 (m, 18H; 3×C<sub>6</sub>H<sub>6</sub>, –PMB, N<sup>6</sup>-H), 6.90 (d, *J* = 8.6 Hz, 2H; –PMB), 6.34 (dd, <sup>3</sup>*J*(1',2') = 4.1, <sup>3</sup>*J*(1',F2') = 17.2 Hz, 1H; H-1'), 5.18 (ddd, <sup>3</sup>*J*(2',3') = 2.3, <sup>3</sup>*J*(1',2') = 4.1, <sup>2</sup>*J*(2',F2') = 52.1 Hz, 1H; H-2'), 5.01 (br s, 1H; –OH), 4.61 (ABq, δ = 24.4 Hz, *J* = 11.3 Hz, 2H; –PMB), 4.51 (ddd, <sup>3</sup>*J*(2',3') = 2.4, <sup>3</sup>*J*(3',4') = 4.9, <sup>3</sup>*J*(3',F2') = 18.7 Hz, 1H; H-3'), 4.09 (q, <sup>3</sup>*J*(4',5') = 3.7, <sup>3</sup>*J*(4',5'') = 3.8, <sup>3</sup>*J*(4',3') = 3.8 Hz, 1H; H-4'), 3.89 (dd, <sup>3</sup>*J*(4',5') = 3.1, <sup>2</sup>*J*(5',5'') = 12.4 Hz, 1H; H-5'), 3.01 (s, 3H; –PMB), 3.70 (dd, <sup>3</sup>*J*(4',5'') = 4.0, <sup>2</sup>*J*(5',5'') = 12.4 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): δ 159.8, 154.3, 152.5, 148.7, 145.0, 139.2 (d, *J* = 4.3 Hz), 129.8, 129.2, 128.9, 128.1, 127.0, 120.5, 114.2, 94.1 (d, <sup>1</sup>*J*(C2',F2') = 195.0 Hz), 84.1 (d, <sup>2</sup>*J*(C1',F2') = 17.4 Hz), 83.2 (d, <sup>3</sup>*J*(C4',F2') = 3.4 Hz), 80.8 (d, <sup>2</sup>*J*(C3',F2') = 25.0 Hz), 72.6, 71.7, 61.5, 55.5; **<sup>19</sup>F NMR** (376 MHz, CDCl<sub>3</sub>): δ –195.8 (dt, <sup>3</sup>*J*(1',F2') = 17.9, <sup>3</sup>*J*(3',F2') = 17.9, <sup>2</sup>*J*(2',F2') = 51.9 Hz).

**N<sup>6</sup>-Trityl-9-[3-deoxy-3-fluoro-2-O-(4-methoxybenzyl)-β-D-xylofuranosyl]adenine (41c)**

This was prepared from ditritylated compound **23** (0.88 g, 1.007 mmol) using general procedure 2. Purification by flash chromatography (SiO<sub>2</sub>, 80:20 EtOAc/hexanes) afforded the product (0.47 g, 75%) as a white solid. *R<sub>f</sub>* = 0.20 (1:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.02, 7.80 (2s, 2H; H-2, H-8), 7.36–7.21 (m, 15H; 3×C<sub>6</sub>H<sub>5</sub>), 7.12 (d, *J* = 8.6 Hz, 2H; –PMB), 7.07 (s, 1H; N<sup>6</sup>-H), 6.77 (d, *J* = 8.6 Hz, 2H; –PMB), 5.97 (d, <sup>3</sup>*J*(1',2') = 4.4 Hz, 1H; H-1'), 5.21 (ddd, <sup>3</sup>*J*(2',3') = 4.2, <sup>3</sup>*J*(3',4') = 5.6, <sup>2</sup>*J*(3',F3') = 53.2 Hz, 1H; H-3'), 4.90 (br s, 1H; –OH), 4.80 (dt, <sup>3</sup>*J*(2',3') = 4.3, <sup>3</sup>*J*(1',2') = 4.3, <sup>3</sup>*J*(2',F3') = 15.7 Hz, 1H; H-2'), 4.61 (ABq, δ = 21.6 Hz, *J* = 11.5 Hz, 2H; –PMB), 4.39 (dq, <sup>3</sup>*J*(3',4') = 4.2, <sup>3</sup>*J*(5',4') = 4.3, <sup>3</sup>*J*(5'',4') = 4.3, <sup>3</sup>*J*(4',F3') = 17.9 Hz, 1H; H-4'), 3.96–3.87 (m, 2H; H-5'/5''), 3.74 (s,

3H; -PMB);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.8, 154.4, 152.3, 148.0, 145.0, 138.9 (d,  $^5J(\text{C8},\text{F3}') = 3.3$  Hz), 129.8, 129.2, 128.8, 128.1, 127.1, 121.6, 114.1, 94.5 (d,  $^1J(\text{C3}',\text{F3}') = 190.1$  Hz), 87.8 (d,  $^3J(\text{C1}',\text{F3}') = 7.1$  Hz), 83.2 (d,  $^2J(\text{C2}',\text{F3}') = 24.0$  Hz), 81.1 (d,  $^2J(\text{C4}',\text{F3}') = 19.9$  Hz), 72.7, 71.7, 60.4 (d,  $^3J(\text{C5}',\text{F3}') = 8.2$  Hz), 55.4;  $^{19}\text{F NMR}$  (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -204.6 (dt,  $^3J(2',\text{F3}') = 16.7$ ,  $^3J(4',\text{F3}') = 16.7$ ,  $^2J(3',\text{F3}') = 53.1$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{37}\text{H}_{35}\text{FN}_5\text{O}_4$  [(M + H) $^+$ ] 632.2668, found 632.2658 ( -1.6 ppm).

#### ***N*<sup>6</sup>-Trityl-9-[3-deoxy-3-fluoro-2-O-(4-methoxybenzyl)- $\beta$ -D-ribofuranosyl]adenine (41d)**

This was prepared from ditritylated compound **27** (0.73 g, 0.835 mmol) using general procedure 2. Purification by flash chromatography ( $\text{SiO}_2$ , 80:20 EtOAc/hexanes) afforded the product (0.39 g, 75%) as a white solid.  $R_f = 0.36$  (1:1 EtOAc/hexanes);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.92, 7.74 (2s, 2H; H-2, H-8), 7.36–7.23 (m, 15H;  $3\times\text{C}_6\text{H}_5$ ), 7.28 (d,  $J = 8.6$  Hz, 2H; -PMB), 7.05 (s, 1H;  $N^6$ -H), 6.86 (dd,  $^5J(\text{OH},\text{F3}') = 2.3$  Hz,  $^3J(\text{OH},5'') = 12.2$  Hz, 1H; 5'-OH), 6.72 (d,  $J = 8.6$  Hz, 2H; -PMB), 5.90 (d,  $^3J(1',2') = 8.1$  Hz, 1H; H-1'), 5.19 (dd,  $^3J(2',3') = 4.0$ ,  $^2J(3',\text{F3}') = 54.7$  Hz, 1H; H-3'), 4.89 (ddd,  $^3J(2',3') = 3.9$ ,  $^3J(1',2') = 8.1$ ,  $^3J(2',\text{F3}') = 24.6$  Hz, 1H; H-2'), 4.49 (d,  $^3J(4',\text{F3}') = 28.4$  Hz, 1H; H-4'), 4.47 (ABq,  $\delta = 53.6$  Hz,  $J = 11.3$  Hz, 2H; -PMB), 3.88 (dm,  $^2J(5',5'') = 12.7$  Hz, 1H; H-5'), 3.74 (s, 3H; -PMB), 3.69 (t,  $^3J(\text{OH},5'') \approx ^2J(5',5'') \approx 12.7$  Hz, 1H; H-5'');  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.8, 154.8, 151.7, 147.3, 144.9, 140.6, 129.8, 129.2, 128.8, 128.1, 127.3, 122.8, 114.0, 91.5 (d,  $^1J(\text{C3}',\text{F3}') = 184.0$  Hz), 89.7, 86.7 (d,  $^2J(\text{C2}',\text{F3}') = 21.5$  Hz), 79.0 (d,  $^2J(\text{C4}',\text{F3}') = 15.9$  Hz), 72.8, 71.8, 62.8 (d,  $^3J(\text{C5}',\text{F3}') = 11.9$  Hz), 55.5;  $^{19}\text{F NMR}$  (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -198.9 (dddd,  $^5J(\text{OH},\text{F3}') = 2.9$ ,  $^3J(2',\text{F3}') = 24.1$ ,  $^3J(4',\text{F3}') = 27.8$ ,  $^2J(3',\text{F3}') = 54.9$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{37}\text{H}_{35}\text{FN}_5\text{O}_4$  [(M + H) $^+$ ] 632.2668, found 632.2615 ( -8.4 ppm).

#### ***N*<sup>6</sup>-Trityl-9-(2,3-dideoxy-2,3-difluoro- $\beta$ -D-xylofuranosyl)adenine (41e)**

This was prepared from ditritylated compound **31** (1.60 g, 2.12 mmol) using general procedure 2. Purification by flash chromatography ( $\text{SiO}_2$ , 80:20 EtOAc/hexanes) afforded the product (0.78 g, 73%) as a white solid.  $R_f = 0.22$  (1:1 EtOAc/hexanes);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05, 7.84 (2s, 2H; H-2, H-8), 7.35–7.22 (m, 15H;  $3\times\text{C}_6\text{H}_5$ ), 7.06 (s, 1H;  $N^6$ -H), 6.12 (dd,  $^3J(1',2') = 4.5$ ,  $^3J(1',\text{F2}') = 15.6$  Hz, 1H; H-1'), 5.85 (ddt,  $^3J(1',2') \approx ^3J(2',3') \approx 4.5$ ,  $^3J(2',\text{F3}') = 13.4$ ,  $^2J(2',\text{F2}') = 52.2$  Hz, 1H; H-2'), 5.44 (dddd,  $^3J(2',3') = 4.4$ ,  $^3J(3',4') = 5.9$ ,  $^3J(3',\text{F2}') = 14.3$ ,  $^2J(3',\text{F3}') = 52.4$  Hz, 1H; H-3'), 4.92–4.89 (m, 1H; 5'-OH), 4.49 (dm,  $^3J(4',\text{F3}') = 15.2$  Hz, 1H; H-4'), 4.01–3.91 (m, 2H; H-5'/5'');  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  154.6, 152.6, 148.1, 144.9, 138.9 (d,  $J = 3.0$  Hz), 129.2, 128.2, 127.2, 121.8, 94.6 (dd,  $^2J(\text{C2}',\text{F3}') = 26.4$ ,  $^1J(\text{C2}',\text{F2}') = 190.3$  Hz), 92.1 (dd,  $^2J(\text{C3}',\text{F2}') = 25.2$ ,  $^1J(\text{C2}',\text{F2}') = 192.4$  Hz), 86.7 (dd,  $^3J(\text{C1}',\text{F3}') = 7.2$ ,  $^2J(\text{C1}',\text{F2}') = 33.6$  Hz), 80.7 (dd,  $^3J(\text{C4}',\text{F2}') = 4.2$ ,  $^2J(\text{C4}',\text{F3}') = 20.3$  Hz), 71.7, 62.3 (d,  $^3J(\text{C5}',\text{F3}') = 7.5$  Hz);  $^{19}\text{F NMR}$  (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -197.8 (dtd,  $^3J(\text{F2}',\text{F3}') = 10.5$ ,  $^3J(3',\text{F2}') = 14.8$ ,  $^3J(1',\text{F2}') = 14.9$ ,  $^2J(2',\text{F2}') = 52.3$  Hz; F-2'), -209.9 (dtd,  $^3J(\text{F2}',\text{F3}') = 10.1$ ,  $^3J(2',\text{F3}') = 13.6$ ,  $^3J(4',\text{F3}') = 14.2$ ,  $^2J(3',\text{F3}') = 52.3$  Hz; F-3'); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{29}\text{H}_{26}\text{F}_2\text{N}_5\text{O}_2$  [(M + H) $^+$ ] 514.2049, found 514.2025 ( -4.6 ppm).

**N<sup>6</sup>-Trityl-9-(2,3-dideoxy-2,3-difluoro-β-D-ribofuranosyl)adenine (41f)**

This was prepared from ditritylated compound **35** (0.81 g, 1.07 mmol) using general procedure 2. Purification by flash chromatography (SiO<sub>2</sub>, 50:50 EtOAc/hexanes) afforded the product (0.51 g, 93%) as a waxy white solid. *R<sub>f</sub>* = 0.40 (3:1 EtOAc/hexanes); <sup>1</sup>H NMR (400 MHz, [D<sub>6</sub>]DMSO): δ 8.52, 7.94 (2s, 2H; H-2, H-8), 7.65 (s, 1H; N<sup>6</sup>-H), 7.35–7.19 (m, 15H; 3×C<sub>6</sub>H<sub>5</sub>), 6.34 (dd, <sup>3</sup>J(1',2') = 5.7 Hz, <sup>3</sup>J(1',F2') = 13.8, 1H; H-1'), 5.95 (ddt, <sup>3</sup>J(1',2') ≈ <sup>3</sup>J(2',3') ≈ 5.8, <sup>3</sup>J(2',F3') = 16.4, <sup>2</sup>J(2',F2') = 50.2 Hz, 1H; H-2'), 5.53 (dm, <sup>2</sup>J(3',F3') = 53.1 Hz, 1H; H-3'), 4.76 (br s, 1H; 5'-OH), 4.38 (dm, <sup>3</sup>J(4',F3') = 23.8 Hz, 1H; H-4'), 3.68 (dd, <sup>3</sup>J(5',4') = 3.5, <sup>2</sup>J(5',5'') = 12.3 Hz, 1H; H-5'), 3.64 (dd, <sup>3</sup>J(5'',4') = 3.5, <sup>2</sup>J(5',5'') = 12.3 Hz, 1H; H-5''); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 154.8, 151.9, 147.4, 144.7, 140.2, 129.1, 128.2, 127.3, 122.6, 91.3 (dd, <sup>2</sup>J(C2',F3') = 13.0, <sup>1</sup>J(C2',F2') = 185.5 Hz), 89.6 (dd, <sup>2</sup>J(C3',F2') = 15.0, <sup>1</sup>J(C2',F2') = 201.7 Hz), 88.1 (d, <sup>2</sup>J(C1',F2') = 30.4 Hz), 86.2 (dd, <sup>3</sup>J(C4',F2') = 3.1, <sup>2</sup>J(C4',F3') = 21.1 Hz), 71.8, 62.3 (d, <sup>3</sup>J(C5',F3') = 11.4 Hz); <sup>19</sup>F NMR (376 MHz, [D<sub>6</sub>]DMSO): δ -204.6 (dddd, <sup>3</sup>J(F2',F3') = 5.0, <sup>3</sup>J(2',F3') = 16.2, <sup>3</sup>J(4',F3') = 22.5, <sup>2</sup>J(3',F3') = 53.4 Hz; F-3'), -212.7 (dm, <sup>2</sup>J(2',F2') = 50.0 Hz; F-2'); HRMS (ESI+): *m/z* calcd for C<sub>29</sub>H<sub>26</sub>F<sub>2</sub>N<sub>5</sub>O<sub>2</sub> [(M + H)<sup>+</sup>] 514.2049, found 514.2075 ( 5.0 ppm).

**General Procedure 3: Azidation**

To a solution of alcohol (1 mmol, 1 equiv) in anhydrous 1,4-dioxane (9 mL) was added DPPA (3 equiv), and DBU (2 equiv) dropwise and the reaction mixture was stirred at rt. The conversion of alcohol to the phosphate intermediate was monitored by TLC, which was usually completed in 1–3 h. NaN<sub>3</sub> (10 equiv) and 15-crown-5 (0.1 equiv) were then added and the reaction mixture was refluxed (oil bath at 110 °C) for 1–24 h. The reaction mixture was allowed to cool to rt, filtered through a short pad of silica, and the silica was washed with EtOAc. The combined filtrate was evaporated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, EtOAc/hexanes gradient) afforded the product.

**N<sup>6</sup>-Trityl-9-[5-azido-2,5-dideoxy-2-fluoro-3-O-(4-methoxybenzyl)-β-D-ribofuranosyl]adenine (42a)**

This was prepared from alcohol **41a** (0.062 g, 0.098 mmol) using general procedure 3. The reaction mixture was refluxed for 17 h. Purification by flash chromatography (SiO<sub>2</sub>, 50:50 EtOAc/hexanes) afforded the product (0.053 g, 83%) as a white solid. *R<sub>f</sub>* = 0.45 (1:1 EtOAc/hexanes); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.00, 7.90 (2s, 2H; H-2, H-8), 7.35–7.20 (m, 17H; 3×C<sub>6</sub>H<sub>6</sub>, -PMB), 7.00 (s, 1H; N<sup>6</sup>-H), 6.87 (d, *J* = 8.6 Hz, 2H; -PMB), 6.12 (dd, <sup>3</sup>J(1',2') = 1.5, <sup>3</sup>J(1',F2') = 19.7 Hz, 1H; H-1'), 5.53 (ddd, <sup>3</sup>J(1',2') = 1.5, <sup>3</sup>J(2',3') = 3.5, <sup>2</sup>J(2',F2') = 52.8 Hz, 1H; H-2'), 4.61 (ddd, <sup>3</sup>J(2',3') = 4.2, <sup>3</sup>J(3',4') = 8.7, <sup>3</sup>J(3',F2') = 20.4 Hz, 1H; H-3'), 4.60 (ABq, δ = 47.6 Hz, *J* = 11.3 Hz, 2H; -PMB), 4.26 (ddd, <sup>3</sup>J(4',5') = 2.9, <sup>3</sup>J(4',5'') = 5.1, <sup>3</sup>J(4',3') = 8.1 Hz, 1H; H-4'), 3.78 (s, 3H; -PMB), 3.63 (dd, <sup>3</sup>J(4',5') = 2.9, <sup>2</sup>J(5',5'') = 13.5 Hz, 1H; H-5'), 3.43 (dd, <sup>3</sup>J(4',5'') = 5.1, <sup>2</sup>J(5',5'') = 13.5 Hz, 1H; H-5''); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 160.0, 154.4, 152.6, 148.2, 145.0, 139.1, 130.2, 129.2, 129.0, 128.1, 127.2, 121.5, 114.2, 91.4 (d, <sup>1</sup>J(C2',F2') = 190.2 Hz), 88.4 (d, <sup>2</sup>J(C1',F2') = 34.5 Hz), 80.2, 75.7 (d, <sup>2</sup>J(C3',F2') = 16.0 Hz), 72.9, 71.7, 55.5, 51.4; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>): δ -202.0 (dt, <sup>3</sup>J(1',F2') = 20.1, <sup>3</sup>J(3',F2') = 20.1, <sup>2</sup>J(2',F2') = 52.9 Hz).

***N*<sup>6</sup>-Trityl-9-[5-azido-2,5-dideoxy-2-fluoro-3-*O*-(4-methoxybenzyl)- $\beta$ -*D*-arabinofuranosyl]adenine (42b)**

This was prepared from alcohol **41b** (0.23 g, 0.364 mmol) using general procedure 3. The reaction mixture was refluxed for 24 h. Purification by flash chromatography (SiO<sub>2</sub>, 50:50 EtOAc/hexanes) afforded the product (0.14 g, 58%) as a white solid. *R<sub>f</sub>* = 0.63 (1:1 EtOAc/hexanes); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.06 (s, 1H; H-2), 8.04 (d, <sup>5</sup>*J*(8,F2') = 2.9 Hz, 1H; H-8), 7.37–7.21 (m, 17H; 3×C<sub>6</sub>H<sub>6</sub>, –PMB), 6.99 (s, 1H; N<sup>6</sup>-H), 6.92 (d, *J* = 8.5 Hz, 2H; –PMB), 6.42 (dd, <sup>3</sup>*J*(1',2') = 3.1, <sup>3</sup>*J*(1',F2') = 21.2 Hz, 1H; H-1'), 5.11 (ddd, <sup>3</sup>*J*(2',3') = 1.5, <sup>3</sup>*J*(1',2') = 3.3, <sup>2</sup>*J*(2',F2') = 52.3 Hz, 1H; H-2'), 4.60 (ABq,  $\delta$  = 35.2 Hz, *J* = 11.5 Hz, 2H; –PMB), 4.27 (ddd, <sup>3</sup>*J*(2',3') = 1.2, <sup>3</sup>*J*(3',4') = 3.9, <sup>3</sup>*J*(3',F2') = 17.9 Hz, 1H; H-3'), 4.16 (q, <sup>3</sup>*J*(4',5') = 4.7, <sup>3</sup>*J*(4',5'') = 4.7, <sup>3</sup>*J*(4',3') = 4.6 Hz, 1H; H-4'), 3.82 (s, 3H; –PMB), 3.58 (dd, <sup>3</sup>*J*(4',5') = 4.5, <sup>2</sup>*J*(5',5'') = 13.2 Hz, 1H; H-5'), 3.43 (dd, <sup>3</sup>*J*(4',5'') = 5.3, <sup>2</sup>*J*(5',5'') = 13.2 Hz, 1H; H-5''); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  160.0, 154.3, 152.6, 148.8, 145.1, 139.4 (d, <sup>4</sup>*J*(C8,F2') = 6.6 Hz), 129.9, 129.2, 128.5, 128.1, 127.1, 120.4, 114.3, 93.2 (d, <sup>1</sup>*J*(C2',F2') = 193.0 Hz), 83.1 (d, <sup>2</sup>*J*(C1',F2') = 17.1 Hz), 81.8 (d, <sup>2</sup>*J*(C3',F2') = 25.6 Hz), 81.2 (d, <sup>3</sup>*J*(C4',F2') = 2.1 Hz), 72.7, 71.6, 55.5, 51.6; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>):  $\delta$  –196.3 (dddd, <sup>5</sup>*J*(8,F2') = 3.0, <sup>3</sup>*J*(3',F2') = 17.7, <sup>3</sup>*J*(1',F2') = 20.8, <sup>2</sup>*J*(2',F2') = 51.3 Hz).

***N*<sup>6</sup>-Trityl-9-[5-azido-3,5-dideoxy-3-fluoro-2-*O*-(4-methoxybenzyl)- $\beta$ -*D*-xylofuranosyl]adenine (42c)**

This was prepared from alcohol **41c** (0.70 g, 1.108 mmol) using general procedure 3. Conversion of alcohol to the phosphate intermediate was completed in 1 h. For the conversion of phosphate to azide, the reaction mixture was refluxed for 24 h. Purification by flash chromatography (SiO<sub>2</sub>, 50:50 EtOAc/hexanes) afforded the product (0.41 g, 57%) as a white solid. *R<sub>f</sub>* = 0.61 (1:1 EtOAc/hexanes); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.07, 7.86 (2s, 2H; H-2, H-8), 7.37–7.21 (m, 17H; 3×C<sub>6</sub>H<sub>5</sub> and –PMB), 6.94 (s, 1H; N<sup>6</sup>-H), 6.83 (d, *J* = 8.6 Hz, 2H; –PMB), 6.27 (d, <sup>3</sup>*J*(1',2') = 1.7 Hz, 1H; H-1'), 5.06 (ddd, <sup>3</sup>*J*(2',3') = 1.1, <sup>3</sup>*J*(3',4') = 2.7, <sup>3</sup>*J*(3',F3') = 51.0 Hz, 1H; H-3'), 4.71 (ABq,  $\delta$  = 48.8 Hz, *J* = 11.6 Hz, 2H; –PMB), 4.50 (dt, <sup>3</sup>*J*(2',3') = 1.4, <sup>3</sup>*J*(1',2') = 1.4, <sup>3</sup>*J*(2',F3') = 15.2 Hz, 1H; H-2'), 4.44 (dtd, <sup>3</sup>*J*(3',4') = 2.7, <sup>3</sup>*J*(5',4') = 6.6, <sup>3</sup>*J*(5'',4') = 6.6, <sup>3</sup>*J*(4',F3') = 28.4 Hz, 1H; H-4'), 3.78 (s, 3H; –PMB), 3.76–3.73 (m, 1H; H-5'), 3.67 (dd, <sup>3</sup>*J*(5',4') = 6.4, <sup>2</sup>*J*(5',5'') = 12.8 Hz, 1H; H-5''); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  159.9, 154.2, 152.7, 148.6, 145.1, 137.7 (d, <sup>5</sup>*J*(C8,F3') = 6.0 Hz), 130.0, 129.2, 128.4, 128.1, 127.1, 121.1, 114.2, 94.0 (d, <sup>1</sup>*J*(C3',F3') = 186.0 Hz), 88.2, 85.0 (d, <sup>2</sup>*J*(C2',F3') = 27.2 Hz), 80.7 (d, <sup>2</sup>*J*(C4',F3') = 19.5 Hz), 72.6, 71.6, 55.5, 48.8 (d, <sup>3</sup>*J*(C5',F3') = 9.3 Hz); <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>):  $\delta$  –202.6 (ddd, <sup>3</sup>*J*(2',F3') = 14.7, <sup>3</sup>*J*(4',F3') = 28.6, <sup>2</sup>*J*(3',F3') = 51.2 Hz); HRMS (ESI+): *m/z* calcd for C<sub>37</sub>H<sub>34</sub>FN<sub>8</sub>O<sub>3</sub> [(M + H)<sup>+</sup>] 657.2732, found 657.2692 (–6.1 ppm).

***N*<sup>6</sup>-Trityl-9-[5-azido-3,5-dideoxy-3-fluoro-2-*O*-(4-methoxybenzyl)- $\beta$ -*D*-ribofuranosyl]adenine (42d)**

This was prepared from alcohol **41d** (0.38 g, 0.602 mmol) using general procedure 3. Conversion of alcohol to phosphate intermediate was completed in 1 h and the conversion of phosphate to azide was completed in 3 h. Purification by flash chromatography (SiO<sub>2</sub>, 50:50 EtOAc/hexanes) afforded the product (0.32 g, 81%) as a white solid. *R<sub>f</sub>* = 0.55 (1:1 EtOAc/



hexanes); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.00, 7.76 (2s, 2H; H-2, H-8), 7.37–7.22 (m, 15H; 3×C<sub>6</sub>H<sub>5</sub>), 7.11 (d, *J* = 8.6 Hz, 2H; –PMB), 6.95 (s, 1H; N<sup>6</sup>-H), 6.78 (d, *J* = 8.6 Hz, 2H; –PMB), 6.02 (d, <sup>3</sup>*J*(1',2') = 6.6 Hz, 1H; H-1'), 5.09 (ddd, <sup>3</sup>*J*(2',3') = 4.5, <sup>3</sup>*J*(1',2') = 6.7, <sup>3</sup>*J*(2',F3') = 18.4 Hz, 1H; H-2'), 5.08 (ddd, <sup>3</sup>*J*(3',4') = 2.2, <sup>3</sup>*J*(3',2') = 4.5, <sup>2</sup>*J*(3',F3') = 54.4 Hz, 1H; H-3'), 4.55 (ABq, δ = 41.2 Hz, *J* = 11.5 Hz, 2H; –PMB), 4.45 (dtd, <sup>3</sup>*J*(3',4') = 2.2, <sup>3</sup>*J*(5',4') = 5.2, <sup>3</sup>*J*(5'',4') = 5.2, <sup>3</sup>*J*(4',F3') = 23.6 Hz, 1H; H-4'), 3.77 (s, 3H; –PMB), 3.68 (dd, <sup>3</sup>*J*(5',4') = 5.4, <sup>2</sup>*J*(5',5'') = 13.1 Hz; H-5'), 3.67 (dd, <sup>3</sup>*J*(5',4') = 4.9, <sup>2</sup>*J*(5',5'') = 13.1 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): δ 159.9, 154.4, 152.5, 148.8, 145.1, 139.6, 129.8, 129.2, 128.7, 128.1, 127.2, 121.9, 114.1, 90.0 (d, <sup>1</sup>*J*(C3',F3') = 189.6 Hz), 87.4, 81.6 (d, <sup>2</sup>*J*(C2',F3') = 24.3 Hz), 77.2 (d, <sup>2</sup>*J*(C4',F3') = 15.5 Hz), 72.9, 71.7, 55.5, 51.6 ppm (d, <sup>3</sup>*J*(C5',F3') = 8.6 Hz); **<sup>19</sup>F NMR** (376 MHz, CDCl<sub>3</sub>): δ –201.8 (ddd, <sup>3</sup>*J*(2',F3') = 18.1, <sup>3</sup>*J*(4',F3') = 23.4, <sup>2</sup>*J*(3',F3') = 54.1 Hz); **HRMS** (ESI+): *m/z* calcd for C<sub>37</sub>H<sub>34</sub>FN<sub>8</sub>O<sub>3</sub> [(M + H)<sup>+</sup>] 657.2732, found 657.2731 (–0.2 ppm).

### N<sup>6</sup>-Trityl-9-(5-azido-2,3-difluoro-2,3,5-trideoxy-β-D-xylofuranosyl)adenine (42e)

This was prepared from alcohol **41e** (0.76 g, 1.48 mmol) using general procedure 3. Conversion of alcohol to phosphate intermediate was completed in 2 h. For the conversion of phosphonate to the azide product, the reaction mixture was refluxed for 24 h. Purification by flash chromatography (SiO<sub>2</sub>, 30:70 EtOAc/hexanes) afforded the product (0.58 g, 73%) as a white solid. *R<sub>f</sub>* = 0.57 (1:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.07, 7.87 (2s, 2H; H-2, H-8), 7.36–7.21 (m, 15H; 3×C<sub>6</sub>H<sub>5</sub>), 6.96 (s, 1H; N<sup>6</sup>-H), 6.38 (d, <sup>3</sup>*J*(1',F2') = 20.4 Hz, 1H; H-1'), 5.57 (dd, <sup>3</sup>*J*(2',F3') = 10.5, <sup>2</sup>*J*(2',F2') = 47.9 Hz, 1H; H-2'), 5.28 (dddd, <sup>4</sup>*J*(3',5') = 1.1, <sup>3</sup>*J*(3',4') = 3.1, <sup>3</sup>*J*(3',F2') = 8.9, <sup>2</sup>*J*(3',F3') = 49.7 Hz, 1H; H-3'), 4.48 (dm, <sup>3</sup>*J*(4',F3') = 28.3 Hz, 1H; H-4'), 3.80 (ddd, <sup>4</sup>*J*(3',5') = 1.1, <sup>3</sup>*J*(5',4') = 6.6, <sup>2</sup>*J*(5',5'') = 13.0 Hz, 1H; H-5'), 3.71 (dd, <sup>3</sup>*J*(5'',4') = 6.5, <sup>2</sup>*J*(5',5'') = 12.8 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): δ 154.3, 153.0, 148.7, 145.0, 137.4 (d, *J* = 6.4 Hz), 129.2, 128.1, 127.2, 121.0, 96.1 (dd, <sup>2</sup>*J*(C2',F3') = 31.5, <sup>1</sup>*J*(C2',F2') = 186.9 Hz), 92.3 (dd, <sup>2</sup>*J*(C3',F2') = 30.4, <sup>1</sup>*J*(C3',F3') = 185.9 Hz), 87.5 (d, <sup>2</sup>*J*(C1',F2') = 36.7 Hz), 80.5 (d, <sup>2</sup>*J*(C4',F3') = 19.7 Hz), 71.7, 48.5 (d, <sup>3</sup>*J*(C5',F3') = 9.1 Hz); **<sup>19</sup>F NMR** (376 MHz, CDCl<sub>3</sub>): δ –192.1 (dddd, <sup>4</sup>*J*(4',F2') = 3.3, <sup>3</sup>*J*(3',F2') = 8.9, <sup>3</sup>*J*(F3',F2') = 14.8, <sup>3</sup>*J*(1',F2') = 20.8, <sup>2</sup>*J*(2',F2') = 48.1 Hz; F-2'), –208.7 (dddd, <sup>3</sup>*J*(2',F3') = 10.5, <sup>3</sup>*J*(F3',F2') = 14.2, <sup>3</sup>*J*(4',F3') = 28.1, <sup>2</sup>*J*(3',F3') = 49.9 Hz; F-3'); **HRMS** (ESI+): *m/z* calcd for C<sub>29</sub>H<sub>24</sub>F<sub>2</sub>N<sub>8</sub>ONa [(M + Na)<sup>+</sup>] 561.1933, found 561.1943 (–1.8 ppm).

### N<sup>6</sup>-Trityl-9-(5-azido-2,3-difluoro-2,3,5-trideoxy-β-D-ribofuranosyl)adenine (42f)

This was prepared from alcohol **41f** (0.41 g, 0.798 mmol) using general procedure 3. Conversion of alcohol to phosphate intermediate was completed in 2 h and the conversion of phosphate to azide was completed in 1 h. Purification by flash chromatography (SiO<sub>2</sub>, 30:70 EtOAc/hexanes) afforded the product (0.40 g, 93%) as a colorless liquid. *R<sub>f</sub>* = 0.54 (1:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]DMSO): δ 8.52, 7.96 (2s, 2H; H-2, H-8), 7.65 (s, 1H; N<sup>6</sup>-H), 7.34–7.19 (m, 15H; 3×C<sub>6</sub>H<sub>5</sub>), 6.38 (dd, <sup>3</sup>*J*(1',2') = 4.8, <sup>3</sup>*J*(1',F2') = 15.2 Hz, 1H; H-1'), 6.15 (ddt, <sup>3</sup>*J*(1',2') ≈ <sup>3</sup>*J*(2',3') ≈ 4.8, <sup>3</sup>*J*(2',F3') = 12.8, <sup>2</sup>*J*(2',F2') = 50.0 Hz, 1H; H-2'), 5.53 (dm, <sup>2</sup>*J*(3',F3') = 52.4 Hz, 1H; H-3'), 4.49 (dm, <sup>3</sup>*J*(4',F3') = 21.2 Hz, 1H; H-4'), 3.73 (dd, <sup>3</sup>*J*(5',4') = 5.6, <sup>2</sup>*J*(5',5'') = 13.2 Hz, 1H; H-5'), 3.52 (dd, <sup>3</sup>*J*(5'',4') = 4.4, <sup>2</sup>*J*(5',5'') =



13.2 Hz, 1H; H-5'');  $^{13}\text{C NMR}$  (100 MHz,  $[\text{D}_6]\text{DMSO}$ ):  $\delta$  153.7, 151.5, 148.2, 144.7, 140.8, 128.6, 127.7, 126.6, 120.8, 89.4 (dd,  $^2J(\text{C}2',\text{F}3') = 14.2$ ,  $^1J(\text{C}2',\text{F}2') = 192.2$  Hz), 88.8 (dd,  $^2J(\text{C}3',\text{F}2') = 13.7$ ,  $^1J(\text{C}3',\text{F}3') = 188.4$  Hz), 84.8 (dd,  $^3J(\text{C}1',\text{F}3') = 2.7$ ,  $^2J(\text{C}1',\text{F}2') = 31.6$  Hz), 79.9 (dd,  $^3J(\text{C}4',\text{F}2') = 2.5$ ,  $^2J(\text{C}4',\text{F}3') = 23.6$  Hz), 70.4, 50.3 (d,  $^3J(\text{C}5',\text{F}3') = 6.3$  Hz);  $^{19}\text{F NMR}$  (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -209.2 (dt,  $^3J(2',\text{F}3') = 13.8$ ,  $^3J(4',\text{F}3') = 13.8$ ,  $^2J(3',\text{F}3') = 51.3$  Hz; F-3'), -210.5 (ddd,  $^3J(3',\text{F}2') = 8.6$ ,  $^3J(1',\text{F}2') = 18.3$ ,  $^2J(2',\text{F}2') = 52.0$  Hz; F-2'); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{29}\text{H}_{25}\text{F}_2\text{N}_8\text{O}$   $[(\text{M} + \text{H})^+]$  539.2114, found 539.2159 ( 8.3 ppm).

***N*<sup>6</sup>-Trityl-9-[2,5-dideoxy-2-fluoro-3-*O*-(4-methoxybenzyl)-5-(*N*-sulfamoyl)amino- $\beta$ -D-ribofuranosyl]adenine (43a)**

To a solution of azide **42a** (0.053 g, 0.081 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added zinc turnings (0.081 g, 1.24 mmol, 15 equiv) and AcOH (43  $\mu\text{L}$ , 0.751 mmol, 9.3 equiv), and the reaction mixture was stirred at rt for 1 h. The reaction mixture was filtered through a short pad of Celite, and the residue was washed with MeOH (3  $\times$  5 mL). The combined filtrates were concentrated and dissolved in EtOAc (5 mL). The EtOAc layer was washed with sat.  $\text{NaHCO}_3$ , brine, dried ( $\text{MgSO}_4$ ), and evaporated to obtain the crude 5'-aminonucleoside. To a solution of the 5'-aminonucleoside in 1,4-dioxane (2 mL) was added sulfamide (0.024 g, 0.250 mmol, 3.0 equiv) and the mixture was refluxed for 2 h. The reaction mixture was evaporated, then partitioned between  $\text{CH}_2\text{Cl}_2$  (5 mL) and  $\text{H}_2\text{O}$  (5 mL). The phases were separated and the organic phase was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated under reduced pressure. Purification by flash chromatography ( $\text{SiO}_2$ , gradient: hexanes to 70% EtOAc in hexanes) afforded the product (0.051 g, 89%) as a white solid.  $R_f = 0.52$  (3:1 EtOAc/hexanes);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.57 (dd,  $^3J(5'',\text{NH}) = 3.4$  Hz,  $^3J(5',\text{NH}) = 8.2$ , 1H; C5'-NH), 8.06, 7.78 (2s, 2H; H-2, H-8), 7.34–7.20 (m, 17H; 3 $\times$ C<sub>6</sub>H<sub>6</sub>, -PMB), 7.01 (s, 1H; N<sup>6</sup>-H), 6.87 (d,  $J = 8.5$  Hz, 2H; -PMB), 6.05 (dd,  $^3J(1',2') = 5.5$ ,  $^3J(1',\text{F}2') = 13.7$  Hz, 1H; H-1'), 5.59 (dt,  $^3J(2',3') = 5.3$ ,  $^3J(1',2') = 5.3$ ,  $^2J(2',\text{F}2') = 51.5$  Hz, 1H; H-2'), 4.64 (ABq,  $\delta = 72.0$  Hz,  $J = 11.0$  Hz, 2H; -PMB), 4.56–4.37 (m, 4H; H-3', H-4', -NH<sub>2</sub>), 3.79 (s, 3H; -PMB), 3.38–3.35 (m, 2H; H-5'/5'');  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.9, 154.7, 152.5, 147.6, 144.8, 139.7, 130.1, 129.4, 129.2, 128.2, 127.2, 122.0, 114.2, 91.2 (d,  $^1J(\text{C}2',\text{F}2') = 196.4$  Hz), 88.4 (d,  $^2J(\text{C}1',\text{F}2') = 31.7$  Hz), 82.9 (d,  $^3J(\text{C}4',\text{F}2') = 2.5$  Hz), 76.5 (d,  $^2J(\text{C}3',\text{F}2') = 14.0$  Hz), 73.5 (d,  $^4J(\text{PMB-CH}_2\text{,F}2') = 2.8$  Hz), 71.7, 55.5, 44.7;  $^{19}\text{F NMR}$  (376 MHz,  $\text{CDCl}_3$ ):  $\delta = -210.0$  (dm,  $^2J(2',\text{F}2') = 51.8$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{37}\text{H}_{36}\text{FN}_7\text{O}_5\text{SNa}$   $[(\text{M} + \text{Na})^+]$  732.2375, found 732.2407 ( 4.4 ppm).

***N*<sup>6</sup>-Trityl-9-[2,5-dideoxy-2-fluoro-3-*O*-(4-methoxybenzyl)-5-(*N*-sulfamoyl)amino- $\beta$ -D-arabinofuranosyl]adenine (43b)**

To a solution of azide **42b** (0.17 g, 0.259 mmol) in  $\text{CH}_2\text{Cl}_2$  (7 mL) was added zinc turnings (0.26 g, 3.978 mmol, 15 equiv) and AcOH (0.14 mL, 2.445 mmol, 9.5 equiv), and the reaction mixture was stirred at rt for 1 h. The reaction mixture was filtered through a short pad of Celite and the residue was washed with MeOH (3  $\times$  5 ml). The combined filtrates were concentrated and dissolved in EtOAc (10 mL). The EtOAc layer was washed with sat.  $\text{NaHCO}_3$ , brine, dried ( $\text{MgSO}_4$ ), and evaporated to obtain the crude 5'-aminonucleoside. To a solution of the 5'-aminonucleoside in 1,4-dioxane (5 mL) was added sulfamide (0.072 g,

0.749 mmol, 2.9 equiv) and the mixture was refluxed for 2 h. The reaction mixture was evaporated then partitioned between CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and water (10 mL). The phases were separated, and the organic layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, gradient: hexanes to 70% EtOAc in hexanes) afforded the product (0.15 g, 83%) as a white solid. *R<sub>f</sub>* = 0.39 (3:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 8.00 (s, 1H; H-2), 7.99 (d, <sup>5</sup>*J*(8,F2') = 2.0 Hz, 1H; H-8), 7.34–7.19 (m, 17H; 3×C<sub>6</sub>H<sub>6</sub>, –PMB), 7.15–7.10 (m, 1H; C5'-NH), 6.95 (s, 1H; N<sup>6</sup>-H), 6.88 (d, *J* = 8.4 Hz, 2H; –PMB), 6.34 (dd, <sup>3</sup>*J*(1',2') = 4.7, <sup>3</sup>*J*(1',F2') = 14.1 Hz, 1H; H-1'), 5.18 (dt, <sup>3</sup>*J*(2',3') = 4.0, <sup>3</sup>*J*(1',2') = 4.0, <sup>2</sup>*J*(2',F2') = 52.2 Hz, 1H; H-2'), 4.80 (s, 2H; –NH<sub>2</sub>), 4.61 (ABq, δ = 30.8 Hz, *J* = 11.3 Hz, 2H; –PMB), 4.47 (ddd, <sup>3</sup>*J*(2',3') = 3.5, <sup>3</sup>*J*(3',4') = 5.6, <sup>3</sup>*J*(3',F2') = 18.1 Hz, 1H; H-3'), 4.08 (q, <sup>3</sup>*J*(4',5') = 4.3, <sup>3</sup>*J*(4',5'') = 4.3, <sup>3</sup>*J*(4',3') = 4.3 Hz, 1H; H-4'), 3.78 (s, 3H; –PMB), 3.40–3.38 (m, 2H; H-5'/5''); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>): δ 159.8, 154.1, 152.5, 148.6, 145.0, 139.8 (d, *J* = 3.9 Hz), 130.2, 129.2, 128.7, 128.1, 127.1, 120.1, 114.3, 94.3 (d, <sup>1</sup>*J*(C2',F2') = 196.4 Hz), 82.9 (d, <sup>2</sup>*J*(C1',F2') = 17.5 Hz), 80.4 (d, <sup>2</sup>*J*(C3',F2') = 23.7 Hz), 79.9 (d, <sup>3</sup>*J*(C4',F2') = 5.4 Hz), 72.7, 71.7, 55.5, 43.7; **<sup>19</sup>F NMR** (376 MHz, CDCl<sub>3</sub>): δ –195.0 (dt, <sup>3</sup>*J*(3',F2') = 16.0, <sup>3</sup>*J*(1',F2') = 16.0, <sup>2</sup>*J*(2',F2') = 52.3 Hz); **HRMS** (ESI+): *m/z* calcd for C<sub>37</sub>H<sub>36</sub>FN<sub>7</sub>O<sub>5</sub>SNa [(M + Na)<sup>+</sup>] 732.2375, found 732.2412 (–5.0 ppm).

#### **N<sup>6</sup>-Trityl-9-[3,5-dideoxy-3-fluoro-2-O-(4-methoxybenzyl)-5-(*N*-sulfamoyl)amino-β-D-xylofuranosyl]adenine (43c)**

To a solution of azide **42c** (0.54 g, 0.822 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (23 mL) was added zinc turnings (0.81 g, 12.393 mmol, 15 equiv) and AcOH (0.35 mL, 6.165 mmol, 7.5 equiv), and the reaction mixture was stirred at rt for 1 h. The reaction mixture was filtered through a short pad of Celite, and the residue was washed with MeOH (3 × 10 mL). The combined filtrates were concentrated and dissolved in EtOAc (10 mL). The EtOAc layer was washed with sat. NaHCO<sub>3</sub>, brine, dried (MgSO<sub>4</sub>), and evaporated to obtain the crude 5'-aminonucleoside. To a solution of the 5'-aminonucleoside in 1,4-dioxane (19 mL) was added sulfamide (0.23 g, 2.393 mmol, 2.9 equiv) and the mixture was refluxed for 2 h. The reaction mixture was evaporated then partitioned between CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and water (10 mL). The phases were separated and the CH<sub>2</sub>Cl<sub>2</sub> layer was washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. Purification by flash chromatography (SiO<sub>2</sub>, gradient: hexanes to 70% EtOAc in hexanes) afforded the product (0.50 g, 86%) as a white solid. *R<sub>f</sub>* = 0.39 (3:1 EtOAc/hexanes); **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]DMSO): δ 8.19, 7.94 (2s, 2H; H-2, H-8), 7.52 (s, 1H; N<sup>6</sup>-H), 7.35–7.20 (m, 17H; 3×C<sub>6</sub>H<sub>5</sub>, –PMB), 6.89 (t, <sup>3</sup>*J*(5'/5'', NH) = 6.1 Hz, 1H; C5'-NH), 6.85 (d, *J* = 8.6 Hz, 2H; –PMB), 6.65 (s, 2H; –NH<sub>2</sub>), 6.09 (d, <sup>3</sup>*J*(1',2') = 2.2 Hz, 1H; H-1'), 5.34 (dd, <sup>3</sup>*J*(3',4') = 2.6, <sup>2</sup>*J*(3',F3') = 51.1 Hz, 1H; H-3'), 4.79 (dd, <sup>3</sup>*J*(1',2') = 3.0, <sup>3</sup>*J*(2',F3') = 15.8 Hz, 1H; H-2'), 4.65 (s, 2H; –PMB), 4.42 (dtd, <sup>3</sup>*J*(3',4') = 2.8, <sup>3</sup>*J*(5',4') = 6.5, <sup>3</sup>*J*(5'',4') = 6.6, <sup>3</sup>*J*(4',F3') = 29.7 Hz, 1H; H-4'), 3.71 (s, 3H; –PMB), 3.35 (dt, <sup>3</sup>*J*(5'',NH) = 6.5, <sup>3</sup>*J*(5'',4') = 6.6, <sup>2</sup>*J*(5'',5') = 13.3 Hz, 1H; H-5'), 3.26 (dt, <sup>3</sup>*J*(5'',4') = 6.5, <sup>3</sup>*J*(5'',NH) = 6.5, <sup>2</sup>*J*(5'',5') = 13.3 Hz, 1H; H-5''); **<sup>13</sup>C NMR** (100 MHz, [D<sub>6</sub>]DMSO): δ 159.0, 153.5, 151.4, 148.2, 144.8, 138.9 (d, <sup>5</sup>*J*(C8,F3') = 4.8 Hz), 129.6, 128.9, 128.6, 127.7, 126.6, 120.6, 113.7, 93.4 (d, <sup>1</sup>*J*(C3',F3') = 183.4 Hz), 87.4, 84.2 (d, <sup>2</sup>*J*(C2',F3') = 27.6 Hz), 80.3 (d, <sup>2</sup>*J*(C4',F3') = 19.3 Hz), 71.4, 70.3, 55.0, 40.5 (d, <sup>3</sup>*J*(C5',F3') = 10.7 Hz); **<sup>19</sup>F NMR** (376 MHz, [D<sub>6</sub>]DMSO): δ –200.8 (ddd, <sup>3</sup>*J*(2',F3') = 15.6, <sup>3</sup>*J*(4',F3') = 29.7, <sup>2</sup>*J*(3',F3') = 50.8

Hz);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -201.8 (ddd,  $^3J(2',\text{F}3') = 16.2$ ,  $^3J(4',\text{F}3') = 23.2$ ,  $^2J(3',\text{F}3') = 52.5$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{37}\text{H}_{37}\text{FN}_7\text{O}_5\text{S}$  [(M + H) $^+$ ] 710.2555, found 710.2576 ( 2.9 ppm).

***N*<sup>6</sup>-Trityl-9-[3,5-dideoxy-3-fluoro-2-O-(4-methoxybenzyl)-5-(*N*-sulfamoyl)amino- $\beta$ -D-ribofuranosyl]adenine (43d)**

To a solution of azide **42d** (0.29 g, 0.442 mmol) in  $\text{CH}_2\text{Cl}_2$  (12 mL) was added zinc turnings (0.43g, 6.624 mmol, 15 equiv) and AcOH (0.19 mL, 3.312 mmol, 7.5 equiv) and the reaction mixture was stirred at rt for 1 h. The reaction mixture was filtered through a short pad of Celite washing with MeOH (3  $\times$  10 mL). The combined filtrates were concentrated and dissolved in EtOAc (10 mL). The EtOAc layer was washed with sat.  $\text{NaHCO}_3$ , brine, dried ( $\text{MgSO}_4$ ), and evaporated to obtain the crude 5'-aminonucleoside. To a solution of the 5'-aminonucleoside in 1,4-dioxane (9 mL) was added sulfamide (0.123 g, 1.284 mmol, 2.9 equiv) and the mixture was refluxed for 2 h. The reaction mixture was evaporated then partitioned between  $\text{CH}_2\text{Cl}_2$  (10 mL) and water (10 mL). The phases were separated and the  $\text{CH}_2\text{Cl}_2$  layer was washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated under reduced pressure. Purification by flash chromatography ( $\text{SiO}_2$ , gradient: hexanes to 70% EtOAc in hexanes) afforded the product (0.26 g, 84%) as a white solid.  $R_f = 0.51$  (3:1 EtOAc/hexanes);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.32 (d,  $^3J(\text{NH},5') = 10.0$  Hz, 1H; C5'-NH), 8.03, 7.73 (2s, 2H; H-2, H-8), 7.36–7.22 (m, 15H; 3 $\times$ C<sub>6</sub>H<sub>5</sub>), 7.05 (s, 1H; N<sup>6</sup>-H), 7.04 (d,  $J = 8.6$  Hz, 2H; -PMB), 6.73 (d,  $J = 8.6$  Hz, 2H; -PMB), 5.85 (d,  $^3J(1',2') = 8.3$  Hz, 1H; H-1'), 5.12 (dd,  $^3J(3',2') = 4.3$ ,  $^2J(3',\text{F}3') = 54.4$  Hz, 1H; H-3'), 4.90 (ddd,  $^3J(2',3') = 4.7$ ,  $^3J(1',2') = 8.3$ ,  $^3J(2',\text{F}3') = 24.2$  Hz, 1H; H-2'), 4.58 (dt,  $^3J(5',4') = 2.3$ ,  $^3J(5'',4') = 2.3$ ,  $^3J(4',\text{F}3') = 28.7$  Hz, 1H; H-4'), 4.45 (ABq,  $\delta = 31.6$  Hz,  $J = 11.2$  Hz, 2H; -PMB), 4.34 (s, 2H; -NH<sub>2</sub>), 3.74 (s, 3H; -PMB), 3.48–3.35 (m, 2H; H-5'/5'');  $^{13}\text{C}$  NMR (100 MHz, [D<sub>6</sub>]DMSO):  $\delta$  158.9, 153.8, 151.1, 147.9, 144.8, 141.3, 129.2, 128.9, 128.6, 127.7, 126.6, 121.3, 113.5, 90.4 (d,  $^1J(\text{C}3',\text{F}3') = 183.0$  Hz), 86.2, 82.1 (d,  $^2J(\text{C}2',\text{F}3') = 23.4$  Hz), 76.8 (d,  $^2J(\text{C}4',\text{F}3') = 15.6$  Hz), 71.1, 70.4, 50.0, 43.7 (d,  $^3J(\text{C}5',\text{F}3') = 10.8$  Hz);  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ ):  $\delta$  -196.1 (ddd,  $^3J(2',\text{F}3') = 24.1$ ,  $^3J(4',\text{F}3') = 28.7$ ,  $^2J(3',\text{F}3') = 53.6$  Hz);  $^{19}\text{F}$  NMR (376 MHz, [D<sub>6</sub>]DMSO):  $\delta$  -196.6 (ddd,  $^3J(2',\text{F}3') = 24.0$ ,  $^3J(4',\text{F}3') = 27.6$ ,  $^2J(3',\text{F}3') = 53.0$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $\text{C}_{37}\text{H}_{36}\text{FN}_7\text{O}_5\text{SNa}$  [(M + Na) $^+$ ] 732.2375, found 732.2421 ( 6.2 ppm).

***N*<sup>6</sup>-Trityl-9-[2,3,5-trideoxy-2,3-difluoro-5-(*N*-sulfamoyl)amino- $\beta$ -D-xylofuranosyl]adenine (43e)**

To a solution of azide **42e** (0.15 g, 0.278 mmol) in MeOH (7 mL) was added 10% Pd/C (0.030 g, 55% wet with water), and the reaction mixture was stirred under H<sub>2</sub> (1 atm) for 1 h. The reaction mixture was filtered through a short pad of Celite washing with MeOH (3  $\times$  5 mL). The combined filtrates were concentrated to obtain the crude 5'-aminonucleoside. To a solution of the crude 5'-aminonucleoside in 1,4-dioxane (5 mL) was added sulfamide (0.09 g, 0.94 mmol) and the mixture was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure. Purification by flash chromatography ( $\text{SiO}_2$ , gradient: hexanes to 70% EtOAc in hexanes) afforded the product (0.14 g, 84%) as a white solid.  $R_f = 0.32$  (3:1 EtOAc/hexanes);  $^1\text{H}$  NMR (400 MHz, [D<sub>6</sub>]DMSO):  $\delta$  8.22 (s, 1H), 7.95 (s, 1H), 7.59 (s,

1H;  $N^6$ -H), 7.35–7.20 (m, 15H;  $3\times C_6H_5$ ), 6.96 (t,  $^3J(5'/5'',NH) = 6.1$  Hz, 1H; C5'-NH), 6.67 (s, 2H;  $-NH_2$ ), 6.29 (dd,  $^3J(1',2') = 2.1$  Hz,  $^3J(1',F2') = 19.7$ , 1H; H-1'), 5.99 (ddt,  $^3J(2',3') \approx ^3J(1',2') \approx 1.7$ ,  $^3J(2',F3') = 12.7$ ,  $^2J(2',F2') = 47.8$  Hz, 1H; H-2'), 5.56 (dddd,  $^3J(2',3') = 1.4$ ,  $^3J(3',4') = 3.2$ ,  $^3J(3',F2') = 10.2$ ,  $^2J(3',F3') = 49.6$  Hz, 1H; H-3'), 4.53 (dm,  $^3J(4',F3') = 28.7$  Hz, 1H; H-4'), 3.41–3.25 (m, 2H; H-5'/5'');  $^{13}C$  NMR (100 MHz,  $[D_6]DMSO$ ):  $\delta$  153.6, 151.6, 148.2, 144.8, 139.0 (d,  $J = 4.5$  Hz), 128.6, 127.7, 126.6, 120.5, 95.5 (dd,  $^2J(C2',F3') = 32.0$ ,  $^1J(C2',F2') = 182.8$  Hz), 92.3 (dd,  $^2J(C3',F2') = 29.6$ ,  $^1J(C3',F3') = 182.9$  Hz), 86.3 (d,  $^2J(C1',F2') = 36.1$  Hz), 79.9 (d,  $^2J(C4',F3') = 19.5$  Hz), 70.3, 40.3 (d,  $^3J(C5',F3') = 10.6$  Hz);  $^{19}F$  NMR (376 MHz,  $[D_6]DMSO$ ):  $\delta$  -193.8 (ddtd,  $^4J(F2',4') = 2.7$ ,  $^3J(F2',3') = 10.4$ ,  $^3J(F2',F3') = 10.4$ ,  $^3J(1',F2') = 20.7$ ,  $^2J(2',F2') = 47.8$  Hz; F-2'), -207.0 (ddt,  $^3J(F2',F3') = 11.6$ ,  $^3J(2',F3') = 11.6$ ,  $^3J(4',F3') = 28.7$ ,  $^2J(3',F3') = 50.7$  Hz; F-3'); **HRMS** (ESI+):  $m/z$  calcd for  $C_{29}H_{27}F_2N_7O_3SNa [(M + Na)^+]$  614.1756, found 614.1786 ( 4.9 ppm).

### ***N*<sup>6</sup>-Trityl-9-[2,3,5-trideoxy-2,3-difluoro-5-(*N*-sulfamoyl)amino- $\beta$ -D-ribofuranosyl]adenine (43f)**

To a solution of azide **42f** (0.32 g, 0.59 mmol) in MeOH (10 mL) was added 10% Pd/C (0.06g, 55% wet with water) and the reaction mixture was stirred under  $H_2$  (1 atm) for 1 h. The reaction mixture was filtered through a short pad of Celite washing with MeOH ( $3 \times 10$  mL). The combined filtrates were concentrated to obtain the crude 5'-aminonucleoside. To a solution of the 5'-aminonucleoside in 1,4-dioxane (9 mL) was added sulfamide (0.18 g, 1.87 mmol, 3.1 equiv) and the mixture was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure. Purification by flash chromatography ( $SiO_2$ , gradient: hexanes to 70% EtOAc in hexanes) afforded the product (0.34 g, 94%) as a white solid.  $R_f = 0.30$  (3:1 EtOAc/hexanes);  $^1H$  NMR (400 MHz,  $[D_6]DMSO$ ):  $\delta$  8.50 (s, 1H), 7.93 (s, 1H), 7.71 (s, 1H;  $N^6$ -H), 7.41 (t,  $^3J(5'/5'',NH) = 6.4$  Hz, 1H; C5'-NH), 7.35–7.20 (m, 15H;  $3\times C_6H_5$ ), 6.72 (s, 2H;  $-NH_2$ ), 6.37 (dd,  $^3J(1',2') = 6.0$  Hz,  $^3J(1',F2') = 13.2$ , 1H; H-1'), 6.00 (dddd,  $^3J(2',3') = 4.8$ ,  $^3J(1',2') = 5.6$ ,  $^3J(2',F3') = 16.4$ ,  $^2J(2',F2') = 49.6$  Hz, 1H; H-2'), 5.51 (dm,  $^2J(3',F3') = 52.8$  Hz, 1H; H-3'), 4.51 (dm,  $^3J(4',F3') = 24.4$  Hz, 1H; H-4'), 3.27–3.24 (m, 2H; H-5'/5'');  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  154.7, 152.3, 147.4, 144.6, 140.1, 129.1, 128.2, 127.3, 122.2, 90.4 (dd,  $^2J(C2',F3') = 12.3$ ,  $^1J(C2',F2') = 185.0$  Hz), 88.4 (dd,  $^2J(C3',F2') = 15.1$ ,  $^1J(C3',F3') = 201.3$  Hz), 87.5 (d,  $^2J(C1',F2') = 29.7$  Hz), 82.9 (dd,  $^3J(C4',F2') = 2.5$ ,  $^2J(C4',F3') = 24.5$  Hz), 71.7, 44.5 (d,  $^3J(C5',F3') = 10.4$  Hz);  $^{19}F$  NMR (376 MHz,  $[D_6]DMSO$ ):  $\delta$  -202.6 (dddd,  $^3J(F2',F3') = 5.1$ ,  $^3J(2',F3') = 16.6$ ,  $^3J(4',F3') = 22.6$ ,  $^2J(3',F3') = 53.1$  Hz; F-3'), -213.9 (dm,  $^2J(2',F2') = 50.0$  Hz; F-2'); **HRMS** (ESI+):  $m/z$  calcd for  $C_{29}H_{27}F_2N_7O_3SNa [(M + Na)^+]$  614.1756, found 614.1779 ( 3.7 ppm).

### **General Procedure 4: Coupling and Deprotection**

To a solution of sulfamide (1.0 mmol, 1.0 equiv) in DMF (20 mL) cooled to 0 °C was added NHS ester **44** (2.0 mmol, 2.0 equiv) followed by  $CS_2CO_3$  (3.0 mmol, 3 equiv) and reaction mixture was stirred at that temperature for 30 min. The reaction mixture was allowed to warm to rt and stirred for 15 h. The reaction mixture was filtered and evaporated under reduced pressure to obtain the crude coupled product. To the crude material was added 80% aqueous TFA (15 mL) and the mixture was stirred for 1 h. The reaction mixture was evaporated and re-evaporated with 5% MeOH in  $CH_2Cl_2$  ( $\times 2$ ) under reduced pressure.

Purification of the residue by flash chromatography (84:15:1 EtOAc/MeOH/Et<sub>3</sub>N) afforded the product with some contamination. Additional purification was performed by preparative reverse-phase HPLC using a Phenomenex Gemini 10 μm C18 110Å (250 × 21.2 mm) column at a flow rate of 21 mL/min with a gradient from 5% to 30% MeCN in 20 mM aqueous triethylammonium bicarbonate (pH 7.5) over 5 min, followed by isocratic elution with 30% MeCN for 15 min. The appropriate fractions containing the product were pooled and lyophilized to afford the final product.

**9-[2,5-Dideoxy-2-fluoro-5-{N-(N-2-hydroxybenzoyl)sulfamoyl}amino-β-D-ribofuranosyl]adenine Triethylammonium Salt (12)**

This was prepared from sulfamide **43a** (0.100 g, 0.141 mmol) using general procedure 4 to afford the title compound (0.033 g, 38%) as a white solid. **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]DMSO): δ 8.33, 8.14 (2s, 2H; H-2, H-8), 7.77 (dd, *J* = 1.8, 7.7 Hz; 1H), 7.34 (br s, 2H; C<sub>6</sub>-NH<sub>2</sub>), 7.22 (ddd, *J* = 1.9, 7.3, 8.3 Hz; 1H), 6.71 (m, 2H), 6.19 (dd, <sup>3</sup>*J*(1',2') = 3.5, <sup>3</sup>*J*(1',F2') = 17.0 Hz, 1H; H-1'), 6.12 (t, <sup>3</sup>*J*(5'/5'',NH) = 7.0 Hz, 1H; 5'-NH), 5.76 (d, <sup>3</sup>*J*(3',OH) = 5.8 Hz, 1H; 3'-OH'), 5.51 (dt, <sup>3</sup>*J*(2',3') ≈ <sup>3</sup>*J*(1',2') = 4.0, <sup>2</sup>*J*(2',F2') = 52.9 Hz, 1H; H-2'), 4.48 (dm, <sup>3</sup>*J*(3',F2') = 16.1 Hz, 1H; H-3'), 4.04 (q, <sup>3</sup>*J*(3',4') ≈ <sup>3</sup>*J*(5',4') ≈ <sup>3</sup>*J*(5'',4') = 5.2 Hz, 1H; H-4'), 3.16 (ddd, <sup>3</sup>*J*(5',4') = 3.8, <sup>3</sup>*J*(5', NH) = 6.5, <sup>2</sup>*J*(5', 5'') = 13.2 Hz, 1H; H-5'), 3.03 (ddd, <sup>3</sup>*J*(5'',4') = 5.9, <sup>3</sup>*J*(5'',NH) = 7.6, <sup>2</sup>*J*(5', 5'') = 13.3 Hz, 1H; H-5''), 2.80 (q, *J* = 7.2 Hz, 6H, Et<sub>3</sub>N), 1.06 (t, *J* = 7.2 Hz, 9H, Et<sub>3</sub>N); **<sup>13</sup>C NMR** (100 MHz, [D<sub>6</sub>]DMSO): δ 170.0, 160.5, 156.1, 152.8, 148.8, 139.7, 132.1, 129.4, 120.4, 119.2, 117.2, 116.5, 92.7 (d, <sup>1</sup>*J*(C2',F2') = 187.9 Hz), 85.7 (d, <sup>2</sup>*J*(C1',F2') = 32.8 Hz), 82.2, 69.7 (d, <sup>2</sup>*J*(C3',F2') = 15.6 Hz), 45.8, 44.8, 9.1; **<sup>19</sup>F NMR** (376 MHz, [D<sub>6</sub>]DMSO): δ -208.5 (dt, <sup>3</sup>*J*(3',F2') = 16.3, <sup>3</sup>*J*(1',F2') = 16.3, <sup>2</sup>*J*(2',F2') = 52.9 Hz); **HRMS** (ESI<sup>-</sup>): *m/z* calcd for C<sub>17</sub>H<sub>17</sub>FN<sub>7</sub>O<sub>6</sub>S [(M - Et<sub>3</sub>NH)<sup>-</sup>] 466.0951, found 466.0988 ( -7.9 ppm).

**9-[2,5-Dideoxy-2-fluoro-5-{N-(N-2-hydroxybenzoyl)sulfamoyl}amino-β-D-arabinofuranosyl]adenine Triethylammonium Salt (13)**

This was prepared from sulfamide **43b** (0.100 g, 0.141 mmol) using general procedure 4 to afford the title compound (0.033 g, 41%) as a white solid. **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]DMSO): δ 8.27 (d, <sup>5</sup>*J*(8,F2') = 2.4 Hz, 1H; H-8), 8.15 (s, 1H; H-2), 7.83 (dd, *J* = 1.8, 8.2 Hz; 1H), 7.35 (br s, 2H; C<sub>6</sub>-NH<sub>2</sub>), 7.23 (td, *J* = 1.9, 7.4, 7.5 Hz; 1H), 6.74–6.71 (m, 2H), 6.38 (dd, <sup>3</sup>*J*(1',2') = 3.7, <sup>3</sup>*J*(1',F2') = 19.0 Hz, 1H; H-1'), 5.98 (d, <sup>3</sup>*J*(3',OH) = 4.6 Hz, 1H; 3'-OH'), 5.91 (t, <sup>3</sup>*J*(5'/5'',NH) = 6.8 Hz, 1H; 5'-NH), 5.06 (dt, <sup>3</sup>*J*(2',3') = 2.9, <sup>3</sup>*J*(1',2') = 2.9, <sup>2</sup>*J*(2',F2') = 52.0 Hz, 1H; H-2'), 4.42 (dq, <sup>3</sup>*J*(2',3') = 3.0, <sup>3</sup>*J*(3',4') = 3.2, <sup>3</sup>*J*(3',OH) = 3.2, <sup>3</sup>*J*(3',F2') = 16.4 Hz, 1H; H-3'), 4.01 (dt, <sup>3</sup>*J*(3',4') = 4.2, <sup>3</sup>*J*(5',4') = 4.3, <sup>3</sup>*J*(5'',4') = 7.9 Hz, 1H; H-4'), 3.15 (ddd, <sup>3</sup>*J*(5',4') = 4.7, <sup>3</sup>*J*(5', NH) = 7.5, <sup>2</sup>*J*(5', 5'') = 12.4 Hz, 1H; H-5'), 3.04 (dt, <sup>3</sup>*J*(5'',NH) = 6.8, <sup>3</sup>*J*(5'',4') = 6.8, <sup>2</sup>*J*(5', 5'') = 13.0 Hz, 1H; H-5''), 2.77 (q, *J* = 7.2 Hz, 6 H, Et<sub>3</sub>N), 1.05 (t, *J* = 7.2 Hz, 9 H, Et<sub>3</sub>N); **<sup>13</sup>C NMR** (100 MHz, [D<sub>6</sub>]DMSO): δ 170.1, 160.5, 156.0, 152.8, 149.0, 139.6 (d, <sup>4</sup>*J*(C8,F2') = 4.2 Hz), 132.1, 129.4, 120.3, 118.2, 117.3, 116.5, 94.7 (d, <sup>1</sup>*J*(C2',F2') = 191.0 Hz), 82.6 (d, <sup>3</sup>*J*(C4',F2') = 3.0 Hz), 82.3 (d, <sup>2</sup>*J*(C1',F2') = 16.5 Hz), 74.2 (d, <sup>2</sup>*J*(C3',F2') = 23.7 Hz), 45.7, 44.9, 10.1; **<sup>19</sup>F NMR** (376 MHz, [D<sub>6</sub>]DMSO): δ -197.6 ppm (dt, <sup>3</sup>*J*(3',F2') = 17.7, <sup>3</sup>*J*(1',F2') = 17.7, <sup>2</sup>*J*(2',F2') = 51.9 Hz);



**HRMS** (ESI+):  $m/z$  calcd for  $C_{17}H_{18}FN_7O_6SNa [(M - Et_3N + Na)^+]$  490.0916, found 490.0905 ( -2.2 ppm).

**9-[3,5-Dideoxy-3-fluoro-5-{N-(N-2-hydroxybenzoyl)sulfamoyl}amino- $\beta$ -xylofuranosyl]adenine Triethylammonium Salt (14)**

This was prepared from sulfamide **43c** (0.48 g, 0.676 mmol) using general procedure 4 to afford the title compound (0.15 g, 39%) as a white solid.  **$^1H$  NMR** (400 MHz,  $[D_6]DMSO$ ):  $\delta$  8.15, 8.05 (2s, 2H; H-2, H-8), 7.81 (dd,  $J = 1.9, 8.0$  Hz, 1H), 7.29 (br s, 2H;  $C_6-NH_2$ ), 7.23 (td,  $J = 1.9, 7.4$  Hz, 1H), 6.74–6.71 (m, 2H), 6.29 (d,  $^3J(2',OH) = 4.6$  Hz, 1H; 2'-OH), 5.95 (t,  $^3J(5'/5'',NH) = 6.7$  Hz, 1H; 5'-NH), 5.90 (d,  $^3J(1',2') = 2.3$  Hz, 1H; H-1'), 5.08 (ddd,  $^3J(3',2') = 1.3$ ,  $^3J(3',4') = 2.8$ ,  $^2J(3',F3') = 51.9$  Hz, 1H; H-3'), 4.72 (dm,  $^3J(2',F3') = 16.1$  Hz, 1H; H-2'), 4.34 (dtd,  $^3J(3',4') = 3.1$ ,  $^3J(5',4') = 6.2$ ,  $^3J(5'',4') = 6.6$ ,  $^3J(4',F3') = 29.1$  Hz, 1H; H-4'), 3.26–3.21 (m, 1H; H-5'), 3.13 (dt,  $^3J(5'',NH) = 6.7$ ,  $^3J(5'',4') = 6.7$ ,  $^2J(5',5'') = 13.2$  Hz, 1H; H-5''), 2.99 (q,  $J = 7.2$  Hz, 6 H,  $Et_3N$ ), 1.13 (t,  $J = 7.2$  Hz, 9 H,  $Et_3N$ );  **$^{13}C$  NMR** (100 MHz,  $[D_6]DMSO$ ):  $\delta$  170.2, 160.5, 156.0, 152.7, 149.1, 138.2 (d,  $^5J(C8,F3') = 5.4$  Hz), 132.1, 129.4, 120.3, 118.8, 117.2, 116.5, 95.7 (d,  $^1J(C3',F3') = 183.9$  Hz), 88.9, 80.2 (d,  $^2J(C2',F3') = 18.9$  Hz), 77.6 (d,  $^2J(C4',F3') = 26.5$  Hz), 45.7, 41.5 (d,  $^3J(C5',F3') = 10.1$  Hz), 9.2;  **$^{19}F$  NMR** (376 MHz,  $[D_6]DMSO$ ):  $\delta$  -200.2 (ddd,  $^3J(2',F3') = 15.2$ ,  $^3J(4',F3') = 28.9$ ,  $^2J(3',F3') = 51.6$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{17}H_{18}FN_7O_6SNa [(M - Et_3N + Na)^+]$  490.0916, found 490.0898 ( -3.7 ppm).

**9-[3,5-Dideoxy-3-fluoro-5-{N-(N-2-hydroxybenzoyl)sulfamoyl}amino- $\beta$ -ribofuranosyl]adenine Triethylammonium Salt (15)**

This was prepared from sulfamide **43d** (0.23 g, 0.324 mmol) using general procedure 4 to afford the title compound (0.08 g, 45%) as a white solid.  **$^1H$  NMR** (400 MHz,  $[D_6]DMSO$ ):  $\delta$  8.37, 8.17 (2s, 2H; H-2, H-8), 7.78 (dd,  $J = 1.8, 7.7$  Hz; 1H), 7.32 (br s, 2H;  $C_6-NH_2$ ), 7.21 (ddd,  $J = 1.9, 7.2, 8.2$  Hz; 1H), 6.77 (t,  $J = 7.0$  Hz, 1H; 5'-NH), 6.70 (td,  $J = 1.2, 9.3$  Hz; 2H), 5.90 (d,  $^3J(2',OH) = 5.7$  Hz, 1H; 2'-OH), 5.88 (d,  $^3J(1',2') = 8.1$  Hz, 1H; H-1'), 5.10 (dd,  $^3J(3',2') = 4.1$ ,  $^2J(3',F3') = 55.6$  Hz, 1H; H-3'), 5.08 (dd,  $^3J(1',2') = 8.5$ ,  $^3J(2',F3') = 26.0$  Hz, 1H; H-2'), 4.34 (ddd,  $^3J(5',4') = 4.1$ ,  $^3J(5'',4') = 5.1$ ,  $^3J(4',F3') = 27.8$  Hz, 1H; H-4'), 3.13–3.10 (m, 2H, H-5'/5''), 2.91 (q,  $J = 7.2$  Hz, 7.8 H,  $Et_3N$ ), 1.10 (t,  $J = 7.2$  Hz, 11.7 H,  $Et_3N$ );  **$^{13}C$  NMR** (100 MHz,  $[D_6]DMSO$ ):  $\delta$  169.9, 160.6, 156.1, 152.7, 149.3, 140.4, 132.0, 129.4, 120.5, 119.4, 117.1, 116.4, 93.2 (d,  $^1J(C3',F3') = 181.2$  Hz), 86.6, 82.0 (d,  $^2J(C2',F3') = 22.7$  Hz), 70.9 (d,  $^2J(C4',F3') = 16.4$  Hz), 45.7, 44.3 (d,  $^3J(C5',F3') = 11.3$  Hz), 9.4;  **$^{19}F$  NMR** (376 MHz,  $[D_6]DMSO$ ):  $\delta$  -197.0 (dt,  $^3J(2',F3') = 27.4$ ,  $^3J(4',F3') = 27.4$ ,  $^2J(3',F3') = 55.3$  Hz); **HRMS** (ESI+):  $m/z$  calcd for  $C_{17}H_{18}FN_7O_6SNa [(M - Et_3N + Na)^+]$  490.0916, found 490.0939 ( 4.7 ppm).

**9-[2,3,5-Trideoxy-2,3-difluoro-5-{N-(N-2-hydroxybenzoyl)sulfamoyl}amino- $\beta$ -xylofuranosyl]adenine Triethylammonium Salt (16)**

This was prepared from sulfamide **43e** (0.360 g, 0.608 mmol) using general procedure 4 to afford the title compound (0.141 g, 50%) as a white solid.  **$^1H$  NMR** (400 MHz,  $[D_6]DMSO$ ):  $\delta$  8.15, 8.07 (2s, 2H; H-2, H-8), 7.79 (dd,  $J = 1.9, 8.1$  Hz, 1H), 7.35 (br s, 2H;  $C_6-NH_2$ ), 7.22 (ddd,  $J = 2.0, 8.0, 9.2$  Hz, 1H), 6.73–6.69 (m, 2H), 6.24 (dd,  $^3J(1',2') =$



2.1,  $^3J(1',F2') = 19.4$  Hz, 1H; H-1'), 5.99 (t,  $J = 6.7$  Hz, 1H; C5'-NH), 5.87 (ddt,  $^3J(2',3') = 1.6$ ,  $^3J(1',2') = 1.7$ ,  $^3J(2',F3') = 12.9$ ,  $^2J(2',F2') = 47.7$  Hz, 1H; H-2'), 5.52 (dddd,  $^3J(2',3') = 1.4$ ,  $^3J(3',4') = 3.2$ ,  $^3J(3',F2') = 10.2$ ,  $^2J(3',F3') = 49.6$  Hz, 1H; H-3'), 4.52 (dm,  $^3J(4',F3') = 28.9$  Hz, 1H; H-4'), 3.28 (ddd,  $^3J(5',4') = 5.8$ ,  $^3J(5',NH) = 7.4$ ,  $^2J(5',5'') = 13.2$  Hz, 1H; H-5'), 3.18–3.12 (m, 1H; H-5'') 2.92 (q,  $J = 7.2$  Hz, 6 H, Et<sub>3</sub>N), 1.10 (t,  $J = 7.2$  Hz, 9 H, Et<sub>3</sub>N); **<sup>13</sup>C NMR** (100 MHz, [D<sub>6</sub>]DMSO):  $\delta$  170.2, 160.5, 156.1, 152.9, 149.0, 138.0 (d,  $J = 4.3$  Hz), 132.2, 129.4, 120.3, 128.8, 127.3, 116.5, 95.7 (dd,  $^2J(C2',F3') = 32.0$ ,  $^1J(C2',F2') = 182.9$  Hz), 92.5 (dd,  $^2J(C3',F2') = 29.6$ ,  $^1J(C3',F3') = 182.7$  Hz), 86.1 (d,  $^2J(C1',F2') = 35.8$  Hz), 80.2 (d,  $^2J(C4',F3') = 19.3$  Hz), 45.7, 41.2 (d,  $^3J(C5',F3') = 9.9$  Hz), 9.2; **<sup>19</sup>F NMR** (376 MHz, [D<sub>6</sub>]DMSO):  $\delta$  -193.82 (ddtd,  $^4J(4',F2') = 2.6$ ,  $^3J(F2',F3') = 10.3$ ,  $^3J(3',F2') = 10.3$ ,  $^3J(1',F2') = 18.8$ ,  $^2J(2',F2') = 47.7$  Hz; F-2'), -206.98 (ddt,  $^3J(F2',F3') = 11.6$ ,  $^3J(2',F3') = 11.6$ ,  $^3J(4',F3') = 28.9$ ,  $^2J(3',F3') = 50.9$  Hz; F-3'); **HRMS** (ESI+):  $m/z$  calcd for C<sub>17</sub>H<sub>17</sub>F<sub>2</sub>N<sub>7</sub>O<sub>5</sub>SNa [(M - Et<sub>3</sub>N + Na)<sup>+</sup>] 492.0872, found 492.0893 ( 4.2 ppm).

### 9-[2,3,5-Trideoxy-2,3-difluoro-5-{N-(N-2-hydroxybenzoyl)sulfamoyl}amino- $\beta$ -*ribo*furanosyl]adenine Triethylammonium Salt (17)

This was prepared from sulfamide **43f** (0.240 g, 0.406 mmol) using general procedure 4 to afford the title compound (0.097 g, 44%) as a white solid. **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]acetone):  $\delta$  8.34, 8.30 (2s, 2H; H-2, H-8), 7.92 (dd,  $J = 1.8, 7.8$  Hz, 1H), 7.22 (ddd,  $J = 1.8, 7.1, 8.2$  Hz, 1H), 6.75–6.70 (m, 2H), 6.33 (ddd,  $^4J(1',F3') = 1.4$ ,  $^3J(1',2') = 6.6$ ,  $^3J(1',F2') = 12.3$  Hz, 1H; H-1'), 6.13 (dddd,  $^3J(2',3') = 4.4$ ,  $^3J(1',2') = 6.6$ ,  $^3J(2',F3') = 18.0$ ,  $^2J(2',F2') = 50.3$  Hz, 1H; H-2'), 5.57 (dm,  $^2J(3',F3') = 53.6$  Hz, 1H; H-3'), 4.58 (dm,  $^3J(4',F3') = 25.8$  Hz, 1H; H-4'), 3.42 (dd,  $^3J(5',4') = 4.1$ ,  $^2J(5',5'') = 13.9$  Hz, 1H; H-5'), 3.35 (dd,  $^3J(5'',4') = 4.4$ ,  $^2J(5',5'') = 13.9$  Hz, 1H; H-5''), 2.89 (q,  $J = 7.2$  Hz, 6H, Et<sub>3</sub>N), 1.15 (t,  $J = 7.2$  Hz, 9H, Et<sub>3</sub>N); **<sup>1</sup>H NMR** (400 MHz, [D<sub>6</sub>]DMSO):  $\delta$  14.02 (s, 1H), 8.41, 8.17 (2s, 2H; H-2, H-8), 7.78 (dd,  $J = 1.8, 7.7$  Hz, 1H), 7.41 (br s, 2H; C<sub>6</sub>-NH<sub>2</sub>), 7.22 (ddd,  $J = 1.9, 7.3, 8.5$  Hz, 1H), 6.73–6.69 (m, 2H), 6.58 (t,  $J = 7.0$  Hz, 1H; C5'-NH), 6.30 (ddd,  $^4J(1',F3') = 1.6$ ,  $^3J(1',2') = 6.7$ ,  $^3J(1',F2') = 12.6$  Hz, 1H; H-1'), 6.07 (dddd,  $^3J(2',3') = 4.2$ ,  $^3J(1',2') = 6.5$ ,  $^3J(2',F3') = 18.2$ ,  $^2J(2',F2') = 50.0$  Hz, 1H; H-2'), 5.52 (dtd,  $^3J(3',4') = 2.1$ ,  $^3J(3',F2') = 3.9$ ,  $^3J(2',3') = 3.9$ ,  $^2J(3',F3') = 53.8$  Hz, 1H; H-3'), 4.45 (dtt,  $^4J(4',F2') = 2.3$ ,  $^3J(3',4') = 2.3$ ,  $^3J(5',4') = 4.5$ ,  $^3J(5'',4') = 4.5$ ,  $^3J(4',F3') = 24.9$  Hz, 1H; H-4'), 3.18–3.14 (m, 2H; H-5'/5'') 3.05 (q,  $J = 7.3$  Hz, 6H, Et<sub>3</sub>N), 1.15 (t,  $J = 7.3$  Hz, 9H, Et<sub>3</sub>N); **<sup>13</sup>C NMR** (100 MHz, [D<sub>6</sub>]DMSO):  $\delta$  170.0, 160.6, 156.2, 153.0, 149.0, 140.0, 132.1, 129.4, 120.4, 119.2, 117.2, 116.5, 90.03 (dd,  $^2J(C3',F2') = 13.4$ ,  $^1J(C3',F3') = 184.0$  Hz), 89.2 (dd,  $^2J(C2',F3') = 14.7$ ,  $^1J(C2',F2') = 194.4$  Hz), 84.3 (d,  $^2J(C1',F2') = 29.9$  Hz), 81.3 (dd,  $^3J(C4',F2') = 3.4$ ,  $^2J(C4',F3') = 22.4$  Hz), 45.8, 43.9 (d,  $^3J(C5',F3') = 9.0$  Hz), 8.8; **<sup>19</sup>F NMR** (376 MHz, [D<sub>6</sub>]DMSO):  $\delta$  -202.03 (dddd,  $^3J(F2',F3') = 5.5$ ,  $^3J(2',F3') = 18.2$ ,  $^3J(4',F3') = 24.4$  Hz,  $^2J(3',F3') = 54.0$  Hz; F-3'), -215.45 (ddtd,  $^4J(4',F2') = 3.1$ ,  $^3J(3',F2') = 3.1$ ,  $^3J(F2',F3') = 5.7$ ,  $^3J(1',F2') = 12.0$ ,  $^1J(2',F2') = 49.9$  Hz; F-2'); **HRMS** (ESI+):  $m/z$  calcd for C<sub>17</sub>H<sub>17</sub>F<sub>2</sub>N<sub>7</sub>O<sub>5</sub>SNa [(M - Et<sub>3</sub>N + Na)<sup>+</sup>] 492.0872, found 492.0899 ( 5.4 ppm).

## Supplementary Material

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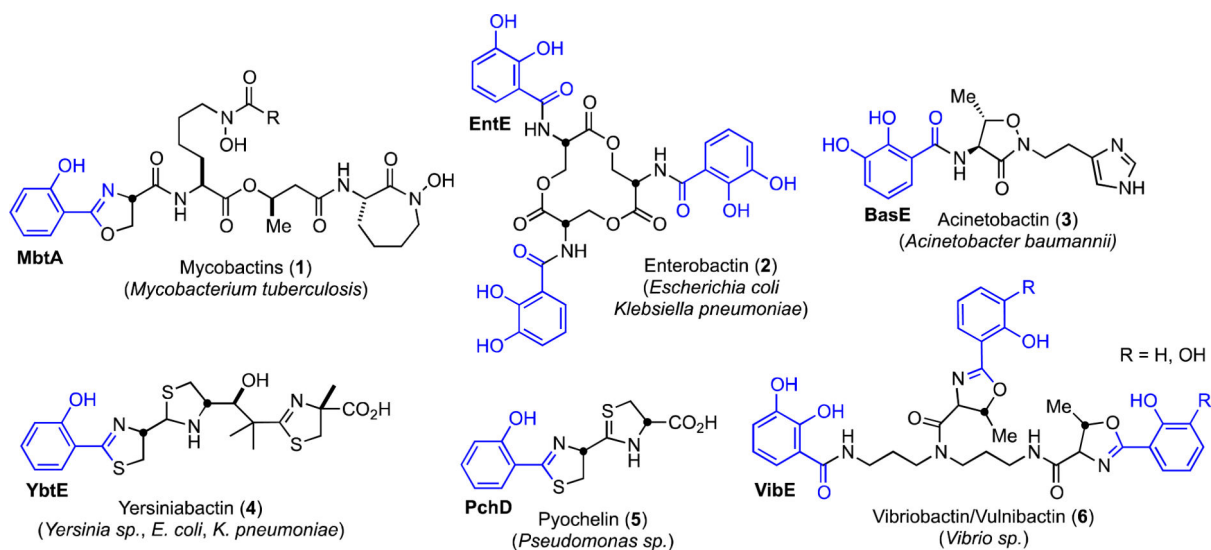
## ACKNOWLEDGMENT

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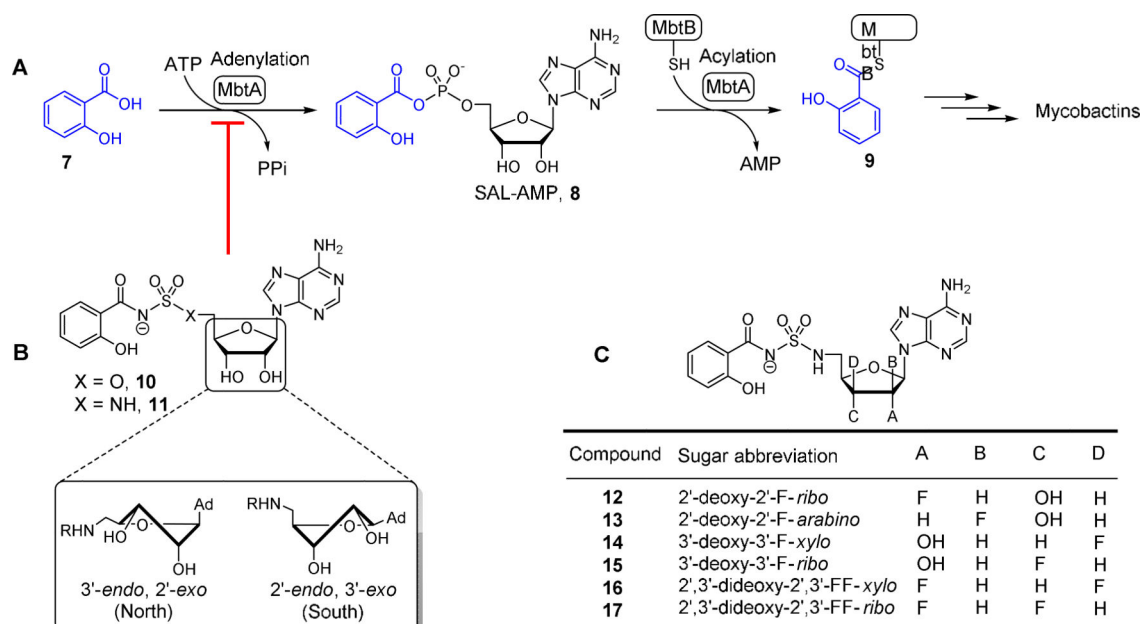
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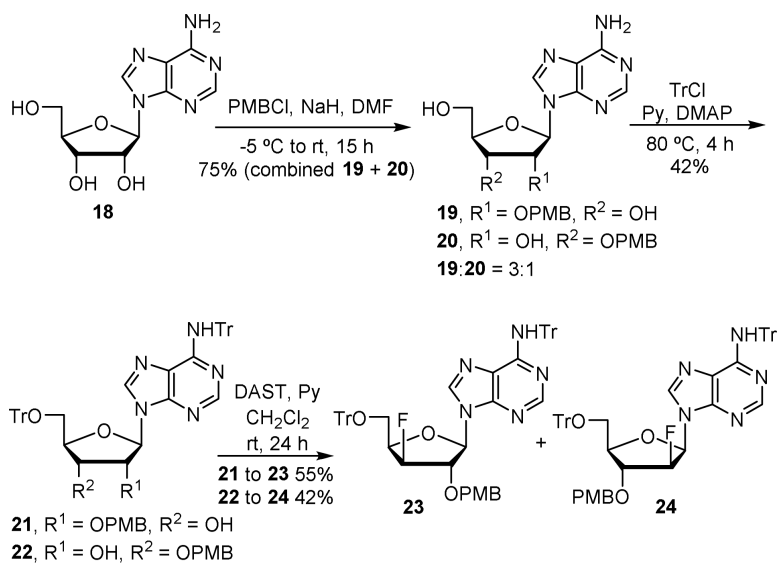
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**Figure 1.**

Structures of representative aryl-capped siderophores. The siderophore name is given below each structure along with the producing organism(s). The aryl caps are highlighted in blue (salicylic acid or 2,3-dihydrobenzoic acid). The aryl acid adenylating enzymes (AAAEs) responsible for incorporation of the respective aryl moieties have high homology at the overall protein level and nearly identical active-site residues (MbtA, EntE, BasE, YbtE, PchD, and VibE).

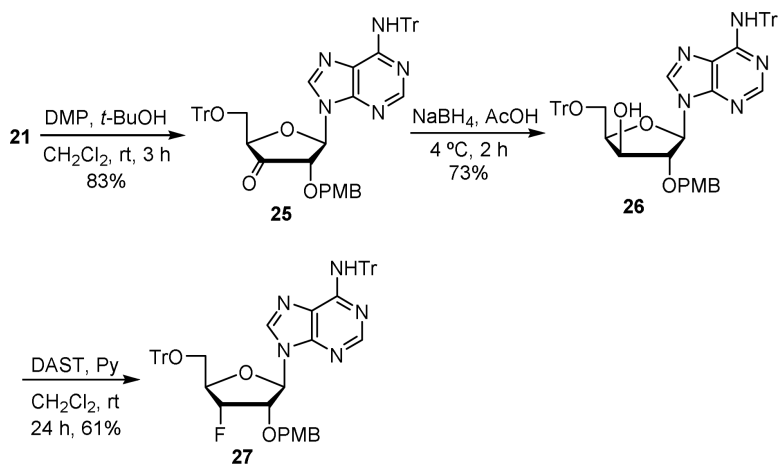
**Figure 2.**

Mycobactin biosynthesis and inhibition. **(A)** The biosynthesis of mycobactins in *M. tuberculosis* is initiated by MbtA that catalyzes two partial reactions at the same active site. In the first half-reaction MbtA condenses salicylic acid (**7**) with ATP to form the reactive mixed anhydride SAL-AMP (**8**). In the second half-reaction MbtA transfers the salicyl moiety onto MbtB, another protein in the biochemical pathway, to afford **9** that is ultimately elaborated to the mycobactins. The initial biosynthetic step of other aryl-capped siderophores is performed by homologous aryl acid adenylation enzymes (AAAEs). **(B)** The inhibitors Sal-AMS **10** and its sulfamide isostere **11** mimic the acyl-adenylate intermediate **8**, thereby blocking siderophore biosynthesis. The sugar can adopt the Northern ( $C3'$ -endo pucker) or Southern ( $C2'$ -endo pucker) conformation as depicted. **(C)** Fluorinated sugar analogs described in this study.

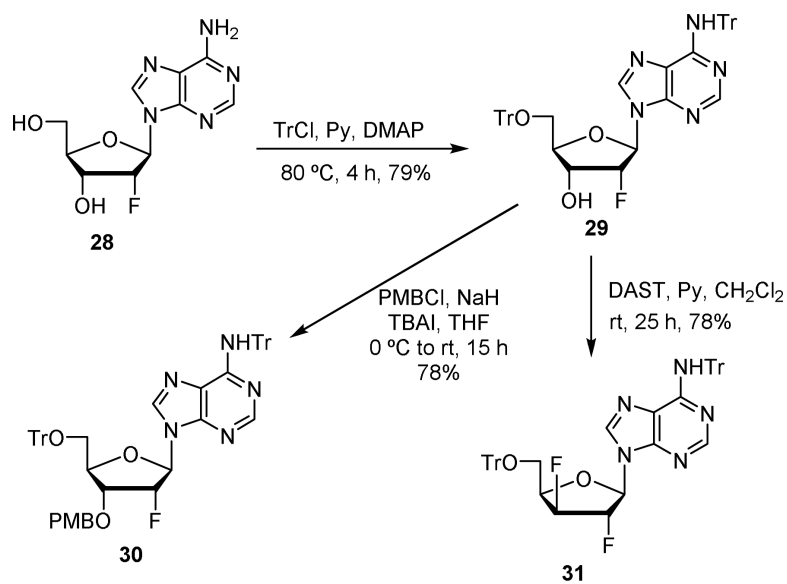
**Scheme 1.**

Synthesis of 3'-deoxy-3'-F-xylo and 2'-deoxy-2'-F-arabino nucleosides

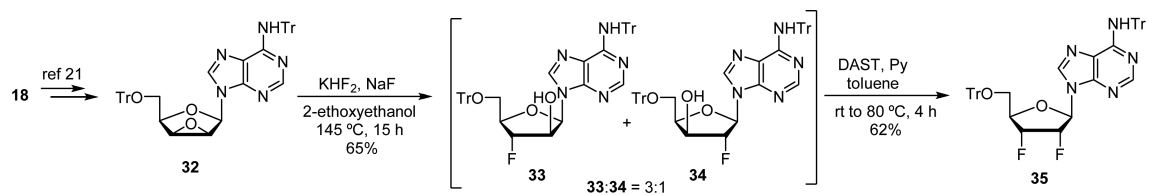




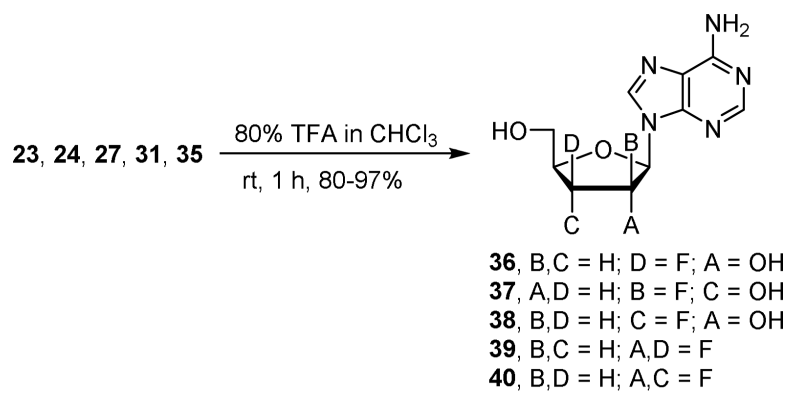
**Scheme 2.**  
Synthesis of 3'-deoxy-3'-F-ribo nucleoside



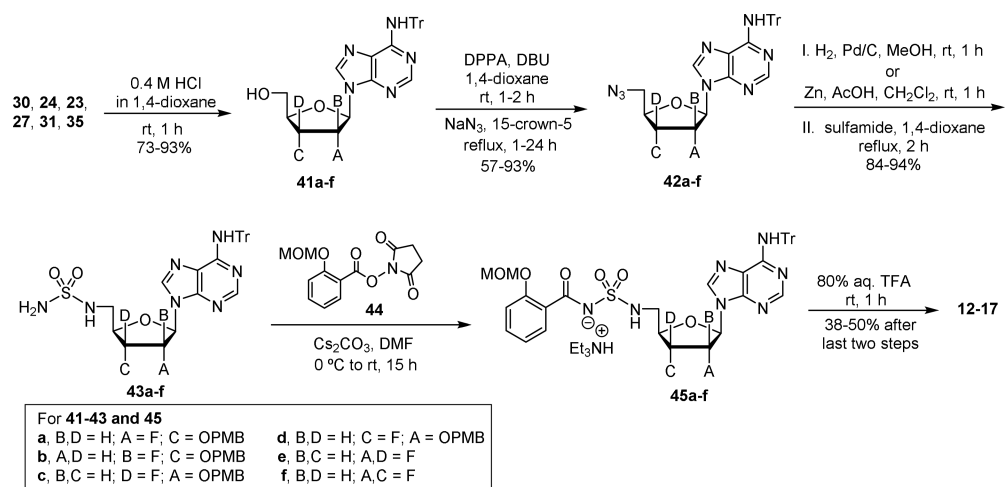
**Scheme 3.**  
Synthesis of 2',3'-dideoxy-2',3'-FF-xylo and 2'-deoxy-2'-F-ribo nucleosides



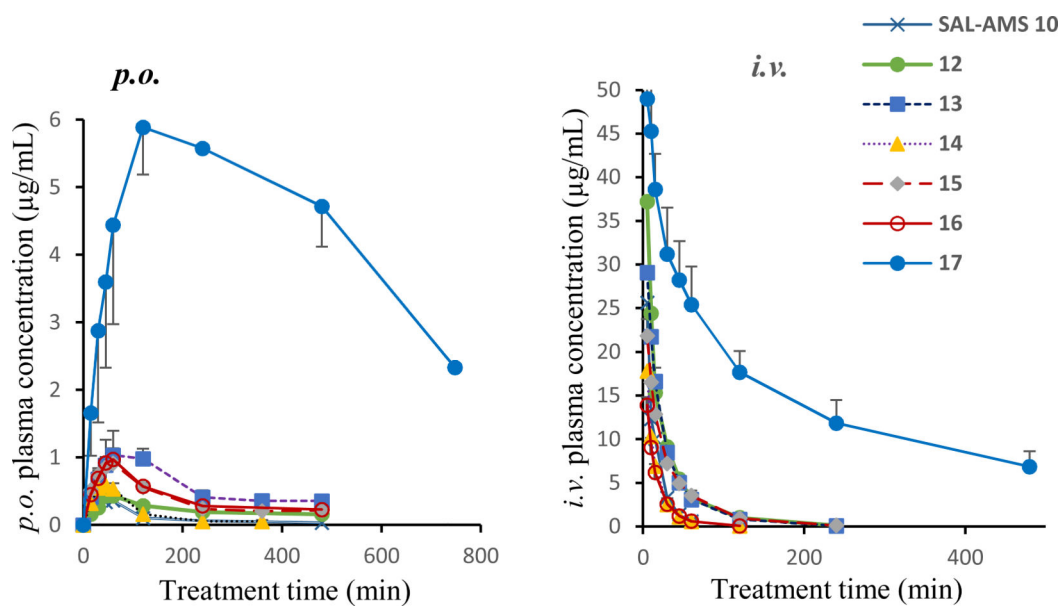
**Scheme 4.**  
Synthesis of 2',3'-dideoxy-2',3'-FF-ribo nucleoside



**Scheme 5.**  
Synthesis of fully unprotected fluorinated nucleoside



**Scheme 6.**  
Completion of synthesis of 7–12

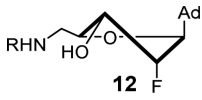
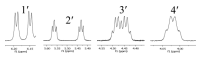
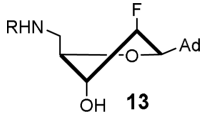
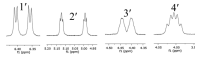
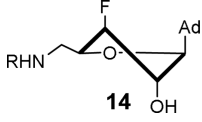
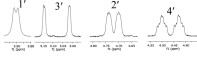
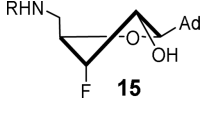
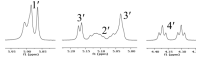
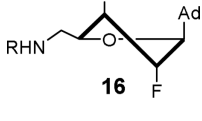
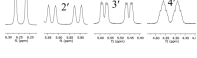
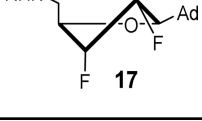
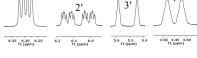


**Figure 3.** Mean plasma concentration versus time curves after single *p.o.* ( $25 \text{ mg.kg}^{-1}$ ) and *i.v.* ( $2.5 \text{ mg.kg}^{-1}$ ) bolus administration of **10** and **12-17** to rats. Error bars represent standard deviation of the mean ( $n = 3$ ).



Table 1

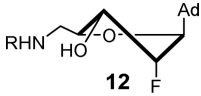
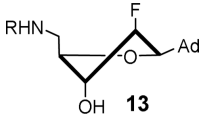
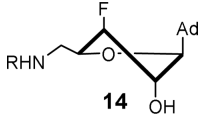
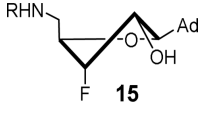
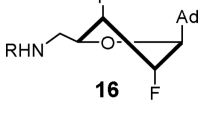
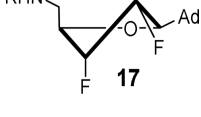
Relationship between bioactivity and sugar ring conformation of inhibitors 12–17

Compound	<sup>1</sup> H NMR resonances of sugar protons <sup>a</sup>	%N (lit.) <sup>b</sup>	K <sub>i</sub> <sup>PPP</sup> (nM)	MIC (μM)
 <b>12</b>		65 (67) <sup>33a,b</sup>	1.7 ± 0.1	0.19
 <b>13</b>		39 (36) <sup>33b</sup>	1.8 ± 0.1	0.15
 <b>14</b>		88 (93) <sup>10</sup>	0.6 ± 0.3	0.60
 <b>15</b>		0 (2) <sup>26a</sup>	6.4 ± 0.3	2.3
 <b>16</b>		96	1.4 ± 0.3	0.78
 <b>17</b>		20 (20) <sup>26a</sup>	139 ± 10	37

<sup>a</sup>NMR was obtained in DMSO-D<sub>6</sub>, peaks are in expansion view and are ordered according to their chemical shifts<sup>b</sup> literature values for free nucleosides are reported in Parenthesis.

Table 2

*In Vivo* pharmacokinetic parameters of inhibitors in female Sprague-Dawley rats ( $n = 3$ , mean  $\pm$  SD) following a single intravenous (*i.v.*) and oral (*p.o.*) administration

Compound	<i>i.v.</i> PK parameters <sup>a</sup>			<i>p.o.</i> PK parameters <sup>b</sup>		
	AUC <sub>0-inf</sub> ( $\mu\text{g}\cdot\text{min}\cdot\text{mL}^{-1}$ )	CL ( $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ )	$t_{1/2}$ (min)	$C_{\text{max}}$ ( $\mu\text{g}\cdot\text{mL}^{-1}$ )	AUC <sub>0-inf</sub> ( $\mu\text{g}\cdot\text{min}\cdot\text{mL}^{-1}$ )	F (%)
10 <sup>c</sup>	519 $\pm$ 115	4.9 $\pm$ 0.9	11.2 $\pm$ 2.2	0.40 $\pm$ 0.20	66 $\pm$ 41	1.3 $\pm$ 0.7
11 <sup>c</sup>	304 $\pm$ 60	8.4 $\pm$ 1.9	10.6 $\pm$ 0.7	0.70 $\pm$ 0.07	100 $\pm$ 14	3.5 $\pm$ 0.2
 12	1040 $\pm$ 330	2.5 $\pm$ 0.9	32.5 $\pm$ 7.4	0.46 $\pm$ 0.30	187 $\pm$ 11	1.8 $\pm$ 0.9
 13	913 $\pm$ 65	2.7 $\pm$ 0.2	31.7 $\pm$ 1.9	1.09 $\pm$ 0.03	422 $\pm$ 70	4.7 $\pm$ 1.1
 14	366 $\pm$ 33	6.9 $\pm$ 0.3	17.4 $\pm$ 1.4	0.64 $\pm$ 0.22	84 $\pm$ 12	2.3 $\pm$ 0.4
 15	807 $\pm$ 78	3.1 $\pm$ 0.3	37.0 $\pm$ 1.7	0.92 $\pm$ 0.04	266 $\pm$ 2	3.5 $\pm$ 0.2
 16	312 $\pm$ 54	8.5 $\pm$ 1.6	17.2 $\pm$ 1.3	0.97 $\pm$ 0.42	362 $\pm$ 85	11.4 $\pm$ 0.3
 17	10100 $\pm$ 2200	0.24 $\pm$ 0.06	267 $\pm$ 36	5.99 $\pm$ 0.56	4730 $\pm$ 540	5.3 $\pm$ 1.3

AUC<sub>0-inf</sub>: area under the plasma concentration-time curve from time 0 to infinity, CL: clearance,  $t_{1/2}$ : terminal elimination half-life,  $C_{\text{max}}$ : maximum plasma concentration, F: relative oral bioavailability calculated as follows  $F = 100 \times [(AUC_{p.o.} \times D_{i.v.}) / (AUC_{i.v.} \times D_{p.o.})]$ .

<sup>a</sup> *i.v.* dose ( $D_{i.v.}$ ) = 2.5 mg/kg

<sup>b</sup> *p.o.* dose ( $D_{p.o.}$ ) = 25 mg/kg

<sup>c</sup> reference 34.