A Concise Synthesis of *β*-Asparaginyladenylate†

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Abstract: Stable analogues of acyladenylate intermediates, such as *N*-acylphosphoramidates, are useful probes of tRNA aminoacylation and enzyme mechanism, and have potential application as enzyme inhibitors. We now report a concise, "one-pot" synthesis of *β*-asparaginyladenylate using a novel coupling protocol that yields the target *N*-acylphosphoramidate in three reactions from readily available precursors. This simple synthetic procedure may represent a general approach for the preparation of functionalized *N*-acylphosphoramidates from amides that do not undergo coupling under the conditions of existing literature protocols.

Stable analogues of acyladenylate intermediates are useful probes of tRNA aminoacylation and enzyme mechanism and have potential application as enzyme inhibitors. Asparagine synthetase (AS),[1] a glutamine-dependent amidotransferase[2] that synthesizes asparagine from aspartic acid, glutamine, and ATP, appears to be intimately linked with progression through the cell cycle[3] and cellular responses to amino acid starvation.[4] Our recent studies on the structure and mechanism of the glutamine-dependent AS (AS−B) encoded by the *asnB* gene in *Escherichia coli* have set the stage for rational approaches to discovering AS inhibitors,[5] which represent novel potential drugs for treating leukemia.[1] The overall transformation catalyzed by glutamine-dependent AS takes place via two half-reactions that take place in two independent active sites,[5] one of which catalyzes the formation of *β*-aspartyl-AMP (*β*AspAMP) **1** (Chart 1). We therefore wished to examine whether stable analogues of *β*AspAMP **1**, which could be prepared using simple synthetic approaches, might behave as potent, selective AS inhibitors. *β*-Asparaginyladenylate **2** was therefore selected for our initial experiments, because of recent progress in methods for the preparation of *N*-acylphosphoramidates,[6,7] and the presence of the *N*-acylphosphoramidate moiety in the antibiotics agrocin-84 **3** and phosmidosine **4**.[8] We now report a concise, "one-pot" preparation of *β*-asparaginyladenylate **2** using a novel coupling protocol that yields the synthetic target in three steps.

Several strategies for the preparation of functionalized *N*-acylphosphoramidates have been reported.[6] Of these, the most general is phosphitylation of primary amides using protected *N*,*N*-chlorodiisopropylphosphoroamidites in the presence of base.[7] Unfortunately, all efforts to couple suitably protected phosphorimidite derivatives of adenosine with the L-asparagine derivative **5**,[9] following literature protocols in which 5-(4-nitrophenyl)-1*H*-tetrazole was used as an activator,[10] failed to give the desired *N*-acylphosphoramidite product. We note, however, that experiments published after these unsuccessful efforts have demonstrated that similar coupling reactions may require the use of alternative activating agents to 5-(4-nitrophenyl)-1*H*-tetrazole, such as 5-(3,5-dinitrophenyl)-1*H*-tetrazole,[7a] in order to avoid the use of extended reaction times and competing hydrolysis of the newly formed P−N bond. In light of the problems with our particular coupling reaction, we elected to employ more reactive phosphine derivatives to prepare intermediates in the synthesis of *β*-asparaginyladenylate **2**. In particular, benzyloxydichlorophosphine **6**, prepared by reaction of PCl_3 with benzyl alcohol at −78 °C in pyridine,[11] appeared suitable for the preparation of functionalized

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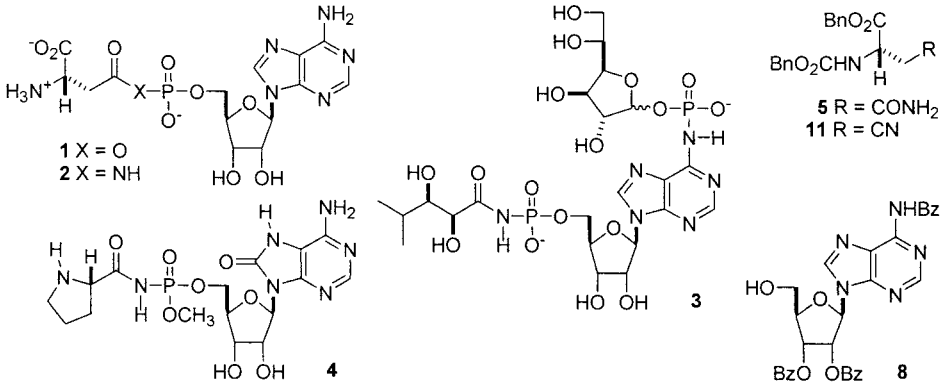
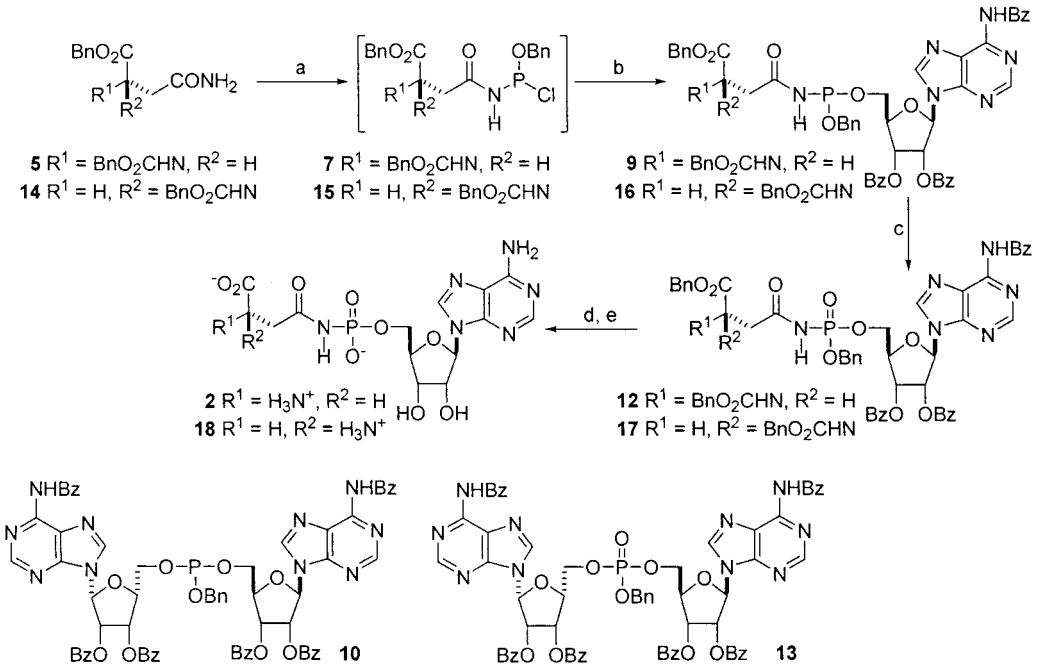
Chart 1



Scheme 1[a]



[a] Reagents and conditions: Bn = $PhCH_2$, Bz = PhCO. (a) $BnOPCl_2$ **6**, $(^iPr)_2NEt$, CH_2Cl_2, −30 °C then RT; (b) **8**, $(^iPr)_2NEt$, CH_2Cl_2, −30 °C, then RT; (c) ^{t}BuOOH, CH_2Cl_2, 0 °C; (d) 10% Pd/C, 1,4-cyclohexadiene, MeOH/EtOH; (e) conc NH_4OH, RT.

phosphoramidites.[11b] As anticipated, this reagent could be employed in a novel "one-pot" procedure to prepare the protected *N*-acylphosphoramidite **9** (Scheme 1), an advanced precursor of *β*-asparaginyladenylate **2**. Thus, the L-asparagine derivative **5** was slowly added to a solution of the electrophilic phosphine **6** in CH_2Cl_2 at low temperature in the presence of diisopropylethylamine. Presumably this procedure yields the monochlorophosphine intermediate **7**, which is sufficiently deactivated to prevent further reaction with primary amide **5**. After this initial step was complete, *N*-benzoyl-2′,3′-di-*O*-benzoyladenosine **8**[12] and additional diisopropylethylamine were added to the solution, allowing the more reactive primary alcohol to couple with **7** to yield **9** (Scheme 1). The ^{31}P NMR spectrum of the crude material confirmed that the coupling reaction had taken place to give **9**.[7a] Remarkably,[13] this highly functionalized phosphine derivative was sufficiently stable for its partial purification from a byproduct that was assigned to be **10** (based on the observation of a single ^{31}P resonance at 141.7 ppm) and the known nitrile **11** (Chart 1).[14] The symmetrical phosphine **10** is presumably formed by reaction of two molecules of **8** with unreacted benzyloxydichlorophosphine **6**, implying that there was incomplete coupling of the amide **5** under our initial conditions. Having established a simple "one-pot" procedure for the preparation of **9**, this compound was easily oxidized using *t*-BuOOH in anhydrous CH_2Cl_2 at 0 °C to give compound **11** in 88% yield from **9**. Using optimized reaction conditions, the

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(13) Similar P(III) intermediates in which the benzyl group is replaced by either a 2-cyanoethylene or 2-trimethylsilyl have been reported to be too unstable for purification by silica chromatography.[7a] In our experiments employing benzyloxy-substituted P(III) intermediates, we have found that their partial purification can be accomplished by rapidly eluting the crude reaction mixture through a short plug of silica using $CHCl_3$/EtOAc.

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stable *N*-acylphosphoramidate **12** could be obtained from **5** in 47% yield, being easily purified from the symmetrically coupled contaminant **13** by column chromatography. We note that the oxidation reaction can, in principle, be performed without isolation of the intermediate *N*-acylphosphorimidate **9**, further simplifying our synthetic approach.

So as to minimize formation of byproducts **10** and **11**, we carried out a series of studies in which solvent, temperature, and base concentration were systematically varied so as to evaluate their effects on the yield of **9**, **10**, and **11**, in our "one-pot" synthetic procedure. In these experiments, since the products were separated by column chromatography, the mixture from the initial coupling reaction was oxidized to **12** and **13** prior to characterization. These studies revealed that the yield of the coupled product **9** was highly dependent upon the ratio of diisopropylethylamine to amide **5** and the reaction temperature at which the amide **5** was reacted with benzyloxydichlorophosphine **6**. Dehydration of **5** to give nitrile **11**, presumably via elimination from an *O*-phosphitylated amide that is the likely kinetic product when the primary amide reacts with benzyloxydichlorophosphine **6**,[15] is a significant side reaction in the presence of excess base and at reaction temperatures higher than RT. Hence, 1.08 equiv of diisopropylethylamine in CH_2Cl_2 at −30 °C to RT gave a better yield of **9** (∼54% from **5**) and reduced amounts of nitrile **11** (42% from **5**). A number of aprotic solvents in which the substrate is soluble, such as CH_2Cl_2, THF, 1,4-dioxane, and DME, were found to be suitable in the coupling reaction. The sequence in which the reagents are added in this "one-pot" synthesis was, however, important to obtaining good yields of the coupled derivative **9**. For example, alcohol **8** must be added to the reaction mixture prior to addition of diisopropylethylamine in the second stage of the coupling procedure. If the order of reagent addition is reversed, then a significant increase in the yield of the nitrile **11** is observed.

Catalytic hydrogenation and subsequent ammonolysis to remove protecting groups afforded the target *N*-acylphosphoramidate **2** in 40% yield (for two steps) after RP-HPLC purification. Cleavage of the C−N or P−N bond by ammonia to yield asparagine was not observed in the final deprotection reaction, supporting the hypothesis that **2** will be an effective AS inhibitor. A similar sequence of reactions, using the N-protected D-asparagine **14** derivative as the starting amide, was used to obtain the diastereoisomeric *N*-acylphosphoramidate **18** in 14% overall yield. This concise "one-pot" approach offers some potential advantages over existing methods for the preparation of *N*-acylphosphoramidates. First, benzyloxydichlorophosphine **6** is sufficiently reactive at low temperatures for the coupling to be performed using reduced reaction times thereby minimizing breakdown of the P−N bond under the coupling conditions. Second, the asparagine derivative **5** appears to be the most highly functionalized amide that has been coupled to form an *N*-acylphosphoramidite derivative of adenosine, suggesting that this procedure may allow an expansion in the variety of functionalized amides that can be used to prepare *N*-acylphosphoramidates. Finally, the presence of the benzyloxy group as a substituent on phosphorus not only simplifies the final deprotection strategy, but also appears to yield P(III) derivatives that are sufficiently stable for purification using column chromatography.

(15) A similar elimination reaction is observed during the acylation of primary amides with acid chlorides in the presence of excess base. For more details, see: Davidson, D.; Skovronek, H. *J. Am. Chem. Soc.* **1958**, *80*, 376−379.

Experimental Section

Melting points were recorded using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured using a Polyscience Model SR-6 polarimeter. ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectra were obtained at 300, 75.4, 121, and 282.2 MHz, respectively. For ^{1}H and ^{13}C, chemical shifts are reported in ppm (δ) downfield of tetramethylsilane as an internal reference (δ 0.0), except for measurements in D_2O for which acetone (δ 2.1) was employed as an external standard. In the case of ^{31}P, 85% H_3PO_4 was used as an external standard (δ 0.0), and $CFCl_3$ was used as an internal standard (δ 0.0) for ^{19}F NMR spectra. Splitting patterns are abbreviated as follows: s, singlet, d, doublet, t, triplet, q, quartet, and m, multiplet. EI, CI and FAB mass spectra were recorded on a Finnegan MAT 25Q (high resolution) spectrometer. Methane was generally employed in obtaining CI mass spectra. Analytical thin-layer chromatography (TLC) was performed on silica gel 60F-254 plates. Flash chromatography was performed using standard procedures[16] on Kieselgel (230−400 mesh), and size exclusion chromatography was performed on Sephadex G-10 resin. Semipreparative reverse-phase (C_{18}) HPLC was carried out on a RAININ system using a DYNAMAX-60A column (2 cm × 30 cm). All reagents were purchased from Aldrich or Fisher Scientific, and were used without further purification except for chromatography solvents, which were distilled before use. Moisture sensitive reactions were carried out under a nitrogen, or argon, atmosphere in glassware that was flame-dried with an inert gas sweep. THF was freshly distilled from sodium benzophenone ketyl before use.

***O*-(6-*N*-Benzoyl-2′,3′-*O*-dibenzoyladenosine-5′-*O*-yl)-*N*-(*O*-benzyl-*N*-benzyloxycarbonyl-L-asparaginyl)-*O*-benzyloxyphosphoramidite (9).** The protected asparagine derivative **5** (340 mg, 0.96 mmol) dissolved in anhydrous CH_2Cl_2 (10 mL) was added to a cooled solution of benzyloxydichlorophosphine **6** (880 mg, 0.96 mmol) in anhydrous CH_2Cl_2 (7 mL) at −30 °C. Diisopropylethylamine (0.18 mL, 1.04 mmol) was then added, and the resulting mixture was stirred for 1 h at −30 °C, before being warmed to RT. After stirring for a further 6 h, the solution was recooled to −30 °C before the sequential addition of the protected adenosine derivative **8** (0.69 g, 1.19 mmol) in anhydrous CH_2Cl_2 (10 mL) and DIPEA (0.36 mL, 2.08 mmol). The resulting solution was slowly warmed to RT and stirred for 4 h, before being concentrated under reduced pressure. The residue was then purified by chromatography (eluant: 3:1 $CHCl_3$/EtOAc to 1:2 $CHCl_3$/EtOAc) giving the known α-cyanoalanine derivative **11** as the first material eluted from the column: 60 mg, 18% (42% based on recovered **5**); mp 105−106 °C (lit.[14a] mp 106−107 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.88 (1 H, dd, J = 17.1, 5.1 Hz), 2.98 (1 H, dd, J = 17.1, 5.1 Hz), 4.57 (1 H, m), 5.10 (2 H, s), 5.22 (2 H, s), 5.86 (1 H, d, J = 6.6 Hz), 7.34 (10 H, m); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 21.56 (t), 50.63 (d), 67.43 (t), 68.39 (t), 115.92 (s), 128.08 (d), 128.31 (d), 128.50 (d), 128.52 (d), 128.68 (d), 128.81 (d), 134.31 (s), 135.60 (s), 155.43 (s), 168.45 (s); MS (FAB) 339 (MH^+, 6), 281 (9), 207 (18), 147 (51), 91 (100); exact mass calcd for MH^+ $C_{19}H_{19}N_2O_4$ requires 339.1345, found 339.1346 (FAB).

Further elution yielded the desired coupled product **9** as a white solid (250 mg, ∼54% based on ^{31}P analysis of this material and the amount of recovered **5**) contaminated with a small amount of the symmetrically coupled material **10**. This material was sufficiently pure for use in the next step, and further efforts to separate these compounds resulted in extensive decomposition. Continued elution gave a pure sample of **10** as a white foam: 310 mg, 40% (58% based on recovered **8**); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.29 (2 H, m), 4.39 (2 H, m), 4.69 (2 H, m), 5.00 (2 H, d, $^3J_{HP}$ = 8.7 Hz), 6.06 (2 H, m), 6.20 (2 H, dd, J = 12.3, 6.3 Hz), 6.67 (2 H, d, J = 6.3 Hz), 7.20−7.53 (23 H, m), 7.76 (4 H,

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m), 7.93 (8 H, m), 8.60 (1 H, s), 8.69 (1 H, s), 8.74 (1 H, s), 8.75 (1 H, s), 9.41 (1 H, s), 9.49 (1 H, s); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 62.10 (dt, $^2J_{CP}$ = 10.5 Hz), 62.22 (dt, $^2J_{CP}$ = 10.5 Hz), 64.86 (dt, $^2J_{CP}$ = 10.5 Hz), 71.76 (d), 71.93 (d), 74.18 (d), 74.49 (d), 82.53 (dt, $^3J_{CP}$ = 4.7 Hz), 82.71 (dt, $^3J_{CP}$ = 4.7 Hz), 85.45 (d), 85.59 (d), 123.08 (s), 127.46 (d), 127.89 (d), 127.92 (d), 128.09 (d), 128.23 (d), 128.37 (d), 128.43 (d), 128.52 (d), 129.62 (d), 132.48 (s), 133.49 (s), 137.20 (s), 137.22 (s), 141.33 (d), 141.62 (d), 149.51 (s), 151.81 (d), 151.90 (d), 152.66 (s), 164.81 (s), 164.91 (s), 165.20 (s); ^{31}P NMR ($CDCl_3$, 121 MHz) δ 141.17 (s); MS (FAB) 1317 (MNa^+, 2), 1295 (MH^+, 1), 281 (10), 240 (38), 201 (100); exact mass calcd for MNa^+ $C_{69}H_{55}N_{10}O_{15}PNa$ requires 1317.3484, found 1317.3464 (FAB).

Samples of the starting materials **8** (210 mg) and **5** (190 mg) were also recovered from the column.

***O*-(6-*N*-Benzoyl-2′,3′-*O*-dibenzoyladenosine-5′-*O*-yl)-*N*-(*O*-benzyl-*N*-benzyloxycarbonyl-L-asparaginyl)-*O*-benzyloxyphosphoramidate (12).** The *N*-acylphosphoramidite **9** (369 mg, 0.34 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and degassed with Ar before being cooled to 0 °C. A solution of *t*-BuOOH (0.34 mL of a 5 M solution in anhydrous CH_2Cl_2, 5 equiv) was added slowly at 0 °C, and the reaction mixture was stirred for 15 min, after which time TLC analysis showed complete consumption of starting material. The solution was then concentrated under reduced pressure, and the residue was purified by chromatography (eluant: 50:1 $CHCl_3$/*i*-PrOH) to afford *N*-acylphosphoramidate **12** as a white foam: 330 mg, 88%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.00 (2 H, m), 4.51−5.17 (10 H, m), 6.00 (1 H, m), 6.10 (1 H, m), 6.55 (1 H, d, J = 8.4 Hz), 6.61 (1 H, d, J = 6.2 Hz), 6.66 (1 H, d, J = 6.0 Hz), 7.15−7.55 (24 H, m), 7.85 (2 H, m), 7.97 (4 H, m), 8.74 (1 H, s), 8.79 (1 H, s), 9.73 (1 H, br. s); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 38.49 (t), 50.41 (d), 66.63 (dt, $^2J_{CP}$ = 13.0 Hz), 66.64 (t), 67.11 (t), 69.89 (dt, $^2J_{CP}$ = 5.6 Hz), 71.49 (d), 74.18 (d), 81.81 (d, $^2J_{CP}$ = 7.0 Hz), 85.20 (d), 122.76 (s), 127.62 (d), 127.72 (d), 127.76 (d), 127.85 (d), 127.97 (d), 128.05(d), 128.08 (d), 128.19 (d), 128.22 (d), 128.29 (d), 128.33 (d), 128.38 (d), 129.63 (d), 129.65 (d), 132.40 (s), 133.58 (s), 134.74 (s), 134.83 (s), 135.06 (d, $^2J_{CP}$ = 6.0 Hz), 135.91 (s), 141.76 (d), 149.63 (s), 151.71 (d), 152.67 (s), 156.02 (s), 164.80 (s), 165.11 (s), 170.88 (s), 170.95 (s), 172.44 (d, $^2J_{CP}$ = 4.5 Hz); ^{31}P NMR ($CDCl_3$, 121 MHz) δ −1.44, −1.69 (s); MS (FAB) 1110 (MNa^+, 4), 240 (22), 201 (61), 105 (80), 91 (100); exact mass calcd for MNa^+ $C_{57}H_{50}N_7O_{14}PNa$ requires 1110.3051, found 1110.3077 (FAB).

Continued elution gave a sample of the symmetrically coupled product **13** (formed by oxidation of a small amount of **10** in the starting material) as a white foam; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.52 (4 H, m), 4.69 (2 H, m), 5.18 (2 H, d, $^3J_{HP}$ = 9.6 Hz), 6.08 (2 H, m), 6.19 (2 H, m), 6.62 (2 H, d, J = 6.0 Hz), 7.24−7.55 (23 H, m), 7.72−7.99 (12 H, m), 8.52 (1 H, s), 8.54 (1 H, s), 8.75 (1 H, s), 8.77 (1 H, s), 9.31 (1 H, br. s), 9.33 (1 H, br. s); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 66.58 (br. dt), 70.15 (dt, $^2J_{CP}$ = 5.7 Hz), 71.02 (d), 71.06 (d), 73.83 (d), 74.11 (d), 81.28 (dt, $^3J_{CP}$ = 4.8 Hz), 81.38 (dt, $^3J_{CP}$ = 4.8 Hz), 85.86 (d), 123.19 (s), 123.26 (s), 127.79 (d), 127.94 (d), 127.97 (d), 127.99 (d), 128.16 (d), 128.25 (d), 128.29 (d), 128.32 (d), 128.37 (d), 128.40 (d), 128.47 (d), 128.62 (d), 129.52 (d), 132.35 (s), 133.36 (s), 133.45 (s), 134.91 (s), 134.99 (s), 141.54 (d), 149.55 (s), 151.62 (d), 152.50 (s), 152.58 (s), 164.69 (s), 164.76 (s), 164.82 (s), 164.87 (s), 165.01 (s), 165.04 (s); ^{31}P NMR ($CDCl_3$, 121 MHz) δ −0.50; MS (FAB) 1333 (MNa^+, 3), 240 (28), 201 (100); exact mass calcd for MNa^+ $C_{69}H_{55}N_{10}PO_{16}Na$ requires 1333.3433, found 1333.3477 (FAB).

***O*-(Adenosine-5′-*O*-yl)-*N*-(L-asparaginyl)phosphoramidate Trifluoroacetate (2)**. The fully protected *N*-acylphosphoramidate **12** (345 mg, 0.32 mmol) was suspended in a solution of MeOH (6 mL) and EtOH (2 mL) containing 1,4-cyclohexadiene (0.91 mL, 9.6 mmol). After the addition of 10% Pd/C (960 mg), the reaction mixture was vigorously stirred at 30 °C for 4 h, before the addition of further portions of 10% Pd/C (400 mg) and 1,4-cyclohexadiene (0.60 mL). Stirring was then continued for another 3 h at this temperature. Distilled water (5 mL) was then added, and the suspension was filtered through Celite. The solid residues were washed well using 50% aq MeOH, and the filtrate was combined with the washings. Removal of the solvent then gave a white solid that was dissolved in concentrated NH_4OH (20 mL) and freshly distilled 1,4-dioxane (20 mL). This solution was stirred for 6 h at RT, under an inert N_2 atmosphere, before being concentrated to dryness under reduced pressure. The resulting white solid was mixed with water (5 mL) and filtered through paper to remove insoluble material. Concentration of the filtrate gave a crude product that was purified on Sephadex G-10 (eluant: H_2O) to afford the ammonium salt of the target *N*-acylphosphoramidate **2**. Further purification by reverse phase HPLC with gradient elution (C_{18} column: solvent flow rate 2 mL/min; from 100:0 H_2O: MeOH + 0.1% TFA to 85:15 H_2O: MeOH + 0.1% TFA over a period of 65 min), with monitoring 260 nm, gave the trifluoroacetate salt of **2** (ret time: 104 min) as a white solid after freeze-drying overnight: 67 mg, 40%; ^{1}H NMR (D_2O, 300 MHz) δ 2.78 (2 H, m), 3.82 (2 H, m), 3.99 (1 H, t, J = 5.1 Hz), 4.04 (1 H, m), 4.11 (1 H, m), 4.40 (1 H, m), 5.83 (1 H, m), 8.10 (1 H, s), 8.30 (1 H, s); ^{13}C NMR (D_2O, 75.4 MHz) δ 48.59 (t), 64.20 (dt, $^2J_{CP}$ = 4.8 Hz), 69.80 (d), 74.14 (d), 83.38 (d), 83.50 (d), 87.45 (d), 115.72 (q, $^1J_{CF}$ = 290.0 Hz), 117.95 (s), 141.97 (d), 144.11 (s), 147.81 (d), 149.31 (s), 162.30 (q, $^2J_{CF}$ = 35.4 Hz), 170.46 (s), 172.08 (s); ^{31}P NMR (D_2O, 121 MHz) δ −5.17; ^{19}F NMR (D_2O, 282.2 MHz) δ −76.22; MS (FAB) 462 (MH^+, 3), 217 (12), 149 (14), 138 (27), 136 (74), 133 (100); exact mass calcd for MH^+ $C_{14}H_{21}N_7O_9P$ requires 462.1138, found 462.1139 (FAB).

***O*-(6-*N*-Benzoyl-2′,3′-*O*-dibenzoyladenosine-5′-*O*-yl)-*N*-(*O*-benzyl-*N*-benzyloxycarbonyl-D-asparaginyl)-*O*-benzyloxyphosphoramidate (17).** The protected derivative of D-asparagine **14** (782 mg, 2.2 mmol) was coupled to the protected adenosine **8** (1.65 g, 2.8 mmol) and the activated phosphine **6** (0.36 mL, 2.2 mmol), using our general "one-pot" procedure, and oxidation of the resulting *N*-acylphosphoramidite intermediate **16** was accomplished by treatment with *t*-BuOOH to give the protected *N*-acylphosphoramidate **17** as a white foam: 711 mg, 30% (based on **14**); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.00 (2 H, m), 4.57 (2 H, m), 4.80−5.15 (6 H, m), 6.00 (2 H, m), 6.13 (1 H, m), 6.26 (1 H, m), 6.60 (1 H, d, J = 6.6 Hz), 6.68 (1 H, d, J = 6.6 Hz), 6.70 (1 H, d, J = 7.2 Hz), 7.10−7.54 (24 H, m), 7.80−7.99 (6 H, m), 8.81 (1 H, s), 8.82 (1 H, s), 9.78 (1 H, br s); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 38.89 (t), 50.29 (d), 66.48 (dt, $^2J_{CP}$ = 10.7 Hz), 66.96 (t), 69.52 (t), 71.43 (dt, $^2J_{CP}$ = 8.9 Hz), 73.83 (d), 74.03 (d), 81.79 (d), 84.84 (d), 122.68 (s), 127.38 (d), 127.52 (d), 127.71 (d), 127.89 (d), 127.93 (d), 128.02 (d), 128.08 (d), 128.12 (d), 128.26 (d), 128.39 (d), 129.51 (d), 132.21 (s), 132.32 (s), 133.47 (s), 134.74 (s), 135.07 (s), 135.89 (s), 141.65 (d), 149.61 (s), 151.65 (d), 152.61 (s), 156.16 (s), 164.63 (s), 164.71 (s), 164.99 (s), 170.87 (s), 172.42 (s); ^{31}P NMR ($CDCl_3$, 121 MHz) δ −1.06, −2.18; MS (FAB) 1088 (MH^+, 8), 240 (14), 201 (98), 105 (26), 91 (100); exact mass calcd for MH^+ $C_{57}H_{51}N_7PO_{14}$ requires 1088.3232, found 1088.3220 (FAB).

***O*-(Adenosine-5′-*O*-yl)-*N*-(D-asparaginyl)phosphoramidate Trifluoroacetate (18).** The protected derivative **17** (230 mg, 0.2 mmol) was converted to *N*-acylphosphoramidate **18** using an identical procedure to that described for the preparation of **2** from **12**. The trifluoroacetate salt of the desired compound was obtained as a white solid after C_{18} RP-HPLC purification: 51 mg, 45%; ^{1}H NMR (D_2O, 300 MHz) δ 2.78 (2 H, m), 3.81 (2 H, m), 4.01 (2 H, m), 4.11 (1 H, t, J = 4.5 Hz), 4.36 (1 H, dd, J = 5.1, 4.8 Hz), 5.82 (1 H, d, J = 4.8 Hz), 8.08 (1 H, s), 8.29 (1 H, s); ^{13}C NMR (D_2O, 75.4 MHz) δ 48.54 (t), 64.14 (dt, $^2J_{CP}$ = 5.0 Hz), 69.67 (d), 74.25 (d), 83.26 (d), 83.39 (d), 87.59 (d), 115.64 (q, $^1J_{CF}$ = 290.0 Hz), 117.92 (s), 142.00 (d), 144.06 (s), 147.71 (d), 149.28 (s), 162.17 (q, $^2J_{CF}$ = 35.6 Hz), 170.28 (s), 172.08 (s); ^{31}P NMR (D_2O, 121 MHz) δ −5.13; ^{19}F NMR (D_2O, 282.2 MHz) δ −76.23; MS (FAB) 462 (MH^+, 6), 263 (14), 217 (72), 136 (42), 133 (13); exact mass calcd for MH^+ $C_{14}H_{21}N_7PO_9$ requires 462.1138, found 462.1150 (FAB).

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra of **2**, **12**, **13**, **17**, and **18**, ^{19}F spectra of **2** and **18**, together with ^{1}H and ^{13}C spectra for **8** and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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