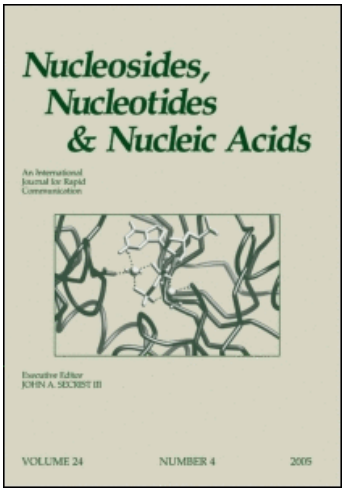

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PREPARATION OF BASE-DEUTERATED 2'-DEOXYADENOSINE NUCLEOSIDES AND THEIR SITE-SPECIFIC INCORPORATION INTO DNA

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PREPARATION OF BASE-DEUTERATED 2′-DEOXYADENOSINE NUCLEOSIDES AND THEIR SITE-SPECIFIC INCORPORATION INTO DNA

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ABSTRACT

We report a four-step synthesis of 2′-deoxy-2-deuteroadenosine from 2′-deoxyadenosine in 38% overall yield. The more accessible 2′-deoxy-8-deuteroadenosine was also prepared and incorporated into DNA by automated solid phase synthesis (80% deuterium) using N^6-benzoyl-2′-deoxy-8-deuteroadenosine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) in combination with acetyl-protected deoxycytidine and phenoxyacetyl-protected purine phosphoramidites.

INTRODUCTION

It is widely accepted that the mechanistic understanding of biomolecular function is based on structure. However, it has become clear that many reported structures represent ground states that may bear little resemblance to the structures of the transition states. Therefore, structural information about ground states needs to be augmented with knowledge about the movements of functionally important residues. Solid state deuterium NMR is a valuable tool to study motions of specific sites in nucleic acids[1–3].

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For example, solid state deuterium NMR was used to detect large amplitude motions in the sugar-phosphate backbone at the GC-AT duplex junction of the *EcoR1* restriction site 5′-d(CGAATTCG)$_2$, precisely where this enzyme cuts[3]. This cleavage reaction can be suppressed by base-methylation at position 5 of 2′-deoxycytidine (dC) and interestingly, the methylation reduces the amplitudes of motion of the sugar-phosphate backbone[3]. It is possible that the origin of the selectivity of *EcoR1* for the non-methylated sequence resides in the change of conformational flexibility at the cleavage site.

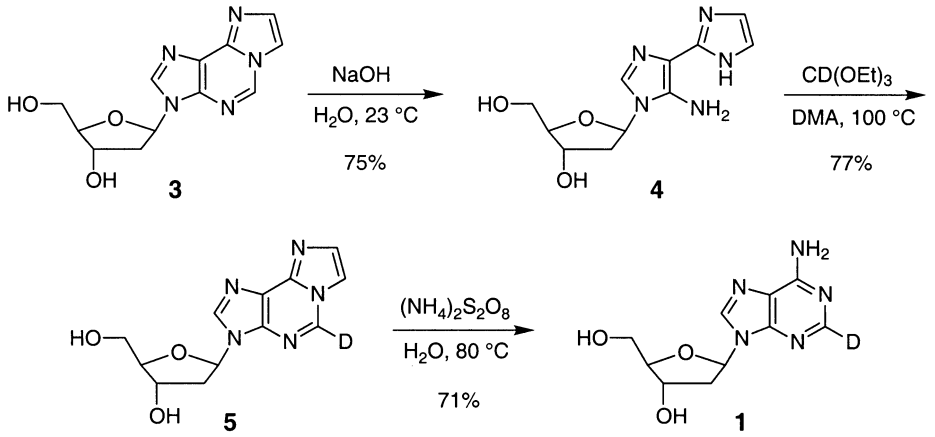
We are interested in studying the role of dynamics in the interactions of proteins with DNA. The study of DNA dynamics by solid state deuterium NMR requires site-specific incorporation of deuterium-labeled nucleotides. The 5′- and 2′-positions of thymidine (T), dC and 2′-deoxyadenosine (dA) have been used for sugar-labeling[4–6] and the 5- and 6-positions have been used for base-labeling of dC and T[1]. The methyl groups of T and 5-methyl dC have also been deuterated[3]. To extend the studies of nucleic acid dynamics by solid state deuterium NMR, we intend to study the base-motions of dA in DNA. The required incorporation of deuterium into either the 2- or 8-position of the adenine base is outlined here.

We focused initially on the 2-position of dA because the proton in the 8-position can exchange with protic solvents[1]. There has been only one report of the synthesis of 2′-deoxy-2-deuteroadenosine (**1**), by oxidation of 2′-deoxyadenosine, followed by ring-opening and subsequent ring-closure using a deuterated derivative of formic acid[7]. However, six synthetic steps were required, giving **1** in only 16% overall yield. Another ring-opening/ring-closure strategy has been reported for the synthesis of 2-deuteroadenosine in four steps via 1-N^6-ethenoadenosine, yielding the desired product in 55% overall yield[8]. We describe here the application of this approach to the preparation of **1** and the corresponding phosphoramidite for its site-specific incorporation into DNA.

Given the synthetic effort required for the preparation of **1** we have also investigated the incorporation of 2′-deoxy-8-deuteroadenosine (**2**) into DNA. Compound **2** can be readily prepared by a proton-exchange reaction in the presence of triethylamine[1]. While this represents an easy route to base-deuterated 2′-deoxyadenosine, the deuteron in the 8-position can proton-exchange with solvent during deprotection and purification of the DNA. We show that this strategy, when coupled with the use of phenoxyacetyl-protected purine nucleosides and acetyl-protected dC, allows incorporation of **2** into DNA with 80% incorporation of deuterium.

RESULTS AND DISCUSSION

Our approach for the synthesis of **1** was to use a ring-opening/ring-closing strategy, similar to that reported for 2-deuteroadenosine[8].

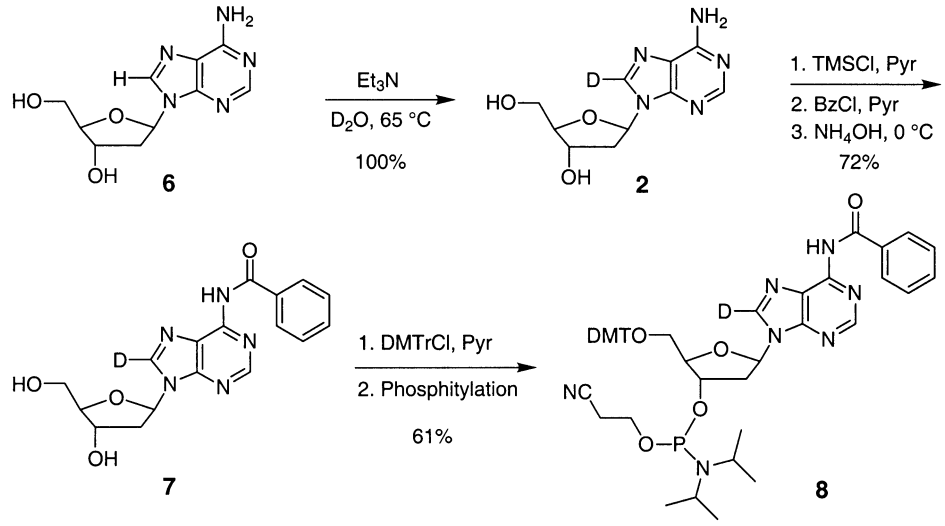


Scheme 1. Synthesis of 2′-deoxy-2-deuteroadenosine.

The synthetic route (Scheme 1) starts with 1,N^6-ethenodeoxyadenosine (**3**), prepared by the reaction of 2′-deoxyadenosine with chloroacetaldehyde using a slight modification of a previously published protocol[9]. Reaction of **3** with NaOH at 23°C resulted in ring opening to yield **4**. We found the optimal concentration of NaOH to be 0.8 M, with a reaction time of 3 h. Reaction of **4** with 1,1,1-triethoxy-1-deuteromethane[10] in *N*,*N*-dimethylacetamide (DMA) resulted in ring closure with incorporation of deuterium (94%) to give **5**. The last step entailed the removal of the etheno-modification using ammonium persulfate[8]. It is critical to maintain the pH close to neutral during this reaction to avoid depurination. We therefore used a high concentration (1 M) of phosphate buffer. The overall yield for the four-step synthesis of **1** from 2′-deoxyadenosine was 38%, a considerable improvement over the six-step synthesis (16% overall yield) reported by Fujii and co-workers[7]. Compound **1** was subsequently converted to a protected phosphoramidite as described in Scheme 1 for 8-deutero-2′-deoxyadenosine.

We also explored the possibility of using 8-deutero-2′-deoxyadenosine (**2**) for solid state deuterium NMR studies. Compound **2**, which has been incorporated into DNA for the chemical shift assignment of triplet repeats,[1] can be conveniently prepared in one step that does not require purification. This approach required using ND_4OD, instead of NH_4OH, for the deprotection of the oligomer. In the solid state NMR experiments, however, the deuterium atom functions as the reporter. Therefore, the ND_4OD deprotection protocol cannot be used due to incorporation of deuterium into other nucleotides. We investigated how much deuterium would be lost by proton-exchange during the synthesis and incorporation of **2** into DNA using non-deuterated reagents.

Compound **2** was prepared by incubation of 2′-deoxyadenosine (**6**) in D_2O in the presence of triethylamine (Scheme 2)[1]. Compound **2** was



Scheme 2. Synthesis of 2′-deoxy-8-deuteroadenosine phosphoramidite.

converted to 2′-deoxy-8-deutero-N^6-benzoyladenosine (**7**) using a transient protection protocol where the hydroxyl groups were protected as trimethylsilyl (TMS) ethers during acylation of the exocyclic amino group[11]. The 5′-alcohol was subsequently protected as a dimethoxytrityl (DMT) ether and the 3′-alcohol phosphitylated to yield phosphoramidite **8**. The level of deuterium incorporation into the phosphoramidite was 90%, as determined by ^{1}H NMR.

During a standard DNA synthesis protocol, the oligomer is deprotected in aqueous ammonia at 55°C, during which time the deuterium atom in **2** would exchange with the solvent. Alternatively, DNA can be synthesized with commercially available phosphoramidites that can be deprotected under much milder conditions (in a methanolic solution of potassium carbonate at 25°C for 2 h)[12]. These phosphoramidites contain phenoxyacetyl(PAC)-protecting groups for the purine nucleosides and an acetyl protecting group for dC. However, the PAC phosphoramidites are not as easy to prepare and are somewhat unstable. Of the normal protecting groups used for automated DNA synthesis, the N^6-benzoyl group of dA is the most easily removed. Therefore, we investigated the possibility of using phosphoramidite **8** in combination with PAC phosphoramidites to reduce the amount of time required for deprotection of the DNA.

The rate of deprotection of the benzoyl group was compared to the loss of deuterium label during the same time period. 2′-Deoxy-N^6-benzoyladenosine was heated at 55°C in aqueous ammonia and shown by RP-HPLC analysis to be $>97\%$ deprotected after 3 h (data not shown). To determine if the rate of deprotection was the same in DNA, the oligomer 5′-d(TTA^{Bz}TT) was prepared and incubated for varying lengths of time under the same

conditions. The ammonia-treated oligomer samples were enzymatically digested with snake venom phosphodiesterase and alkaline phosphatase. The resulting mixture of nucleosides was subsequently analyzed by RP-HPLC (data not shown). Interestingly, the benzoyl group was removed faster in the oligomer, where deprotection was complete after 1 h (data not shown).

The rate of exchange for the deuteron in position 8 was determined by incubation of **2** in aqueous ammonia at 55°C for different periods of time (Fig. 1). The level of deuterium in the samples was determined by ^{1}H-NMR analysis. There was still 78% of deuterium left in the 8-position of dA after incubation for 3 h. To verify that the same results would be obtained in DNA, the oligomer 5′-d(TTA^{Bz}TT) was synthesized using phosphoramidite **8** and deprotected as described above. ^{1}H-NMR analysis showed that there was indeed 80% deuterium present in the oligomer, which makes this a practical approach for site-specific deuteration of deoxyadenosine nucleotides in DNA. The same procedure was also used to make sequences containing A, T, C, and G (data not shown).

In conclusion, we have described an improved synthesis of **1** for incorporation into DNA. This nucleoside will be valuable for solid state NMR studies that require prolonged incubation under conditions where there might be appreciable proton exchange in the 8-position. However, most experiments can be performed with deuterated DNA prepared from **2**, which is substantially easier to prepare. We have shown that site-specifically deuterated DNA can be synthesized by using a combination of an N^6-benzoyl-protected phosphoramidite of **2**, and phosphoramidites that can be deprotected under mild conditions, with 80% incorporation of deuterium. The results of solid state deuterium NMR studies of samples prepared by the protocols described here will be reported in due course.

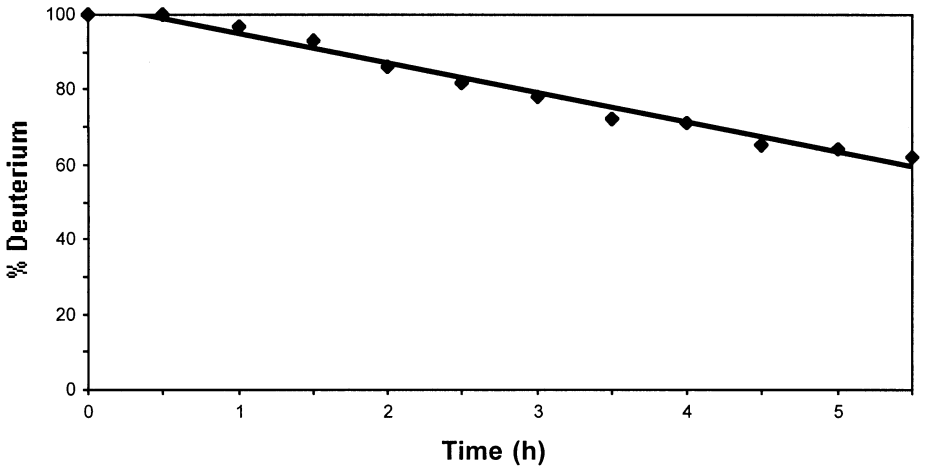


Figure 1. Deuterium exchange from **2** in aqueous ammonia at 55°C.

EXPERIMENTAL SECTION

Materials and Methods

2′-Deoxyadenosine was purchased from Biosearch Research Chemicals. Chloroacetaldehyde (50% wt. solution in water), *N*, *N*-dimethylacetamide (DMA), and ammonium persulfate were purchased from Aldrich. Aqueous ammonia (29.4%) was purchased from Fisher. CH_2Cl_2 and pyridine were distilled from calcium hydride. All reactions were run under anhydrous conditions and an inert argon atmosphere unless otherwise noted. DNA oligomers were synthesized on an ABI 394 synthesizer, using phosphoramidites purchased from Glen Research. Flash column chromatography was carried out with EM type 60 (230–400 mesh) silica gel and neutral alumina, Brockmann activity grade 1 (50–200 μm). ^{1}H NMR and ^{13}C NMR were recorded on a Bruker DRX 500 MHz spectrometer. Chemical shifts are reported in ppm relative to DMSO-d_6 (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C) or $CDCl_3$ (7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C), unless otherwise noted. High resolution (HRMS), fast atom bombardment (FAB) mass spectra were obtained on a JEOL HX-110 mass spectrometer for new compounds (**4** and **5**). UV spectra were recorded on a Perkin Elmer, Lambda BIO 20 spectrophotometer.

Preparative RP-HPLC separations were performed using a Dynamax C18 column (Rainin, 25 cm, 4.6 mm ID, 5 μm, 300 Å) and the solvent gradient was run at 10 mL/min as follows: Solvent A 100 mM Et_3NHOAc (pH 7.0); solvent B, acetonitrile; isocratic 98% A for 13 min, 12 min linear gradient to 85% A, 10 min linear gradient to 75% A, isocratic for 10 min, 5 min linear gradient to initial conditions. Analytical RP-HPLC analysis was performed using an Econosphere C18 column (Alltech, 25 cm, 2.4 mm ID, 5 μm, 300 Å) and the solvent gradient was run at 1 mL/min as follows: Solvent A 50 mM Et_3NHOAc (pH 7.0); solvent B, 30% solvent A in acetonitrile; isocratic 88.6% A for 7 min, 10 min linear gradient to 57.1% A, 5 min linear gradient to 14.3% A, 5 min linear gradient to 0.0% A, isocratic for 5 min, then 3 min linear gradient to initial conditions.

Deprotection of N^6-benzoyl-2′-deoxyadenosine

a) *Nucleoside*. Twelve different solutions containing N^6-Benzoyl-2′-deoxyadenosine (1.0 mg each, 0.0028 mmol) in aqueous ammonia (0.25 mL) were heated at 55°C. Every half-hour (over a 5.5 h period) a sample was removed from the heat block and the solvent evaporated in vacuo, followed by HPLC analysis. b) *Oligomer*. The oligomer 5′-d(TTA^{Bz}TT) was prepared by automated DNA 5′-DMT-on synthesis on a 1-μmol scale. The oligomer was not cleaved from the resin. Seven different mixtures containing resin-bound DNA (3.0 mg, 5% of a 1 μmol synthesis) in aqueous ammonia

(0.25 mL) were incubated at 55°C. Time points (10 min, 30 min, 1 h, 2 h, 3 h, 4 h and 5 h) were collected by removing a sample from the heat block, filtering off the resin and evaporating the solvent in vacuo. Each sample was purified by preparative RP-HPLC, the DMT group removed (see below), and the oligomer enzymatically digested (see below) and analyzed by analytical RP-HPLC.

Deuterium Exchange of 2′-deoxy-8-deuteroadenosine with Aqueous Ammonia

Twelve solutions containing 2′-deoxy-8-deuteroadenosine (7.5 mg each, 0.0297 mmol) in aqueous ammonia (0.63 mL) were heated at 55°C. Every 0.5 h (over a 5.5 h period) a sample was removed from the heat block, cooled down to 0°C and the solvent evaporated in vacuo, followed by ^{1}H-NMR analysis. For the 0 h time point, the aqueous ammonia was added and immediately evaporated.

Determination of Deuterium Incorporation into DNA

The oligomer 5′-d(TTATT) containing a 5′-DMT group was synthesized on a 2-μmol scale using phosphoramidite **8** and deprotected in aqueous ammonia (2.0 mL) at 55°C for 3 h. The sample was then filtered and the solvent removed in vacuo. After purification by RP-HPLC, the DMT group was removed by treatment with aqueous acetic acid (0.5 mL, 80%) for 15 min at 23°C, followed by removal of the solvent in vacuo. The oligomer was dissolved in water (300 μL), extracted with $CHCl_3$ (3 X 300 μL) and the solvent removed in vacuo from the aqueous phase. The DNA was dissolved in D_2O and the ^{1}H NMR spectrum recorded using HOD as an internal standard at 4.8 ppm. Integration of the H8 proton of dA (δ 8.36), relative to the H2 proton (δ 8.06), revealed 80% incorporation of deuterium.

Enzymatic Digestion

The oligonucleotide (1.3 nmol) in a TRIS buffer (50 mM, 50 μL, pH 8.5 @ 20°C) containing $MgCl_2$ (1 mM) was digested with snake venom phosphodiesterase I (0.4 U) and calf intestinal alkaline phosphatase (6.4 U) for 1 h at 37°C, followed by RP-HPLC analysis.

2′-Deoxy-1,N^6-ethenoadenosine (3). 2′-Deoxyadenosine (2.51 g, 10.0 mmol) was stirred at 30°C for 12 h in a 2 M aqueous solution of chloroacetaldehyde (5.4 mL, 10.7 mmol). The reaction mixture was maintained at pH 4.5 by the addition of aqueous NaOH (4 M). After completion of the reaction, the pH was adjusted to 7.2 and the solvent was removed in vacuo.

The residue was purified by flash column chromatography on silica gel with $MeOH/CH_2Cl_2$ (3:17) to give **3** in 92% yield as a white solid. ^1H NMR (DMSO-d_6): δ 9.30 (1H, s, H2), 8.53 (1H, s, H8), 8.08 (1H, d, J = 1.5 Hz, H11), 7.55 (1H, d, J = 1.5 Hz, H10), 6.48 (1H, t, J = 7.0 Hz, H1′), 5.37 (1H, d, J = 4.0 Hz, 3′-OH), 4.97 (1H, t, J = 5.5 Hz, 5′-OH), 4.44 (1H, m, H3′), 3.90 (1H, m, H4′), 3.62 (1H, m, H5′or H5″), 3.54 (1H, m, H5′or H5″), 2.72 (1H, m, H2′or H2″), 2.38 (1H, m, H2′or H2″).

3-β-D-2′-Deoxyribofuranosyl-4-amino-5-(imidazol-2-yl)imidazole (4). Compound **3** (0.500 g, 1.82 mmol) in aqueous NaOH (0.8 N, 12.5 mL, 9.99 mmol), was stirred at 23°C for 3 h. The reaction mixture was cooled to 0°C and neutralized by dropwise addition of concentrated hydrochloric acid. The solvent was removed in vacuo and the solid residue was purified by flash column chromatography on neutral alumina with $MeOH/CH_2Cl_2$ (3:7) to give **4** in 76% yield as a pale yellow solid. ^1H NMR (DMSO-d_6): δ 7.46 (1H, s, H2), 6.98 (2H, s, H8 and H9), 5.97 (1H, t, J = 7.5 Hz, H1′), 5.76 (2H, bs, NH_2), 5.31 (1H, d, J = 4.0 Hz, OH), 5.12 (1H, t, J = 4.0 Hz, OH), 4.35 (1H, m, H3′), 3.52 (1H, m, H4′), 3.82 (2H, m, H5′ and H5″), 2.48 (1H, m, H2′ or H2″), 2.17 (1H, m, H2′ or H2″); ^{13}C NMR (DMSO-d_6): δ 141.0, 139.5, 130.0, 114.5, 89.0, 85.5, 81.5, 62.0, 64.0; FAB-HRMS: m/z 266.1255 $(M+H)^+$ (calcd. 266.1255 for $C_{11}H_{15}N_5O_3$).

2-Deutero-2′-deoxy-1,N^6-ethenoadenosine (5). 1,1,1-Triethoxy-1-deuteromethane (1.961 mL, 11.7 mmol) was added dropwise to a stirred solution of imidazole **4** (0.7348 g, 2.769 mmol) in *N*,*N*-dimethylacetamide (DMA) (12 mL) at 23°C. The reaction mixture was stirred at 100°C for 12 h. The solvent was removed in vacuo and the crude solid was purified by flash column chromatography on neutral alumina with $MeOH/CH_2Cl_2$ (1:19 to 3:7) to give **5** in 77% yield as a white solid (94% deuterium). ^1H NMR (DMSO-d_6): δ 9.30 (0.06H, s, H2), 8.53 (1H, s, H8), 8.08 (1H, d, J = 1.5 Hz, H11), 7.55 (1H, d, J = 1.5 Hz, H10), 6.48 (1H, t, J = 7.0 Hz, H1′), 5.37 (1H, d, J = 4.0 Hz, 3′-OH), 4.97 (1H, t, J = 5.5 Hz, 5′-OH), 4.44 (1H, m, H3′), 3.90 (1H, m, H4′), 3.62 (1H, m, H5′or H5″), 3.54 (1H, m, H5′or H5″), 2.72 (1H, m, H2′or H2″), 2.38 (1H, m, H2′or H2″); ^{13}C NMR (DMSO-d_6): δ 141.4, 140.5, 140.4, 138.9, 133.6, 123.4, 113.0, 88.8, 84.7, 71.5, 62.4; FAB-HRMS: m/z 277.1159 $(M+H)^+$ (calcd. 277.1159 for $C_{12}H_{13}N_5O_3$).

2-Deutero-2′-deoxyadenosine (1). Compound **5** (0.1326 g, 0.4799 mmol) and ammonium persulfate (0.2190 g, 0.9597 mmol) were dissolved in an aqueous phosphate buffer (10 mL, 1 M, pH 7.2 @ 23°C) and stirred at 80°C for 3 h. The solvent was removed in vacuo and the solid residue was purified by flash column chromatography on silica with $MeOH/CH_2Cl_2$ (5:95) to give **1** as a white solid in 71% yield (94% deuterium). ^1H NMR (DMSO-d_6): δ 8.33 (1H, s, H8), 8.15 (0.06H, s, H2), 7.31 (2H, s, NH_2),

6.48 (1H, m, H1′), 5.37 (1H, d, J = 4.0 Hz, 3′-OH), 5.25 (1H, t, J = 6 Hz, 5′-OH), 4.41 (1H, m, H3′), 3.88 (1H, m, H4′), 3.65 (1H, m, H5′ or H5″), 3.55 (1H, m, H5′ or H5″), 2.71 (1H, m, H2′ or H2″), 2.35 (1H, m, H2′ or H2″).

2′-Deoxy-8-deuteroadenosine (2). 2′-Deoxyadenosine (5.0043 g, 18.589 mmol), deuterium oxide (250 mL), and triethylamine (2.58 mL, 18.6 mmol) were stirred at 65°C for 65 h. The solvent was removed in vacuo to give **2** as a white solid in quantitative yield (> 98% deuterium). ^{1}H NMR (DMSO-d_6): δ 8.08 (1H, s, H2), 7.30 (2H, s, NH_2), 6.33 (1H, m, H1′), 5.27 (1H, d, J = 4.0 Hz, 3′-OH), 5.24 (1H, d, J = 6 Hz, 5′-OH), 4.42 (1H, m, H3′), 3.87 (1H, m, H4′), 3.58 (2H, m H5′ and H5″), 2.69 (1H, m, H2′ or H2″), 2.35 (1H, m, H2′ or H2″).

***N*^{6}-Benzoyl-8-deutero-2′-deoxyadenosine (7).** Compound **2** (5.0029 g, 19.84 mmol) was co-evaporated with pyridine (3 × 20 mL) and suspended in 50 mL of pyridine. The mixture was cooled to 0°C and chlorotrimethylsilane (12.5 mL, 98.5 mmol) was slowly added. The reaction mixture was stirred for 30 min at 0°C, followed by addition of benzoyl chloride (11.0 mL, 94.5 mmol). The ice bath was removed and the reaction was stirred at 23°C for 2 h. The mixture was cooled again in an ice-bath and water (20 mL) was added. After 20 min, aqueous ammonia (40 mL) was added, the ice bath removed and the reaction mixture stirred for 30 min. The solvent was removed *in vacuo* and the residue was dissolved in water (100 mL) and washed with ethyl acetate (40 mL). The aqueous solution was refrigerated (4°C) for 2 h and the white solid collected and dried to give **7** in 72% yield (97% deuterium). ^{1}H NMR ($CDCl_3$): δ 8.82 (1H, s, H2), 8.15 (0.03H, s, H8), 6.86 (5H, m, Bz), 6.51 (1H, m, H1′), 4.74 (1H, s, H3′), 4.17 (1H, s, H4′), 3.45 (2H, m, H5′ and H5″), 2.93 (1H, m, H2′ or H2″), 2.62 (1H, m, H2′ or H2″).

5′-*O*-(Dimethoxytrityl)-*N*^{6}-benzoyl-8-deutero-2′-deoxyadenosine. Compound **7** (0.4397 g, 1.235 mmol) was co-evaporated with pyridine (3 × 5 mL), suspended in pyridine (8 mL), and cooled to 0°C. 4,4-Dimethoxytrityl chloride (0.5023 g, 1.482 mmol) was added and the solution was allowed to warm up to 23°C. After 12 h, the solution was cooled to 0°C and methanol (2 mL) was added. The solvent was removed in vacuo and the crude product was purified by flash column chromatography on silica gel with MeOH/CH_2Cl_2 (0:10 to 3:7) giving the desired compound as a white solid in 83% yield (90% deuterium). ^{1}H NMR ($CDCl_3$): δ 8.75 (1H, s, H2), 8.18 (0.1H, s, H8), 8.04–6.82 (18H, ArH), 6.51 (1H, m, 1H′), 4.73 (1H, m, H3′), 4.18 (1H, m, H4′), 3.81 (3H, s, OCH_3), 3.44 (2H, m, H5′ or H5″), 2.93 (1H, m, H2′ or H2″), 2.56 (1H, m, H2′ or H2″).

5′-*O*-(Dimethoxytrityl)-*N*^{6}-benzoyl-8-deutero-2′-deoxyadenosine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (8). The trityl derivative of

compound **7** (0.3640 g, 0.5527 mmol) was dried by co-evaporation with pyridine (3 × 5 mL) and stored in vacuo overnight. The residue was dissolved in CH_2Cl_2 (5 mL) was treated dropwise with *N*, *N*-diisopropyl ethylamine (0.21 mL, 1.2 mmol) and 2-cyanoethyl diisopropylchloro-phosphoramidite (0.14 mL, 0.61 mmol) while stirring at 23°C. After 20 min, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed sequentially with saturated aqueous sodium bicarbonate (25 mL) and brine (20 mL). The aqueous solutions were extracted with CH_2Cl_2 (25 mL). The organic phases were combined, dried over sodium sulfate, and concentrated in vacuo. The solid residue was purified by flash column chromatography on silica gel with hexane/EtOAc /Et_3N (20:80:0.5) to give **8** as a white solid in 73% yield (**91%** deuterium). ^{1}H NMR ($CDCl_3$): δ 8.92 (1H, s, H2, diasteromer 1), 8.77 (1H, s, H2, diasteromer 2), 8.23 (0.09H, s, H8, diasteromer 1), 8.21 (0.09H, s, H8, diastereomer 2), 8.04–6.82 (18H, ArH), 6.53 (1H, m, H1′), 4.82 (1H, m, H3′), 4.37 (1H, m, H4′), 3.80 (6H, s, OCH_3), 3.63 (4H, m, C_2H_4CN), 3.42 (2H, m, CH), 3.44 (2H, m, H5′), 2.93, (1H, m, H2′ or H2″), 2.56 (1H, m, H2′ or H2″), 1.28 (12H, m, CH_3).

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