**Data of 6-substituted deoxyadenosine analogues for modifications on 8-17 DNAzymes**

**Shanshan Du1, Yang Li1, Zhilong Chai2, Weiguo Shi1, Junlin He\*1**

1. State Key Laboratory of Toxicology and Medical Countermeasures, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China

2. School of Pharmaceutical Sciences, Guizhou University, Guizhou 550025, China

**Corresponding author:** Junlin He (hejunlin@bmi.ac.cn) –State Key Laboratory of Toxicology and Medical Countermeasures, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China

**Abstract**

The synthesis of the phosphoramidites of 6-substituted 2’-deoxyadenosine analogues, N6-(6-aminopropyl)-2’-deoxyadenosine (2), N6-*{*2-[N,N-bis(2-aminoethyl)ethyl}-2’-deoxyadenosine (3), N6-(3-guanidinopropyl)-2’-deoxyadenosine (4), and N6-[3-(imidazo-1-yl)propyl]-2’-deoxyadenosine (5) were reported. The characterization data of 1H, 13C and 31P NMR as well as high-resolution mass spectra of new compounds were presented. The sequences of 17 new modified DNAzymes based on 8-17 and 17E were reported with ESI-MS data. CD spectra of modified DNAzyme-substrate complexes in the presence of Mg2+ or Ca2+ were collected. The thermal stability data of DNAzyme-substrate complexes in the presence of Mg2+ were presented. These new 2’-deoxyadenosine analogues were functionalized at adenine with amino, guanidino, and imidazolyl groups in three adenine residues in 8-17 and 17E DNAzymes, which are active functional groups found in protein enzymes and are capable of hydrogen bonding and proton transfer. At A15 of both DNAzymes, compound 1 was demonstrated to be favourable for the catalytic activity, and it was capable of modulating metal ion-dependence of the DNAzymes. Especially, two much more efficient DNAzymes were obtained with Ca2+-dependence. These nucleoside analogues are potential molecular tools for optimization of other functional nucleic acids, including DNAzymes, aptamers. The effective DNAzymes and the synthesis methods of the phosphoramidites were helpful for the researchers in the fields of nucleic acid chemistry, genetic therapeutics and biosensors.

**Keywords**

8-17 DNAzyme, 17E DNAzyme, amino, guanidino, imidazolyl, CD spectra, thermal stability

**Specifications Table** [Every section of this table is mandatory. Please enter information in the right-hand column and remove all the instructions]

|  |  |
| --- | --- |
| **Subject** | Organic chemistry |
| **Specific subject area** | Nucleic acid chemistry used in the optimization of DNAzymes and aptamers for better performance in genetic therapeutics and biosensing platform. |
| **Type of data** | Table  Figure |
| **How data were acquired** | 1H, 13C, and 31P NMR spectra were recorded on a JNM-ECA400 (JEOL, Tokyo, Japan) spectrometer.  HRMS data on a Agilent TOF G6230A (Agilent Technologies, Santa Clara, CA, USA)  Thermal stability measured on a Varian Bio 100 spectrometer (Varian, USA).  CD spectra recorded on a MOS-450 spectropolarimeter (Biologic, France).  The cleavage percentage P% of the substrate was obtained by denaturing PAGE separation (20%, 8 M urea) of samples and quantitated on a PhosphoImager (Cyclone Plus Phosphor Scanning System Molders C43120, PerkinElmer, USA). The observed rate constants were calculated according to P % = P∞%–C exp [-*k*obst], where P is the percentage of the cleaved product at time t, C is the difference of P% between t = ∞ and t = 0, and P∞ is 90%. |
| **Parameters for data collection** | 1H, 13C, and 31P NMR were recorded at 400 MHz, 200 MHz, and 160 MHz, respectively. Chemical shifts were reported in ppm.  Tm: The sample solution was cooled at 1 ºC/min and the UV absorbance at 260 nm was recorded simultaneously.  CD spectra: A scanning rate of 100 nm/min and bandwidth of 1 nm were set for the scanning for each sample.  In the calculation of *k*obs with P % = P∞%–C exp [-*k*obst], three parameters were used, P is the percentage of the cleaved product at time t, C is the difference of P% between t = ∞ and t = 0, and P∞ is the endpoint, an endpoint of 90% product was assumed. The data was the averaged result of at least two or three independent experiments with a variation of < 20%. |
| **Description of data collection** | 1H and 13C NMR were referenced to the solvent peak tetramethylsilane (TMS), and 31P NMR was referenced to 80% H3PO4.  Tm: The UV absorbance vs temperature curve was derivatized to obtain the Tm value.  CD spectra: three scans for each sample were averaged and smoothed.  For the *k*obs of each reaction, the cleavage percentage P% was obtained by denaturing PAGE separation (20%, 8 M urea) and quantitated on a PhosphoImager. |
| **Data source location** | Beijing Institute of Pharmacology and Toxicology  Beijing  China |
| **Data accessibility** | With the article |

**Value of the Data**

* The 2’-deoxyadenosine analogues were modified with active functional groups at nucleobases, which could be used for the optimization of functional nucleic acids (DNAzymes, aptamers, et al).
* Scientists in the fields of nucleic acid chemistry, genetic therapeutics and biosensors could benefit from these data, as more efficient functional nucleic acids is always required to realize the potential applications of DNAzymes and aptamers.
* These data could be referenced for the optimization of other DNAzymes and synthesis of other 6-substituted 2’-deoxyadenosine analogues.

**Data Description**

1. Synthesis of phosphoramidites of 2’-deoxyadenosine analogues, according to Scheme 1 and 2.



Scheme 1. Synthesis of 6-substituted 2’-deoxyadenosine analogues and phosphoramidites. Conditions: (i) 1,6-diaminohexane, in isopropanol, 50 ºC; ethyl trifluoroacetate, triethylamine, in methanol, r.t.; (ii) DMTCl, in pyridine r.t.; (iii) diethylpropylammonium tetrazolide, (NCCH2CH2O)[(iPr)2N]2P, (iPr)2EtN, in CH2Cl2; (iv) triaminoethylamine, in isopropanol; 50 ºC, ethyl trifluoroacetate, triethylamine, in methanol, r.t.; (v) N-(3-aminopropyl)imidazole, Et3N, in n-BuOH, at 80 ºC; 0.2 M CH3ONa/CH3OH.



Scheme 2. Synthesis of guanidinyl group-attaching nucleoside analogue and the phosphoramidite. Conditions: (i) aq. ammonia/methylamine (v/v = 1:1); (ii) N,N'-di-Boc-N''-triflylguanidine, Et3N, CH2Cl2, 0 ºC (30 min) to r.t. (30 min); (iii) diethylpropylammonium tetrazolide, (NCCH2CH2O)[(iPr)2N]2P, (iPr)2EtN, in CH2Cl2, at r.t.

1. Kinetic measurements of DNAzymes

In Table 1, 8-17 DNAzyme and its modified versions were listed. 15 modified DNAzymes were evaluated under single-turnover conditions, in the presence of 20 mM Mg2+. The observed rate constants were calculated according to equation P % = P∞%–C exp [-*k*obst], where P is the percentage of the cleaved product at time t, C is the difference of P% between t = ∞ and t = 0, and P∞ is the endpoint, an endpoint of 90% product was assumed. The data was the averaged result of at least two or three independent experiments with a variation of < 20%.

Table 1. The observed rate constants of modified 8-17 DNAzyme under single-turnover conditions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| DNAzyme | substituent | *k*obs (min-1) | DNAzyme | substituent | *k*obs (min-1) |
| 8-17DZ |  | 0.0027±0.0002 |  |  |  |
| 8-17DZ-A6-1 | A6 = 1 | -*a* | 8-17DZ-A12-1 | A12 = 1 | 0.0005±0.00006 |
| 8-17DZ-A6-2 | A6 = 2 | - *a* | 8-17DZ-A12-2 | A12 = 2 | 0.0018±0.0001 |
| 8-17DZ-A6-3 | A6 = 3 | - *a* | 8-17DZ-A12-3 | A12 = 3 | - *a* |
| 8-17DZ-A6-4 | A6 = 4 | - *a* | 8-17DZ-A12-4 | A12 = 4 | 0.0100±0.0008 |
| 8-17DZ-A6-5 | A6 = 5 | - | 8-17DZ-A12-5 | A12 = 5 | 0.0017±0.00006 |
|  |  |  |  |  |  |
| 8-17DZ-A15-1 | A15 = 1 | 0.0096±0.0003 | 8-17DZ-A15-4 | A15 = 4 | 0.0033±0.0004 |
| 8-17DZ-A15-2 | A15 = 2 | 0.0010±0.00006 | 8-17DZ-A15-5 | A15 = 5 | 0.0012±0.00006 |
| 8-17DZ-A15-3 | A15 = 3 | 0.0008±0.00005 |  |  |  |

*a*Too slow to be measured under present conditions (50 mM Tris-HCl, pH 7.2, 20 mM Mg2+).

In Table 2, the selected DNAzymes were evaluated in the presence of Ca2+. 20 mM Ca2+ was used for 8-17, 8-**17DZ-A12-1**, **8-17DZ-A12-4**, and **8-17DZ-A15-4**; 5 mM Ca2+ was used for **8-17DZ-A15-1**. The rate increase of each DNAzyme for the two metal ions was compared as *k*obs (Ca2+)/ *k*obs (Mg2+). The thermal stability of the DNAzymes in the presence of Ca2+and Mg2+ was measured.

Table 2. Comparison between Ca2+- and Mg2+-promoted cleavage reactions of modified 8-17 DNAzymes under single-turnover conditions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| DNAzyme | *k*obs (min-1)  (20 mM Ca2+) | *k*obs (Ca2+)/  *k*obs (Mg2+) | Tm  (20 mM Mg2+)*c* | Tm  (20 mM Ca2+)*c* |
| 8-17 | 0.0049±0.0003 | 1.8 | 57.0 | 57.0 |
| 8-17DZ-A12-1 | 0.0070±0.0004 | 16.6 | 57.5 | 56.7 |
| 8-17DZ-A15-1 | 0.1181±0.0218 *a* | 53.7 *b* | 55.0 | 51.5 |
| 8-17DZ-A12-4 | 0.0959±0.0082 | 9.6 | 61.1 | 55.2 |
| 8-17DZ-A15-4 | 0.0694±0.0053 | 21.0 | 51.5 | 50.8 |

*a* *k*obs measured at 5 mM Ca2+.

*b k*obs (Ca2+)/ *k*obs (Mg2+) at 5 mM metal ions.

*c* Metal ion concentration: 20 mM

In Table 3, 17E and its modified DNAzymes were measured for *k*obs under single turnover conditions, in the presence of 20 mM Mg2+ or 20 mM Ca2+. 5 mM Ca2+ was used for the reaction of **17E-A15-1**.

Table 3. Properties of 17E and its modified DNAzymes in the presence of Ca2+ or Mg2+

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| DNAzyme | *k*obs (min-1)  (20 mM Mg2+) | *k*obs (min-1)  (20 mM Ca2+) | *k*obs (Ca2+)/  *k*obs (Mg2+) | Tm  (20 mM Mg2+) | Tm  (20 mM Ca2+) |
| 17E | 0.0096±0.0006 | 0.0201±0.0008 | 2.1 | 56.2 | 59.2 |
| 17E-A15-1 | 0.0039±0.0003 | 0.2156±0.0049*a* | 55.3*b* | 53.6 | 56.9 |
| 17E-A15.0-1 | 0.0020±0.0002 | 0.0131±0.0005 | 6.2 | 54.5 | 55.2 |

*a k*obs measured at 5 mM Ca2+.

*b k*obs (Ca2+)/ *k*obs (Mg2+) at 5 mM metal ions.

In Table 4, the *k*obs of two fast DNAzymes **8-17DZ-A15-1** and **17E-A15-1** were measured in the presence of different concentrations of Mg2+ or Ca2+, respectively.

Table 4. The observed rate constants of two modified DNAzymes in the presence of metal ions with different concentrations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 8-17DZ-A15-1 | | 17E-A15-1 | |
| Concentration (mM) | Mg2+ | Ca2+ | Mg2+ | Ca2+ |
| 0.625 | -*a* | 0.0017±0.0002 | -*a* | 0.0041±0.0004 |
| 1.25 | -*a* | 0.0149±0.0037 | 0.0008±0.00007 | 0.0218±0.0024 |
| 2.5 | 0.0014±0.0001 | 0.0427±0.0021 | 0.0022±0.0003 | 0.1104±0.0030 |
| 5 | 0.0022±0.0002 | 0.1181±0.0218 | 0.0039±0.0003 | 0.2156±0.0049 |
| 10 | 0.0050±0.0006 | -*b* | 0.0096±0.0004 | -*b* |
| 20 | 0.0090±0.0007 | -*b* | 0.0151±0.0017 | -*b* |

*a* Too slow reactions not measured.

*b* Too fast to be measured by manual pipetting.

In Figure 2, the metal ion dependence of 8-17DZ, 17E, and two modified DNAzymes 8-17DZ-A15-1 and 17E-A15-1 were compared.





Fig. 2. The cleavage reaction profiles of 8-17 DNAzyme and 17E and their modified DNAzymes with the influence of Mg2+ and Ca2+ compared.

In Figure 3, the concentration-dependence of the observed rate constants of two modified DNAzymes 8-17DZ-A15-1 and 17E-A15-1 were presented.



Fig. 3. Mg2+ or Ca2+ concentration-dependence of the observed rate constants of DNAzymes under single turnover conditions.

1. Characterization of DNAzymes

In Table S1, ESI-MS for all the DNAzymes were measured, the results were listed.

Table S1 Characterization results of DNAzymes by ESI-MS

|  |  |  |  |
| --- | --- | --- | --- |
| DNAzyme | sequence | MW (calc) | MW (found) |
| **8-17DZ** | 5’-d(agg atc tat CCG AGC CGG ACG A ggc tcc at)-3’ | 9216.9 | 9216.2 |
| **8-17DZ-A6-1** | 5’-d(agg atc tat CCG **1**GC CGG ACG A ggc tcc at)-3’ | 9274.0 | 9275.4 |
| **8-17DZ-A12-1** | 5’-d(agg atc tat CCG AGC CGG **1**CG A ggc tcc at)-3’ | 9274.0 | 9281.8 |
| **8-17DZ-A15-1** | 5’-d(agg atc tat CCG AGC CGG ACG**1** ggc tcc at)-3’ | 9274.0 | 9276.1 |
| **8-17DZ-A6-2** | 5’-d(agg atc tat CCG **2**GC CGG ACG A ggc tcc at)-3’ | 9311.7 | 9317.3 |
| **8-17DZ-A12-2** | 5’-d(agg atc tat CCG AGC CGG **2**CG A ggc tcc at)-3’ | 9311.7 | 9317.1 |
| **8-17DZ-A15-2** | 5’-d(agg atc tat CCG AGC CGG ACG **2** ggc tcc at)-3’ | 9311.7 | 9317.7 |
| **8-17DZ-A6-3** | 5’-d(agg atc tat CCG **3**GC CGG ACG A ggc tcc at)-3’ | 9346.14 | 9348.9 |
| **8-17DZ-A12-3** | 5’-d(agg atc tat CCG AGC CGG **3**CG A ggc tcc at)-3’ | 9346.14 | 9352.0 |
| **8-17DZ-A15-3** | 5’-d(agg atc tat CCG AGC CGG ACG **3** ggc tcc at)-3’ | 9346.14 | 9349.1 |
| **8-17DZ-A6-4** | 5’-d(agg atc tat CCG **4**GC CGG ACG A ggc tcc at)-3’ | 9316.0 | 9315.2 |
| **8-17DZ-A12-4** | 5’-d(agg atc tat CCG AGC CGG **4**CG A ggc tcc at)-3’ | 9316.0 | 9314.2 |
| **8-17DZ-A15-4** | 5’-d(agg atc tat CCG AGC CGG ACG **4** ggc tcc at)-3’ | 9316.0 | 9315.6 |
| **8-17DZ-A6-5** | 5’-d(agg atc tat CCG **5**GC CGG ACG A ggc tcc at)-3’ | 9326.1 | 9323.4 |
| **8-17DZ-A12-5** | 5’-d(agg atc tat CCG AGC CGG **5**CG A ggc tcc at)-3’ | 9326.1 | 9323.6 |
| **8-17DZ-A15-5** | 5’-d(agg atc tat CCG AGC CGG ACG **5** ggc tcc at)-3’ | 9326.1 | 9323.9 |
| **17E** | 5’-d(agg atc tat CCG AGC CGG TCG AA ggc tcc at)-3’ | 9521.0 | 9521.0 |
| **17E-A15-1** | 5’-d(agg atc tat CCG AGC CGG TCG 1A ggc tcc at)-3’ | 9578.2 | 9578.8 |
| **17E-A15.0-1** | 5’-d(agg atc tat CCG AGC CGG TCG A1 ggc tcc at)-3’ | 9578.2 | 9578.0 |

1. Thermal stability of modified DNAzyme-substrate complexes.

In Table S2, the thermal stabilities (Tm values) were measured for the DNAzyme-substrate complexes under the reaction conditions.

Table S2. Thermal stabilities of DNAzyme-substrate complexes *a*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| DNAzyme | substitution | Tm (ºC)(ΔTm) | DNAzyme | substitution | Tm (ºC) (ΔTm) |
| **8-17DZ** |  | 57.0 |  |  |  |
| **8-17DZ-A6-1** | A6 = 1 | 58.3 (1.3) | **8-17DZ-A12-1** | A12 = 1 | 57.5 (0.5) |
| **8-17DZ-A6-2** | A6 = 2 | 56.5 (-0.5) | **8-17DZ-A12-2** | A12 = 2 | 56.4 (-0.6) |
| **8-17DZ-A6-3** | A6 = 3 | 54.7 (-2.3) | **8-17DZ-A12-3** | A12 = 3 | 53.3 (-3.7) |
| **8-17DZ-A6-4** | A6 = 4 | 59.3 (2.3) | **8-17DZ-A12-4** | A12 = 4 | 61.1 (4.1) |
| **8-17DZ-A6-5** | A6 = 5 | 57.6 (0.6) | **8-17DZ-A12-5** | A12 = 5 | 58.8 (1.8) |
|  |  |  |  |  |  |
| **8-17DZ-A15-1** | A15 = 1 | 55.0 (-2) | **8-17DZ-A15-4** | A15 = 4 | 51.5 (-5.5) |
| **8-17DZ-A15-2** | A15 = 2 | 53.8 (-3.2) | **8-17DZ-A15-5** | A15 = 5 | 51.2 (-5.8) |
| **8-17DZ-A15-3** | A15 = 3 | 54.7 (-2.3) | **D18S+D18** |  | 57.9 (0.9) |

*a* Equamolar mixture of DNAzyme (5 μM) and the full-DNA substrate or the dsDNA (D18S+D18) in the solution (50 mM Tris-HCl, pH 7.5, 20 mM Mg2+), with a cooling rate of 1.0 ºC, with an error of ±1.0 ºC.

1. CD spectra of modified DNAzyme-substrate complexes.

In Fig.S1, the CD spectra of 8-17DZ and modified DNAzymes were evaluated for their overall conformation when forming with the complementary substrate D18, in the presence of 20 mM Mg2+ or Ca2+. In the presence of Mg2+, DNAzymes with modified A6 with residues 1-6 were compared, DNAzymes with modified A12 with residues 1-6 were compared, and DNAzymes with modified A15 with residues 1-6 were compared. CD spectra of 8-17DZ, 8-17DZ-A15-1, 8-17DZ-A12-1, 8-17DZ-A15-4, and 8-17DZ-A15-4 were compared in the presence of Mg2+ or Ca2+.

 



Fig. S1 CD spectra of DNAzyme-substrate complexes. The reaction solution conditions (50 mM Tris–HCl, pH 7.5, 20 mM Mg2+) were used, and The effect of Mg2+ and Ca2+ (20 mM) on the CD spectra of modified DNAzymes compared.

In Fig. S2, the CD spectra of 8-17DZ, 8-17DZ-A15-1, 8-17DZ-A12-1, 8-17DZ-A15-4, and 8-17DZ-A15-4 were assembled for comparison in the presence of Mg2+ or Ca2+. The CD spectra of 17E and modified DNAzymes were compared, in the presence of Mg2+ or Ca2+.





Fig. S2 CD spectra of 17E and modified DNAzymes in the presence of Mg2+ (20 mM) or Ca2+ (20 mM).

**Experimental Design, Materials, and Methods**

*Oligonucleotides*

Oligonucleotides were synthesized on a 392 DNA/RNA synthesizer (Biosystem, USA), on a 1 μmol scale. The coupling of the modified nucleoside phosphoramidites were conducted for 3 min. The DMT-off sequences was cleaved by conc. ammonia and the solutions were sealed in a bottle and heated at 55 ºC for 16 h, for a complete deprotection. Then the solutions were concentrated for denaturing PAGE (20%, 8 M urea). The product was extracted from gel and desalted on SEP-PAK column. Quantification was made by UV absorption at 85 ºC, and the molar extinction coefficients of modified 2’-deoxyadenosine analogues were assumed to be the same as that of 2’-deoxyadenosine. The extinction coefficients of DNAzymes were calculated by the nearest neighbor model and the concentration of oligonucleotides were determined from UV absorbance at λ = 260 nm. D18 and D18S were purchased from TSINGKE Bio. Tech. (Beijing, China).

*Tm measurement*

The DNAzyme-substrate complex in the reaction buffers (50 mM Tris-HCl, pH 7.0, 20 mM Mg2+ or Ca2+) was used for the measurement of the thermal stability, on a Varian Bio 100 spectrometer (Varian, USA). The solution was incubated at 85 ºC for 5 min, followed by cooling at 1 ºC/min and the UV absorbance at 260 nm was recorded simultaneously. The UV-temperature curve was derivatized to obtain the Tm value.

*Circular dichroism*

On a MOS-450 spectropolarimeter (Biologic, France), the DNAzyme-substrate complex solution (0.5 μM, 50 mM Tris-HCl, pH 7.0, 20 mM Mg2+ or Ca2+) in quartz cuvette was used for CD spectra measurement. Each sample was incubated at 85 ºC for 10 min and naturally cooled to r.t., followed by staying overnight at 4 ºC. A scanning rate of 100 nm/min and bandwidth of 1 nm were set for the scanning, and three scans were averaged and smoothed.

*Catalytic reactions*

Firstly, the chimeric substrate 5’-d(ATG GAG CC)-r(AG)-d(TAG ATC CT)-3’ was radioactively labelled with [γ-32P]ATP and TK4 polymerase. The substrate and DNAzyme in 50 mM Tris-HCl (pH 7.0) were mixed, and metal ion at the same solution was added to initiate the cleavage reaction (the reaction concentration of metal ions were reached). Samples were taken from the reaction mixture at appropriate time points, and quenched by stopping solution (100 mM EDTA and 8 M urea). The cleavage pattern was obtained by denaturing PAGE separation (20%, 8 M urea) and quantitated on a PhosphoImager (Cyclone Plus Phosphor Scanning System Molders C43120, PerkinElmer, USA). The observed rate constants were calculated with the equation P % = P∞%–C exp [-*k*obst], where P is the percentage of the cleaved product at time t, C is the difference of P% between t = ∞ and t = 0, and P∞ is the endpoint, an endpoint of 90% product was assumed. The data was the averaged result of at least two or three independent experiments with a variation of < 20%.

**Synthesis of phosphoramidites**

*General*

All chemicals were used without purification. The NMR spectra were recorded on a JNM-ECA400 (JEOL, Tokyo, Japan) spectrometer. 1H, 13C, and 31P NMR spectra were recorded at 400 MHz, 200 MHz, and 160 MHz, respectively. Chemical shifts were reported in ppm, referenced to the solvent peak tetramethylsilane (TMS) for 1H and 13C, and 85% H3PO4 at 0.00 ppm for 31P. HRMS was performed on a Agilent TOF G6230A (Agilent Technologies, CA, USA). Column chromatography was performed using silica gel (200-300 mesh) and TLC on GF254.

*N6-(6-trifluoroacetylaminohexyl)-2’-deoxyadenosine (****2a****)*

The solution of 3’,5’-di-p-chlorobenzoyl)-6-chloropurine 2’-deoxynucleoside (1 g, 1.8 mmol) in isopropanol (4 mL) was added dropwise to a solution of 1,6-diaminohexane (4.7 g, 40.47 mmol) in isopropanol (10 mL), at 50 ºC. After stirring for 4 h, the reaction was completed. The reaction mixture was cooled and concentrated, and the residue was dissolved in methanol (10 mL), triethylamine (3.5 mL) and ethyl trifluoroacetate (7 mL) was added to the solution. Stirring was continued for 2 h. The solution was concentrated for flash chromatography to obtain the compound **2a** as a white solid (0.5 g, 61% for two steps). Rf (CH2Cl2/CH3OH, 20/1), 0.58. 1H NMR (400 MHz, DMSO-*d*6): δ (ppm) 1.28 (m, 4 H, 2 CH2), 1.45 (m, 2 H, CH2), 1.58 (m, 2 H, CH2), 2.24, 2.71 (2 m, 2 H, C2’-H), 3.15 (m, 2 H, CH2), 3.43-3.64 (m, 4 H, C5’-H, CH2), 3.86 (m, 1 H, C4’-H), 4.39 (m, 1 H, C3’-H), 5.31 (d, J = 3.9, C3’-OH), 5.27 (m, 1 H, C5’-OH), 6.33 (m, 1 H, C1’-H), 8.18 (s, 1 H, C2-H), 7.88 (m, 1 H, 6-NH), 8.31 (s, 1 H, C8-H), 9.40 (m, 1 H, NH). 13C NMR (200 MHz, DMSO-*d*6): δ (ppm) 26.9, 29.1, 29.9, 62.9, 71.9, 84.9, 89.0, 115.5, 118.3, 120.6, 140.2, 149.0, 153.3, 155.6, 156.9, 157.2. HRMS for C18H25F3N6O4+H+, calcd: 447.1962; found: 447.1962.

*5’-O-(4,4’-Dimethoxytrityl)-N6-(6-trifluoroacetylaminohexyl)-2’-deoxyadenosine (****2b****)*

Compound **2a** (0.5 g, 1.1 mmol) was coevaporated with dried pyridine (5 mL) for three times and the residue was dissolved in dried pyridine (3 mL), DMTCl (1.4 g, 4.13 mmol) was added in portions, at r.t. After stirring for 2 h, methanol (3 mL) was added and the solution was stirred for 30 min. By concentration in vacuum, the residue was applied for flash chromatography, the product was obtained as white foam (0.3 g, 36%). Rf (CH2Cl2/CH3OH, 20/1) 0.42. 1H NMR (400 MHz, DMSO-*d*6): δ (ppm) 1.28 (m, 4 H, 2 CH2), 1.44 (m, 2 H, CH2), 1.56 (m, 2 H, CH2), 2.30, 2.86 (2 m, 2 H, C2’-H), 3.13 (m, 4 H, 2 CH2), 3.43 (m, 2 H, C5’-H), 3.69 (s, 6 H, 2 OCH3), 3.95 (m, 1 H, C4’-H), 4.47 (m, 1 H, C3’-H), 5.36 (m, 1 H, C3’-OH), 6.34 (t, J = 6.4, 1 H, C1’-H), 6.77, 7.13-7.31 (m, 13 H, arom.H), 7.82 (m, 1 H, NH), 8.13 (s, 1 H, C2-H), 8.21 (s, 1 H, C8-H), 9.40 (m, 1 H, NH). 13C NMR (200 MHz, DMSO-*d*6): δ (ppm) 14.2, 26.1, 28.4, 29.1, 55.1, 64.2, 70.8, 83.4, 85.5, 85.9, 113.2, 119.7, 126.7, 127.8, 129.8, 135.7, 135.8, 139.3, 145.0, 148.4, 152.6, 154.7, 156.1, 156.4, 158.1. HRMS for C39H43F3N6O6+H+, calcd: 749.3269; found: 749.3268.

*5’-O-(4,4’-Dimethoxytrityl)-N6-(6-trifluoroacetylaminohexyl)-2’-deoxyadenosine 3’-O-[(2-cyanoethyl)-N,N’-diisopropylphosphoramidite] (****2c****)*

Compound **2b** (1.12 g, 1.58 mmol) was dissolved in distilled dichloromethane (20 mL) and stirred at r.t., N,N-diethylammonium tetrazolide (0.17 g, 2.39 mmol), 2-cyanoethyl N,N,N’,N’-tetraisopropylphosphorodiamidite [(NCCH2CH2O)[(iPr)2N]2P] (0.3 mL) were added to the solution. After stirring for 40 min, the solution was diluted with dichloromethane (30 mL), and washed with cold 5% sodium bicarbonate and brine. The organic solution was dried with anhydr. Na2SO4, and concentrated for flash chromatography. The product was obtained as white foam (0.85 g, 40%). Rf (CH2Cl2/CH3OH, 20/1) 0.55. 1H NMR (400 MHz, CDCl3): δ (ppm) 1.20 {m, 12 H, N[CH(C*H*3)2]2}, 1.43 (m, 4 H, CH2CH2), 1.57 (m, 2 H, CH2), 1.69 (m, 2 H, CH2), 2.56-2.91 (m, 4 H, C2’-H, OCH2C*H*2CN), 3.19-3.80 {m, 16 H, CH2CH2, C5’-H, 2 OCH3, OC*H*2CH2CN, N[C*H*(C*H*3)2]2}, 4.38 (m, 1 H, C4’-H), 4.76 (m, 1 H, C3’-H), 5.73 (br, 6-NH), 6.44 (m, 1 H, C1’-H), 6.79 (m, 4 H, arom. H), 7.22-7.41 (m, 9 H, arom. H), 7.95 (m, 1 H, C2-H), 7.88 (m, 1 H, 6-NH), 8.32 (s, 1 H, C8-H). 31P NMR (160 MHz, CDCl3): δ (ppm) 149.21, 149.30. HRMS for C48H60F3N8O7P+H+, calcd: 949.4347; found: 949.4347.

*N6-{2-[N,N-bis(2-trifluoroacetylaminoethyl]ethyl}-2’-deoxyadenosine (****3a****)*

With the similar procedure for compound **2a**, compound **3a** was obtained from the reaction between 3’,5’-di-p-chlorobenzoyl)-6-chloropurine 2’-deoxynucleoside (2.5 g, 4.5 mmol）and triaminoethylamine (15 mL, 101 mmol). The reaction was monitored by TLC, Rf (CH2Cl2 : CH3OH saturated with NH3, 5:1) 0.49. The reaction mixture was concentrated and the residue was dissolved in methanol (20 ml). Triethylamine (3.5 mL) and ethyl trifluoroacetate (7 mL) were added to the solution. The mixture was concentrated for flash chromatography to obtain the product as a white solid (2 g, 77.8%). Rf (CH2Cl2/CH3OH, 9/1) 0.33. 1H NMR (400 MHz, DMSO-*d*6): δ (ppm) 2.26 (m, 1 H, C2’-H), 2.70 (m, 7 H, C2’-H, 3 CH2), 3.26 (m, 4 H, 2 CH2), 3.48-3.64 (m, 4 H, C5’-H, CH2), 3.88 (m, 1 H, C4’-H), 4.41 (m, 1 H, C3’-H), 5.26 (m, 1 H, C5’-OH), 5.33 (d, J = 4.3, 1 H, C3’-OH), 6.35 (m, 1 H, C1’-H), 7.72 (m, 1 H, NH), 8.21 (s, 1 H, C2-H), 8.34 (s, 1 H, C8-H), 9.31 (m, 2 H, NH). 13C NMR (200 MHz, DMSO-*d*6): δ (ppm) 38.6, 53.1, 62.9, 72.0, 85.0, 89.1, 115.6, 118.4, 140.4, 153.4, 155.6, 157.1, 157.5, 157.8. HRMS for C20H26F6N8O5+H+ calcd: 573.2003; found: 573.2003.

*N6-{2-[N,N-bis(2-trifluoroacetylaminoethyl]ethyl}-5’-O-(4,4’-dimethoxytrityl)-2’-deoxyadenosine (****3b****)*

With the same procedure for compound **2b**, the reaction between compound **3a** (1.2 g, 2.1 mmol) and DMT-Cl (0.86 g, 2.5 mmol) was conducted in anhydr. pyridine (2 mL) at r.t. With flash chromatography, the product was purified as white foam (0.6 g, 32.6%). Rf (CH2Cl2/CH3OH, 20:1) 0.38. 1H NMR (400 MHz, DMSO-*d*6): δ (ppm) 2.32 (m, 1 H, C2’-H), 2.69 (m, 6 H, 3 CH2), 2.87 (m, 1 H, C2’-H), 3.16-3.29 (m, 6 H, 3 CH2), 3.53 (m, 2 H, C5’-H), 3.98 (m, 1 H, C4’-H), 3.71, 3.72 (2 s, 6 H, 2 OCH3), 4.48 (m, 1 H, C3’-H), 5.38 (d, J = 4.5, C3’-OH), 6.37 (t, J = 6.5, 1 H, C1’-H), 6.80 (m, 4 H, arom. H), 7.17-7.34 (m, 9 H, arom.H, 6-NH), 7.69 (br, 1 H, NH), 8.16 (br, 1 H, C2-H), 8.24 (s, 1 H, C8-H), 9.30 (m, 2 H, NH). 13C NMR (200 MHz, DMSO-*d*6): δ (ppm) 38.5, 53.0, 55.9, 65.0, 71.6, 84.3, 86.3, 86.7, 114.0, 118.3, 127.5, 128.7, 136.5, 140.3, 145.8, 153.4, 157.0, 157.4, 159.0. HRMS for C41H44F6N8O7+H+, calcd: 875.3310; found: 875.3310.

*N6-{2-[N,N-bis(2-trifluoroacetylaminoethyl]ethyl}-5’-O-(4,4’-dimethoxytrityl)-2’-deoxyadenosine 3’-O-[(2-cyanoethyl)-N,N’-diisopropylphosphoramidite] (****3c****)*

With the same procedure for compound **2c**, from the reaction between **3b** (0.5 g, 0.57 mmol) and 2-cyanoethyl N,N,N’,N’-tetraisopropylphosphorodiamidite (0.1 ml) in distilled dichloromethane (10 mL), in the presence of N,N-diethylammonium tetrazolide (0.15 g, 0.86 mmol), the product was obtained as white foam (0.23 g, 37.7%). Rf (CH2Cl2/CH3OH, 20/1) 0.48. 1H NMR (400 MHz, CDCl3): δ (ppm) 1.11-1.19 (m, 12 H, 4 CH3), 2.44-2.90 (m, 12 H, C2’-H, 4 CH2, OCH2C*H*2CN), 3.29-3.85 (m, 20 H, 2 CH, 4 CH2, C5’-H, 2 OCH3, OC*H*2CH2CN), 4.27 (m, 1 H, C4’-H), 4.79 (m, 1 H, C3’-H), 6.45 (m, 1 H, C1’-H), 6.77 (m, 4 H, arom.H), 7.18-7.38 (m, 9 H, arom.H), 7.97 (m, 1 H, C2-H), 8.06 (br, 1 H, 6-NH), 8.32 (m, 1 H, C8-H). 13C NMR (200 MHz, CDCl3): δ (ppm) 24.6, 38.3, 43.2, 53.9, 54.3, 55.2, 63.0, 63.2, 84.4, 85.5, 86.5, 113.1, 117.1, 117.7, 119.7, 126.9, 127.8, 128.2, 130.0, 135.6, 138.5, 144.5, 153.2, 154.4, 157.6, 158.0, 158.5. 31P NMR (160 MHz, CDCl3): δ (ppm) 149.38, 149.46. HRMS for C50H61F6N10O8P+H+, calcd: 1074.4316; found: 1075.4387.

*N6-(3-aminopropyl)-5’-Dimethoxytrityl-2’-deoxyadenosine (****4b****)*

Compound **4a** (2.72 g, 3.85 mmol) [1] was dissolved in a solution of conc. ammonia (15 mL) and aq. methylamine (40%) (15 mL), and sealed in a vessel. The mixture was heated at 60 ºC for 1 h. After cooling, it was concentrated to obtain a residue for next step, without purification.

*5’-O-(4,4’-Dimethoxytrityl)-N6-[3-(N,N’-di-tert-butoxycarbonylguanidinopropyl)]-2’-deoxyadeno-sine (****4c****)*

N,N’-di-Boc-N’’-triflylguanidine [2] (0.28 g, 0.72 mmol) was dissolved in dichloromethane, followed by the addition of triethylamine (0.1 mL) and cooled at 0 ºC. To the solution was added compound **4b** (0.43 g, 0.71 mmol). After stirring for 1 h at 0 ºC and 1 h at r.t, the reaction mixture was diluted with dichloromethane, and the solution was washed with sat. sodium bicarbonate and then brine. The organic layer was dried over Na2SO4 and concentrated for flash chromatography to obtain the product as white solid (0.41 g, 70.1%). Rf (CH2Cl2/CH3OH, 20/1) 0.46. 1H NMR (400 MHz, DMSO-*d*6) δ : 11.53 (s, 1 H, NH-Boc), 8.67 (1 br, 1 H, NH), 8.24, 8.18 (2 s, 2 H, C8-H, C2-H), 7.94 (s, 1 H, NH), 7.34 - 7.13 (m, 9 H, arom-H), 6.79 (m, 4 H, arom-H), 6.36 (t, *J* = 6.4 Hz, 1 H, C1’-H), 5.38 (d, *J* = 4.4 Hz, 1 H, C3’-OH), 4.48 (m, 1 H, C4’-H), 3.95 (m, 1 H, C3’-H), 3.72, 3.71 (2 s, 6 H, 2 OCH3), 3.16 (m, 6 H, C5’-H, NC*H*2CH2C*H*2), 2.45 – 2.35 (m, 6 H, -NCH2CH2CH2), 2.88 (m, 1 H, C2’-H), 2.32 (m, 1 H, C2’-H), 1.76 (m, 2 H, NCH2C*H*2CH2), 1.38, 1.48 (2 s, 18 H, 2 C(CH3)3). 13C NMR (200 MHz, DMSO-*d*6): δ 164.1, 158.9, 156.3, 155.8, 153.4, 152.9, 145.9, 140.3, 136.6, 136.5, 130.6, 128.6, 127.5, 120.6, 114.0, 86.6, 86.3, 84.4, 83.7, 79.0, 71.6, 65.0, 55.9, 46.6, 29.0, 28.6, 12.7. HRMS for C45H56N8O9+H+, calcd: 853.4243; found, 853.4241.

*5’-O-(4,4’-Dimethoxytrityl)-N6-[3-(N,N’-di-tert-butoxycarbonylguanidinopropyl)]-2’-deoxyadenosine 3’-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (****4d****)*

With the same procedure for compound **2c**, from the reaction of **4c** (0.5 g, 0.58 mmol) and 2-cyanoethyl-N,N,N’,N’-diisopropylphosphoramidite (0.5 mL) as well as N，N-diisopropylammonium tetrazolium (0.18 g, 1.06 mmol), the product was obtained as white foam (0.48 g, 81%). Rf (CH2Cl2/CH3OH, 20/1) 0.6. 1H NMR (400 MHz, CDCl3): δ 11.54 (s, 1 H, NH-Boc), 8.71 (br, 1 H, NH), 8.38 (br, 1 H, NH), 7.96, 7.94 (2 s, 2 H, C8-H, C2-H), 7.44 - 7.18 (m, 9 H, arom. H), 6.81 (m, 4 H, arom. H), 6.46 (m, 1 H, C1’-H), 4.77 (m, 1 H, C4’-H), 4.30 (m,1 H, C3’-H), 3.96-3.30 (m, 15 H, C5’-H, 2 OCH3, NC*H*2CH2C*H*2NH, NCCH2C*H*2O, NCH), 2.90-2.46 (m, 4 H, C2’-H, NCC*H*2CH2O), 1.93 (m, 2 H, -CH2C*H*2CH2), 1.52 (2 s, 18 H, 2 C(CH3)3)], 1.28 - 1.12 (m, 12 H, 4 CH3), 13C NMR (200 MHz, CDCl3): δ 163.7, 158.6, 156.6, 155.1, 153.3, 153.2, 144.6, 138.1, 135.8, 130.2, 128.3, 128.2, 127.9, 127.0, 113.2, 86.5, 85.8, 85.6, 84.3, 83.1, 79.4, 77.5, 77.1, 76.8, 74.2, 73.6, 63.6, 63.4, 58.5, 58.3, 55.3, 45.7, 43.3, 39.6, 37.7, 30.1, 28.4, 28.2, 24.7, 23.2, 22.3, 20.3. 31P NMR (160 MHz, CDCl3): δ (ppm) 149.29, 149.30. HRMS for C54H73N10O10P+H+, calcd: 1053.5322; found, 1053.5325.

*N6-[3-(imidazo-1-yl)propyl]-2’-deoxyadenosine (****5a****)*

To the solution of 3’,5’-di-p-chlorobenzoyl)-6-chloropurine 2’-deoxynucleoside (2 g, 3.7 mmol) in n-butanol (30 mL) was added N-(3-aminopropyl)-imidazole (0.55 g, 4.4 mmol) and triehtylamine (6 mL, 44.4 mmol). The reaction mixture was heated at 80 ºC for 3 h. After concentration, the residue was dissolved in 0.2 M CH3ONa/CH3OH (40 mL) and stirred for 1 h at r.t. Then, the solution was neutralized with acetic acid and concentrated for flash chromatography to obtain the product as a white solid (1.2 g, 90%). Rf (CH2Cl2/CH3OH saturated with ammonia, 9/1) 0.2. 1H NMR (400 MHz, DMSO-*d*6): δ8.36 (s, 1 H, C8-H), 8.22 (s, 1 H, C2-H), 8.03 (br, 1 H, NH), 7.64 (d, J = 20.2 Hz, 1 H, Im-H), 7.19 (d, J = 23.8 Hz, 1 H, Im-H), 6.89 (d, J = 2.9 Hz, 1 H, Im-H), 6.36 (t, J = 6.9, 1 H, C1’-H), 5.32 (br, 2 H, J = 3.9 Hz, C5’-OH, C3’-OH), 4.42 (m, 1 H, C3’-H), 4.04 (m, 4 H, CH2CH2*CH2*Im), 3.89 (m, 1 H, C4’-H), 3.66-3.44 (m, 4 H, C5’-H, C*H*2*C*H*2*CH2Im), 2.75 (m, 1 H, C2’-H), 2.28 (m, 1 H, C2’-H), 2.04 (m, 2 H, CH2*CH2*CH2Im). 13C NMR (200 MHz, DMSO-*d*6): δ 155.1, 152.8, 148.7, 140.1, 140.0, 137.9, 137.8, 131.4, 128.9, 127.9, 120.2, 119.9, 119.8, 88.6, 84.5, 71.5, 62.4, 44.3, 44.0, 38.4, 37.6, 33.8, 31.3, 34.08, 31.49. HRMS for C16N21N7O3+H+, calcd: 360.1779; found: 360.1779.

*5’-O-(4,4’-Dimethoxytrityl)-N6-[3-(imidazo-1-yl)propyl]-2’-deoxyadenosine (****5b****)*

With the same procedure for compound **2b**, from the reaction between **5a** (0.89 g, 2.48 mmol) and DMT-Cl (1.7 g, 5.01 mmol) in anhydr. pyridine (2 mL), the product was obtained as a white solid (1.1 g, 66.8%). Rf (CH2Cl2/CH3OH, 20/1) 0.5. 1H NMR (400 MHz, DMSO-*d*6): δ 8.32 (s, 1 H, C8-H), 8.21 (s, 1 H, C2-H), 8.05 (br, 1 H, NH), 7.72 (s, 1 H, Im-H), 7.38 (d, J = 1.5, 1 H, Im-H), 7.37 (d, J = 1.5, 1 H, Im-H), 7.31 – 7.19 (m, 8 H, arom.H), 6.94 (s, 1 H, Im-H), 6.84 (m, 4 H, arom.H), 6.43 (t, J = 6.4 Hz, 1 H, C1’-H), 5.46 (d, J = 4.5 Hz, 1 H, C3’-OH), 4.54 (m, 1 H, C4’-H), 4.08 (m, 3 H, CH2CH2*CH2*Im, C3’-H), 3.76, 3.75 (2 s, 6 H, 2 OCH3), 3.51 (m, 2 H, C5’-CH2), 3.22 (m, 2 H, *CH2*CH2CH2Im), 2.94 (m, 1 H, C2’-H), 2.38 (m, 1 H, C2’-H), 2.08 (m, 2 H, CH2*CH2*CH2Im). 13C NMR (200 MHz, DMSO- *d*6): δ 158.5, 155.0, 153.0, 145.5, 139.9, 137.9, 136.1, 130.2, 128.9, 128.3, 128.2, 127.1, 119.9, 113.6, 86.3, 85.9, 83.9, 71.2, 64.6, 55.5, 44.3, 37.6, 31.3. HRMS for C37H39N7O5+Na+, calcd: 684.2905; found: 684.2901.

*N6-[3-(imidazo-1-yl)propyl]-5’-O-(4,4’-dimethoxytrityl)-2’-deoxyadenosine 3’-O-[(2-cyanoethyl)-N,N’-diisopropylphosphoramidite] (****5c****)*

With the same procedure for compound **2c**, the reaction was conducted between **8b** (0.46 g, 0.71 mmol) and 2-cyanoethyl N,N,N’,N’-tetraisopropylphosphorodiamidite (0.12 mL), in the presence of N,N-diethylammonium tetrazolide (0.18 g，1.06 mmol). After stirring for 40 min at r.t., another portion of the phosphoramidite reagent (0.3 mL) was added. The product was obtained as white foam (0.49 g, 80%). 1H NMR (400 MHz, CDCl3): δ 8.33 (s, 1 H, C8-H), 8.01 (s, 1 H, C2-H), 7.53 (s, 1 H, Im-H), 7.34 -7.16 (m, 9 H, arom.H), 7.07 (s, 1 H, Im-H), 6.95 (d, J = 1.2 Hz, 1 H, Im-H), 6.84 – 6.74 (m, 4 H, arom.H), 6.44 (m, 1 H, C1’-H), 6.00 (m, 1 H, NH), 5.05, 4.76 (2 m, 1 H, C3’-H), 4.28 (m, 1 H, C4’-H), 4.08-3.28 (m, 16 H, O*CH2*CH2CN, C*H*2CH2*CH2*Im, 2 OCH3, C5’H, 2 NCH), 2.88 (m, 1 H, C2’-H), 2.68 – 2.54 (m, 3 H, C2’-H, OCH2*CH2*CN), 2.18 (m, 2 H, CH2*CH2*CH2Im), 1.28-1.10 (m, 12 H, 4 CH3). 13C NMR (200 MHz, CDCl3): δ 158.6, 154.9, 153.1, 144.6, 144.5, 138.6, 138.4, 137.3, 135.8, 135.7, 130.2, 129.7, 128.3, 128.2, 128.0, 127.0, 126.9, 120.3, 118.9, 117.7, 117.6, 117.3, 113.2, 86.6, 86.5, 85.9, 85.6, 84.5, 84.4, 84.3, 74.2, 74.1, 73.5, 73.3, 73.0, 72.9, 72.8, 63.5, 63.3, 63.1, 59.5, 59.3, 58.5, 58.3, 58.2, 56.9, 55.3, 44.5, 43.4, 43.2, 39.6, 37.8, 31.5, 29.8, 24.7, 20.5, 20.3, 17.0. HRMS for C46H56N9O6P+Na+, calcd: 884.3983; found: 884.3992.

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**Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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