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2'-DEOXYADENYLYL-(3'→5')-ISODIDEOXYADENOSINE, A UNIQUE DINUCLEOTIDE: SYNTHESIS, ENZYMOLOGY, AND CONFORMATIONAL STUDIES

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Abstract: The synthesis, enzymatic stability toward nucleases, and conformational properties of a unique dinucleotide, 2'-deoxyadenylyl-(3'→5')-isodideoxyadenosine (**2**), a model system for the terminus of HIV viral DNA on incorporation of the L-related isomeric dideoxynucleoside, (S,S)-IsoddA, is reported. This is the first example of a dinucleotide bearing an isomeric nucleoside component.

A key viral enzyme encoded by the human immunodeficiency virus (HIV) is the polymerase, HIV reverse transcriptase (RT).[1] Selective nucleoside inhibitors of this enzyme are of intense chemical and biological interest.[2] The antiviral activity of known dideoxynucleoside inhibitors is through their cellularly produced triphosphates which act as inhibitors of HIV RT by incorporation and termination of the growing viral DNA chain.[3] Levorotatory (*S,S*)-isodideoxyadenosine or (*S,S*)-IsoddA, **1,** is an isomeric dideoxynucleoside where the base has been transposed from the natural 1'-position to the isomeric 2'-position. This compound, previously synthesized by us,[4] has been discovered to have anti-HIV activity against HIV-1, HIV-2 and AZT-resistant clinical isolates. The cellularly active compound, IsoddATP, also synthesized by us, is a potent inhibitor of HIV RT (K_i = 16 nM). This communication reports on the synthesis, enzymatic stability and conformational properties of a unique dinucleotide, 2'-deoxyadenylyl-(3'→5')-isodideoxyadenosine (**2**), a model system designed to represent the terminus of viral DNA on incorporation of the L-related isomeric dideoxynucleoside, (S,S)-IsoddA. This is the first example of a dinucleotide bearing an isomeric nucleoside component.

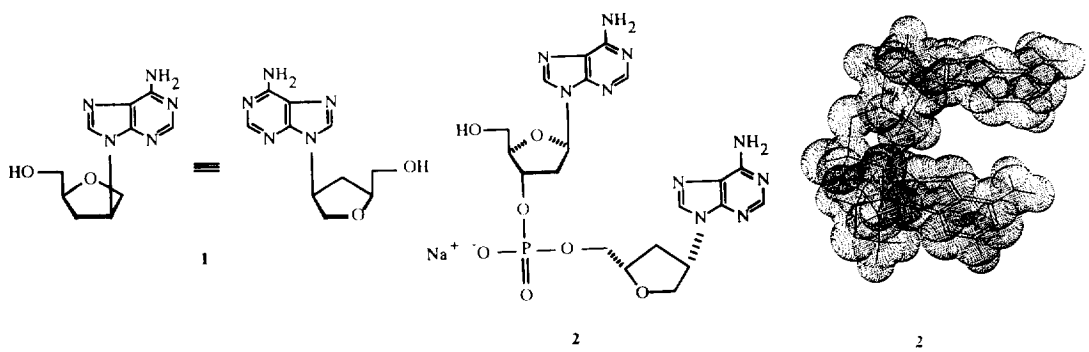
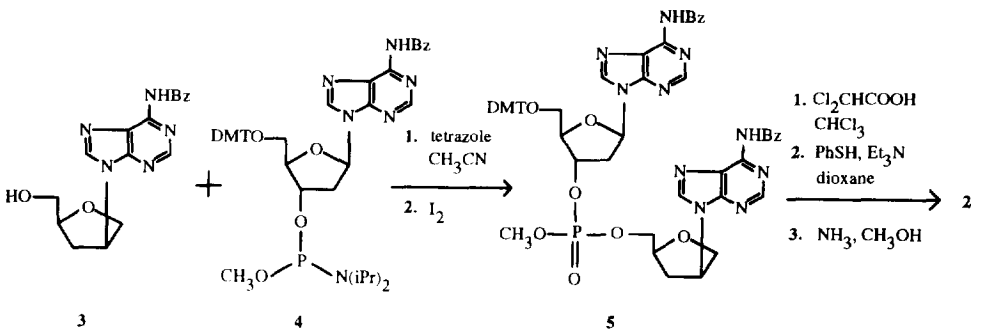


Figure 1. (S,S)-IsoddA (**1**), 2'-Deoxyadenylyl-(3'→5')-Isodideoxyadenosine (**2**) and Stereoelectronic View of **2**

The solution phase phosphoramidite methodology for oligonucleotides[5] was used for the synthesis of **2** (see Scheme 1). IsoddA was protected[6] and prepared for coupling in three steps (55% overall yield). Protected IsoddA (**3**) was coupled with two equivalents of N-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine-O-methylphosphoramidite (**4**) in the presence of tetrazole. Subsequent oxidation with iodine gave **5** (75% yield).

Deprotection of the dimethoxytrityl group with dichloroacetic acid was quantitative. Further deprotections with thiophenol and triethylamine in dioxane followed by methanolic ammonia gave the target molecule **2** in 45 % yield (from **5**). All intermediates were separated by flash or radial chromatography on silica gel. Dinucleotide **2** (as its sodium salt) was purified by reversed phase HPLC on a C_{18} column (water/methanol elution). Structural confirmation of the dinucleotide came from UV and multinuclear NMR data and elemental analysis.[7]



3 4 5

Scheme 1

Use of dinucleotides as conformational models for DNA is established and spectroscopic methods have been used for such studies including hypochromicity[8] and circular dichroism.[9] The UV spectrum of **2** (Table 1) exhibits clear evidence of hypochromicity, an indication of base-stacking interaction between the chromophores.

Table 1. UV Maxima and Molar Extinction Coefficients (in H_2O) of dA, IsoddA and **2**

Compound	λ_{max}	ε
Deoxyadenosine	259	15,000
IsoddA	260	14,700
2	258	24,000

Circular dichroism data can also give information on stacking interactions in nucleic acids, in systems even as small as dinucleoside phosphates.[9] Figure 2 shows the CD spectra of **2** compared to the CD spectra of the known deoxydinucleotide, 2'-deoxyadenylyl-(3'→5')-2'-deoxyadenosine (dApdA) at temperatures from 5°C to 55 °C. Both sets of spectra show the existence of the following: (i) well-defined isodichroic points; (ii) changes in the intensity of the CD minima and maxima; and, (iii) λ_{max} and λ_{min} values which are independent of temperature. These features indicate the two-state stack/unstack equilibrium. A view of the base stacking of **2** is shown in Figure 1.

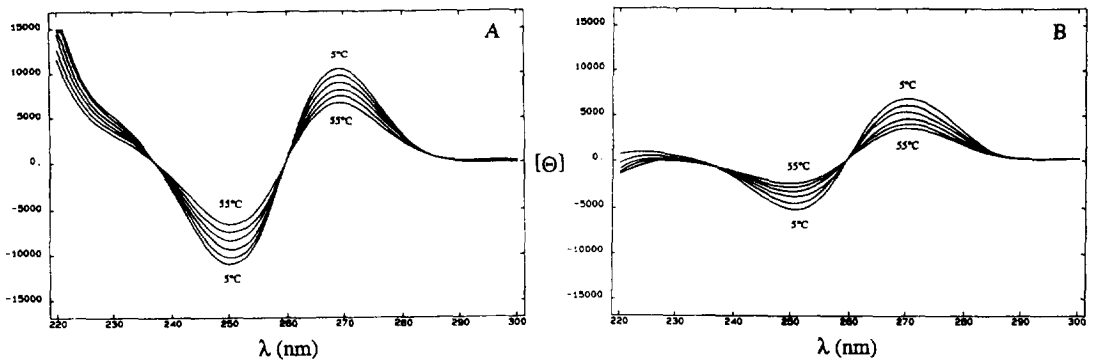


Figure 2. The CD Spectra of dApdA (A) and Dinucleotide **2** (B) in H_2O

The parent nucleoside, IsoddA, **1**, is known to exhibit enzymatic and glycosidic bond hydrolytic stability.[4] Enzymatic stability of the dinucleotide **2** was assessed with nucleases and compared to the natural analogue, dApdA, using reversed HPLC (C_{18}). Two nucleases were studied: snake venom phosphodiesterase [SV PDE, (3'-5')-exonuclease] and bovine spleen phosphodiesterase [BS PDE, (5'-3')-exonuclease]. The dinucleotide **2** showed markedly increased resistance to degradation by these exonucleases compared to dApdA (Table 2).

Table 2. Enzymatic Cleavage of Dinucleotides By Exonucleases

Compound	$t_{1/2}$ (min.) BS PDE	$t_{1/2}$ (min.) SV PDE
dApdA	1.5	<1
2	50	>550

In summary, compound **2** is the first example of a unique dinucleotide which incorporates a natural (D) and an isomeric (L-related) nucleoside within its structure. Our initial results suggest that the incorporation of (-) IsoddA within the terminus of the viral DNA structure would not interfere significantly with the conformational stability associated with base-stacking. The presence of this base-stacking was noted both in the hypochromicity from the UV spectral studies and also from the CD spectral data. In addition, introduction of the isomeric structure resulted in marked resistance in internucleotide bond cleavage with exonucleases. Further studies on the incorporation of isomeric nucleosides within DNA structures are in progress.

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7. Spectral data for **2**: UV (H_2O) λ_{max} 258 nm (ε 24000); ^1H NMR (D_2O with DSS as external ref.) δ 2.20 (m, 1H), 2.46 (m, 2H), 2.76 (m, 1H), 3.72 (m, 3H), 4.02 (m, 3H), 4.15 (m, 2H), 4.30 (m, 1H), 5.05 (m, 1H), 6.10 (t, 1H), 7.85 (s, 1H), 7.87 (s, 1H), 8.07 (s, 1H), 8.15 (s, 1H); ^{13}C NMR (D_2O with DSS as external ref.) δ 35.5, 40.6 (d, J=3.4 Hz), 57.5, 63.9, 68.3 (d, J=5.2 Hz), 74.9, 77.8 (d, J=5.4 Hz), 80.6 (d, J=9.2 Hz), 87.0, 88.7 (d, J=6.7 Hz), 120.5, 121.3, 142.3, 142.7, 150.3, 150.7, 154.5, 154.7, 157.4, 157.7; ^{31}P NMR (D_2O) (85% H_3PO_4 external reference) δ 1.26. Anal. Calcd. for $C_{20}H_{24}N_{10}O_7PNa$ C, 42.11; H, 4.24; N, 24.55. Found C, 41.74; H, 4.30; N, 24.12.
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