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Discovery of a Nuclease-Resistant, Non-natural Dinucleotide that Inhibits HIV-1 Integrase

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Abstract—Integration of HIV viral DNA into human chromosomal DNA catalyzed by HIV integrase is essential for the replication of HIV. Discovery of novel inhibitors of HIV integrase is of considerable significance in approaches to the development of therapeutic agents against AIDS. We have synthesized a new dinucleotide **1** with an internucleotide phosphate bond that is unusually resistant to exonucleases. This compound exhibits potent anti-HIV-1 integrase activity.

The retroviral enzyme, HIV-1 integrase, incorporates HIV double helical DNA (the replicative form of the HIV genome synthesized by HIV reverse transcriptase) into host chromosomal DNA. This viral enzyme first catalyzes the excision of two terminal nucleotides of the 3′-end of each strand of viral DNA (endonuclease activity, 3′-processing) leaving recessed ends that terminate with xxCA-OH (Fig. 1). In the next steps (strand transfer, integration) nucleophilic attack of the terminal 3′-OH of the tailored HIV DNA on a specific internucleotide phosphodiester functionality results in cleavage of host DNA and subsequent integration of the tailored HIV DNA into host DNA.[1–4] The integration process is essential for the replication for HIV and there is no functional equivalent of HIV integrase in human cells.

Some oligonucleotides of natural origin are capable of interfering with the integration process by competing with viral DNA for binding to HIV integrase.[5] This type of protein–nucleotide binding is important in other steps in the replication cycle of HIV, for example, in the recognition and binding of Tat protein to HIV-1 TAR RNA.[6] However, small oligonucleotides are rapidly cleaved by cellular nuclease activity. In addition, increasing nuclease resistance by chemical alteration of the internucleotide phosphate bond results in decreased integrase activity.[5] We have designed and synthesized a conceptually new dinucleotide **1** with a conformationally unusual internucleotide phosphate bond and overall conformation that exhibits complete resistance to mammalian 3′- and 5′-exonucleases and that has potent anti-HIV-1 integrase activity. This dinucleotide is superior in activity and stability toward nucleases than a compound previously reported by us.[7]

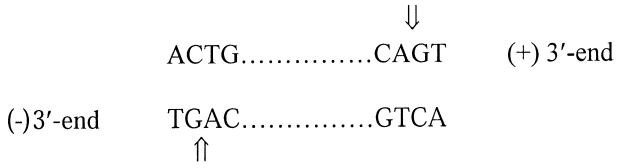

Figure 1. Tailoring of HIV double helical DNA prior to integration into host DNA.

The preparation of dinucleotide **1** (Scheme 1) utilized the deoxyisouridine **2**[8,9] as the starting point of the synthesis. This precursor can be synthesized in several steps from ribose.[8] Compound **2** can be tailored for coupling by its conversion to **3** in three steps (5′-dimethoxytritylation, 3′-acetylation, and 5′-detritylation). Treatment of **3** with **4**, the protected 2′-deoxycytidine phosphate, in the presence of TPS-TAZ and pyridine followed by 5′-deprotection with dichloroacetic acid gave the coupled compound **5** (two diastereoisomeric forms) in 72% overall yield. In the next step, 5′-phosphorylation was carried out with our new phosphorylating reagent, 2-*O*-(4,4′-dimethoxytrityl) sulfonyldiethanol

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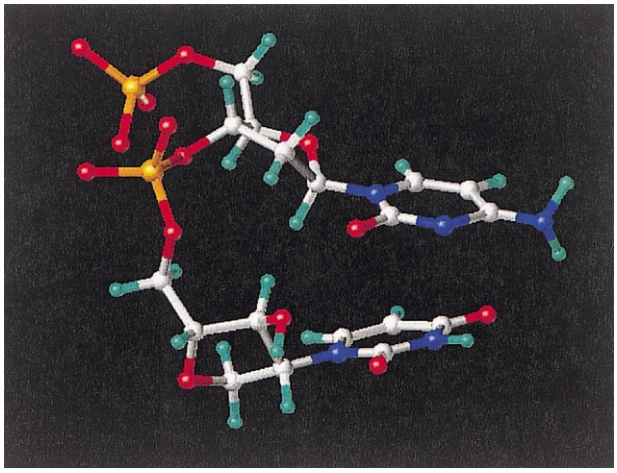
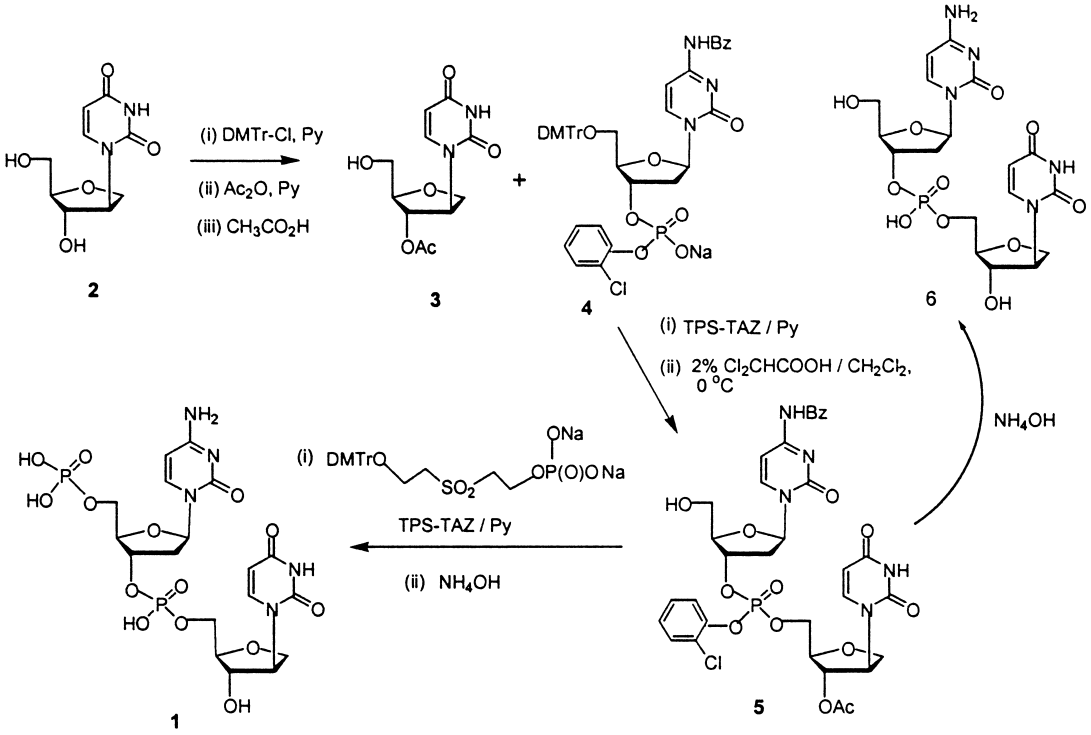


Figure 2. A preferred conformation of pdCpIsodU.

phosphate[7] in the presence of TPS-TAZ and pyridine followed by deprotection. Following purification by HPLC, target compound **1** was obtained in 79% yield. Its structure was confirmed by multinuclear NMR data (^{1}H, ^{13}C, ^{31}P, COSY, HMQC, and HMBC), quantitative UV and CD spectra, and electrospray HRMS data [calculated for **1**: 596.0795 $(M–H)^-$, found: 596.0790]. The CD and UV data [λ_{max} 268 (ε 15,900)] and observed hypochromicity suggested the presence of base stacking in preferred conformations and implied the presence of a conformationally unusual internucleotide phosphate bond because of the spatial arrangement of the two sugar rings to accommodate base stacking. The molecular modeling data showed a preferred conformation (Fig. 2) that was consistent with these results. This unusual structure of the internucleotide sugar phosphate diester linkage and the overall molecular conformation of **1** appears to manifest itself in the total resistance of the internucleotide bond of **1** towards hydrolytic cleavage catalyzed by exonucleases (PDE I and II from bovine intestinal mucosa and bovine spleen, respectively). Interestingly, even the 'precursor' dinucleotide **6** (i.e., compound **1** without the 5′-phosphate group) was totally resistant to cleavage by PDE II and almost so by PDE I ($V_{max}/K_m = 0.61\%$ of dCpdU). A comparison of these data for PDE I (5′-exonuclease) for **6** and the natural counterpart (dCpdU) is illustrated in Figure 3.

Integrase inhibition assays were conducted with purified recombinant HIV-1 integrase using a 21-mer oligonucleotide substrate as described by Pommier and coworkers.[10] The data showed that compound **1** has strong inhibitory activity against recombinant wild-type HIV integrase in reproducible assays [$IC_{50} = 7.5\,\mu M$ (3′-processing); $IC_{50} = 5.9\,\mu M$ (strand transfer)]. The data for this compound are superior to that obtained for mononucleotides[7,11,12] and are similar to that of the closest known natural dinucleotide, pdCpdT ($IC_{50} = 8$ and $6\,\mu M$).[5] However, pdCpdT and other natural dinucleotides are rapidly cleaved by 5′-exonucleases while **1** is totally resistant to both 3′- and 5′-exonucleases. The inhibition data and the nuclease resistance properties for **1** are also more compelling than that exhibited by the non-natural compound, pIsodApdC ($IC_{50} = 19$ and $25\,\mu M$), previously reported by us.[7] Compound **1** inhibited all enzymatic activities of integrase and DNA–integrase cross-linking in the same range as indicated



Scheme 1. Key steps in the synthesis of dinucleotide **1**.

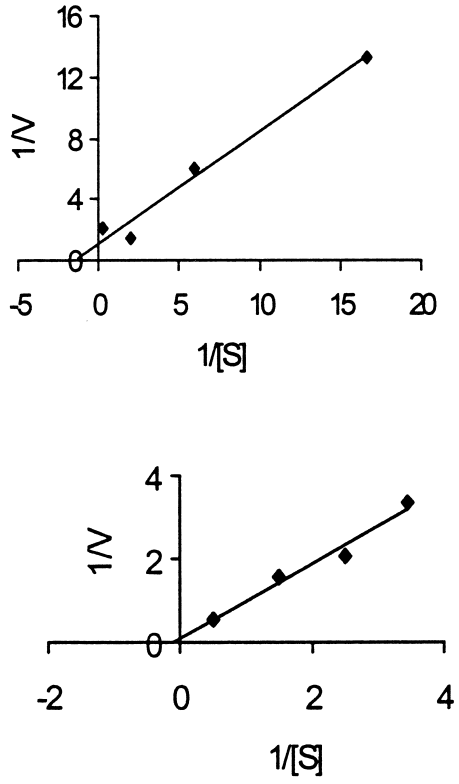


Figure 3. Lineweaver–Burk plots (1/V vs 1/[S], mM^{-1}) of the substrate activities of the natural compound, dCpdU (top) and the novel non-natural compound, dCpIsodU (bottom) with bovine 5′-exonuclease (PDE I).

above when Mn^{+2} or Mg^{+2} was used as a cofactor. This suggests that the inhibitor binds to the catalytic core of integrase and the inhibition is metal-independent. The base sequence recognition (CU) by HIV integrase is significant in that both bases C and U appear to be recognized. This recognition of U in other small oligonucleotide inhibitors has not been previously reported. The remarkable nuclease stability and the strong anti-HIV integrase activity suggests that this compound is worthy of further investigation as a potential antiviral therapeutic agent and this and related studies are currently in progress.

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