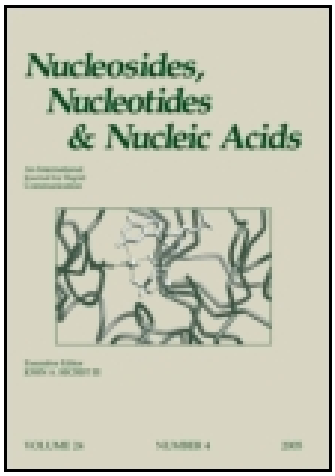

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DESIGN AND SYNTHESIS OF SPECIFIC INHIBITORS OF THE 3′-PROCESSING STEP OF HIV-1 INTEGRASE

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▫ *The novel dinucleotide 5′-phosphate, [(L,D)-pIsodApdC], discovered in our laboratory, is a strong inhibitor of HIV-1 integrase for both the 3′-processing and the strand transfer steps. The rationale used in this molecular design was that residues immediately upstream of the dinucleotide cleavage site in the 3′-processing step might provide critical recognition/binding sites on integrase. The rationale for the second type of inhibitors was based on the elimination products (linear and cyclic dinucleotides) of 3′-processing. However, while the linear dinucleotide 5′-phosphate (pdGpdT) was active, its cyclic counterpart was inactive against both wild-type and mutant HIV integrase.*

Keywords HIV-1 Integrase, Cyclic Dinucleotide, Inhibitors

INTRODUCTION

Integration of HIV DNA into the host cell genome occurs by an ordered sequence of DNA tailoring (3′ -processing) and coupling (integration) reactions.[1–4] In the 3′-processing step, endonuclease activity removes two nucleotides from each 3′-end of double helical viral DNA to produce a truncated viral DNA with new *CA*OH-3′ termini and an elimination product, pdGpdT, which is apparently produced in both the cyclic and linear forms. During the 3′-processing step, it is suggested that water, glycerol, and the viral DNA 3′-end hydroxyl can act as the nucleophile to cleave the internucleotide phosphodiester bond.[5] With the viral DNA 3′-end hydroxyl as the nucleophile, the cyclic dinucleotide will be produced.[5] We have shown that linear dinucleotides, with adenine and cytosine (A and C) as bases (Figure 1, **1** and **2**), are inhibitors of HIV-1 integrase.[6,7] In order to determine whether another product of the 3′-processing step, i.e., a cyclic

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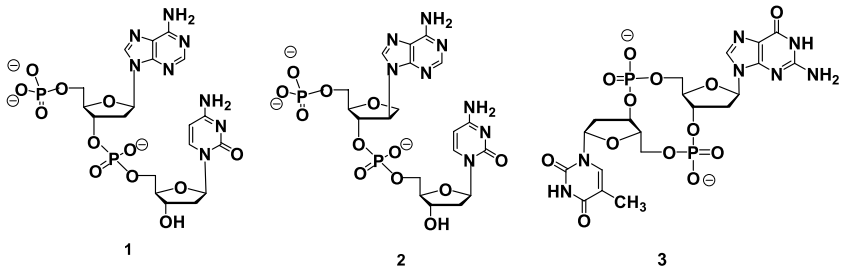
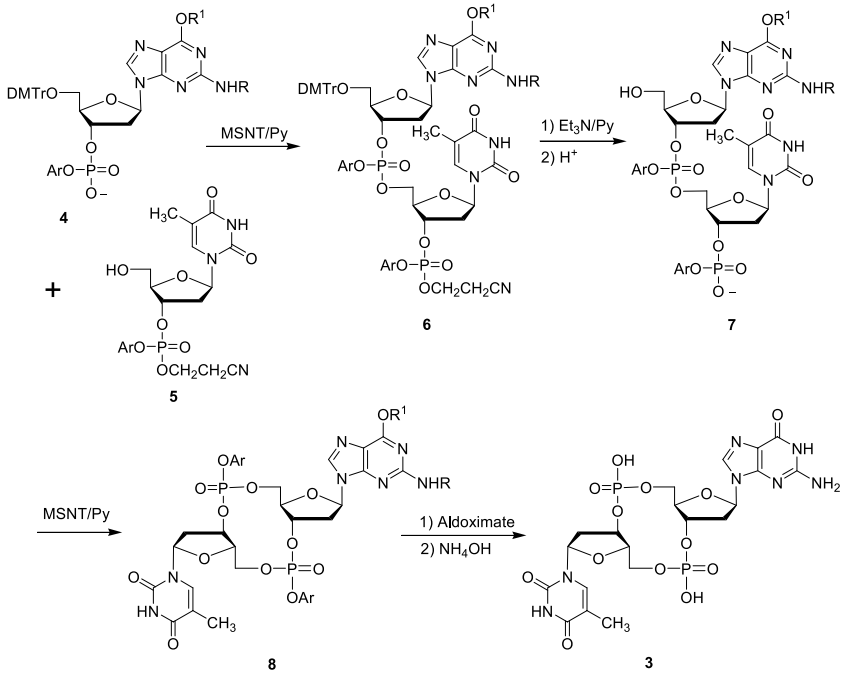


FIGURE 1 Structures of compounds 1, 2 and 3.

dinucleotide, could also be an inhibitor of HIV integrase, we have investigated a specific cyclic dinucleotide, cyclic pdGpdT **3**, as a potential inhibitor of HIV-1 integrase.

RESULTS AND DISCUSSION

Several methods have been reported for the synthesis of cyclic nucleotides including the phosphotriester method[8–10] and the H-phosphonate method.[11] We



Ar = 2-chlorophenyl; R = isobutyryl, R^1 = 2-(4-nitrophenyl)-ethyl; MSNT = 1-mesitylene-sulfonyl-3-nitro-1,2,4-triazole; Aldoximate = *syn*-2-pyridinealdoxime tetramethylguanidine

SCHEME 1 Synthesis of cyclic dinucleotide 3.

TABLE 1 Anti-HIV-1 Integrase Data for Dinucleotides

Compounds	3′-Processing IC_{50} (μM)	Strand transfer IC_{50} (μM)
1	6	3[6]
2	19	25[7]
3	>1000	>1000

chose to utilize the phosphotriester method in this synthesis (Scheme 1). Thus, the fully protected dinucleotide **6** was synthesized from **4**[9,12] and **5**[12] in 92% yield by a condensation reaction in the presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) in pyridine. Selective removal of the cyanoethyl protecting group of **6** with triethylamine and pyridine and subsequent deprotection of the trityl group with 2% dichloroacetic acid in CH_2Cl_2 afforded partially deblocked dimer **7**. Intramolecular cyclization of **7** under conditions of high dilution with MSNT in pyridine gave the fully protected cyclic dimer **8** (44% yield for 2 steps). Protecting group removal using *syn*-2-pyridinealdoximine and tetramethyl-guanidine followed by treatment with ammonium hydroxide[13] gave **3** in 46% yield. The complete structure of **3** was established by multinuclear NMR spectral data, HRMS, and quantitative UV data.* Consistent with the absence of base stacking, no observed hypochromicity could be discerned from the UV data. Support of the cyclic nature of **3** also came from the NMR data, through observation of the downfield shift of both H-3′ hydrogens compared to the uncyclized dinucleotide and from the splitting of the carbon resonances for both C-5′ carbons to doublets.

Integrase inhibition assays were conducted with purified recombinant HIV-1 integrase using a 21-mer oligonucleotide substrate.[6,7] The data (Table 1) clearly showed that the cyclic dinucleotide **3** was not an inhibitor of HIV-1 integrase, in contrast to its non-cyclic counterparts **1** and **2**.

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*HNMR (D_2O): 7.91 (s, 1H), 7.54 (s, 1H), 6.13 (m, 1H), 6.07 (m, 1H), 4.88 (m, 1H), 4.75 (m, 1H), 4.00–4.09 (m, 4H), 3.89–3.93 (m, 2H), 2.80 (m, 1H), 2.44–2.61 (m, 3H), 1.68 (s, 3H). ^{13}CNMR (D_2O): 166.5, 159.0, 153.9, 151.6, 151.0, 137.4 (two carbons, T-6, G-8), 116.4, 111.4, 84.4, 83.1, 82.5, 82.2, 71.5, 70.5, 62.4, 62.1, 38.3, 38.1, 11.6. ^{31}PNMR (D_2O): −0.075, −0.29. FAB-HRMS: $[M + Na]^+$ calcd. for $C_{20}H_{25}N_7NaO_{13}P_2$ 656.0883, found 656.0905. UV (H_2O): λ_{max} 256 (ε 18,900).

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