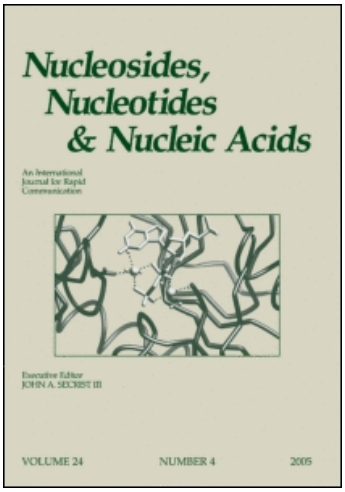

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Design and Synthesis of Specific Inhibitors of the 3'-Processing Step of HIV-1 Integrase

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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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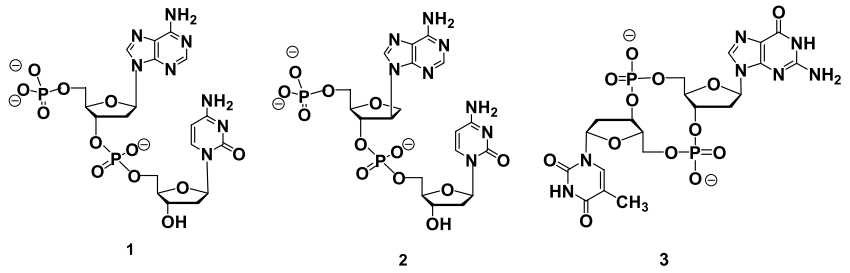
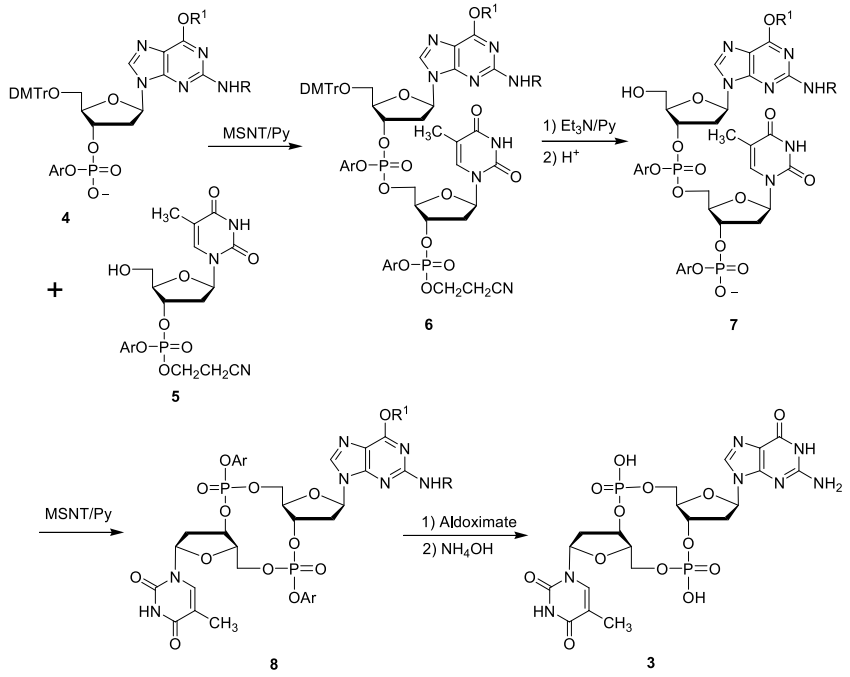


FIGURE 1 Structures of compounds 1, 2 and 3.

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**.

REFERENCES

1. Frankel, A.D.; Young, J.A.T. HIV-1: fifteen proteins and an RNA. Annu. Rev. Biochem. **1998**, *67*, 1–25.
2. Asante-Appiah, E.; Skalka, A.M. HIV-1 integrase: structural organization, conformational changes, and catalysis. Adv. Virus Res. **1999**, *52*, 351–369.
3. Esposito, D.; Craigie, R. HIV integrase structure and function. Adv. Virus Res. **1999**, *52*, 319–333.
4. Nair, V. HIV integrase as a target for antiviral chemotherapy. Rev. Med. Virol. **2002**, *12*, 179–193.
5. Engelman, A.; Mizuuchi, K.; Craigie, R. HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell **1991**, *67*, 1211–1221.
6. Mazumder, A.; Uchida, H.; Neamati, N.; Sunder, S.; Jaworska-Maslanka, M.; Wickstrom, E.; Zeng, F.; Jones, R.A.; Mandes, R.F.; Chenault, H.K.; Pommier, Y. Probing interactions between viral DNA and human immunodeficiency virus type 1 integrase using dinucleotides. Mol. Pharmacol. **1997**, *51*, 567–575.

*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).

7. Taktakishvili, M.; Neamati, N.; Pommier, Y.; Pal, S.; Nair, V. Recognition and inhibition of HIV integrase by novel dinucleotides. J. Am. Chem. Soc. **2000**, *122*, 5671–5677.
8. Hsu, C.-Y.; Dennis, D.; Jones, R.A. Synthesis and physical characterization of bis- 3′, 5′ cyclic dinucleotides (Np–Np): RNA polymerase inhibitors. Nucleosides Nucleotides **1985**, *4*, 377–389.
9. Barbato, S.; De Napoli, L.; Mayol, L.; Piccialli, G.; Santacroce, C. A polymer-nucleotide linkage useful for the solid-phase synthesis of cyclic oligodeoxyribonucleotides. Tetrahedron **1987**, *45*, 4523–4536.
10. Capobianco, M.L.; Carcuro, A.; Tondelli, L.; Garbesi, A.; Bonora, G.M. One pot solution synthesis of cyclic oligodeoxyribonucleotides. Nucleic Acids Res. **1990**, *18*, 2661–2669.
11. Zeng, F.; Jones, R.A. Synthesis of cyclic dinucleotides by an H-phosphonate method in solution. Nucleosides Nucleotides **1996**, *15*, 1679–1686.
12. Sproat, B.S.; Gait, M.J. *Solid-phase Synthesis of Oligodeoxyribonucleotides by the Phosphotriester Method in Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984; 94–96.
13. Reese, C.B.; Titmas, R.C.; Yau, L. Oximate ion promoted unblocking of oligonucleotide phosphotriester intermediates. Tetrahedron Lett. **1978**, *19*, 2727–2730.