Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 249–251

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Recognition and Inhibition of HIV Integrase by a Novel Dinucleotide

Michael Taktakishvili,[a] Nouri Neamati,[b] Yves Pommier[b] and Vasu Nair[a,*]

[a]*Department of Chemistry, The University of Iowa, Iowa City, IA 52242, USA*
[b]*Laboratory of Pharmacology, National Cancer Institute, NIH, Bethesda, MD 20892, USA*

Received 26 October 1999; accepted 22 November 1999

Abstract—The viral enzyme, HIV integrase, is involved in the integration of viral DNA into host cell DNA. In the quest for a small nucleotide system with nuclease stability of the internucleotide phosphate bond and critical structural features for recognition and inhibition of HIV-1 integrase, we have discovered a conceptually novel dinucleotide, pIsodApdC, which is a potent inhibitor of this key viral enzyme.

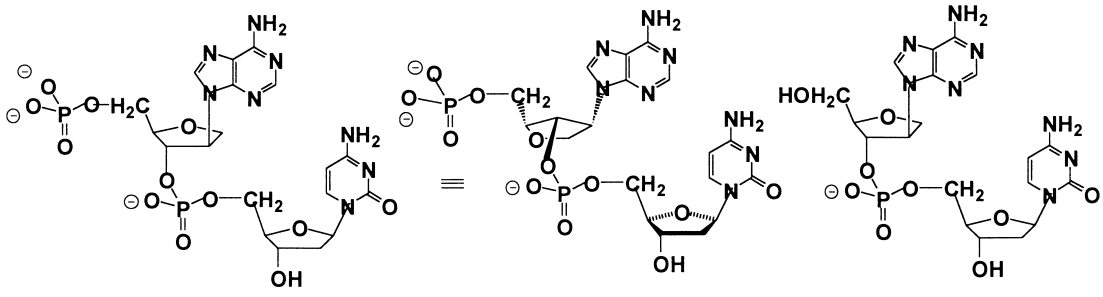
The viral enzyme, HIV integrase, is involved in the integration of viral DNA into host cell DNA, a biological process that occurs by a sequence involving DNA splicing (3′-processing) and coupling (integration) reactions.[1–4] The enzyme apparently recognizes specific sequences (5′-ACTG...CAGT-3′) in the LTRs of viral DNA. In the first step which involves 3′-processing (or cleavage step), specific endonuclease activity removes two nucleotides from each end of the double helical viral DNA producing new 3′-hydroxyl ends (CAOH-3′). This truncated viral DNA is coupled in the next steps to host cell DNA (integration) which includes the DNA strand transfer reaction. Both the 3′-processing and strand transfer steps involve transesterifications. The integration process is essential for the replication of HIV. Although studies on the search for clinically useful anti-integrase agents are relatively recent, the screening of inhibitors against purified recombinant integrase has contributed to the identification of some interesting lead compounds which include small and large nucleotides.[5–8] In the quest for a small nucleotide system with nuclease stability of the internucleotide phosphate bond and critical structural features for recognition and inhibition of HIV integrase, we have discovered a novel dinucleotide, pIsodApdC (**1**), which is a potent inhibitor of this key viral enzyme.

Compound **1** is an unusual phosphorylated dinucleotide which is composed of an L-related isodeoxynucleoside[9–13] and a D-deoxynucleoside. The stereochemical implications of such a structure are illustrated in **1** (right). Dinucleotide **1** and its precursor **2** were synthesized in several steps from protected (*S,S*)-isodeoxyadenosine[14] in an overall yield of over 10% (Scheme 1). These target compounds were purified by reversed-phase HPLC on a C_{18} column (ethanol/water elution) and initially identified by comparison of ion-exchange retention times with natural dinucleotides (Partisil-10 SAX ion-exchange HPLC column, phosphate buffer system, retention times for **1** and **2**: 40 and 70 min, respectively). Their complete structures were established by multinuclear NMR data (^{1}H, ^{13}C, ^{31}P NMR spectra and COSY, HMQC and HMBC data), quantitative UV spectra, CD spectra, and electrospray (ESI) HRMS data (calculated for **1**: 619.1067 $(M–H)^-$, found: 619.1056; calculated for **2**: 539.1404 $(M–H)^-$, found: 539.1396). The quantitative UV spectral data (λ_{max} 263 nm, ε 19,500 and 19,400) gave evidence of hypochromicity in these molecules. This and the CD data suggested the existence of base stacking interactions. In order for stacking interactions to occur, the carbohydrate moieties must assume an orthogonal relationship, which results in an unusual internucleotide phosphate linkage. The consequences of this with respect to nuclease stability are discussed below.

HIV integrase inhibition assays were conducted with purified recombinant integrase using a 21-mer oligonucleotide substrate as described previously by Pommier

Keywords: antivirals; nucleotides; enzyme inhibitors.
*Corresponding author. Tel.: +1-319-335-1364; fax: +1-319-353-2621; e-mail: vasu-nair@uiowa.edu

0960-894X/00/$ - see front matter
PII: S0960-894X(99)00677-0



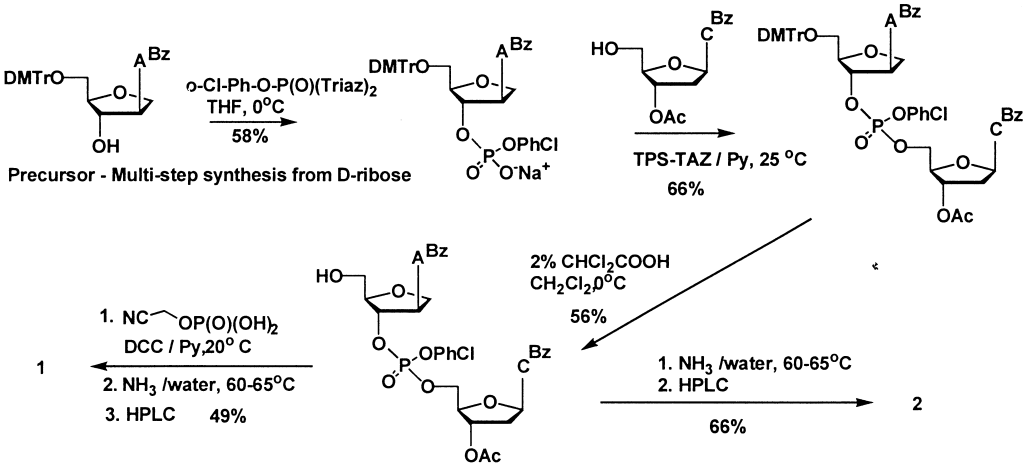
1 2

and co-workers.[15] The data are summarized in Table 1. Compound **1** was found to have strong inhibitory activity against recombinant wild type HIV integrase in reproducible assays (IC_{50} 19 μM for 3′-processing and 25 μM for strand transfer). The activity is much greater than for dideoxynucleoside monophosphates[5,6] and is close to the activity of the corresponding "natural dinucleotide" pdApdC.[16] The significant anti-integrase activity of dinucleotide **1** suggests some sequence selectivity, which is consistent with the catalytic mechanism of 3′-processing. Molecular recognition by the integrase of the ultimate and penultimate bases (5′-AC-3′) at the 5′-end of the minus strand of non-cleaved viral DNA may result in stable complex formation before the strand transfer reaction.[17] Thus, the potent inhibitor activity of **1** may reflect the affinity that HIV integrase has for this dinucleotide sequence and other data[16] also suggest that two neighboring bases may fulfill a substantial part of the essential interaction requirements when integrase recognizes its viral DNA substrate. The terminal 5′-phosphate appears to be essential for activity as the precursor of **2**, i.e. the dinucleotide that is devoid of the 5′-phosphate group, is much less potent. Compounds **1** and **2** inhibited all enzymatic activities of integrase and DNA-integrase cross-linking in the same range as indicated in Table 1 when Mn^{+2} or Mg^{+2} was used as a cofactor (data not shown). This suggests that compound **1** binds to the catalytic core of integrase and the inhibition of integrase by **1** is metal-independent.

Another remarkable aspect of compounds **1** and **2** is that the internucleotide bond exhibits resistance to cleavage by mammalian 5′- and 3′-exonucleases (phosphodiesterases (PDE)). For example, for compound **2**, cleavage of the internucleotide phosphate bond is approximately 33% of that for the natural dinucleotide, dApdC, with PDE I and approximately 20% with PDE II. The results are graphically represented in Figures 1 and 2. The resistance to internucleotide phosphate bond cleavage is not associated with chemical alteration of the phosphate bond, as is commonly found with nuclease-resistant compounds, but with the structural distortion of this phosphate bond as illustrated in **1**. Further studies of conceptually-new, nuclease-resistant dinucleotides as inhibitors of HIV integrase are in progress.

Table 1. Anti-integrase inhibition data for **1** and **2** and some selected compounds

Compound	3′-processing (μM)	Strand transfer (μM)
PIsodApdC **1**	19	25
IsodApdC **2**	200	200
(*S*,*S*)-IsoddAMP	>200	>200
L-ddCMP[5]	50	45
AZTMP[6]	>110	140
pdApdC[13]	6	3



Scheme 1.

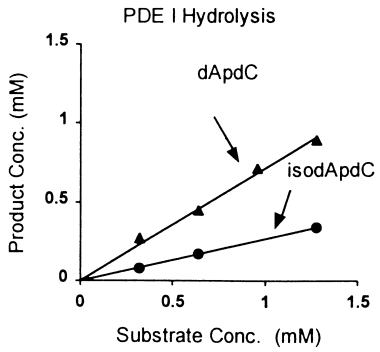


Figure 1. Hydrolysis of isodApdC in comparison with the natural dinucleotide, dApdC, with PDE I from bovine intestinal mucosa.

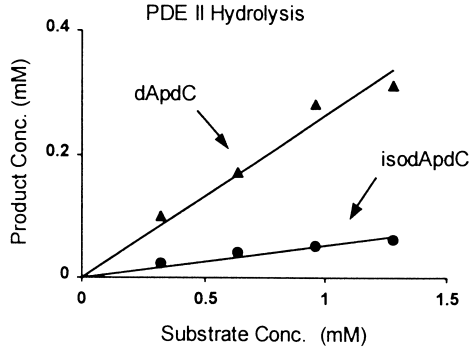


Figure 2. PDE II from bovine spleen.

Acknowledgments

We thank Dr. Suresh Pal for help with the PDE studies, Dr. Lynn Teesch for the ESI HRMS data, and Mr. Guisen Zhao for the preparation of isodeoxyadenosine. We gratefully acknowledge support of this research by the National Institutes of Health (AI 43181).

References and Notes

1. Drake, R.; Neamati, N.; Hong, H.; Pilon, A. A.; Sunthankar, P.; Hume, S. D.; Milne, G. W. A.; Pommier, Y. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4170.
2. Katz, R. A.; Skalka, A. M. *Ann. Rev. Biochem.* **1994**, *63*, 133.
3. Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Engelman, A.; Craigie, R.; Davies, D. R. *Science* **1994**, *266*, 1981.
4. Vaishnav, Y. N.; Wong-Staal, F. *Ann. Rev. Biochem.* **1991**, *60*, 577.
5. Mazumder, A.; Neamati, N.; Sommadossi, J.-P.; Gosselin, G.; Schinazi, R. F.; Imbach, J.-L.; Pommier, Y. *Mol. Pharmacol.* **1996**, *49*, 621.
6. Mazumder, A.; Cooney, D.; Agbaria, R.; Gupta, M.; Pommier, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5771.
7. Ojwang, J. O.; Buckheit, R. W.; Pommier, Y.; Mazumder, A.; De Vreese, K.; Este, J. A.; Reymen, D.; Pallansch, L. A.; Lackman-Smith, C.; Wallace, T. L.; De Clercq, E.; McGrath, M. S.; Rando, R. F. *Antimicrob. Agents Chemother.* **1995**, *39*, 2426.
8. Mazumder, A.; Neamati, N.; Ojwang, J. O.; Sunder, S.; Rando, R. F.; Pommier, Y. *Biochemistry* **1996**, *35*, 13762.
9. Nair, V.; Jahnke, T. S. *Antimicrob. Agents and Chemother.* **1995**, *39*, 1017.
10. Bolon, P. J.; Sells, T. B.; Nuesca, Z. M.; Purdy, D. F.; Nair, V. *Tetrahedron* **1994**, *50*, 7747.
11. Nair, V.; Nuesca, Z. M. *J. Am. Chem. Soc.* **1992**, *114*, 7951.
12. Nair, V.; Jahnke, T. S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2235.
13. Nair, V.; St. Clair, M.; Reardon, J. E.; Krasny, H. C.; Hazen, R. J.; Paff, M. T.; Boone, L. R.; Tisdale, M.; Najera, I.; Dornsife, R. E.; Everett, D. R.; Borroto-Esoda, K.; Yale, J. L.; Zimmerman, T. P.; Rideout, J. L. *Antimicrob. Agents and Chemother.* **1995**, *39*, 1993.
14. Nair, V.; Wenzel, T. *Bioconjugate Chem.* **1998**, *9*, 683.
15. Mazumder, A.; Neamati, N.; Sundar, S.; Owen, J.; Pommier, Y. In *Methods in Cellular and Molecular Biology: Antiviral Evaluation*; Kinchington, D., Schinazi, R., Eds.; Humana: Totowa, 1998. Integrase Assays. In brief, IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, (pH 7.5), 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (wt/vol), 7.5 mM $MnCl_2$, 0.1 mg of bovine serum albumin per ml, 10 mM 2-mercaptoethanol, 10% DMSO, and 25 mM MOPS (morpholinepropanesulfonic acid) (pH 7.2) at 30 °C for 30 min. Then, the 5′-end ^{32}P-labeled linear oligonucleotide substrate (20 nM) was added, and incubation was continued for 1 h. Reactions were quenched by the addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, 0.025% bromophenol blue). An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M Tris–borate (pH 8.3), 2 mM EDTA, 20% acrylamide, 8 M urea). Gels were dried, exposed in a PhosphorImager cassette, and analyzed with a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Percent inhibition was calculated using the following equation: inhibition = $100\times[1-(D-C)/(N-C)]$, where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The 50% inhibitory concentrations (IC_{50}) were determined by plotting the log of drug concentration versus percent inhibition and identifying the concentration which produced an inhibition of 50%.
16. 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. *Mol. Pharmacol.* **1997**, *51*, 567.
17. Ellison, V.; Brown, P. O. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7316.