Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4815–4817
Bioorganic & Medicinal Chemistry Letters

Inhibition of the strand transfer step of HIV-1 integrase by non-natural dinucleotides

Guochen Chi,[a] Nouri Neamati[b] and Vasu Nair[a,*]

[a]*Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30602, USA*
[b]*Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90089, USA*

Received 19 June 2004; revised 22 July 2004; accepted 23 July 2004
Available online 13 August 2004

Abstract—New, non-natural dinucleotide 5′-monophosphates, with a surrogate isonucleoside component of L-related stereochemistry at the 'terminal' position, have been synthesized. Structures of **2a–c** were confirmed by multinuclear NMR spectra (^{1}H, ^{13}C, ^{31}P, COSY), UV hypochromicity and FAB HRMS data. These compounds are totally resistant to cleavage by 3′- and 5′-exonucleases. The dinucleotides showed remarkable selectivity for inhibition of the strand transfer step of HIV-1 integrase. To the best of our knowledge, these compounds represent only the second example of selective strand transfer inhibitors of HIV integrase.

The retroviral enzyme, HIV-1 integrase, incorporates HIV double helical DNA into host chromosomal DNA.[1–7] This viral enzyme first catalyzes the endonucleatic removal of two terminal nucleotides at the 3′-end of each strand of viral DNA (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 this is followed by integration of the tailored HIV DNA into host DNA.[1–3] The integration process is essential for the replication of HIV and there is apparently 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.[8] Protein–nucleotide interactions appear to be of importance in other steps of the replication cycle of HIV such as the recognition and binding of Tat protein to HIV-1 TAR RNA.[9] However, small oligonucleotides of natural origin 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.[8] A non-natural dinucleotide with a conformationally unusual internucleotide phosphodiester bond that joins a D-deoxynucleoside and an L-related isodeoxynucleoside, **1** (Fig. 2),

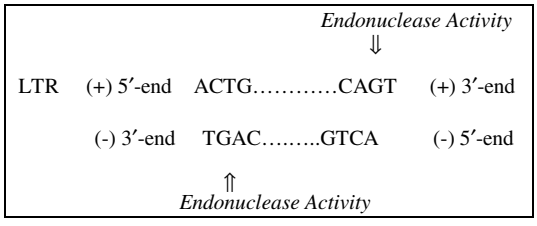


Figure 1. Processing of HIV DNA prior to integration into host DNA.

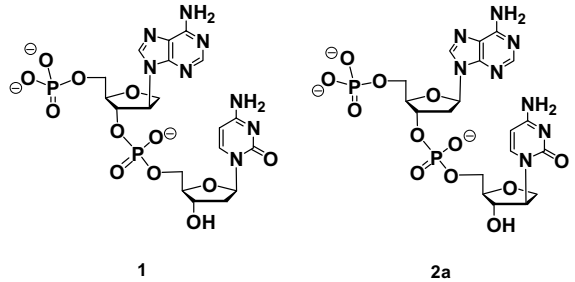
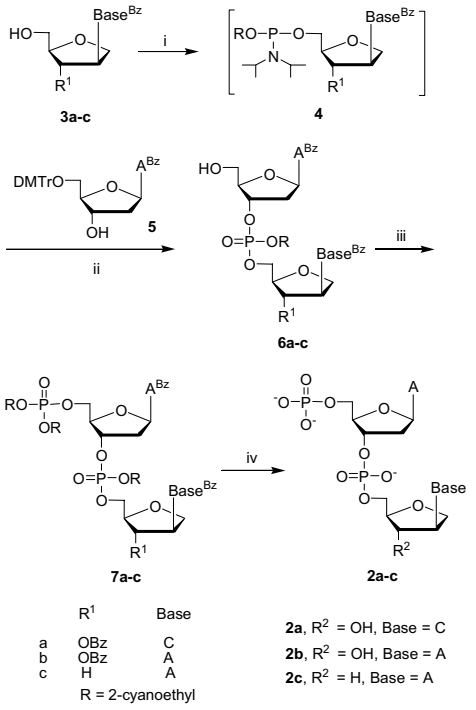


Figure 2. Structure of the anti-HIV integrase compound **1** and its isomer **2a** in which the position of the surrogate isonucleoside is changed.

Keywords: HIV integrase; Dinucleotides; Strand transfer inhibitors.
* Corresponding author. Tel.: +1-706-542-6293; fax: +1-706-583-8283; e-mail: vnair@rx.uga.edu

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.07.050



Scheme 1. Reagents and conditions: [i] $(i\text{-}Pr_2N)_2POCH_2CH_2CN$, 1H-tetrazole, CH_2Cl_2, rt, 1 h; [ii] (1) 1H-tetrazole, 3 h, (2) I_2/THF/H_2O/Py; (3) 2% $CHCl_2COOH$ in CH_2Cl_2; [iii] (1) $i\text{-}Pr_2NP(OCH_2CH_2CN)_2$, 1H-tetrazole, CH_2Cl_2, 1 h; (2) I_2/THF/H_2O/Py; [iv] concd NH_4OH, rt, 24 h.

previously synthesized by us,[10] exhibits resistance to mammalian 3′- and 5′-exonucleases.[11] This compound is an inhibitor of wild-type HIV-1 integrase activity, inhibiting both the 3′-processing and strand transfer steps.[10] We report here some new results that suggest that changing the position of the surrogate nucleoside component of the dinucleotide (e.g., compound **2a**) can dramatically change the mode of inhibitory activity from both key steps of integrase action to just the strand transfer step.

The isonucleosides were prepared from ribitol cyclic sulfate as the precursor[12] and then were appropriately tailored for the coupling steps. Dinucleotides were synthesized through the phosphoramidite method Scheme 1).[10] Thus, isodeoxynucleoside **3** with a free 5′-hydroxyl group was condensed with the phosphorylating reagent [$(i\text{-}Pr_2N)_2POCH_2CH_2CN$] in the presence of 1H-tetrazole to give the phosphoramidite **4**, which was immediately coupled through the free 3′-hydroxyl group of deoxynucleoside **5**. Iodine oxidation and selective removal of the trityl group using dichloroacetic acid[10] provided the phosphotriester **6**. Phosphorylation of dinucleotide **6** was performed using di(2-cyanoethyl)*N*,*N*-diisopropyl-phosphoramidite as the phosphorylating reagent.[13] After deprotection, the final products were purified by HPLC. The structures of **2a**–**c** were confirmed by multinuclear NMR data (^1H, ^{13}C, ^{31}P, COSY) and FAB HRMS data.[14] The observed hypochromicity (12–18%) from the quantitative UV data [**2a**, λ_{max} 262 (ε 19,900); **2b**, λ_{max} 259 (ε 26,400); **2c**, λ_{max} 259 (ε 24,500)] suggested the presence of base stacking in the preferred conformations. Base stacking implies the presence of conformationally unusual internucleotide phosphate bonds because of the spatial arrangement of the two sugar rings that is necessary to accommodate this assembly of the bases (Fig. 2).

Table 1. Anti-HIV-1 integrase data for dinucleotides

Compounds	3′-Processing, IC_{50} (μM)	Strand transfer, IC_{50} (μM)
2a	>1000	65
2b	>1000	41
2c	>1000	114
1	19	25

Integrase inhibition assays were conducted with purified recombinant HIV-1 integrase using a 21-mer oligonucleotide substrate.[15] The data (Table 1) clearly showed that compounds **2a**–**c** have strand transfer inhibitory activity against wild-type HIV-1 integrase but do not exhibit inhibition of the 3′-processing step. This is in sharp contrast to compound **1** (pIsodApdC), which showed strong inhibition of both key steps of the integrase mechanism of action. The major structural difference between dinucleotide **1** and its counterpart **2a** (or the related compound **2b**) is the position of the surrogate isonucleoside component. The inhibition of integrase by **1** is likely the result of base recognition and binding by the viral enzyme. Thus, it is remarkable that this apparently small structural change in the counterpart of **1** can produce such a major impact on the mode of inhibition of integrase. The only reported selective inhibitor of strand transfer is a class of diketo containing compounds,[16] and, to the best of our knowledge, the compounds described in this communication represent the second examples. Finally, compounds **2a**–**c** are resistant to cleavage of the internucleotide phosphodiester linkage by mammalian 3′- and 5′-exonucleases.

Acknowledgements

The project described was supported by Grant Number RO1 AI 43181 (to V.N.) from the National Institutes of Health. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. One of us (V.N.) thanks the Georgia Research Alliance for an award toward the purchase of the 500 MHz NMR spectrometer used in this project.

References and notes

1. Frankel, A. D.; Young, J. A. T. *Annu. Rev. Biochem.* **1998**, *67*, 1.
2. Esposito, D.; Craigie, R. *Adv. Virus Res.* **1999**, *52*, 319.
3. Haren, L.; Ton-Hoang, B.; Chandler, M. *Annu. Rev. Microbiol.* **1999**, *53*, 245.
4. Dyda, F.; Hickman, A. B.; Jenkins, T. M.; Engelman, A.; Craigie, R.; Davies, D. R. *Science* **1994**, *266*, 1981.
5. Mazumder, A.; Neamati, N.; Ojwang, J. O.; Sunder, S.; Rando, R. F.; Pommier, Y. *Biochemistry* **1996**, *35*, 13762.
6. Nair, V. *Rev. Med. Virol.* **2002**, *12*, 179.
7. Nair, V. *Curr. Pharm. Des.* **2003**, *9*, 2553.

8. 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.
9. Tao, J.; Frankel, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2723.
10. Taktakishvili, M.; Neamati, N.; Pommier, Y.; Pal, S.; Nair, V. *J. Am. Chem. Soc.* **2000**, *122*, 5671.
11. Nair, V.; Pal, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 289.
12. Bera, S.; Nair, V. *Tetrahedron Lett.* **2001**, *42*, 5813.
13. Zhu, X. F.; Scott, A. I. *Nucleosides Nucleotides* **2001**, *20*, 197.
14. Experimental data: **2a**, ^{1}H NMR (D_2O): 8.40 (s, 1H), 8.20 (s, 1H), 7.74 (d, 1H, J = 7.5), 6.37 (t, 1H, J = 6.5), 6.04 (d, 1H, J = 7.5), 4.82 (m, 1H), 4.73 (m, 1H), 4.28 (m, 2H), 3.98–4.09 (m, 3H), 3.89–3.93 (m, 3H), 3.81 (m, 1H), 2.71 (m, 1H), 2.61 (m, 1H). ^{13}C NMR (D_2O): 159.2, 150.5, 149.2, 148.3, 146.4, 145.5, 142.4, 118.5, 95.2, 85.5, 84.7, 84.3, 75.98, 75.92, 69.0, 65.1, 64.8, 64.1, 38.8. ^{31}P NMR: 0.94, −0.030. FAB-HRMS: $[M+1]^+$ calcd for $C_{19}H_{27}N_8O_{12}P_2$ 621.1224, found 621.1217. UV (H_2O): λ_{max} 262 (ε 19,900).
2b,^{1}H NMR (D_2O): 8.37 (s, 1H), 8.26 (s, 1H), 8.19 (s, 1H), 8.16 (s, 1H), 6.29 (t, 1H, J = 7.0), 4.98 (m, 1H), 4.80 (s, br, 1H), 4.47 (m, 1H), 4.21–4.28 (m, 3H), 3.90–4.04 (m, 5H), 2.63–2.66 (m, 1H), 2.55–2.58 (m, 1H). ^{13}C NMR (D_2O): 150.2, 150.1, 148.5, 148.0, 145.1, 144.8, 142.8, 142.4, 118.4, 118.1, 85.3, 84.6, 84.0, 76.1, 75.8, 69.8, 64.6, 64.1, 62.8, 38.7. ^{31}P NMR: 0.065, −0.75. FAB-HRMS: $[M+1]^+$ calcd for $C_{20}H_{27}N_{10}O_{11}P_2$ 645.1336, found 645.1325. UV (H_2O): λ_{max} 259 (ε 26,400).
2c,^{1}H NMR (D_2O): 8.39 (s, 1H), 8.34 (s, 1H), 8.19 (s, 1H), 8.16 (s, 1H), 6.30 (t, 1H, J = 6.5), 5.21 (m, br, 1H), 4.82 (s, br, 1H), 4.27 (s, br, 1H), 4.21 (s, br, 1H), 4.14 (d, 1H, J = 10.5), 4.04 (m, 1H), 3.98 (dd, 1H, J = 10.5, 5.5), 3.86–3.92 (m, 3H), 2.67 (m, 2H), 2.58 (m, 1H), 2.11 (m, 1H). ^{13}C NMR (D_2O): 150.4, 150.1, 148.4, 148.2, 145.3, 144.8, 142.9, 142.4, 118.5, 118.1, 85.5, 84.7, 78.3, 75.9, 72.3, 65.9, 64.8, 55.7, 38.9, 33.6. ^{31}P NMR (D_2O): 1.00, 0.25. FAB-HRMS: $[M+1]^+$ calcd for $C_{20}H_{27}N_{10}O_{10}P_2$ 629.1387, found 629.1412. UV (H_2O): λ_{max} 259 (ε 24,500).
15. Mazumder, A. M.; Neamati, N.; Sunder, S.; Owen, J.; Pommier, Y. In *Methods in Cellular and Molecular Biology: Antiviral Evaluation*; Kinchington, D., Schinazi, R., Eds.; Humana: Totowa, 1998.
16. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L. Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.