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INHIBITION OF HIV INTEGRASE BY NOVEL NUCLEOTIDES BEARING TRICYCLIC BASES

Jianzhong Zhang,[a] Nouri Neamati,[b] Yves Pommier,[b] and Vasu Nair[a]*

[a] *Department of Chemistry, The University of Iowa, Iowa City, IA 52242, U.S.A.*

[b] *Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, Bethesda, MD 20892, U.S.A.*

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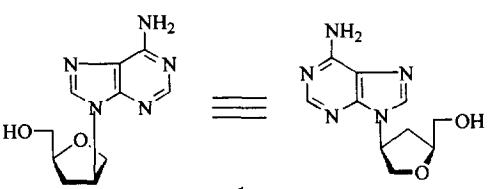
Abstract: 5'-Monophosphates of several novel dideoxynucleosides bearing tricyclic nucleobases were synthesized. Both linear and angular ring-extended analogs of isomeric dideoxyadenosine 5'-monophosphate were discovered to have moderate to good inhibition of the viral-encoded enzyme, HIV integrase. The results suggest that the nucleotide binding site of HIV integrase can accommodate major modifications in the nucleobase, which is in stark contrast to the nucleotide binding site on HIV reverse transcriptase.

Introduction

The viral encoded enzyme, human immunodeficiency virus (HIV) integrase, catalyzes the incorporation of double-stranded viral DNA resulting from reverse transcription of viral RNA, into the host cell DNA genome.[1,2] The integration process is essential for the replication of HIV. Studies on the discovery of clinically useful agents targeted against the enzyme are relatively recent.[3,4] Rapid screening of inhibitors against purified recombinant integrase has helped in identifying some lead compounds. For example, it was shown that HIV-1 integrase can accommodate a variety of structurally dissimilar phosphoryl donor and acceptor substrate molecules in the active site, which suggests that nucleotides could also be inhibitors of HIV-1 integrase.[5,6] Indeed, AZTMP and other nucleotides are known to inhibit the 3'-processing and strand transfer processes of HIV-1 integrase.[5,7] In a recent report, Drake et al. have also shown that a photoaffinity analog of AZTMP crosslinks to a peptide region corresponding to amino acids 158-170 of HIV-1 integrase, which appears to be part of the active site of the enzyme.[8] As a continuation of our efforts in the discovery of new antiviral agents targeted against key enzymes of HIV-1 replication, we synthesized novel ring extended analogs of the anti-HIV active compound **1**[9,10] and investigated their inhibitory activity against HIV-1 integrase.[11]



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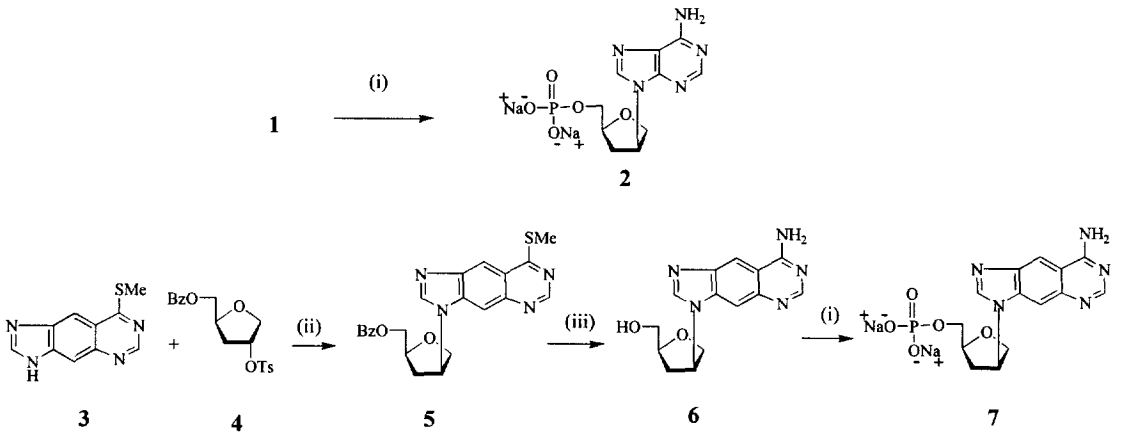
Results and Discussion

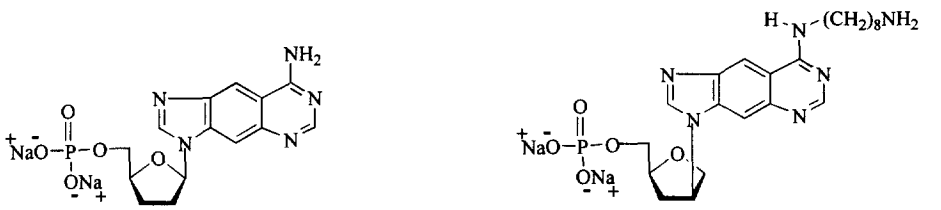
Compound **2** was prepared by phosphorylation of **1**[10] with $POCl_3$ at 0 °C followed by purification using reversed-phase HPLC. Its linear ring-extended analog, isodideoxy-lin-benzoadenosine 5'-mono-phosphate (**7**), was prepared by coupling of tosylate **4**, synthesized in several steps from D-xylose,[10] with benzopurine thiomethyl ether **3**[12] followed by treatment of the product **5** (45% yield) with CH_3OH/NH_3 (90% yield) and phosphorylation (34%). Compound **7** exhibited moderate inhibitory activity against HIV integrase in the strand transfer step (IC_{50} = 68 μM), and served as a lead compound in the search for more potent inhibitors.



1 2 3 4 5 6 7

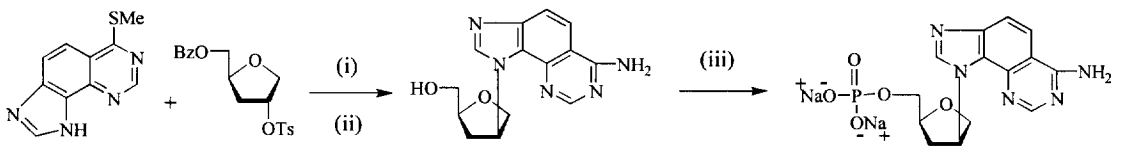
Reagents: (i) $POCl_3$, $(EtO)_3PO$, 0 °C; (ii) K_2CO_3, 18-crown-6-ether, 95 °C; (iii) NH_3, MeOH

Two analogues of **7**, compounds **8** and **9**, were subsequently synthesized. Compound **8**, the normal analog of **7** (i.e. the compound with the base at the 1'-position), was prepared through the Vorbruggen coupling method (26%) followed by treatment with methanolic ammonia (60%) and phosphorylation (53%). It was synthesized for direct comparison with the activity of **7**. Compound **9**, prepared as described for **7**, was designed with the expectation that the long alkyl chain could act as a DNA minor-groove binder. However, both compounds were found to exhibit very low inhibitory activity against HIV integrase (e.g. for compound **8**, IC_{50} = 750 μM for 3'-processing and IC_{50} = 550 μM for strand transfer).



8 9

In the search for more active compounds related to **7**, an angular tricyclic nucleoside **11** was synthesized from the condensation of the angular benzopurine **10** (prepared from 4-chloroanthranilic acid[12,13]) with tosylate **4** followed by treatment with methanolic ammonia. The site of attachment of the sugar moiety on nucleobase **10** was apparent from the observation of a large downfield shift (~1.0 ppm) of the 2'-proton chemical shift in **11** compared to the chemical shift of its counterpart proton in **6**, probably resulting from the anisotropic effect of the pyrimidine ring in **11**. Extensive NOE studies also supported the assigned regiochemistry. Compound **12**,[14] the monophosphate of **11**, displayed good inhibition of HIV integrase (IC_{50} = 75 μM for 3'-processing and IC_{50} = 53 μM for strand transfer).[15,16] Its activity is comparable to the best known mononucleotide inhibitors of the enzyme,[5] but this compound and its linear analog **7** represent the first examples of nucleotides with ring-extended bases that have been found to have anti-integrase activity.



10 **4** **11** **12**

Reagents: (i) K_2CO_3, 18-crown-6-ether, 95 °C; (ii) NH_3, MeOH; (iii) $POCl_3$, $(EtO)_3PO$, 0 °C

In summary, we have discovered that HIV integrase is inhibited by the 5'-monophosphate of certain isomeric dideoxynucleosides bearing tricyclic nucleobases. Our results suggest that the nucleotide binding site of HIV integrase can accommodate modifications in the nucleobase including a ring-extended system. This is in contrast to the nucleotide binding site on HIV reverse transcriptase (polymerase active site of the p66 subunit) which exhibits little tolerance for modified bases. Further structure-activity studies on the inhibition of HIV integrase by nucleotides with linearly and angularly extended bases are in progress.

Acknowledgment. We thank the National Institutes of Health for support of this investigation.

References and Notes

1. Katz, R. A.; Skalka, A. M. *Ann. Rev. Biochem.* **1994**, *63*, 133.
2. Rice, P.; Craigie, R.; Davies, D. R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 76.
3. Pommier, Y.; Pilon, A.; Bajaj, K.; Mazumder, A.; Neamati, N. *Antiviral Chem. Chemother.* **1997**, *8*, 463.
4. Neamati, N.; Sunder, S.; Pommier, Y. *Drug Discovery Today* **1997**, *2*, 487.
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.; 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.
7. Mazumder, A.; Cooney, D.; Agbaria, R.; Gupta, M.; Pommier, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*,

5771.
8. Drake, R.; Neamati, N.; Hong, H.; Pilon, A. A.; Sunthankar, P.; Hume, S. D.; Milne, G. W. A.; Pommier, Y. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 4170.
9. Nair, V.; St. Clair, M. H.; Reardon, J. E.; Krasny, H. C.; Hazen, R. J.; Paff, M. T.; Boone, L. R.; Tisdale, M.; Najera, I.; Dornsife, R. E.; Averett, D. R.; Borroto-Esoda, K.; Yale, J. L.; Zimmerman, T. P.; Rideout, J. L. *Antimicrob. Agents Chemother.* **1995**, *39*, 1993.
10. Bolon. P. J.; Sells, T. B.; Nuesca, Z. M.; Purdy, D. F.; Nair, V. *Tetrahedron* **1994**, *50*, 7747; Nair, V. and Nuesca, Z. M. *J. Am. Chem. Soc.* **1992**, *114*, 7951.
11. Part of the synthetic work described in this manuscript was presented at the 214th American Chemical Society National Meeting in Las Vegas, Nevada, September, 1997.
12. Leonard, N. J.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* **1975**, *40*, 356.
13. Morrice, A. G.; Sprecker, M. A.; Leonard, N. J. *J. Org. Chem.* **1975**, *40*, 363.
14. 4(*S*)-(6-Amino-1*H*-imidazo [4,5-*h*] quinazolin-1-yl) -2(*S*)-tetrahydrofuranmethyl phosphate **12**: A mixture composed of **4**[10] (120 mg, 0.319 mmol), **10**[12,13] (83 mg, 0.382 mmol), K_2CO_3 (138 mg, 0.638 mmol) and 18-crown-6 ether in 10 mL of dry DMF was heated at 95 °C for 5 h. Removal of DMF in vacuo resulted in a viscous oily residue which was chromatographed with 4% MeOH/$CHCl_3$ on silica gel plates to afford the coupled product (87 mg, 65% yield) as a light yellow viscous oil: UV (MeOH) 243 nm, 274, 302, 314, 327; ^{1}H NMR ($CDCl_3$) δ 2.18 (m, 1H), 2.73 (s, 3H), 3.00 (m, 1H), 4.28 (dd, J = 10.2 Hz, 5.7 Hz, 1H), 4.48 (m, 3H), 4.61 (dd, J = 8.7 Hz, 1.2 Hz, 1H), 6.71 (m, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.56 (t, J = 7.2 Hz, 1H), 7.94 (m, 4H), 8.42 (s, 1H), 9.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.7, 37.0, 57.6, 65.5, 73.5, 77.2, 118.0, 120.5, 121.5, 126.5, 128.4, 129.5, 129.6, 133.2, 138.9, 141.5, 146.4, 152.4, 166.3, 170.9. This coupled product was allowed to react with methanolic ammonia at 120 °C for 22 h to afford **11** in 86% yield after purification on silica gel plates (10% MeOH/$CHCl_3$): mp 278 - 280 °C; $[\alpha]_D$ -46.0° (*c* 0.01, MeOH); UV (MeOH) 227 nm, 251, 284 (sh), 295, 307, 321; ^{1}H NMR (DMSO-d_6) δ 2.15 (m, 1H), 2.69 (m, 1H), 3.59 (m, 2H), 4.08 (m, 3H), 4.98 (br, 1H, D_2O exchangeable), 6.56 (m, 1H), 7.73 (d, J = 9.0 Hz, 3H, D_2O partially exchangeable, NH_2), 7.97 (d, J = 8.7 Hz, 1H), 8.49 (s, 1H, NOE 6% when 2'-H at 6.56 ppm was irradiated), 8.59 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 34.9, 57.1, 62.4, 73.0, 79.7, 109.3, 117.1, 118.4, 126.3, 140.6, 142.4, 145.6, 154.6, 161.7. Compound **11** was phosphorylated to afford **12** in 38% yield: mp 228-230 °C (lyophilized powder); $[\alpha]_D$ -51.0° (*c* 0.01, H_2O); UV (H_2O, pH 7) 220 nm (ε 26700), 252 (50700), 294 (12000), 308 (10400), 320 (6600); ^{31}P NMR (D_2O + DSS) δ 0.6; ^{1}H NMR (D_2O + DSS) δ 2.12 (m, 1H), 2.74 (m, 1H), 4.18 (m, 5H), 5.64 (m, 1H), 6.96 (s, 1H), 7.19 (s, br, 1H), 7.94 (s, 1H), 8.52 (s, br, 1H); ^{13}C NMR (D_2O + DSS) δ 36.7, 60.2, 68.2 (d, J = 0.7 Hz), 75.8 (d, J = 4.2 Hz), 81.2, 104.6, 111.2, 119.8, 120.7, 127.8, 140.9, 145.6, 153.5, 161.8; MS (FAB) calcd for $C_{14}H_{15}N_5O_5P$: 364 (M - H)$^-$. Found: 364.
15. Integrase Assays.[16] 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 X [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.; 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; In Press.