# Metal-Chelating Inhibitors of a Zinc Finger Protein HIV-EP1. Remarkable Potentiation of Inhibitory Activity by Introduction of SH Groups

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HIV-EP1 is a $C_2H_2$ type zinc finger protein which binds to DNA $\kappa$B site present in the long terminal repeat of HIV provirus. Previously we have reported zinc chelators having histidine−pyridine−histidine skeleton and were successful in inhibiting the DNA binding of HIV-EP1 by removing zinc from the zinc finger domain. Aiming at the potentiation of the inhibitory activity of our previous zinc chelators, herein synthesized were novel chelators comprising pyridine and aminoalkanethiol. These showed marked inhibitory activity on the DNA binding of HIV-EP1. In particular, one of them having a bis(2-mercaptoethyl)amino side chain showed inhibitory activity ($IC_{50}$, ~4 $\mu$M) 10 times stronger than that of the strongest inhibitor that we reported previously. It appeared that these inhibited the DNA binding of HIV-EP1 by a mechanism distinct from that of the previous histidine-based inhibitors.

## Introduction

Zinc finger proteins constitute a major group of transcription factor and play important roles in the gene expression at the terminus of cellular signal transduction. Our interest has been focused on a $C_2H_2$ type zinc finger protein HIV-EP1[1] (also designated as PRDII-BF1[2] or MBP1[3]) which binds to DNA $\kappa$B site (5′-GGGAC-TTTCC-3′)[4] present in the long terminal repeat of HIV provirus to activate the HIV-1 gene expression.[5] Inhibition of HIV-EP1 would lead to the interference of the replication of AIDS virus. Recently we presented a new strategy for the inhibition of zinc finger proteins, i.e. removal of zinc from the finger domain by use of a chelator, and demonstrated this concept to be applicable to the case of HIV-EP1.[6] Thus, heterocyclic ligands comprising (dimethylamino)pyridine and histidine units such as **1** (Figure 1) were designed and synthesized.[6] These compounds exhibited remarkable zinc-binding capability and showed marked inhibitory effect on the DNA binding activity of HIV-EP1.[6] Indeed, the inhibition seemed to be due to the abstraction of zinc from the zinc finger sites of HIV-EP1 because the DNA-binding capability of HIV-EP1 was restored by adding extra zinc during or after the treatment with the inhibitor.[6]

For the regulation of actual cellular biochemical process, it was desired to gain specificity and efficiency of the inhibitors. We made some progress in the issue of specificity by developing inhibitors which discriminate two $\kappa$B site binding proteins HIV-EP1 and NF-$\kappa$B both being induced by the same extracellular stimuli.[7] Efficiency problem is the subject of the present study, and described herein is our success in the remarkable potentiation of activity of the inhibitor.

## Results and Discussion

**Chemistry.** Our previous metal chelators contain imidazole, secondary amine, pyridine, and carboxylic ester groups as potential metal chelating sites in a common histidine−pyridine−histidine skeleton as envisaged in the inhibitor **1**. Introduction of a trityl group into each imidazole ring and hydrolysis of the ester groups resulted in the marked improvement of zinc-binding affinity of the ligand.[6] However, the increase of zinc-binding affinity did not always lead to the enhancement in the inhibition against HIV-EP1, presumably because of the bulkiness and hydrophobicity of the molecule arising from the introduction of two trityl groups onto the imidazoles. In order to overcome this constraint in the imidazole-based ligands, we needed a new system less bulky and less hydrophobic. To this end, we intended to replace the imidazole by a mercapto group considering that all known zinc finger proteins contain mercapto group as a key ligating residue. Mercapto compounds, in fact, display unique and strong metal binding property due to back-donation of electron from metal d$\pi$ orbital to sulfur d$\pi$ or p$\pi$ orbital[8] as exemplified by cystein−zinc complex which has stability constant greater than that of other amino acids, e.g. histidine.[9,10] Thus, it was considered that replacement of the imidazole moiety of our previous synthetic chelators by a mercapto group would alter the

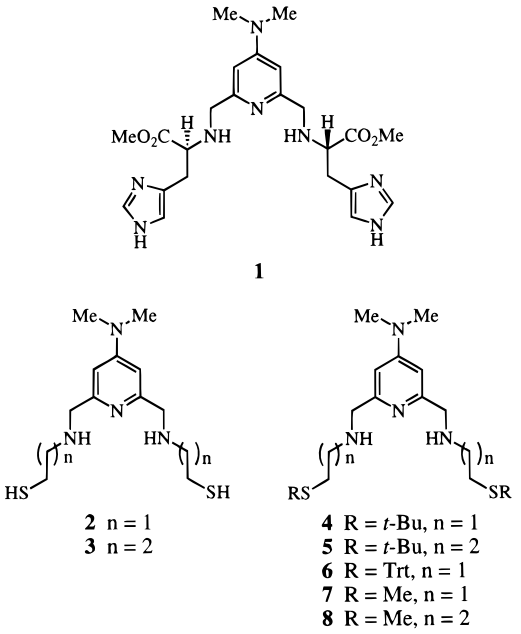


**Figure 1.** Structure of metal-binding inhibitors of HIV-EP1.

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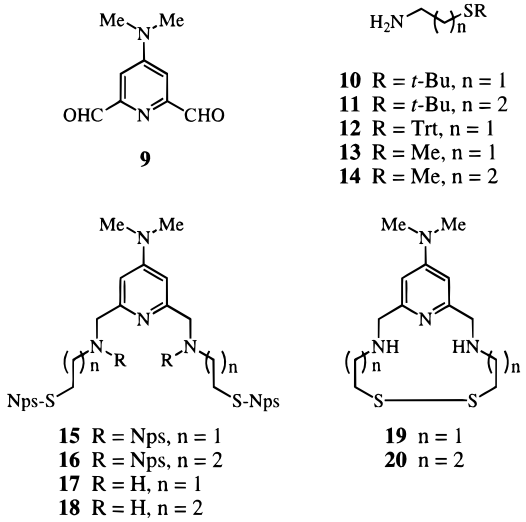


**Figure 2.** Synthetic intermediates for inhibitors and related compounds.

fundament of the metal-binding characteristics, and hence we designed novel chelators **2** and **3** and some related sulfur-containing ligands (Figure 1).

These were prepared as follows (Figure 2). *S*-Alkyl ligands **4**−**8** were obtained by the Schiff base formation between dialdehyde **9**[11] and (alkylthio)ethylamine **10**−**14**[12−15] followed by *in situ* reduction with $NaBH_3CN$. Compounds **4** and **5** were further transformed to mercapto ligands **2** and **3**, respectively. Treatment of **4** with 2-nitrobenzenesulfenyl chloride (Nps-Cl) afforded **15** in 77% yield (similarly, compound **6** also gave **15** in 70% yield). The 2-nitrophenylthio (Nps) group on the secondary amino nitrogen of **15** was selectively removed by the treatment with aqueous HCl−3-methylindole to give disulfide **17** in 88% yield. Reduction of **17** with $NaBH_4$ proceeded with color change from yellow to orange, and subsequent careful extractive workup at pH 6−7 afforded the desired thiol **2** in 75% yield. Compound **3** was prepared by the same procedure starting with **5**.

Compounds **2** and **3** were found to be easily autoxidized under basic conditions (pH > 7), resulting in the formation of disulfides whose main constituents were those assignable to **19** and **20**, respectively, based on the HRMS data (**19**, HRMS calcd for $C_{13}H_{22}N_4S_2$ 298.1286, found 298.1307; **20**, HRMS calcd for $C_{15}H_{26}N_4S_2$ 326.1598, found 326.1573). Special care had to be taken to prevent **2** and **3** from the autoxidation and to maintain their reduced form. We found disulfides **17**−**20** to be stable in air, easy to handle, and quantitatively reducible by dithiothreitol (DTT) to generate either **2** or **3** *in situ*.[16] Therefore, we employed **17**−**20** as practical equivalents for **2** and **3** in the biochemical experiments described below.

**Improved Inhibition of DNA Binding of HIV-EP1.** Figures 3 and 4 show the comparison of inhibitory effects of imidazole compound **1**, mercapto compounds **2** and **3**, alkylthio compounds **4**−**8**, and other mercapto compounds on the DNA binding of HIV-EP1 as demonstrated by electrophoretic mobility shift assay. Compound **2** or **3** generated from **17** or **18** was indistinguishable from that obtained from **19** or **20** in terms of the inhibitory activity. Mercapto compounds **2** and **3** exhibited remarkable inhibitory effect (Figure 3, lanes 3−6), much stronger than that of imidazole compound **1** (lane 2). The most potent was compound **2**, which inhibited DNA binding of HIV-EP1 almost completely at 30 $\mu$M concentration, whereas 300 $\mu$M of **1** was required for the effective inhibition (Figure 4).[6] The $IC_{50}$ of **2** was ~4 $\mu$M (Figure 4, second column). Thus, inhibitory activity of compound **2** was shown to be 10 times stronger than that of **1**. *tert*-Butylthio, tritylthio, and methylthio analogues **4**−**8**, and other thiols, e.g. 2-aminoethanethiol, glutathione, and DTT, showed markedly lowered inhibitory effect at 30 $\mu$M concentration (Figure 4).[17] It should be noted that the inhibitory effect of 2-aminoethanethiol was small but significant because this constitutes the side chain of the inhibitor **2**, demonstrating the effect of assembling the 2-aminoethanethiol units on a pyridine ring in potentiating the inhibitory activity.

As previously reported,[6,7] compound **1** was shown to abstract zinc from the zinc finger site of HIV-EP1 because the DNA−HIV-EP1 binding was restored by the

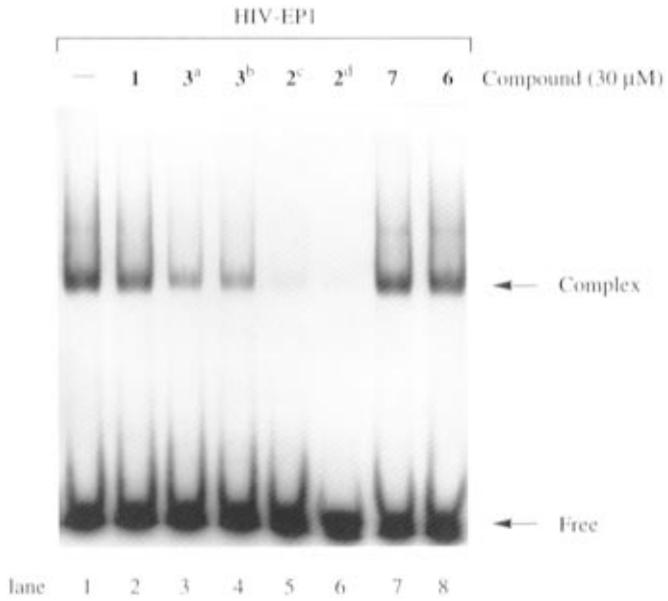


**Figure 3.** Effect of synthetic chelators (30 $\mu$M) on the DNA binding of HIV-EP1. After HIV-EP1 was incubated with each chelator in the presence of poly(dI-dC) at room temperature for 30 min, a radioactive DNA probe containing a $\kappa$B site from the mouse $\kappa$ light-chain enhancer was added. Sample was loaded onto a polyacrylamide band shift gel, and the gel electrophoresis was run. [a]Generated from **20**. [b]Generated from **18**. [c]Generated from **19**. [d]Generated from **17**.

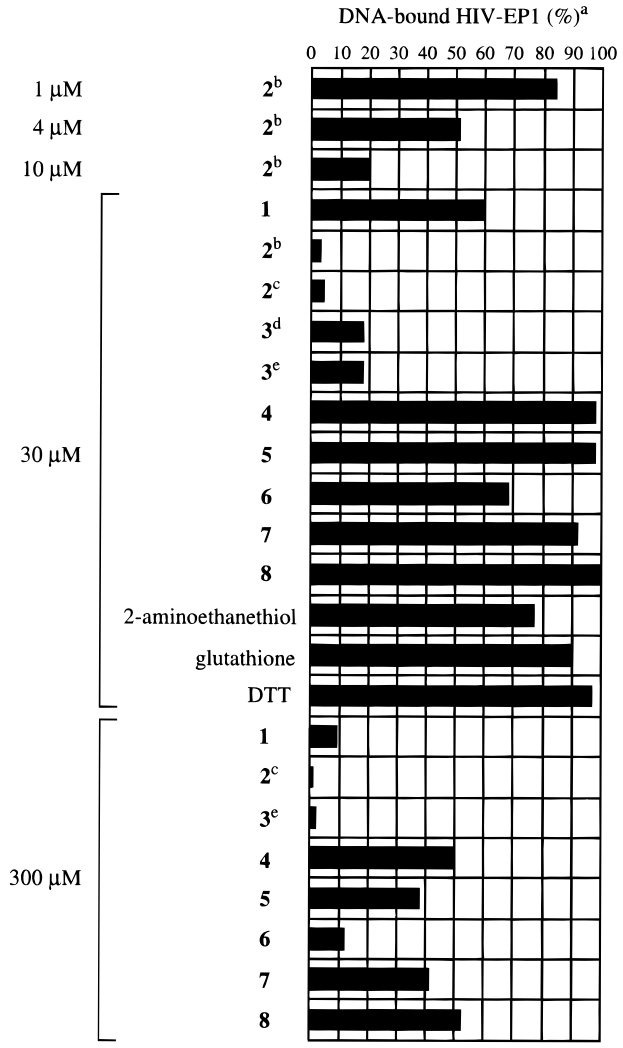


**Figure 4.** Effect of synthetic chelators and mercapto compounds on the DNA binding of HIV-EP1. [a]Quantitation of radioactivity of the electrophoretic band was conducted using an image analyzer. [b]Generated from **17**. [c]Generated from **19**. [d]Generated from **18**. [e]Generated from **20**.

addition of zinc before or after the inhibition reaction (Table 1, numbers 1−7). In contrast, when zinc was introduced after the DNA binding inhibition reaction with **2**, virtually no or limited recovery of HIV-EP1−DNA complex was observed depending on the dose of **2** (30 $\mu$M, 300 $\mu$M, and 1.0 mM) (Table 1, numbers 8−12, 14, 15; Figure 5, lane 4). Addition of zinc prior to the inhibition reaction also resulted in no recovery of DNA binding (Table 1. number 13; Figure 5, lane 3). Compound **3** gave the same result (Table 1, numbers 16, 17). These results can hardly be explained solely by the zinc abstraction mechanism,[18] and alternate consistency could be attained by invoking, for example, literature precedents that certain mercapto compounds inhibit zinc enzymes not by abstracting zinc but by binding to the zinc site of the enzymes.[19,20]

## Conclusion

Herein we prepared several sulfur-containing ligands by replacing the histidine moiety of our previous imidazole-based compounds. Thus, we were successful in the 10-fold potentiation of the inhibitory activity of synthetic inhibitor of HIV-EP1 by introducing mercapto groups. The mechanism of the inhibition, seemingly distinct from that of our previous inhibitors, could be a new clue to the specificity issue to distinguish zinc finger proteins involved in the cellular signal crosstalk, which is our next subgoal.

**Table 1.** Effect of Zinc in the Inhibition of the DNA Binding of HIV-EP1 by Compounds **1**−**3**

| no. | compound | Zn | DNA-bound HIV-EP1 (%)[a] |
|---|---|---|---|
| 1 | **1** (0.7 mM) | | 0[f] |
| 2 | **1** (0.7 mM) | 0.7 mM[b] | 100[f] |
| 3 | **1** (0.7 mM) | 2.1 mM[c] | 100[f] |
| 4 | **1** (1.0 mM) | | 0 |
| 5 | **1** (1.0 mM) | 1.0 mM[b] | 99 |
| 6 | **1** (1.0 mM) | 1.0 mM[c] | 77 |
| 7 | **1** (1.0 mM) | 2.0 mM[c] | 94 |
| 8 | **2**[d] (30 $\mu$M) | | 4[g] |
| 9 | **2**[d] (30 $\mu$M) | 90 $\mu$M[c] | 23 |
| 10 | **2**[d] (300 $\mu$M) | | 1[g] |
| 11 | **2**[d] (300 $\mu$M) | 900 $\mu$M[c] | 22 |
| 12 | **2**[d] (1.0 mM) | | 0 |
| 13 | **2**[d] (1.0 mM) | 1.0 mM[b] | 4 |
| 14 | **2**[d] (1.0 mM) | 2.0 mM[c] | 0 |
| 15 | **2**[d] (1.0 mM) | 3.0 mM[c] | 2 |
| 16 | **3**[e] (1.0 mM) | | 0 |
| 17 | **3**[e] (1.0 mM) | 3.0 mM[c] | 12 |

[a] Quantitation of radioactivity of the electrophoretic band was conducted using an image analyzer. [b] $Zn^{2+}$ was added before addition of HIV-EP1. [c] $Zn^{2+}$ was added after addition of HIV-EP1. [d] Generated from **19**. [e] Generated from **20**. [f] Reference 6. [g] Figure 4.

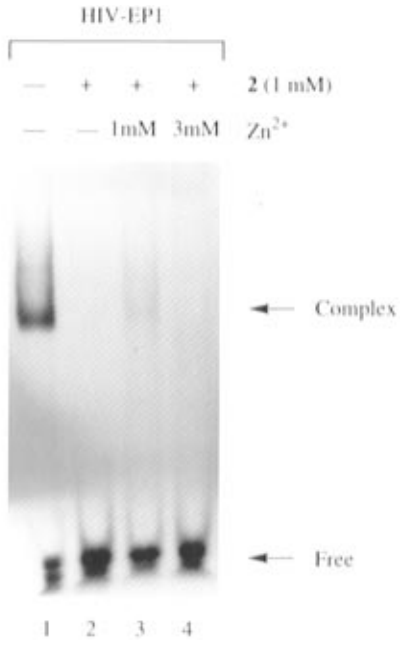


**Figure 5.** Effect of zinc in the inhibition of the DNA binding of HIV-EP1 by compound **2** (1 mM) generated from **19**. $Zn^{2+}$ was introduced before (lane 3) or after (lane 4) the addition of HIV-EP1.

## Experimental Section

**General Procedures.** Melting points were determined with a Yanaco MP-J3 apparatus and are uncorrected. NMR spectra were measured on Varian VXR-200 specteometers using TMS as the internal standard. IR spectra were obtained using a Hitachi 260−50. Mass spectra were measured on a JEOL JMS-DX300. Fast atom bombardment high-resolution mass spectra were determined on a JEOL JMS-SX102A mass spectrometer by courtesy of JEOL Ltd. Elemental analyses were performed with a Yanaco CORDER MT-5. Homogeneity of new compounds **2**−**8** and **15**−**18** were confirmed by HPLC, TLC, and $^1$H NMR.

**[2-(*tert*-Butylthio)ethyl]amine (10).**[12] To a solution of 2-aminoethanethiol hydrochloride (3.97 g, 34.9 mmol) in 2 N aqueous HCl (16 mL) was added *tert*-butyl alcohol (4.3 mL,

46 mmol). The mixture was heated under reflux for 11 h and concentrated in vacuo. The residue was dried over $P_2O_5$, crushed, and washed with acetone (20 mL × 4). The solid was crystallized from 4 N aqueous HCl to afford white crystal **10**·HCl·$^1/_3H_2O$ (1.19 g, 19%): mp 190−193 °C; $^1$H NMR ($CD_3OD$) $\delta$ 1.32 (s, 9H), 2.79 (t, $J$ = 7.0 Hz, 2H), 3.07 (t, $J$ = 7.0 Hz, 2H); IR (KBr) 2950, 1600, 1450, 1360, 1250, 1160, 1130, 1070, 930, 910, 730 cm$^{-1}$; MS(FAB) $m/z$ 134 $(M + H)^+$. Anal. Calcd for $C_6H_{15}NS$·HCl·$^1/_3H_2O$: C, 41.01; H, 9.56; N, 7.97. Found: C, 41.32; H, 9.47; N, 8.21.

**4-(Dimethylamino)-2,6-bis[[[2-(*tert*-butylthio)ethyl]amino]methyl]pyridine (4).** To a solution of 4-(dimethylamino)-pyridine-2,6-dicarbaldehyde (**9**)[11] (102 mg, 0.572 mmol) and **10**·HCl·$^1/_3H_2O$ (195 mg, 1.11mmol) in methanol (3.0 mL) was added sodium cyanoborohydride (95%, 91.0 mg, 1.38 mmol). The mixture was stirred at room temperature for 36 h. Water (2 mL) was added, and then the mixture was stirred at room temperature for 5 h and concentrated in vacuo. The residue was dried over $P_2O_5$ and chromatographed on silica gel (eluted with $CH_2Cl_2$:MeOH = 30:1 → 5:1) to afford colorless oil **4** (113 mg, 49%): $^1$H NMR ($CDCl_3$) $\delta$ 1.30 (s, 18H), 2.76 (t, $J$ = 6.0 Hz, 4H), 2.90 (t, $J$ = 6.0 Hz, 4H), 3.19 (s, 6H), 4.01 (s, 4H), 5.92 (brs, 2H), 6.65 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 28.7, 32.2, 40.6, 44.1, 50.5, 53.3, 106.3, 155.6, 158.9; IR (KBr) 2960, 2320, 1610, 1550, 1460, 1390, 1360, 1160, 1120 cm$^{-1}$; HRMS (FAB) calcd for $C_{21}H_{41}N_4S_2$ 413.2773, found 413.2740.

**4-(Dimethylamino)-2,6-bis[[*N*-[(2-nitrophenyl)thio]-*N*-[2-[(2-nitrophenyl)dithio]ethyl]amino]methyl]pyridine (15).** To a solution of **4** (10.5 mg, 0.0254 mmol) in acetic acid (0.9 mL) was added 2-nitrobenzenesulfenyl chloride (58.0 mg, 0.306 mmol). The mixture was stirred at room temperature for 16 h and concentrated in vacuo. Dichloromethane (10 mL) and water (5 mL) were added, and the mixture was neutralized with aqueous $NaHCO_3$. The organic layer was separated and concentrated in vacuo. The residue was chromatographed on silica gel (eluted with hexane:EtOAc = 10:1 → EtOAc → $CH_2Cl_2$:MeOH = 10:1) to afford yellow solid **15** (17.8 mg, 77%). Compound **15** was also obtained from **6** according to the same procedure: mp 88−91 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.93 (t, $J$ = 6.0 Hz, 4H), 3.03 (s, 6H), 3.51 (br, 4H), 4.34 (br, 4H), 6.50 (s, 2H), 7.19−7.39 (m, 4H), 7.53−7.76 (m, 4H), 8.01−8.33 (m, 8H); $^{13}$C NMR ($CDCl_3$) $\delta$ 36.7, 39.3, 55.6, 63.6, 104.6, 124.8, 125.1, 125.9, 126.0, 126.1, 127.1, 133.9, 134.1, 137.2, 142.1, 145.1, 145.3, 155.6, 156.4; IR (KBr) 3420, 1590, 1510, 1330, 1300, 730 cm$^{-1}$; HRMS (FAB) calcd for $C_{37}H_{37}O_8N_8S_6$ 913.1059, found 913.1068.

**4-(Dimethylamino)-2,6-bis[[*N*-[2-[(2-nitrophenyl)dithio]-ethyl]amino]methyl]pyridine (17).** 3-Methylindole (17.9 mg, 0.136 mmol) and 0.5 N aqueous HCl (0.6 mL, 0.3 mmol) were successively added to a solution of **15** (17.8 mg, 0.0195 mmol) in dichloromethane−methanol (2:3, 2.5 mL) at 5 °C. The mixture was stirred at room temperature for 9 h, neutralized with aqueous $NaHCO_3$, and concentrated in vacuo. Dichloromethane (30 mL) and water (5 mL) were added to the mixture. The organic layer was separated and concentrated in vacuo. The residue was chromatographed on silica gel (eluted with EtOAc → $CH_2Cl_2$:MeOH = 10:1) to afford yellow solid **17** (10.4 mg, 88%): $^1$H NMR ($CDCl_3$) $\delta$ 2.90 (t, $J$ = 7.0 Hz, 4H), 2.92 (t, $J$ = 7.0 Hz, 4H), 3.06 (s, 6H), 3.57 (brs, 2H), 3.84 (s, 4H), 6.46 (s, 2H), 7.26−7.42 (m, 2H), 7.62−7.75 (m, 2H), 8.20−8.39 (m, 4H); $^{13}$C NMR ($CD_3OD$) $\delta$ 39.3, 40.3, 48.8, 55.5, 105.9, 127.9, 128.5, 129.3, 136.2, 138.8, 147.8, 158.2, 159.7; IR (KBr) 3420, 2920, 1600, 1510, 1340, 1300, 730 cm$^{-1}$; HRMS (FAB) calcd for $C_{25}H_{31}O_4N_6S_4$ 607.1290, found 607.1264.

**4-(Dimethylamino)-2,6-bis[[(2-mercaptoethyl)amino]-methyl]pyridine (2).** To a solution of **17** (43.6 mg, 0.0718 mmol) in methanol (11 mL) was added sodium borohydride (40.9 mg, 1.08 mmol). The mixture was stirred at room temperature for 1 h and concentrated in vacuo. Water (14 mL) was added, and then the solution was acidified to pH 1 with 1 N aqueous HCl and washed with dichloromethane (45 mL × 4). The aqueous solution was neutralized (pH 6−7) with aqueous $NaHCO_3$, and the mixture was extracted with dichloromethane (45 mL × 5). The organic layer was concentrated in vacuo to afford colorless oil **2** (16.3 mg, 75%). HCl salt of **2** (pale yellow solid, hygroscopic) was also prepared by concentrating the acidic aqueous solution obtained after the acidification and dichloromethane washing of the above reaction mixture. **2**: $^1$H NMR ($CDCl_3$) $\delta$ 2.65 (t, $J$ = 6.5 Hz, 4H), 2.89 (t, $J$ = 6.5 Hz, 4H), 3.01 (s, 6H), 3.84 (s, 4H), 6.34 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 25.4, 40.3, 53.6, 55.1, 105.9, 158.4, 159.2; HRMS calcd for $C_{13}H_{24}N_4S_2$ 300.1442, found 300.1432. HCl salt of **2**: $^1$H NMR ($CD_3OD$) $\delta$ 2.93 (t, $J$ = 7.0 Hz, 4H), 3.30 (s, 6H), 3.39 (t, $J$ = 7.0 Hz, 4H), 4.48 (s, 4H), 7.29 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 21.8, 41.7, 49.5, 52.7, 110.6, 145.9, 159.9; IR (KBr) 3390, 2920, 2740, 1640, 1570, 1410 cm$^{-1}$; HRMS (FAB) calcd for $C_{13}H_{25}N_4S_2$ 301.1521, found 301.1528.

**4-(Dimethylamino)-2,6-bis[[*N*-[2-[(triphenylmethyl)thio]ethyl]amino]methyl]pyridine (6).** Compound **6** was synthesized from [2-[(triphenylmethyl)thio]ethyl]amine hydrochloride hemihydrate (**12**)[14] according to the same procedure as that for **4**. Compound **6** was obtained as white solid (41% yield): mp 65−67 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.35 (t, $J$ = 7.5 Hz, 4H), 2.38 (t, $J$ = 7.5 Hz, 4H), 3.14 (s, 6H), 3.70 (s, 4H), 4.72 (br s, 2H), 6.57 (s, 2H), 7.0−7.5 (m, 30H); $^{13}$C NMR ($CD_3$-OD) $\delta$ 33.3, 40.8, 49.5, 52.4, 68.8, 106.0, 128.7, 129.8, 131.5, 146.9, 155.2, 159.4; IR (KBr) 3430, 1630, 1600, 1440, 1120, 1030, 740, 700, 450 cm$^{-1}$; HRMS (FAB) calcd for $C_{51}H_{53}N_4S_2$ 785.3712, found 785.3712.

**[3-(*tert*-Butylthio)propyl]amine (11).**[13] *tert*-Butanethiol (9.3 mL, 82 mmol) and a solution of sodium hydroxide (5.7 g, 143 mmol) in methanol (5 mL) and water (10 mL) were successively added to a solution of 3-chloropropylamine hydrochloride (1.08 g, 8.31 mmol) in methanol (10 mL) and water (10 mL) at 5 °C. The mixture was stirred at room temperature for 1 d, and methanol was removed in vacuo. The pH of the solution was adjusted to 8 with 1 N aqueous HCl, and the resulting mixture was extracted with dichloromethane (200 mL × 2). The organic layer was dried over $MgSO_4$, acidified with 1.0 M HCl solution in diethyl ether (20 mL), and concentrated in vacuo to afford white solid **11**·HCl·$^1/_3H_2O$ (0.984 g, 62%): mp 142−143 °C; $^1$H NMR ($CD_3OD$) $\delta$ 1.33 (s, 9H), 1.91 (quint, $J$ = 7.0 Hz, 2H), 2.63 (t, $J$ = 7.0 Hz, 2H), 3.02 (t, $J$ = 7.0 Hz, 2H); IR (KBr) 2960, 1600, 1490, 1440, 1360, 1170, 1010, 960, 840 cm$^{-1}$; MS(FAB) $m/z$ 148 $(M + H)^+$. Anal calcd for $C_7H_{17}NS$·HCl·$^1/_3H_2O$: C, 44.31; H, 9.92; N, 7.38. Found: C, 44.60; H, 9.81; N, 7.07.

**4-(Dimethylamino)-2,6-bis[[[3-(*tert*-butylthio)propyl]-amino]methyl]pyridine (5).** Compound **5** was synthesized from **11**·HCl·$^1/_3H_2O$ according to the same procedure as that for **4**. Compound **5** was obtained as pale purple oil (39% yield): $^1$H NMR ($CDCl_3$) $\delta$ 1.29 (s, 18H), 1.95 (quint, $J$ = 7.0 Hz, 4H), 2.62 (t, $J$ = 7.0 Hz, 4H), 2.95 (t, $J$ = 7.0 Hz, 4H), 3.12 (s, 6H), 4.03 (s, 4H), 6.54 (s, 2H), 6.69 (br s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 27.1, 29.5, 32.2, 40.4, 43.9, 49.3, 53.6, 106.7, 154.6, 158.4; IR (KBr) 2950, 2310, 1610, 1540, 1460, 1390, 1360, 1160, 1110 cm$^{-1}$; HRMS (FAB) calcd for $C_{23}H_{45}N_4S_2$ 441.3086, found 441.3081.

**4-(Dimethylamino)-2,6-bis[[*N*-[(2-nitrophenyl)thio]-*N*-[3-[(2-nitrophenyl)dithio]propyl]amino]methyl]-pyridine (16).** Compound **16** was synthesized from **5** according to the same procedure as that for **15**. Compound **16** was obtained as yellow solid (61% yield): mp 51−54 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.99 (quint, $J$ = 6.5 Hz, 4H), 2.72 (t, $J$ = 6.5 Hz, 4H), 2.97 (s, 6H), 3.32 (br, 4H), 4.27 (br, 4H), 6.42 (s, 2H), 7.17−7.35 (m, 4H), 7.52−7.67 (m, 4H), 7.93−8.04 (m, 2H), 8.13−8.32 (m, 6H); $^{13}$C NMR ($CDCl_3$) $\delta$ 28.1, 35.8, 39.3, 55.7, 64.2, 104.4, 124.7, 125.1, 126.0, 126.1, 127.2, 133.7, 134.0, 137.6, 142.2, 145.5, 145.9, 155.5, 157.4; IR (KBr) 3410, 1590, 1510, 1330, 1300, 730 cm$^{-1}$; HRMS (FAB) calcd for $C_{39}H_{40}N_8S_6O_8$ 941.1372, found 941.1368.

**4-(Dimethylamino)-2,6-bis[[*N*-[3-[(2-nitrophenyl)dithio]-propyl]amino]methyl]pyridine (18).** Compound **18** was synthesized from **16** according to the same procedure as that for **17**. Compound **18** was obtained as yellow solid (90% yield): $^1$H NMR ($CDCl_3$) $\delta$ 1.90 (quint, $J$ = 7.0 Hz, 4H), 2.74 (t, $J$ = 7.0 Hz, 4H), 2.82 (t, $J$ = 7.0 Hz, 4H), 2.91 (br s, 2H), 3.00 (s, 6H), 3.76 (s, 4H), 6.39 (s, 2H), 7.27−7.42 (m, 2H), 7.62−7.76 (m, 2H), 8.21−8.34 (m, 4H); $^{13}$C NMR ($CD_3OD$) $\delta$ 30.5, 37.5, 40.3, 48.9, 55.6, 105.9, 127.9, 128.5, 129.3, 136.2, 138.8, 147.8, 158.3, 159.2; IR (KBr) 3430, 2920, 1600, 1510, 1330, 1300, 730 cm$^{-1}$; HRMS (FAB) calcd for $C_{27}H_{35}O_4N_6S_4$ 635. 1602, found 635.1594.

**4-(Dimethylamino)-2,6-bis[[(3-mercaptopropyl)amino]-methyl]pyridine (3).** Compound **3** was synthesized from **18**

according to the same procedure as that for **2** except that dithiothreitol (2.3 equiv) was used instead of sodium borohydride. Compound **3** was obtained as a colorless oil (67% yield). **3**: $^1$H NMR ($CDCl_3$) $\delta$ 1.85 (quint, $J$ = 7.0 Hz, 4H), 2.63 (t, $J$ = 7.0 Hz, 4H), 2.76 (t, $J$ = 7.0 Hz, 4H), 3.00 (s, 6H), 3.75 (s, 4H), 6.42 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 23.5, 35.0, 40.3, 49.1, 55.5, 106.0, 158.3, 158.8; HRMS calcd for $C_{15}H_{28}N_4S_2$ 328.1755, found 328.1743. HCl salt of **3**: pale yellow solid, hygroscopic; $^1$H NMR ($CD_3OD$) $\delta$ 2.11 (quint, $J$ = 6.5 Hz, 4H), 2.67 (t, $J$ = 6.5 Hz, 4H), 3.3−3.4 (m, 4H), 3.33 (s, 6H), 4.47 (s, 4H), 7.34 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 22.7, 32.1, 41.8, 48.9, 49.6, 110.7, 145.7, 160.1; IR (KBr) 3390, 2920, 2760, 1640, 1570, 1420 cm$^{-1}$; HRMS (FAB) calcd for $C_{15}H_{29}N_4S_2$ 329.1834, found 329.1840.

**4-(Dimethylamino)-2,6-bis[[[2-(methylthio)ethyl]amino]methyl]pyridine (7).** Compound **7** was synthesized from [2-(methylthio)ethyl]amine hydrochloride (**13**)[15] according to the same procedure as that for **4**. Compound **7** was obtained as pale pink oil (19% yield): $^1$H NMR ($CDCl_3$) $\delta$ 2.12 (s, 6H), 2.75 (t, $J$ = 6.0 Hz, 4H), 2.97 (t, $J$ = 6.0 Hz, 4H), 3.21 (s, 6H), 4.07 (s, 4H), 5.13 (br s, 2H), 6.66 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 15.9, 34.2, 40.5, 48.8, 53.4, 106.3, 155.9, 158.9; IR (KBr) 2910, 2320, 1610, 1540, 1430, 1390, 1120 cm$^{-1}$; HRMS (FAB) calcd for $C_{15}H_{29}N_4S_2$ 329.1834, found 329.1821.

**4-(Dimethylamino)-2,6-bis[[[3-(methylthio)propyl]amino]methyl]pyridine (8).** Compound **8** was synthesized from [3-(methylthio)propyl]amine hydrochloride (**14**) according to the same procedure as that for **4**. Compound **8** was obtained as pale blue oil (13% yield): $^1$H NMR ($CDCl_3$) $\delta$ 2.03 (quint, $J$ = 6.0 Hz, 4H), 2.10 (s, 6H), 2.61 (t, $J$ = 6.0 Hz, 4H), 3.02 (t, $J$ = 6.0 Hz, 4H), 3.10 (s, 6H), 4.09 (s, 4H), 5.94 (br s, 2H), 6.53 (s, 2H); $^{13}$C NMR ($CD_3OD$) $\delta$ 16.0, 28.3, 32.8, 40.4, 50.2, 53.6, 106.6, 154.7, 158.5; IR (KBr) 2910, 2310, 1610, 1540, 1430, 1390, 1110 cm$^{-1}$; HRMS calcd for $C_{17}H_{32}N_4S_2$ 356.2068, found 356.2050.

**Electrophoretic Mobility Shift Assay (EMSA).** Double-stranded oligonucleotide containing a $\kappa$B site from the mouse immunoglobulin $\kappa$ light-chain enhancer

5′-AGCTTCAGAGGGGACTTTCCGAGAGG-3′

3′-AGTCTCCCCTGAAAGGCTCTCCAGCT-5′

was phosphorylated with polynucleotide kinase in the presence of [$\gamma$-$^{32}$P]ATP (Du Pont, >5000 Ci/mmol) and purified by a G-50 Sephadex spin column. The DNA binding domain of HIV-EP1 was expressed as a fusion protein with $\beta$-galactosidase in bacteria. About 500 ng of HIV-EP1 was used for EMSA. After incubation of the each reaction mixture containing pH 7.0 binding buffer (15 mM Tris·HCl, pH 7.0, 75 mM NaCl, 1.5 mM EDTA,[7] 1.5 mM dithiothreitol, 7.5% glycerol, 0.3% NP-40, 1 $\mu$g/$\mu$L BSA), 4% methanol, 2.4 $\mu$g of poly(dI-dC), HIV-EP1, and each compound at room temperature for 30 min, labeled DNA probe (30 000 cpm) was added, and the mixture was further incubated at room temperature for 15 min. The sample in a volume of 24 $\mu$L was loaded onto 4% polyacrylamide gels and electrophoresed at 150 CV.

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(16) Reduction of **17** or **19** with 3 equiv of DTT proceeded immediately and completely to afford **2**. Similarly, **18** or **20** gave **3**.

(17) Compounds **4**, **5**, **7**, and **8** at 300 $\mu$M concentration and compound **6** at 30 $\mu$M concentration also showed some inhibitory effect on the DNA binding of HIV-EP1 (Figure 4).

(18) NMR monitoring of zinc titration of compound **2** in the binding buffer used in the EMSA assay suggested that compound **2** evidently forms a zinc complex when equimolar zinc was added. On the basis of the NMR spectra, it was not likely that compound **2** tightly bound 3 equiv of extra zinc added after the inhibition reaction to interfere the recovery of DNA binding.

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