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RESEARCH ARTICLE

Synthesis and anti-HIV activity of L-2′,3′-Dideoxy-4′-selenonucleosides (L-4′-Se-ddNs)

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Abstract Based on the potent anti-HIV activity of L-2′,3′-dideoxycytidine (L-ddC), L-2′,3′-dideoxy-4′-selenonucleosides (L-4′-Se-ddNs) have been synthesized from natural chiral template, L-glutamic acid, using Pummerer-type condensation as a key step. All synthesized compounds were assayed for anti-HIV-1 activity, but none of them did show any significant antiviral activity up to 100 μM, probably due to conformational differences between L-ddC and L-4′-Se-ddC, induced by the bulky selenium atom, which might play an important role in phosphorylation by cellular kinase.

Keywords Antiviral · L-2′,3′-Dideoxy-4′-selenonucleosides · L-Nucleoside · Pummerer-type condensation · L-4′-Se-ddC

Introduction

An estimated 36 million people are infected with human immunodeficiency virus (HIV) worldwide. The HIV is a member of the retroviridae family, which is transmitted as single stranded, positive-sense enveloped RNA virus (Deeks et al. 2015). The viral RNA genome is reverse transcribed into double-stranded DNA by a virally encoded enzyme, reverse transcriptase (RT) (Deeks et al. 2015). Thus, the inhibition of RT makes it impossible to replicate the viral RNA genome, resulting in potent anti-HIV activity. Nucleos(t)ide reverse transcriptase inhibitors (NRTIs) bind to the catalytic site of RT after being converted to the triphosphates and compete with natural substrate, nucleoside triphosphate (NTP) against viral DNA polymerase (Sluis-Cremer et al. 2000). So far, total 8 different NRTIs have been approved by the Food and Drug Administration (FDA) and these are 2′,3′-dideoxyinosine (ddI, didanosine), 2′,3′-dideoxycytidine (ddC, zalcitabine), 3′-azido-3′-deoxythymidine (AZT, zidovudine), 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T, stavudine), (−)-2′,3′-dideoxy-3′-thiacytidine (lamivudine, (−)-3TC), (−)-5-fluoro-2′,3′-dideoxy-3′-thiacytidine (emtricitabine, (−)-FTC), abacavir, and tenofovir (De Clercq 2009).

D-2′,3′-Dideoxycytidine (D-ddC) is one of the most potent anti-HIV-1 nucleosides (Mitsuya and Broder 1986). It exerts the anti-HIV-1 activity by inhibiting RT competitively and/or terminating viral DNA chain after incorporation (Mitsuya and Broder 1986). Based on the potent anti-HIV-1 activity of D-ddC, we reported the synthesis of the corresponding D-2′,3′-dideoxy-4′-selenocytidine (D-4′-Se-ddC) with anti-HIV-1 activity (Jeong et al. 2008a, b). To our disappointment, D-4′-Se-ddC did not exhibit any anti-HIV-1 activity up to 100 μM although it showed the same South conformation as D-ddC. No antiviral activity might be attributed to the conformational differences, resulting from bulky selenium atom whose steric effects overwhelm the electronic (gauche) effects (Jeong et al. 2008a, b; Sahu et al. 2014, 2015).

On the other hand, since the approval of lamivudine and emtricitabine for the treatments of HIV-1 and HBV infections, the unnatural or L-nucleosides have been paid much attentions for the developments of novel antiviral

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agents because of less toxicity than the corresponding D-nucleosides, while maintaining the antiviral activity (Mathé and Gosselin 2006). For example, β-L-2′,3′-dideoxycytidine (L-ddC) exhibited potent anti-HIV-1 and anti-HBV activities without the inhibition against mitochondrial DNA synthesis up to 100 μM, but the corresponding D-ddC inhibited the mitochondrial DNA synthesis in CEM cells with an IC_{50} value of 0.022 μM (Lin et al. 1994). Another L-nucleoside, β-L-thymidine (L-dT, Tyzeka® or Telvivo®) was also approved for the treatment of HBV (Nash 2009). It was turned out to be more effective than lamivudine and less likely to cause resistance in clinical trials.

Thus, as L-ddC was also reported to exhibit potent anti-HIV-1 and anti-HBV activities without cytotoxicity endowed with D-ddC, it is very interesting to synthesize the corresponding L-4′-Se-ddNs such as L-4′-Se-ddC (**2**), L-4′-Se-ddU (**3**), and L-4′-Se-ddT (**4**) and to evaluate them for anti-HIV-1 activity (Fig. 1). Herein, we report the synthesis and anti-HIV-1 activity of L-4′-Se-ddNs, starting from L-glutamic acid.

Materials and methods

Chemical synthesis

^{1}H NMR (400 MHz, Varian Unity Inova) and ^{13}C NMR (100 MHz, Varian Unity Inova) spectra were measured in $CDCl_3$ or CD_3OD and chemical shifts are recorded as parts per million (δ) relative to the solvent peak. Coupling constants (*J*) are reported in hertz (Hz). Optical rotations were determined on Jasco III (Jasco, Sapporo, Japan) in appropriate solvent. UV spectra were recorded on U-3000 (Hitachi, Tokyo, Japan) in methanol or water. Melting points were measured on melting point apparatus B-540 (Büchi Labortechnik AG, Flawil, Switzerland). Elemental analysis (C, H, and N) were measured on automatic elemental analyzer (ThermoQuest Italia S. p. A.) to determine the purity of all synthesized compounds, and the results were within ± 0.4% of the calculated values, confirming ≥ 95% purity. Mass spectra obtained with VG Trio-2 GC-MS instrument (a VG instruments group, Altrincham, UK). Reactions were checked with TLC (Merck precoated 60F254 plates). Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck). Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. All solvents were purified and dried by standard techniques just before use.

(S)-5-(Hydroxymethyl)dihydrofuran-2(3H)-one (6)

Cyclization

To a cooled (0 °C) suspension of L-glutamic acid (20.0 g, 135.93 mmol) in H_2O (50 mL) and 1 *N* H_2SO_4 (200 mL) was dropwise added a solution of $NaNO_2$ (14.1 g, 204.35 mmol) in H_2O (30 mL). After stirring at room temperature for 15 h, the solvent was evaporated at reduced pressure and diluted with H_2O (50 mL) and EtOAc (150 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (100 mL × 2). The combined organic layers were washed successively with H_2O and brine, dried over anhydrous $MgSO_4$, and evaporated to give the crude **5** (14.6 g) as yellowish syrup, which was used directly for the next step without purification.

Reduction

To a cooled (0 °C) solution of the crude **5** (14.6 g) in THF (200 mL) was dropwise added BH_3·Me_2S (148 mL, 2 M solution in THF, 295.90 mmol) under N_2. After stirring at room temperature for 1 h, the reaction mixture was quenched with MeOH (200 mL) and evaporated. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 30/1) to give **6** (10.4 g, 61%) as yellowish syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.61–4.66 (m, 1 H, *H*-4), 3.92 (dd, *J* = 2.8, 12.4 Hz, 1 H, *H*-5), 3.67 (dd, *J* = 4.8, 12.4 Hz, 1 H, *H*-5), 2.51–2.67 (m, 2 H, *H*-2, *H*-2), 2.23–2.32 (m, 1 H, *H*-3), 2.10–2.20 (m, 1 H, *H*-3); LRMS (FAB) m/z 117 $[M+H]^+$; ^{1}H NMR and mass spectral data are consistent with the literature values (Wrona et al. 2010).

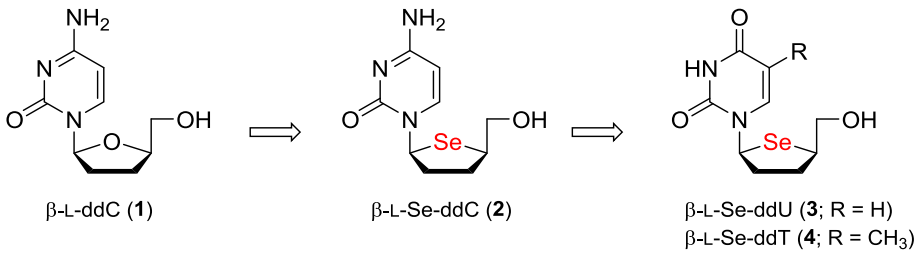


Fig. 1 The rationale for the design of the target nucleosides **2**–**4**

(S)-5-(((tert-Butyldiphenylsilyl)oxy)methyl) dihydrofuran-2(3H)-one (7)

To a solution of **6** (2.38 g, 20.45 mmol) in anhydrous CH_2Cl_2 (100 mL) were successively added triethylamine (7.13 mL, 51.13 mmol), 4-(dimethylamino)pyridine (0.50 g, 4.09 mmol) and *tert*-butyl(chloro)diphenylsilane (7.85 mL, 30.68 mmol) at room temperature under N_2. After stirring at the same temperature for 2 h, the reaction mixture was quenched with H_2O (100 mL), and the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL × 2). The combined organic layers were washed successively with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 8/1) to give **7** (6.74 g, 93%) as a white solid: mp 75.9–77.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.68 (m, 4 H, *phenyl* in TBDPS), 7.38–7.47 (m, 6 H, *phenyl* in TBDPS), 4.58–4.63 (m, 1 H, *H*-4), 3.89 (dd, *J* = 3.6, 11.2 Hz, 1 H, *H*-5), 3.70 (dd, *J* = 3.6, 11.2 Hz, 1 H, *H*-5), 2.64–2.73 (m, 1 H, *H*-2), 2.47–2.56 (m, 1 H, *H*-2), 2.18–2.34 (m, 2 H, *H*-3, *H*-3), 1.06 (s, 9 H, 3 × CH_3-*tert*-butyl in TBDPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 135.8, 135.7, 133.2, 132.8, 130.1, 128.1, 80.2, 65.7, 28.8, 27.0, 23.9, 19.4; LRMS (FAB) m/z 355 $[M+H]^+$; $[\alpha]_D^{24}$ = + 27.42 (*c* 0.06, CH_3OH); Calcd for $C_{21}H_{26}O_3Si$: C, 71.15; H, 7.39. Found: C, 71.05; H, 7.34.

(S)-5-(tert-Butyldiphenylsilanyloxy)pentane-1,4-diol (8)

To cooled (0 °C) solution of **7** (35.9 g, 101.21 mmol) in THF (360 mL) was added lithium borohydride (4.64 g, 202.42 mmol) in one portion under N_2 (g). After heating at 40 °C (bath temperature) with stirring for 2 h, the reaction mixture was quenched with H_2O (100 mL), and diluted with EtOAc. The organic layer was separated, and the aqueous layer was extracted with EtOAc (50 mL × 2). The combined organic layers were washed successively with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography (silica gel, hexanes/EtOAc, 2/1) to give **8** (33.8 g, 93%) as colorless syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66–7.69 (m, 4 H, *phenyl* in TBDPS), 7.38–7.46 (m, 6 H, *phenyl* in TBDPS), 3.75–3.79 (m, 1 H, *H*-4), 3.50–3.67 (m, 4 H, *H*-1, *H*-1, *H*-5, *H*-5), 2.98 (bs, 2 H, 2 × OH), 1.63–1.68 (m, 2 H, *H*-2, *H*-2), 1.53–1.56 (m, 1 H, *H*-3), 1.43–1.50 (m, 1 H, *H*-3), 1.08 (s, 9 H, 3 × CH_3-*tert*-butyl in TBDPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.1, 135.9, 135.7, 133.29, 133.27, 130.0, 128.0, 127.9, 127.8, 72.1, 68.1, 62.9, 29.9, 29.8, 27.0, 19.4; LRMS (FAB) m/z 359 $[M+H]^+$; $[\alpha]_D^{24}$ = − 5.20 (*c* 6.87, CH_3OH); Calcd for $C_{21}H_{30}O_3Si$: C, 70.35; H, 8.43. Found: C, 70.47; H, 8.28.

(S)-5-((tert-Butyldiphenylsilyl)oxy)pentane-1,4-diyl dimethanesulfonate (9)

To a cooled (0 °C) solution of **8** (4.09 g, 11.41 mmol) in anhydrous CH_2Cl_2 (40 mL) were successively added 4-(dimethylamino)pyridine (0.279 g, 2.28 mmol), triethylamine (12.72 mL, 91.28 mmol) and methanesulfonyl chloride (3.53 mL, 45.64 mmol) under N_2 (g). After stirring for 0.5 h at room temperature, the mixture was quenched with saturated $NaHCO_3$ solution and diluted with CH_2Cl_2. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL × 2). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 3/1) to give **9** (5.17 g, 88%) as colorless syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.67 (m, 4 H, *phenyl* in TBDPS), 7.39–7.48 (m, 6 H, *phenyl* in TBDPS), 4.74–4.78 (m, 1 H, *H*-4), 4.22–4.27 (m, 2 H, *H*-5, *H*-5), 3.83 (dd, *J* = 6.0, 11.2 Hz, 1 H, *H*-1), 3.74 (dd, *J* = 4.0, 11.6 Hz, 1 H, *H*-1), 2.99 (d, *J* = 5.2 Hz, 6 H, 2 × CH_3-methanesulfonyl), 1.79–1.89 (m, 4 H, *H*-2, *H*-2, *H*-3, *H*-3), 1.07 (s, 9 H, 3 × CH_3-*tert*-butyl in TBDPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.8, 135.7, 132.9, 132.7, 130.35, 130.29, 128.2, 82.7, 69.3, 65.5, 38.9, 37.6, 27.7, 27.1, 24.9, 19.4; LRMS (FAB) m/z 515 $[M+H]^+$; $[\alpha]_D^{23}$ = − 7.75 (*c* 0.528, CH_3OH); Calcd for $C_{23}H_{34}O_7S_2Si$: C, 53.67; H, 6.66. Found: C, 53.85; H, 6.23.

(R)-tert-Butyldiphenyl((tetrahydroselenophen-2-yl)methoxy)silane (10)

To a cooled (0 °C) suspension of selenium powder (2.31 g, 29.21 mmol) in EtOH (250.0 mL) was added sodium borohydride (3.31 g, 87.63 mmol) in 10 portions, until the color of the reaction mixture changed from black suspension to colorless solution. To the above-generated solution was dropwise added dimesylate **9** (10.02 g, 19.47 mmol) in THF (150 mL). After being heated at 60 °C (bath temperature) with stirring for 15 h, the reaction mixture was cooled to room temperature and evaporated, and diluted with H_2O and EtOAc (100 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed successively with H_2O and saturated brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 20/1) to give **10** (6.92 g, 88%) as pale yellow syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67–7.70 (m, 4 H, *phenyl* in TBDPS), 7.37–7.45 (m, 6 H, *phenyl* in TBDPS), 3.69–3.78 (m, 3 H, *H*-4, *H*-5, *H*-5), 2.85–2.89 (m, 2 H, *H*-1, *H*-1), 1.93–2.04 (m, 4 H, *H*-2, *H*-2, *H*-3, *H*-3), 1.08 (s, 9 H, 3 × CH_3-*tert*-butyl in TBDPS); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 135.8, 135.7, 133.8, 133.7, 129.8, 127.8, 68.1, 46.1, 35.1, 31.6, 27.0, 25.0, 19.5; LRMS (FAB) m/z 403 $[M+H]^+$; $[\alpha]_D^{24} = -33.26$ (*c* 5.12, CH_3OH); Calcd for $C_{21}H_{28}OSeSi$: C, 62.51; H, 6.99. Found: C, 62.73; H, 6.60.

(R)-tert-Butyldiphenyl((tetrahydroselenophen-2-yl)methoxy)silane-1-oxide (11)

To a cooled (− 78 °C) solution of **10** (3.11 g, 7.72 mmol) in anhydrous CH_2Cl_2 (60 mL) was dropwise added a solution of 3-chloroperbenzoic acid (1.90 g, 8.49 mmol, 77%) in anhydrous CH_2Cl_2 (30 mL) and the reaction mixture was stirred at the same temperature for 45 min. The reaction mixture was quenched with saturated $NaHCO_3$ solution and diluted with CH_2Cl_2. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL × 2). The combined organic layers were washed successively with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, CH_2Cl_2/CH_3OH, 15/1) to give **11** (3.17 g, 98%) as colorless syrup, which was immediately used for next step.

General procedure for the synthesis of 12–15

To a suspension of N^4-benzoylcytosine, uracil or thymine (1 equiv) in anhydrous toluene (0.23 M) were added triethylamine (4 equiv) and trimethylsilyl trifluoromethanesulfonate (8 equiv) under N_2 (g). After stirring at room temperature for 1 h, anhydrous CH_2Cl_2 (0.6 M) was added. To a cooled (0 °C) solution of above generated-reaction mixture was added a solution of **11** (1 equiv) in CH_2Cl_2 (0.6 M) followed by triethylamine (4 equiv) in anhydrous toluene (0.6 M). The reaction mixture was heated at 70 °C (bath temperature) with stirring for 15 h, and then cooled, quenched with saturated $NaHCO_3$ solution, and diluted with CH_2Cl_2. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were filtered through Celite, washed successively with H_2O and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc) to give **12–15**.

*N-(1-((2S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl) tetrahydroselenophen-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**12**)*

Yield = 8%, UV λ_{max} (CH_3OH) 260.0 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 7.6 Hz, 1 H, *H*-6), 7.90–7.92 (m, 2 H, *H*-5, *phenyl* in Bz), 7.68–7.73 (m, 4 H, *phenyl* in Bz), 7.40–7.61 (m, 10 H, *phenyl* in TBDPS), 6.57 (pseudo t, *J* = 4.0 Hz, 1 H, *H*-1′), 3.83–4.13 (m, 3 H, *H*-4′, *H*-5′, *H*-5′), 2.33–2.42 (m, 1 H, *H*-2′), 2.14–2.24 (m, 2 H, *H*-2′, *H*-3′), 1.86–1.92 (m, 1 H, *H*-3′), 1.10 (s, 9 H, 3 × CH_3-*tert*-butyl in TBDPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 149.1, 135.9, 135.8, 133.9, 130.4, 130.3, 130.0, 128.5, 128.1, 96.8, 67.1, 61.3, 50.4, 39.4, 31.8, 27.1, 27.0, 19.5; LRMS (FAB) m/z 618 $[M+H]^+$; $[\alpha]_D^{23} = +40.00$ (*c* 1.78, CH_2Cl_2); Calcd for $C_{32}H_{35}N_3O_3SeSi$: C, 62.32; H, 5.72; N, 6.81. Found: C, 62.72; H, 5.48; N, 6.99.

*N-(1-((2R,5R)-5-(((tert-butyldiphenylsilyl)oxy) methyl)tetrahydroselenophen-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**13**)*

Yield = 8%, UV λ_{max} (CH_3OH) 260.5 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 7.6 Hz, 1 H, *H*-6), 7.90-7.92 (m, 2 H, *phenyl* in Bz), 7.37–7.69 (m, 14 H, *phenyl* in Bz, *phenyl* in TBDPS, *H*-5), 6.57 (t, *J* = 6.0 Hz, 1 H, *H*-1′), 4.09–4.16 (m, 1 H, *H*-4′), 3.84 (dd, *J* = 7.2, 10.4 Hz, 1 H, *H*-5′), 3.73 (dd, *J* = 7.2, 10.4 Hz, 1 H, *H*-5′), 2.42–2.45 (m, 2 H, *H*-2′, *H*-2′), 2.02–2.15 (m, 2 H, *H*-3′, *H*-3′), 1.07 (s, 9 H, 3 × CH_3-*tert*-butyl in TBDPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 151.3, 135.82, 135.76, 133.3, 133.2, 130.2, 129.4, 128.1, 96.1, 67.1, 61.1, 50.0, 39.2, 32.1, 30.0, 27.0, 19.5; LRMS (FAB) m/z 618 $[M+H]^+$; $[\alpha]_D^{23} = +110.02$ (*c* 1.43, CH_2Cl_2); Calcd for $C_{32}H_{35}N_3O_3SeSi$: C, 62.32; H, 5.72; N, 6.81. Found: C, 62.66; H, 5.44; N, 7.01.

*The mixture of 1-((2R,5R)-5-(((tert-butyldiphenylsilyl) oxy)methyl)tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (α of **14**) and 1-((2S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (β of **14**)*

Yield = 13%, (α + β mixture); pale brownish syrup; UV λ_{max} (CH_3OH) 265.5 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 10.37 (s, 2 H), 7.34–7.81 (m, 20 H), 6.48–6.53 (m, 2 H), 5.80 (d, *J* = 8.0 Hz, 1 H), 5.62 (d, *J* = 8.0 Hz, 1 H), 3.68–4.13 (m, 6 H), 1.89–2.40 (m, 10 H), 1.07 (s, 9 H), 1.05 (s, 9 H); Calcd for $C_{25}H_{30}N_2O_3SeSi$: C, 58.47; H, 5.89; N, 5.45. Found: C, 58.77; H, 5.75; N, 5.85.

*The mixture of 1-((2R,5R)-5-(((tert-butyldiphenylsilyl)oxy) methyl)tetrahydroselenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (α of **15**) and 1-((2S,5R)-5-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydroselenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (β of **15**)*

Yield = 16%, α + β mixture; pale brownish syrup; UV λ_{max} (CH_2Cl_2) 271.5 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65–7.71 (m, 8 H), 7.58 (d, *J* = 1.2 Hz, 2 H) 7.38–7.47 (m, 2 H), 6.48–6.54 (m, 2 H), 4.10–4.17 (m, 1 H), 3.84–3.91 (m, 4 H), 3.70–3.74 (m, 1 H), 2.09–2.46 (m, 8 H), 1.83–2.02 (m, 6 H), 1.08 (s, 9 H), 1.06 (s, 9 H); Calcd for

$C_{26}H_{32}N_2O_3SeSi$: C, 59.19; H, 6.11; N, 5.31. Found: C, 58.99; H, 6.32; N, 5.11.

The mixture of 1-((2R,5R)-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (α of 16) and 1-((2S,5R)-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (β of 16)

To a solution of **14** (0.48 g, 0.93 mmol) in anhydrous THF (20 mL) was dropwise added tetra-*n*-butylammonium fluoride solution (0.74 mL, 1 M in THF) under N_2 (g). After stirring at room temperature for 0.5 h, the reaction mixture was evaporated and the residue was purified by flash column chromatography (silica gel, CH_2Cl_2/CH_3OH, 20/1) to give **16** (0.24 g, 91%, α + β mixture) as white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.4 Hz, 1 H), 8.03 (d, *J* = 8.0 Hz, 1 H), 6.46 (t, *J* = 6.8 Hz, 1 H), 6.42 (t, *J* = 6.0 Hz, 1 H), 5.76 (d, *J* = 6.0 Hz, 1 H), 5.74 (d, *J* = 6.0 Hz, 1 H), 4.07–4.14 (m, 1 H), 3.74–3.93 (m, 4 H), 3.57–3.62 (m, 1 H), 2.43–2.51 (m, 1 H), 2.17–2.39 (m, 5 H), 2.06–2.13 (m, 1 H), 1.91–2.00 (m, 1 H); Calcd for $C_9H_{12}N_2O_3Se$: C, 39.28; H, 4.40; N, 10.18. Found: C, 39.45; H, 4.16; N, 10.58.

*The mixture of 1-((2R,5R)-5-(hydroxymethyl) tetrahydroselenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (α of **17**) and 1-((2S,5R)-5-(hydroxymethyl)tetrahydroselenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (β of **17**)*

Compound **15** (0.55 g, 1.04 mmol) was converted to compound **17** (0.28 g, 95%, α + β mixture) using a similar procedure to that used in the preparation of compound **16**; white foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1 H), 7.80 (s, 1 H), 6.48 (t, *J* = 6.8 Hz, 1 H), 6.43 (t, *J* = 6.0 Hz, 1 H), 4.12–4.15 (m, 1 H), 3.75–3.93 (m, 4 H), 3.58–3.63 (m, 1 H), 2.13–2.49 (m, 8 H), 1.88–1.93 (m, 6 H); Calcd for $C_{10}H_{14}N_2O_3Se$: C, 41.53; H, 4.88; N, 9.69. Found: C, 41.35; H, 4.47; N, 9.34.

((2R,5S)-5-(3-Benzoyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydroselenophen-2-yl)methyl benzoate (18a) and ((2R,5R)-5-(3-benzoyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydroselenophen-2-yl)methyl benzoate (18b)

To a solution of **16** (0.23 g, 0.84 mmol) in pyridine (12 mL) was dropwise added benzoyl chloride (0.48 mL, 4.20 mmol) under N_2 (g). After heating at 70 °C (bath temperature) with stirring for 15 h, the reaction mixture was evaporated and the residue was purified by column chromatography (silica gel, hexane/EtOAc, 3/1) to give β-isomer **18a** (174 mg, 43%) as white foam and α-isomer **18b** (175 mg, 43%) as white foam.

For β-Isomer (**18a**): UV λ_{max} (CH_3OH) 252.5 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–8.07 (m, 2 H, *phenyl* in Bz), 7.99 (d, *J* = 8.4 Hz, 1 H, *H*-6), 7.91–7.93 (m, 2 H, *phenyl* in Bz), 7.58–7.66 (m, 2 H, *phenyl* in Bz), 7.45–7.50 (m, 4 H, *phenyl* in Bz), 6.52 (t, *J* = 6.4, 1 H, *H*-1′), 5.79 (d, *J* = 8.0 Hz, 1 H, *H*-5), 4.72 (dd, *J* = 7.2, 11.6 Hz, 1 H, *H*-5′), 4.57 (dd, *J* = 6.4, 11.6 Hz, 1 H, *H*-5′), 4.07–4.11 (m, 1 H, *H*-4′), 2.42–2.48 (m, 1 H, *H*-2′), 2.26–2.33 (m, 2 H, *H*-2′, *H*-3′), 2.13–2.18 (m, 1 H, *H*-3′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 168.8, 166.4, 162.3, 149.6, 141.7, 135.4, 133.8, 133.7, 131.5, 130.7, 130.3, 129.8, 129.7, 129.5, 128.8, 128.6, 102.6, 67.0, 59.3, 45.1, 38.1, 32.9; LRMS (FAB) m/z 483 $[M+H]^+$; $[\alpha]_D^{23}$ = + 2.50 (*c* 3.58, CH_2Cl_2); Calcd for $C_{23}H_{20}N_2O_5Se$: C, 57.15; H, 4.17; N, 5.80. Found: C, 57.55; H, 4.45; N, 5.47.

For α-Isomer (**18b**): UV λ_{max} (CH_3OH) 253.5 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02–8.05 (m, 2 H, *phenyl* in Bz), 7.89–7.94 (m, 2 H, *phenyl* in Bz), 7.63–7.67 (m, 1 H, *phenyl* in Bz), 7.56–7.61 (m, 1 H, *phenyl* in Bz), 7.44–7.51 (m, 5 H, *phenyl* in Bz, *H*-6), 6.58 (t, *J* = 6.4 Hz, 1 H, *H*-1′), 5.90 (d, *J* = 8.0 Hz, 1 H, *H*-5), 4.58 (dd, *J* = 7.2, 10.8 Hz, 1 H, *H*-4′), 4.31–4.40 (m, 2 H, *H*-5′, *H*-5′), 2.58–2.63 (m, 1 H, *H*-2′), 2.34–2.39 (m, 1 H, *H*-2′), 2.04–2.18 (m, 2 H, *H*-3′, *H*-3′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 166.2, 162.0, 149.6, 141.6, 135.4, 133.5, 131.5, 130.7, 129.8, 129.8, 129.4, 128.7, 102.9, 67.6, 58.6, 44.9, 38.7, 33.6; LRMS (FAB) m/z 483 $[M+H]^+$; $[\alpha]_D^{23}$ = − 21.79 (*c* 0.98, CH_3OH); Calcd for $C_{23}H_{20}N_2O_5Se$: C, 57.15; H, 4.17; N, 5.80. Found: C, 57.54; H, 4.02; N, 5.73.

*((2R,5S)-5-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydroselenophen-2-yl)methyl benzoate (**19a**) and ((2R,5R)-5-(3-benzoyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydroselenophen-2-yl)methyl benzoate (**19b**)*

Compound **17** (0.28 g, 0.97 mmol) was converted to compound **19a** (193 mg, 40%) and **19b** (202 mg, 42%) using a similar procedure to that used in the preparation of compound **18a** and **18b**; white foam; For β-Isomer (**19a**): UV λ_{max} (CH_3OH) 253.5 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–8.08 (m, 2 H, *phenyl* in Bz), 7.90–7.93 (m, 2 H, *phenyl* in Bz), 7.73 (s, 1 H, *H*-6), 7.58–7.66 (m, 2 H, *phenyl* in Bz), 7.46–7.50 (m, 4 H, *phenyl* in Bz, *H*-1′), 6.57 (t, *J* = 6.8 Hz, 1 H, *H*-4′), 4.71 (dd, *J* = 8.0, 11.2 Hz, 1 H, *H*-5′), 4.60 (dd, *J* = 6.8, 11.6 Hz, 1 H, *H*-5′), 2.46–2.51 (m, 1 H, *H*-2′), 2.22–2.30 (m, 3 H, *H*-2′, *H*-3′, *H*-3′), 1.93 (s, 3 H, *H*-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 169.0, 166.5, 162.8, 149.7, 137.1, 135.2, 133.9, 133.6, 131.7, 130.7, 130.4, 129.9, 129.8, 129.3, 128.8, 128.7, 111.7, 67.3, 58.6, 44.8, 37.5, 33.0, 12.9; LRMS (FAB) m/z 497 $[M+H]^+$;

$[\alpha]_D^{23}$ = + 55.36 (*c* 6.34, CH_3OH); Calcd for $C_{24}H_{22}N_2O_5Se$: C, 57.95; H, 4.46; N, 5.63. Found: C, 57.86; H, 4.58; N, 5.43; For: α-Isomer (**19b**): UV λ_{max} (CH_3OH) 254.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03–8.06 (m, 2 H, *phenyl* in Bz), 7.91–7.93 (m, 2 H, *phenyl* in Bz), 7.57–7.67 (m, 3 H, *phenyl* in Bz, *H*-6), 7.44–7.51 (m, 4 H, *phenyl* in Bz), 6.63 (t, *J* = 6.8 Hz, 1 H, *H*-1′), 4.58–4.62 (m, 1 H, *H*-4′), 4.34–4.42 (m, 2 H, *H*-5′, *H*-5′), 2.60-2.64 (m, 1 H, *H*-2′), 2.40–2.45 (m, 2 H, *H*-2′, *H*-3′), 2.10–2.17 (m, 1 H, *H*-3′), 1.99 (s, 3 H, *H*-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 166.2, 162.8, 149.6, 137.1, 135.3, 133.5, 131.7, 130.7, 129.8, 129.4, 128.7, 111.7, 67.9, 58.1, 45.0, 38.7, 33.8, 13.0; LRMS (FAB) m/z 497 $[M+H]^+$; $[\alpha]_D^{23}$ = − 97.98 (*c* 0.79, CH_3OH); Calcd for $C_{24}H_{22}N_2O_5Se$: C, 57.95; H, 4.46; N, 5.63. Found: C, 57.78; H, 4.34; N, 5.78.

4-Amino-1-((2S,5R)-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidin-2(1H)-one (2)

To a solution of β-isomer **12** (0.46 g, 0.74 mmol) in anhydrous THF (46 mL) was dropwise added tetra-*n*-butylammonium fluoride (1.11 mL, 1 M in THF) under N_2 (g). After stirring at room temperature for 0.5 h, the reaction mixture was evaporated. To this residue, methanolic ammonia (5 mL) was added and stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2/CH_3OH, 30/1) to give **2** (0.17 g, 83%) as a white solid: mp 143.0–146.3 °C (from CH_3OH/ether); UV λ_{max} (CH_3OH) 277.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (d, *J* = 7.6 Hz, 1 H, *H*-6), 6.45 (t, *J* = 5.6 Hz, 1 H, *H*-1′), 5.92 (d, *J* = 7.6 Hz, 1 H, *H*-5), 3.83–3.92 (m, 2 H, *H*-5′, *H*-5′), 3.75 (dd, *J* = 6.0, 10.4 Hz, 1 H, *H*-4′), 2.30–2.36 (m, 2 H, *H*-2′, *H*-2′), 2.20–2.26 (m, 1 H, *H*-3′), 2.04–2.07 (m, 1 H, *H*-3′); ^{13}C NMR (100 MHz, CD_3OD) δ 167.5, 158.7, 144.5, 96.3, 66.8, 60.5, 50.3, 38.7, 33.7; LRMS (FAB) m/z 276 $[M+H]^+$; $[\alpha]_D^{23}$ = + 188.79 (*c* 0.11, CH_3OH); Calcd for $C_9H_{13}N_3O_2Se$: C, 39.43; H, 4.78; N, 15.33. Found: C, 39.87; H, 4.98; N, 15.18.

*4-Amino-1-((2R,5R)-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidin-2(1H)-one (**2a**)*

Compound **13** (0.46 g, 0.746 mmol) was converted to compound **2a** (173 mg, 85%) using a similar procedure to that used in the preparation of compound **2**; Yield = 85%; white solid; mp 203.7–205.8 °C (from CH_3OH/ether); UV λ_{max} (CH_3OH) 277.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (d, *J* = 7.6 Hz, 1 H, *H*-6), 6.49 (t, *J* = 6.4 Hz, 1 H, *H*-1′), 5.94 (d, *J* = 7.6 Hz, 1 H, *H*-5), 4.04–4.10 (m, 1 H, *H*-4′), 3.78 (dd, *J* = 6.8, 10.8 Hz, 1 H, *H*-5′), 3.60 (dd, *J* = 7.2, 11.2 Hz, 1 H, *H*-5′), 2.42–2.50 (m, 1 H, *H*-2′), 2.15–2.32 (m, 2 H, *H*-2′, *H*-3′), 1.94–2.02 (m, 1 H, *H*-3′); ^{13}C NMR (100 MHz, CD_3OD) δ 167.4, 158.5, 144.5, 96.6, 67.3, 59.7, 50.0, 38.8, 34.0; LRMS (FAB) m/z 276 $[M+H]^+$; $[\alpha]_D^{23}$ = − 248.24 (*c* 0.28, CH_3OH); Calcd for $C_9H_{13}N_3O_2Se$: C, 39.43; H, 4.78; N, 15.33. Found: C, 39.83; H, 4.98; N, 14.89.

1-((2S,5R)-5-(Hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (3)

The solution of **18a** (0.38 g, 0.79 mmol) in methanolic ammonia (5 mL) was stirred at room temperature for 15 h. The reaction mixture was evaporated and the residue was purified by column chromatography (silica gel, CH_2Cl_2/CH_3OH, 30/1) to give **3** (0.19 g, 90%) as a white solid: mp 166.5-168.6 °C (from CH_3OH/ ether); UV λ_{max} (CH_3OH) 266.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 8.13 (d, *J* = 8.0 Hz, 1 H, *H*-6), 6.42 (t, *J* = 6.0 Hz, 1 H, *H*-1′), 5.74 (d, *J* = 8.0 Hz, 1 H, *H*-5), 3.84–3.93 (m, 2 H, *H*-5′, *H*-5′), 3.76 (dd, *J* = 5.6, 10.4 Hz, 1 H, *H*-4′), 2.31–2.37 (m, 2 H, *H*-2′, *H*-2′), 2.19–2.24 (m, 1 H, *H*-3′), 2.08–2.11 (m, 1 H, *H*-3′); ^{13}C NMR (100 MHz, CD_3OD) δ 166.2, 152.5, 144.3, 102.8, 66.7, 59.4, 50.6, 38.5, 33.7; LRMS (FAB) m/z 276 $[M+H]^+$; $[\alpha]_D^{23}$ = + 96.20 (*c* 0.37, CH_3OH); Calcd for $C_9H_{12}N_2O_3Se$: C, 39.28; H, 4.40; N, 10.18. Found: C, 39.48; H, 4.84; N, 9.98.

*1-((2R,5R)-5-(Hydroxymethyl)tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (**3a**)*

Compound **18b** (175 mg, 0.362 mmol) was converted to compound **3a** (92 mg, 92%) using a similar procedure to that used in the preparation of compound **3**; white solid; mp 188.3–190.2 °C (from CH_3OH/ether); UV λ_{max} (CH_3OH) 266.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (d, *J* = 8.4 Hz, 1 H, *H*-6), 6.46 (t, *J* = 6.8 Hz, 1 H, *H*-1′), 5.75 (d, *J* = 7.6 Hz, 1 H, *H*-5), 4.08–4.14 (m, 1 H, *H*-4′), 3.79 (dd, *J* = 6.8, 10.8 Hz, 1 H, *H*-5′), 3.60 (dd, *J* = 7.2, 11.2 Hz, 1 H, *H*-5′), 2.43–2.51 (m, 1 H, *H*-2′), 2.27–2.35 (m, 1 H, *H*-2′), 2.17–2.25 (m, 1 H, *H*-3′), 1.91–2.00 (m, 1 H, *H*-3′); ^{13}C NMR (100 MHz, CD_3OD) δ 166.2, 152.5, 144.3, 103.1, 67.3, 58.6, 50.5, 38.8, 34.2; LRMS (FAB) m/z 276 $[M+H]^+$; $[\alpha]_D^{23}$ = − 167.94 (*c* 0.57, CH_3OH); Calcd for $C_9H_{12}N_2O_3Se$: C, 39.28; H, 4.40; N, 10.18. Found: C, 39.48; H, 4.33; N, 10.40.

*1-((2S,5R)-5-(Hydroxymethyl)tetrahydroselenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**4**)*

Compound **19a** (193 mg, 0.388 mmol) was converted to compound **4** (95 mg, 85%) using a similar procedure to that used in the preparation of compound **3**; white solid; mp 147.7–149.9 °C (from CH_3OH/ether); UV λ_{max} (CH_3OH)

271.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (s, 1 H, *H*-6), 6.43 (t, *J* = 6.4 Hz, 1 H, *H*-1′), 3.84–3.93 (m, 2 H, *H*-4′, *H*-5′), 3.77 (dd, *J* = 5.6, 10.4 Hz, 1 H, *H*-5′), 2.30–2.35 (m, 2 H, *H*-2′, *H*-2′), 2.13–2.20 (m, 2 H, *H*-3′, *H*-3′), 1.90 (s, 3 H, *H*-7); ^{13}C NMR (100 MHz, CD_3OD) δ 166.4, 152.7, 139.8, 111.7, 66.6, 59.2, 50.5, 38.4, 33.7, 12.6; LRMS (FAB) m/z 289 $[M+H]^+$; $[\alpha]_D^{23}$ = + 67.83 (*c* 0.31, CH_3OH); Calcd for $C_{10}H_{14}N_2O_3Se$: C, 41.53; H, 4.88; N, 9.69. Found: C, 41.66; H, 4.84; N, 9.54.

*1-((2R,5R)-5-(Hydroxymethyl)tetrahydroselenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**4a**)*

Compound **19b** (202 mg, 0.406 mmol) was converted to compound **4a** (103 mg, 88%) using a similar procedure to that used in the preparation of compound **3**; white solid; mp 210.2–215.3 °C (from CH_3OH/ ether); UV λ_{max} (CH_3OH) 270.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (s, 1 H, *H*-6), 6.48 (t, *J* = 6.8 Hz, 1 H, *H*-1′), 4.10–4.15 (m, 1 H, *H*-4′), 3.80 (dd, *J* = 6.8, 12.8 Hz, 1 H, *H*-5′), 3.61 (dd, *J* = 7.6, 11.2 Hz, 1 H, *H*-5′), 2.43-2.50 (m, 1 H, *H*-2′), 2.31–2.39 (m, 1 H, *H*-2′), 2.17–2.26 (m, 1 H, *H*-3′), 1.85–1.94 (m, 4 H, *H*-3′, *H*-7); ^{13}C NMR (100 MHz, CD_3OD) δ 139.6, 112.1, 67.4, 58.2, 50.6, 38.7, 34.4, 12.6; LRMS (FAB) m/z 289 $[M+H]^+$; $[\alpha]_D^{23}$ = − 131.61 (*c* 0.37, CH_3OH); Calcd for $C_{10}H_{14}N_2O_3Se$: C, 41.53; H, 4.88; N, 9.69. Found: C, 41.91; H, 4.99; N, 9.48.

Anti-HIV-1 activity

Anti-HIV-1 assay was performed as described previously (Singh et al. 2016). HIV-1 (HTLV-IIIB strain) was amplified in MT-4 (HTLV-1-infected human T lymphocytes) cells and grown in RPMI 1640 medium supplemented with 10% FBS and 4 μg/mL gentamycin. This medium was used for making dilutions of the drugs and maintenance of the cultures during the assays. Aliquots of the virus stocks were stored as culture supernatants at − 70 °C until used. The virus-induced-CPE inhibition assay was used to measure the anti-HIV activity. Log-phase MT-4 cells were plated and infected with the virus at a multiplicity of infection of 20 to 100 $CCID_{50}$ (50% cell culture inhibitory dose) per well. The cells were immediately resuspended in RPMI 1640 plus 10% FBS at a concentration of 105 cells/mL. Aliquots of 100 μL of the resuspended cells were placed in the wells of a 96-well plate containing 100 μL of twofold-concentrated test samples. After 5 days of incubation at 37 °C, the cells were observed microscopically, and cell viability was quantified using the MTT assay (Mosmann 1983; Pauwels et al. 1988), which is based on the mitochondrial reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The effective antiviral concentration was expressed as the EC_{50}, which is the concentration of compound required to inhibit virus-induced CPE by 50%. The cytotoxic concentration is expressed as the CC_{50}, which is the concentration of the compound that killed 50% of the mock-infected cells. 2′,3′-Dideoxycytidine (ddC) (Sigma) and azidothymidine (AZT) (Sigma) were used as references in the anti-HIV tests. All compounds were dissolved in 100% dimethyl sulfoxide (DMSO) at a stock concentration of 20 mg/mL.

X-ray structure determination

A suitable crystal of L-β-Se-ddC (**2**) was selected and determined on a SuperNova, Dual, Cu at home/near, AtlasS2 diffractometer. The crystal was kept at 294.8(7) K during data collection. Using Olex2 (Dolomanov et al. 2009), the structure was solved with the ShelXT (Sheldrick 2015a, b) structure solution program using Intrinsic Phasing and refined with the ShelXL (Sheldrick 2015a, b) refinement package using Least Squares minimization.

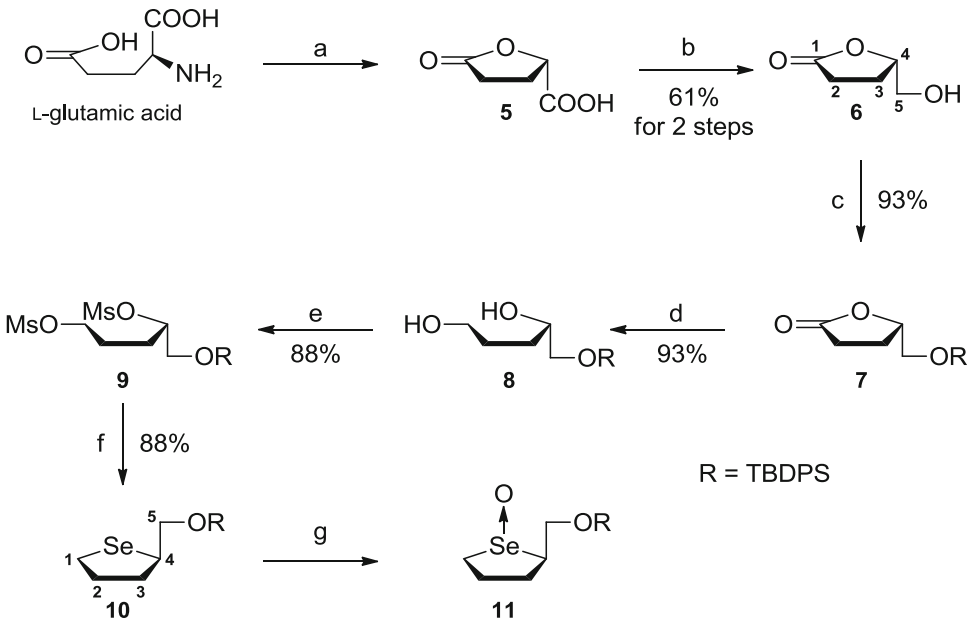
Crystal Data for $C_9H_{13}N_3O_2Se$ (M = 274.18 g/mol): orthorhombic, space group $P2_12_12_1$(no. 19), a = 9.2920(7) Å, b = 10.9925(9) Å, c = 11.3141(13) Å, V = 1155.65(18) Å^3, Z = 4, T = 294.8(7) K, μ(MoKα) = 3.235 mm^{-1}, *Dcalc* = 1.576 g/cm^3, 9541 reflections measured (5.166° ≤ 2Θ ≤ 59.404°), 2825 unique (R_{int} = 0.0391, R_{sigma} = 0.0391) which were used in all calculations. The final R_1 was 0.0326 (I > 2σ(I)) and wR_2 was 0.0771 (all data). Further details of the crystal structure investigation(s) may be obtained from the Cambridge Crystallographic Data Centre (CCDC, 12 Union Road, Cambridge, CB2 1EZ (UK); Tel.: (+44)1223-336-408, fax: (+44)1223-336-033, e-mail: deposit@ccdc.cam.ac.uk) on quoting the Depository No. CSD-1862312.

Results and discussion

Our synthetic strategy to the final nucleosides **2**–**4** is to synthesize the glycosyl donor from the chiral template and then to condense it with silylated pyrimidine bases under the Pummerer rearrangement conditions, using the same procedure used in the preparation of the corresponding D-nucleosides (Jeong et al. 2008a, b). Firstly, the glycosyl donor **11** was synthesized from L-glutamic acid, as shown in Scheme 1.

Diazotization of L-glutamic acid with sodium nitrite ($NaNO_2$) in the presence of c-H_2SO_4 yielded the γ-lactone **5** as a single stereoisomer (Wrona et al. 2010). Reduction of the carboxylic acid of **5** with borane-dimethylsulfide complex afforded the primary alcohol **6** (Wrona et al. 2010). The primary alcohol of **6** was protected with TBDPS group to give lactone **7**, which was reduced with $LiBH_4$ to yield the diol **8**. Diol **8** was dimesylated with MsCl to give **9**, which was treated with selenium powder in

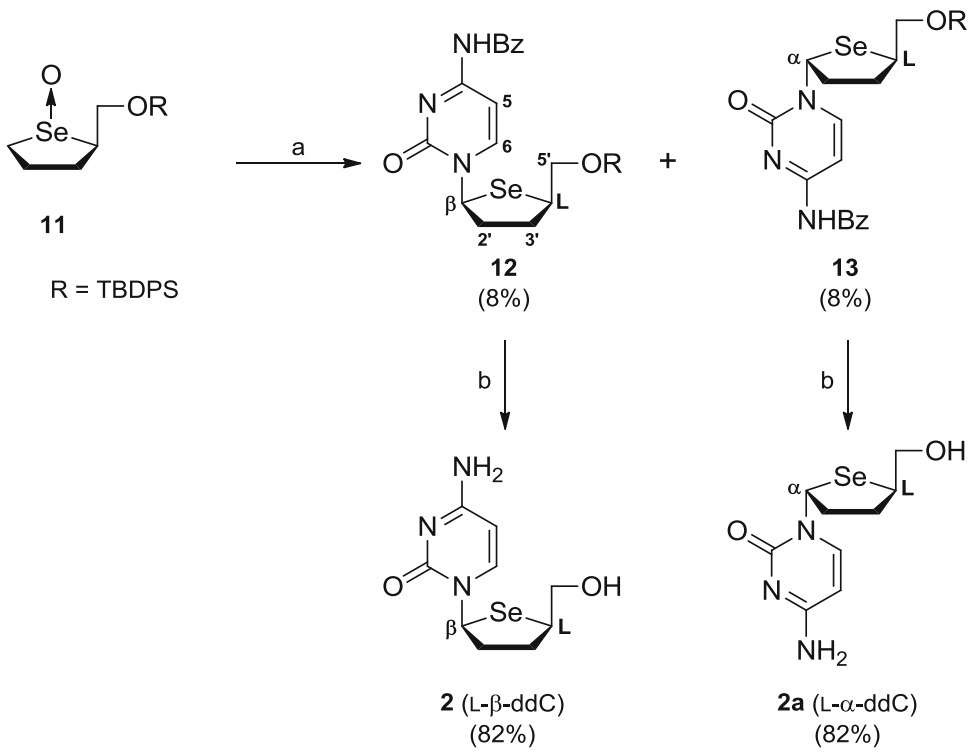
Scheme 1 Synthesis of the glycosyl donor **11**



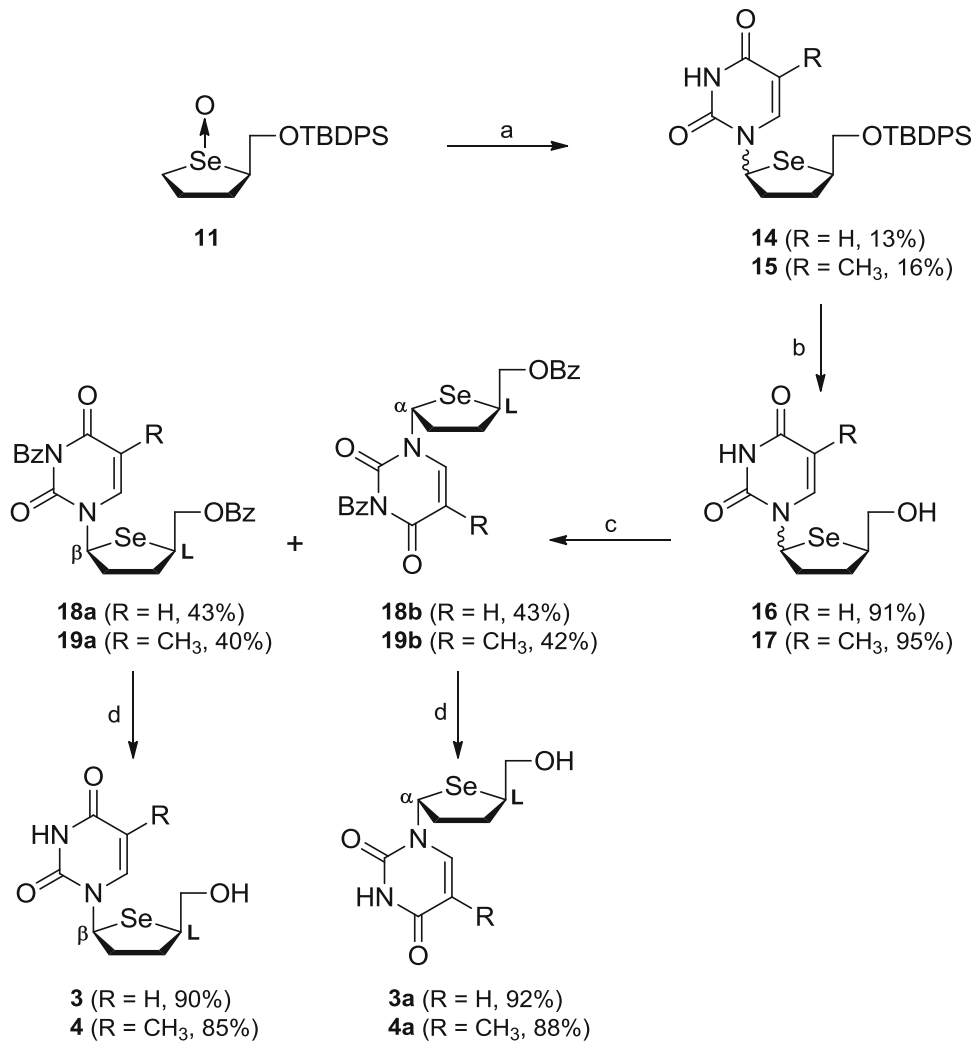
the presence of $NaBH_4$ to give 4-selenosugar **10** with L-configuration. Oxidation of **10** with mCPBA afforded the L-glycosyl donor **11**, which is the condensation substrate for Pummerer rearrangement conditions.

The L-glycosyl donor **11** was condensed with silylated N^4-benzoylcytosine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and triethylamine (Et_3N) to give the L-β-isomer **12** and L-α-isomer **13** (Scheme 2) (α:β = 1:1). Treatment of **12** and **13** with tetra-*n*-butylammonium fluoride (TBAF) followed by further treatment with methanolic ammonia afforded the L-β-Se-ddC (**2**) and its L-α-Se-isomer **2a**, respectively. The structure of anomers **2** and **2a** were confirmed from their spectral features and comparison with the spectral data of their enantiomers D-Se-ddC (Jeong et al. 2008a, b). The ^1H NMR spectrum of L-β-Se-ddC **2** and L-α-Se-ddC **2a** were identical with those of D-β-Se-ddC and D-α-Se-ddC, respectively.

Scheme 2 Synthesis of L-β-Se-ddC (**2**) and its L-α-isomer **2a**



Scheme 3 Synthesis of L-β-Se-ddU (**3**) and L-β-Se-ddT (**4**) and their L-α-isomers **3a** and **4a**



Next, the L-glycosyl donor **11** was condensed with silylated uracil and thymine under the same conditions to give **14** and **15**, respectively as inseparable α/β-mixtures (Scheme 3). The removal of TBDPS group of **14** and **15** with TBAF yielded **16** and **17**, respectively, but they were still obtained as inseparable α/β-mixtures. For the isolation of inseparable α/β-mixtures, compound **16** was benzoylated to give the N^3-benzoates, which were separated by silica gel column chromatography to yield the β-benzoate **18a** and the α-benzoate **18b**. Similarly, compound **17** was converted to the β-benzoate **19a** and the α-benzoate **19b**. The β-anomers **18a** and **19a** were treated with methanolic ammonia to afford the final L-β-Se-ddU (**3**) and L-β-Se-ddT (**4**), respectively. Similarly, the α-anomers **18b** and **19b** were converted the corresponding L-α-isomers **3a** and **4a**, respectively.

All final compounds **2**–**4** and **2a**–**4a** were assayed for their anti-HIV-1 activities in MT-4 cells. Unfortunately, none of the synthesized compounds did show any antiviral activity up to 100 μM, indicating that they are not converted into the corresponding triphosphates by cellular kinases, maybe due to steric repulsion between bulky selenium atom and cellular kinase (Sahu et al. 2014, 2015).

The conformation of a representative synthesized 4′-seleno compound, L-β-Se-ddC (**2**) was analyzed using spectral data and X-ray crystallography. The X-ray crystal structure of **2** was obtained as illustrated in Fig. 2. Compound **2** exhibited a 2′-endo/3′-exo (South) conformation, which is the same of that of L-ddC (Birnbaum 1988). Thus, as in the case of 4′-Se-d4T, one-carbon homologation of 4′-Se-ddNs might reduce steric repulsion between bulky selenium atom and cellular kinase, and restore the antiviral activity by phosphorylating them by cellular kinases.

Conclusions

All final compounds **2**–**4** and **2a**–**4a** were assayed for their anti-HIV-1 activity in MT-4 cells. Disappointedly, all synthesized compounds showed neither anti-HIV-1 activity

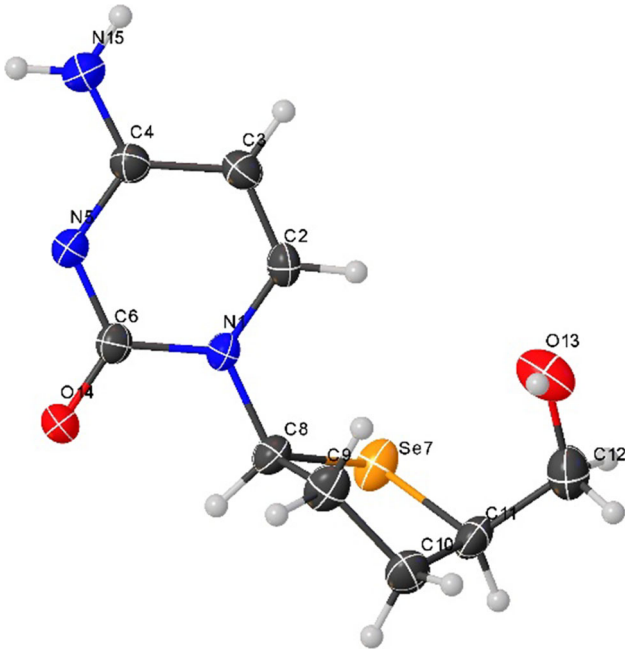


Fig. 2 X-ray crystal structure of L-β-Se-ddC (**2**)

nor cytotoxicity up to 100 μM, indicating that they are not converted into the corresponding triphosphates by cellular kinases, maybe due to steric repulsion between bulky selenium atom and cellular kinases. Thus, as in the case of 4′-Se-d4T (Qu et al. 2016), one-carbon homologation of 4′-Se-ddNs or phosphoramidate prodrug approach might restore the antiviral activity by phosphorylating them by cellular kinases.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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