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A Divergent Approach for the Synthesis of D- and L-4′-Ethynyl Dioxolane Nucleosides with Potent Anti-HIV Activity

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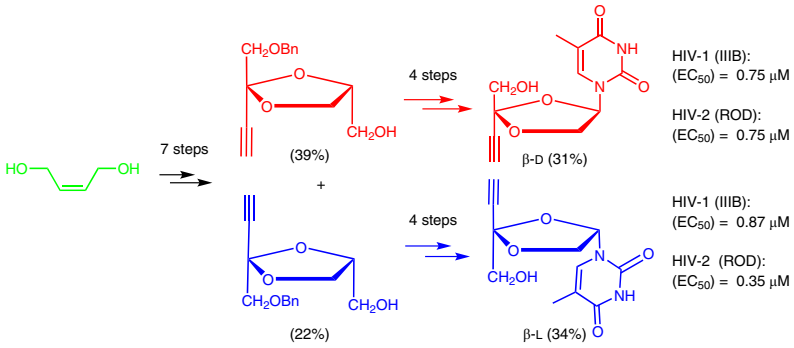

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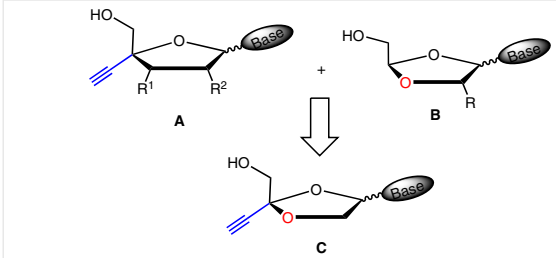
Abstract Novel 4′-*C*-ethynyl isomeric dioxolane nucleoside analogues (β-D, α-D, β-L, and α-L, respectively) are successfully synthesized via a divergent strategy from the common starting material, (*Z*)-but-2-ene-1,4-diol, and are characterized and evaluated for their anti-HIV-1 and anti-HIV-2 activities. The β-D and β-L products display potent *in vitro* activities against HIV-1 (IIIB) with EC_{50} values of 0.75 and 0.87 μM, respectively, and against HIV-2 (ROD) with EC_{50} values of 0.75 and 0.35 μM, respectively, being better in comparison with 3TC [EC_{50}, 5.27 μM (HIV-1) and 1.30 μM (HIV-2)]. The β-D and β-L nucleosides also potently inhibit different drug-resistant strains of the HIV-1 virus (L100I, K103N, Y181C, and V106A). The selectivity indices and cytotoxic profiles of the β-D and β-L nucleosides are much better than those of the standard drugs AZT and d4T.

Key words anti-HIV, AIDS, dioxolane nucleosides, 4′-acetylene nucleosides, divergent synthesis, asymmetric synthesis

Acquired immune deficiency syndrome (AIDS), which is caused by human immunodeficiency virus 1 (HIV-1) and human immunodeficiency virus 2 (HIV-2) infection, is a serious worldwide health concern.[1,2] Conventional treatment[3] of HIV infection is based on combinatorial antiretroviral therapy (cART), which uses combinations of three or more antiretroviral agents to control rapidly HIV replication.[4,5] In cART, nucleoside analogues as reverse transcriptase inhibitors play a pivotal role along with other protease inhibitors and integrase inhibitors. However, the effectiveness of cART is compromised by the rapid development of multidrug-resistant strains of HIV and cumulative cytotoxic effects observed during prolonged uses of this therapy. Thus, there is a continuous need of better anti-HIV agents having increased potency and improved pharmacokinetic profile.[6]

It is evident from the literature that acetylene-containing nucleosides have shown potent anti-HIV activities.[7] In addition, 1,3-dioxalane-[8] and 1,3-oxathiolane-based[9] nucleosides have good potential as shown by their promising antiviral activities. Lamivudine (3TC) and emtricitabine (FTC) have been approved by the FDA as anti-HIV agents.[9] Until now, there have been no reports of 4′-*C*-ethynyl dioxolane nucleosides in the literature. Thus, it is of great interest to combine both structures to prepare 4′-*C*-ethynyl dioxolane



Scheme 1 Rational design of 4′-*C*-ethynyl dioxolane nucleosides

nucleosides in order to develop novel nucleoside reverse transcriptase inhibitors as shown in Scheme 1. Since the FDA-approved anti-HIV drugs AZT and d4T contain thymine as an integral part of their structures, we initially planned to synthesize ethynyl dioxolane nucleosides with thymine as the base to test the plausibility of this template.

Herein, the syntheses and anti-HIV activity of four isomeric 4′-*C*-ethynyl dioxolane nucleoside analogues **15a**–**d** are reported (Figure 1).

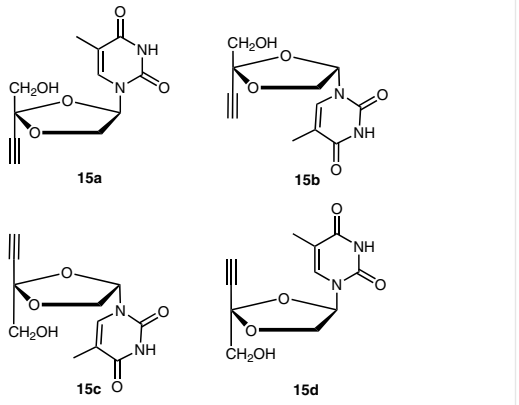
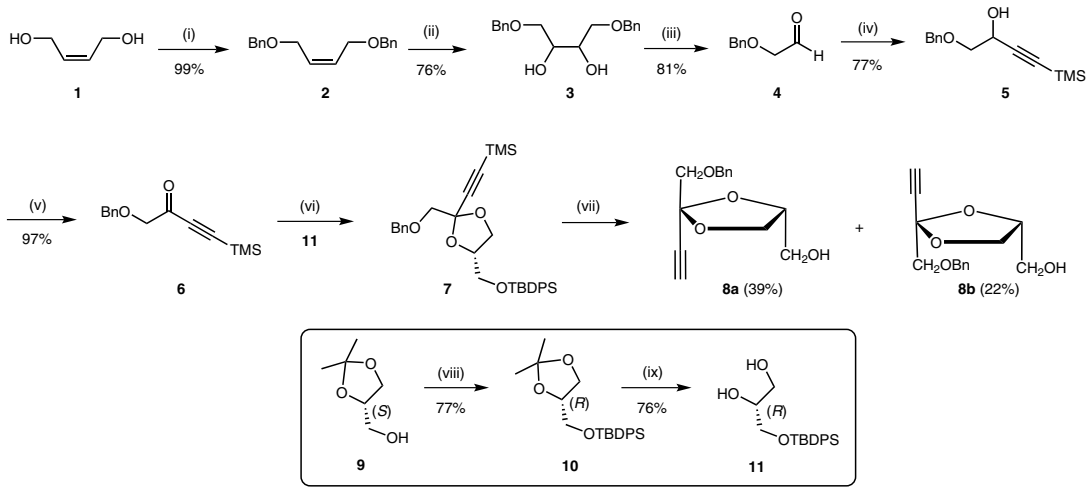


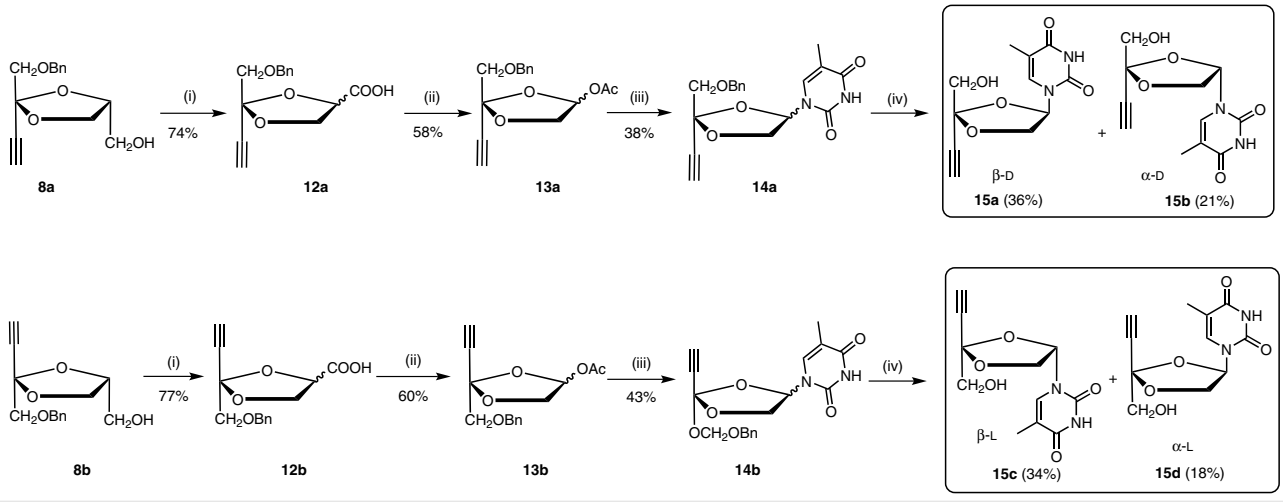
Figure 1 Structures of compounds **15a**–**d**

Compounds **15a**–**d** were synthesized using the divergent strategies illustrated in Schemes 2 and 3. Initially, the hydroxy groups of (*Z*)-but-2-ene-1,4-diol (**1**) were protected with benzyl groups using benzyl bromide and NaH in DMF (Scheme 2). Subsequent *cis*-hydroxylation with OsO_4/NMO in acetone/H_2O gave diol **3** in 76% yield. Cleavage of compound **3** was performed with $NaIO_4$ affording aldehyde **4** in 81% yield, followed by immediate reaction with trimethylsilylacetylene in THF using *n*-BuLi as the base. The resulting alkyne **5** was then oxidized to give compound **6** using NaOCl as the oxidizing agent. The freshly prepared compound **6** was then immediately coupled with compound **11** in benzene using a catalytic amount of PTSA under reflux conditions employing a Dean–Stark assembly. Silyl group deprotection of resulting compound **7** with TBAF in THF afforded diastereomeric compounds **8a** and **8b** in 39% and 22% yields, respectively.

Next, the CH_2OH group of compound **8a** was transformed into a COOH group using PDC as the oxidizing agent in DMF to give compound **12a** as a diastereomeric mixture. Compound **12a**, without any purification, was directly converted into acetate **13a** using $Pb(OAc)_4$ and pyridine. Compound **13a** was then coupled with thymine to give product **14a** as a mixture of diastereomers. Benzyl group deprotection in compound **14a** with $AlCl_3$ and anisole afforded the desired products **15a** and **15b** in 36% and 21% yields, respectively. A similar synthetic strategy was used for the synthesis of compounds **15c** and **15d** starting from compound **8b** (Scheme 3). At this point, it is important to mention that debenzylation of compounds **14a** and **14b** was very challenging. All the available conditions for debenzylation failed leading to decomposition of the dioxolane ring due to its instability under acidic conditions, but finally, deprotection with $AlCl_3$/anisole afforded the desired products. The different reaction conditions employed for the debenzylation of compound **14a** are given in the Supporting Information.



Scheme 2 *Reagents and conditions*: (i) BnBr, NaH, DMF, 0 °C to r.t.; (ii) OsO_4, NMO, acetone/H_2O; (iii) $NaIO_4$, MeOH, 0 °C to r.t.; (iv) *n*-BuLi, trimethylsilylacetylene, THF, –78 °C; (v) 5% NaOCl, $NaHCO_3$, KI, TEMPO, CH_2Cl_2, r.t.; (vi) **11**, PTSA, benzene, 90 °C; (vii) TBAF, THF, 0 °C; (viii) TBDPSCl, DMAP, Et_3N, CH_2Cl_2, 0 °C; (ix) AcOH, H_2O, 90 °C.



Scheme 3 *Reagents and conditions*: (i) PDC, DMF, r.t.; (ii) $Pb(OAc)_4$, py, THF, r.t.; (iii) thymine, 2,4,6-collidine, TMSI, TBDMSOTf, CH_2Cl_2, 40 °C; (iv) $AlCl_3$, anisole, CH_2Cl_2, 0 °C.

Attempts were also made to assign the absolute configurations of nucleosides **15a**–**d**. Compound **15d** was able to be recrystallized, and X-ray crystal structure analysis revealed that it had α-L configuration (Figure 2).

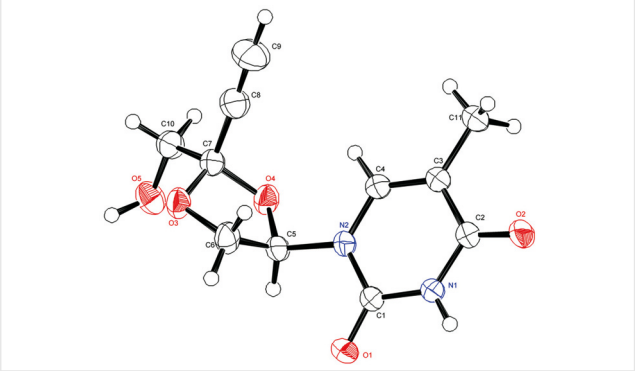


Figure 2 X-ray crystal structure of compound **15d** (CCDC 1479802)

The NMR spectra, HPLC retention times, R_f values (TLC) and melting points of **15a**,**c** and **15b**,**d** were the same, indicating their enantiomeric relationships with each other. On the basis of these experiments, the absolute configurations of compounds **15a**, **15b**, **15c** and **15d** were assigned as β-D, α-D, β-L, and α-L, respectively.

Compounds **15a**–**d** were then evaluated for their activities against HIV-1 and HIV-2 using the IIIB and ROD strains, respectively. The results are shown in Table 1, which also includes the activities of the standard drugs AZT, 3TC and d4T against these viral strains. As is evident from Table 1, nucleosides **15a** and **15c** with β-configurations displayed potent anti-HIV-1 and anti-HIV-2 activities, whereas compounds **15b** and **15d** having α-configurations were found to be inactive. The activities of both compounds **15a** and **15c** were better than the standard drug 3TC. All the compounds also showed excellent cytotoxic and selectivity index profiles suggesting their scope for further development.

Table 1 Anti-HIV Activities of Compounds **15a**–**d**

Compound	Toxicity CC_{50}	Anti-viral activity (EC_{50}) (μM)		Selectivity index	
		HIV-1 strain (IIIB)	HIV-2 strain (ROD)	IIIB	ROD
15a	>100	0.75	0.75	>562.32	>562.32
15b	>100	14.78	16.18	>26.82	>24.48
15c	>100	0.87	0.35	>447.16	>1132.43
15d	>100	112.0	39.80	>3.54	> 9.96
AZT	3.522	0.015	0.0015	864	8427
3TC	>100	5.27	1.30	>82.64	>333.33
d4T	7.55	0.27	0.024	127	1373

Table 2 Anti-HIV Activities of Compounds **15a** and **15c** against Drug-Resistant Strains of the HIV-1 Virus

Compound	Anti-viral activity (EC_{50}) (HIV-1) (μM)							
	RTMDR1	L100I	K103N	V106A	Y181C	Y188H	Y188L	G190A
15a	5.94	0.24	0.24	0.47	0.25	0.28	0.31	0.194
15c	5.590	0.21	nd[a]	nd[a]	nd[a]	0.30	0.30	0.225
ddC	1.65	0.33	0.33	0.57	0.331	0.01	0.09	0.014
AZT	0.13	0.0026	0.0067	0.012	0.0052	0.011	0.011	0.0063

[a] Not determined.

As the emergence of resistant viral strains is a major challenge and a matter of concern, we evaluated the activities of compounds **15a** and **15c** against different drug-resistant strains of the HIV-1 virus. The results are shown in Table 2. Compounds **15a** and **15c** were able to potently inhibit different drug-resistant strains of the HIV-1 virus. The activity of **15a** was better than ddC against the L100I, K103N, V106A and Y181C strains of virus. Similarly, compound **15c** also displayed better activity than ddC against the L100I virus strain.

In summary, all four isomers of novel 4′-*C*-ethynyl dioxolane nucleosides **15a**–**d** have been successfully synthesized (and characterized) via a divergent strategy from the same starting material, (*Z*)-but-2-ene-1,4-diol (**1**). Compounds **15a** and **15c** displayed potent *in vitro* activities against HIV-1 and HIV-2 viruses with no cytotoxic effects. Compounds **15a** and **15c** also displayed potent activities against different drug-resistant strains of the HIV-1 virus. These results warrant further study on the mode of action and the derivatization of these analogues to search for more potent anti-HIV agents.

TLC was performed on Merck silica gel 60 F254 plates. Column chromatography was conducted using silica gel 60 N (Kanto, 100e210 mm) or silica gel 60 (Kanto, 40e50 mm). All melting points were determined using a Yamato melting point apparatus (model MP-J3). The NMR spectra were recorded on a JEOL JNM-ECA-500 spectrometer. The chemical shifts (δ) are reported in parts per million relative to TMS (0.0 ppm) as the internal standard, and the signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). The values of the coupling constants (*J*) are given in hertz. The HRMS spectra were obtained using JEOL JMS-HX110, JEOL JMS-700TZ, and JEOL AccuTOF LC-plus systems with electrospray ionization (ESI) operating in positive modes.

Viruses and Cell Lines

The virus strains used in these anti-HIV tests are as follows: (1) human immunodeficiency virus type 1 (HIV-1), strain IIIb and the type 2 (HIV-2), strain ROD were kindly provided by the National Institute for Biological Standards and Control (NIBSC), U.K., as part of the MRC AIDS Reagents Program. (2) Drug-resistant strains of HIV-1 having mutation(s) on the reverse transcriptase gene were obtained from the Centralized Facility for AIDS Reagents, NIBSC according to the EU program EVA (Framework V), U.K. Medical Research Council.

All of the HIV strains were amplified in MT-4 (HTLV-1-infected human T lymphocytes) cells, which were kindly provided by N. Yamamoto of the Tokyo Medical and Dental University, Japan, and grown in RPMI 1640 medium with 10% FBS and 4 μg/mL gentamycin. The aforementioned 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.

Standard Compounds

The following standard compounds: 2′,3′-dideoxycytidine (ddC) (Sigma), 3TC (Sigma), d4T (Sigma) and azidothymidine (AZT) (Sigma) were used as references in the anti-HIV tests. All of the compounds were dissolved in 100% dimethyl sulfoxide (DMSO) at a stock concentration of 20 mg/mL.

Antiviral Assays

The virus-induced-CPE inhibition assay[10] was used to measure the anti-HIV activities. Log-phase MT-4 cells were plated and infected with the virus at a multiplicity of infection of 20~100 $CCID_{50}$ (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 that contained 100 μL of twofold-concentrated test samples. After 5 d of incubation at 37 °C, the cells were observed microscopically, and cell viability was quantified using the MTT assay, which is based on the mitochondrial reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.[11] 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.

(*Z*)-1,4-Bis(benzyloxy)but-2-ene (2)

To a mixture of 55% NaH (13.0 g, 298 mmol) and DMF (250 mL) was slowly added a solution of **1** (10.0 g, 114 mmol) in DMF (50 mL) at 0 °C. After stirring the mixture for 1 h at r.t., benzyl bromide (35.3 mL, 398 mmol) was added dropwise. The resulting mixture was stirred for 4 h at r.t. The reaction was quenched by the addition of sat. aq NH_4Cl solution, followed by extraction with EtOAc. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated to afford crude compound **2**. The crude was purified by silica gel column chromatography (hexane–EtOAc, 8:1) to give **2** (28.4 g, 99%) as a colorless oil.

^1H NMR (500 MHz, $CDCl_3$): δ = 4.15–4.16 (d, *J* = 2.5 Hz, 4 H), 4.58 (s, 4 H), 5.89 (br s, 2 H), 7.36–7.42 (m, 10 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 66.0, 72.3, 127.8, 127.9, 128.6, 129.7, 138.6.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{21}O_2$: 269.1542; found: 269.1536.

meso-1,4-Bis(benzyloxy)butane-2,3-diol (3)

To a solution of **2** (25.0 g, 93 mmol) in acetone (200 mL) and H_2O (200 mL), NMO (12 g, 102 mmol) and a 0.02 M solution of OsO_4 in *t*-BuOH (5 mL) were added at r.t. The mixture was stirred at r.t. for 12–14 h. The work-up was performed by diluting the mixture with EtOAc and washing with sat. aq $Na_2S_2O_4$ solution, H_2O, and brine, respectively. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (hexane–EtOAc, 1:1) to give **3** (21.3 g, 76%) as a white solid; mp 58–59 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.69–2.71 (m, 2 H), 3.60–3.67 (m, 4 H), 3.81–3.82 (br s, 2 H), 4.53 (s, 4 H), 7.24–7.35 (m, 10 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 71.2, 71.6, 73.5, 127.8, 128.0, 128.5, 137.9.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{18}H_{22}O_4Na$: 325.1416; found: 325.1417.

1-(Benzyloxy)-4-(trimethylsilyl)but-3-yn-2-ol (5)

To a solution of **3** (12 g, 39.6 mmol) in MeOH (90 mL), $NaIO_4$ (12.76 g, 59.52 mmol) was added at 0 °C. The resulting mixture was stirred for 8 h at r.t., followed by removal of precipitates by filtration. Most of the solvent was removed using a rotary evaporator and the residual oil (**4**) (9.62 g, 81%) was used as such for further transformation. In another round-bottom flask, a solution of trimethylsilylacetylene (8.4 mL, 59.35 mmol) in THF (50 mL) was stirred at –78 °C. To this solution was added *n*-BuLi (37.5 mL, 2.5 M solution in hexanes, 87.88 mmol) slowly, and the resulting mixture was stirred at –78 °C for 30 min. To this solution was added compound **4** (9 g, 59.92 mmol) in THF (30 mL) dropwise, and the resulting mixture was further stirred for 5 min at –78 °C and then at 0 °C for 2 h. Sat. NH_4Cl (50 mL) was added to the mixture at 0 °C, followed by extraction with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (100 mL) and dried over $MgSO_4$. The solvent was removed by evaporation and the resulting residue was purified by column chromatography (EtOAc–hexane, 1:3) to afford racemic propargylic alcohol (±)-**5** as a pale yellow oil (11.5 g, 77% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.00 (9 H, s), 3.35–3.44 (m, 3 H), 4.36–4.40 (m, 3 H), 7.09–7.15 (m, 5 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 0.0, 62.1, 73.4, 73.8, 90.2, 104.1, 127.9, 128.0, 128.4, 128.6, 137.9.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{14}H_{20}O_2SiNa$: 271.1130; found: 271.1125.

[(2*S*,4*S*)-2-(Benzyloxymethyl)-2-ethynyl-1,3-dioxolan-4-yl]methanol (8a) and [(2*R*,4*S*)-2-(Benzyloxymethyl)-2-ethynyl-1,3-dioxolan-4-yl]methanol (8b)

In a round-bottom flask containing **5** (9.4 g, 37.8 mmol) and CH_2Cl_2 (142 mL) was added 5% aq $NaHCO_3$ solution (75.2 mL). After stirring the mixture for 10 min, TEMPO (58.28 mg, 0.372 mmol), KI (628 mg, 3.78 mmol), and NaOCl (65 mL, 5% solution) were added to the biphasic mixture. The mixture was further stirred for 1.5 h and then extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and evaporated to yield **6** as a light yellow oil (9.02 g, 97%), which was immediately used for further transformation.

In a two-neck round-bottom flask containing a solution of compound **11** (7 g, 21.21 mmol) and dry benzene (120 mL) was added freshly prepared compound **6** (4.45 g, 18.06 mmol) and PTSA (343.2 mg, 1.80 mmol). The mixture was heated at reflux temperature (90 °C) overnight with continuous removal of in situ formed H_2O using a Dean–Stark apparatus. The reaction was worked up by extracting the mixture with EtOAc. The organic layer was washed with H_2O and dried over $MgSO_4$, the solvent evaporated and the residue was purified through a silica column to give crude compound **7** (4 g, 40%).

Crude compound **7** (3.5 g, 9.65 mmol) was dissolved in dry THF (100 mL) and stirred for 5 min at 0 °C. To this solution was added TBAF (14.45 mL, 1 M solution in THF, 14.45 mmol). The mixture was stirred for 1 h at 0 °C and worked up by extraction with EtOAc. The organic layer was washed with H_2O, dried over $MgSO_4$ and evaporated to give a crude mixture of diastereomers **8a** and **8b** which were separated by column chromatography (EtOAc–hexane, 3:1).

Compound 8a

Compound **8a** (0.60 g, 39%) was obtained as a pale yellow solid; mp 36–37 °C.

Purity = 99% [determined by HPLC on a 5 µM Fortis C18 column (150 × 4.6 mm), MeCN–H_2O, 20:80; flow rate = 0.6 mL/min, λ = 254 nm]; t_R = 12.07 min.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.60 (s, 1 H), 3.32 (br s, 1 H), 3.48 (dd, *J* = 15, 5 Hz, 1 H), 3.68–3.76 (m, 3 H), 4.01–4.04 (m, 1 H), 4.12–4.15 (m, 1 H), 4.35–4.37 (m, 1 H), 4.60–4.67 (m, 2 H), 7.31–7.34 (m, 5 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 63.1, 66.8, 72.5, 73.7, 74.0, 77.0, 80.0, 102.0, 126.4, 127.85, 127.91, 128.3, 128.5, 137.2.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{14}H_{16}O_4Na$: 271.0946; found: 271.0949.

Compound 8b

Compound **8b** (0.34 g, 22%) was obtained as a pale yellow solid; mp 34–35 °C.

Purity = 99% [determined by HPLC on a 5 µM Fortis C18 column (150 × 4.6 mm), MeCN–H_2O, 20:80; flow rate = 0.6 mL/min, λ = 254 nm]; t_R = 11.68 min.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.47 (br s, 1 H), 2.62 (s, 1 H), 3.67–3.80 (m, 4 H), 3.99–4.02 (m, 1 H), 4.17–4.20 (m, 1 H), 4.26–4.28 (m, 1 H), 4.68 (s, 2 H), 7.33–7.34 (m, 5 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 62.2, 67.3, 73.4, 73.92, 73.93, 78.2, 81.0, 102.2, 127.7, 128.4, 137.8.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{14}H_{16}O_4Na$: 271.0946; found: 271.0941.

(*R*)-3-(*tert*-Butyldiphenylsilyloxy)propane-1,2-diol (11)[12]

In a round-bottom flask containing a solution of compound **9** (24 g, 181.5 mmol) and dry CH_2Cl_2 (150 mL) was added Et_3N (30.39 mL, 217.8 mmol) and DMAP (1.10 g, 9 mmol). The mixture was stirred at 0 °C for 10 min and then TBDPSCl (50 g, 181.5 mmol) was added to the mixture dropwise. The mixture was slowly allowed to warm to r.t. and was further stirred for 24 h. The mixture was worked up by extraction with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, evaporated under vacuum and purified through a silica column to afford compound **10** (51.81 g, 77%).

Compound **10** (10 g, 26.98 mmol) was added to a round-bottom flask containing 20% aq AcOH (50 mL) and the mixture was heated at reflux temperature (90 °C) overnight. Excess AcOH was evaporated under vacuum and the residue was purified by column chromatography (EtOAc–hexane, 1:9) to afford product **11** as a white solid (7.36 g, 76%); mp 58–60 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.10 (s, 9 H), 2.23 (s, 1 H), 2.72 (s, 1 H), 3.60–3.79 (m, 5 H), 7.37–7.56 (m, 6 H), 7.64–7.63 (m, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 19.2, 26.9, 63.9, 65.2, 71.9, 127.9, 130.0, 132.9, 135.5.

(*R*)-1-{2-[(Benzyloxy)methyl]-2-ethynyl-1,3-dioxolan-4-yl}-acetate (13a)

To a two-neck round-bottom flask were added compound **8a** (800 mg, 3.22 mmol), PDC (18.18 g, 48.33 mmol) and DMF (50 mL). The mixture was stirred at r.t. for 24 h and worked up by washing with H_2O (40 mL) and extraction with Et_2O (4 × 40 mL). The organic layer was dried over $MgSO_4$ and evaporated to afford **12a** (622.3 mg, 74%). Compound **12a** was used directly for the next transformation without any purification.

Compound **12a** (300 mg, 1.14 mmol) was taken up in a two-neck round-bottom flask containing dry THF (12 mL). To this mixture was added $Pb(OAc)_4$ (760 mg, 1.71 mmol) and pyridine (0.15 mL, 1.86 mmol) under inert conditions. The mixture was stirred for 40 min under nitrogen and worked up by adding EtOAc (50 mL) to the mixture. The mixture was filtered and purified to give acetate **13a** (184.3 mg, 58%) as a pale yellow liquid (~1:1 mixture of diastereomers).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.96 (s, 1.5 H), 2.11 (s, 1.5 H), 2.60 (s, 0.5 H), 2.63 (s, 0.5 H), 3.70–3.76 (m, 2 H), 4.15–4.31 (m, 2 H), 4.67 (s, 1 H), 4.71 (s, 1 H), 6.39–6.41 (br s, 1 H), 7.25–7.37 (m, 5 H).

^{13}C NMR (125 MHz, MeOD): δ = 20.9, 21.0, 71.0, 71.3, 73.0, 73.1, 73.8, 74.0, 74.2, 79.1, 79.5, 94.2, 94.9, 103.4, 104.2, 127.7, 128.3, 128.4, 137.7, 137.8, 170.0, 170.1.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{15}H_{16}O_5Na$: 299.0895; found: 299.0893.

(*S*)-1-{2-[(Benzyloxy)methyl]-2-ethynyl-1,3-dioxolan-4-yl}-acetate (13b)

Compound **13b** (189 mg; 60%) was obtained as a pale yellow liquid using a similar strategy to that described above starting from compound **8b** (~1:1 mixture of diastereomers).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.99 (s, 1.5 H), 2.11 (s, 1.5 H), 2.60 (s, 0.5 H), 2.63 (s, 0.5 H), 3.71–3.77 (m, 2 H), 4.16–4.32 (m, 2 H), 4.67 (s, 1 H), 4.71 (s, 1 H), 6.40 (br s, 1 H), 7.27–7.37 (m, 5 H).

^{13}C NMR (125 MHz, MeOD): δ = 20.9, 21.0, 71.0, 71.4, 73.0, 73.2, 73.9, 74.1, 74.2, 79.1, 79.5, 94.2, 94.9, 103.5, 104.3, 127.7, 127.8, 128.3, 128.4, 137.7, 137.8, 170.0, 170.2.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{15}H_{16}O_5Na$: 299.0895; found: 299.0892.

(*R*)-1-{2-[(Benzyloxy)methyl]-2-ethynyl-1,3-dioxolan-4-yl}-thymine (14a)

To a perfectly dry round-bottom flask were added dry CH_2Cl_2 (20 mL) and thymine (102.2 mg, 0.81 mmol). After 2 min of stirring, TBDMSOTf (0.37 mL, 1.61 mmol) was added slowly to the mixture which was allowed to stir for 2 min. Next, 2,4,6-collidine (0.21 mL, 1.62 mmol) was added and the mixture was allowed to stir for 30 min at 40 °C. To this clear solution was added a solution of compound **13a** (148 mg, 0.53 mmol) and dry CH_2Cl_2 (10 mL) slowly, followed by TMSI (0.21 mL). The mixture was allowed to stir for 3–4 h and then quenched with sat. $Na_2S_2O_3$ solution, followed by extraction with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$ and evaporated to give crude **14a** as a diastereomeric mixture. The crude product was purified by column chromatography (EtOAc–hexane, 3:7) to afford pure **14a** (102 mg, 38%) as a white solid (~2:1 mixture of diastereomers).

^{1}H NMR (500 MHz, $CDCl_3$): [mixture of diastereomers (~2:1 ratio)]: δ = 1.56 (s , 1 H), 1.94 (s, 2 H), 2.62 (s, 0.35 H), 2.77 (s, 0.65 H), 3.74 (s, 1.30 H), 3.93 (s, 0.70 H), 4.08-4.18 (m, 2 H), 4.49–4.52 (m, 0.35 H), 4.57–4.60 (m, 0.65 H), 4.63–4.69 (s, 2 H), 6.32–6.34 (m, 0.65 H), 6.53–6.54 (d, *J* = 5 Hz, 0.35 H), 7.30–7.37 (m, 4 H), 7.59–7.60 (d, *J* = 7 Hz, 1 H), 9.10 (s, 0.35 H), 9.19 (s, 0.65 H).

^{13}C NMR (125 MHz, MeOD): δ = 12.0, 12.6, 70.4, 71.1, 71.6, 73.5, 74.1, 74.4, 74.8, 75.9, 78.1, 78.9, 80.3, 83.2, 103.4, 104.3, 111.3, 111.7, 127.7, 127.9, 128.1, 128.5, 128.6, 135.3, 135.7, 137.0, 137.3, 150.7, 150.8, 163.8, 163.9.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{18}H_{18}N_2O_5Na$: 365.1113; found: 365.1123.

(*S*)-1-{2-[(Benzyloxy)methyl]-2-ethynyl-1,3-dioxolan-4-yl}-thymine (14b)

Compound **14b** (115 mg, 43%) was obtained as a white solid (~2:1 mixture of diastereomers) using the same sequence of steps as described above starting from compound **13b** (148 mg scale).

^{1}H NMR (500 MHz, $CDCl_3$): [mixture of diastereomers (~2:1 ratio)]: δ = 1.56 (s, 1 H), 1.95 (s, 2 H), 2.62 (s, 0.3 5H), 2.77 (s, 0.65 H), 3.74 (s, 1.30 H), 3.94 (s, 0.70 H), 4.10–4.18 (m, 2 H), 4.49-4.53 (m, 0.35 H), 4.57–4.60 (m, 0.65 H), 4.63–4.69 (s, 2 H), 6.32–6.34 (m, 0.65 H), 6.52–6.54 (dd, *J* = 5.5, 1.5 Hz, 0.35 H), 7.29–7.38 (m, 4 H), 7.58–7.60 (m, 1 H), 8.68 (s, 0.35 H), 8.83 (s, 0.65 H).

^{13}C NMR (125 MHz, MeOD): δ = 12.0, 12.6, 14.2, 21.0, 60.3, 70.4, 71.6, 73.5, 74.1, 74.4, 75.9, 83.2, 103.4, 104.3, 111.3, 127.7, 127.9, 128.0, 128.1, 128.5, 135.3, 137.3, 150.7, 163.8.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{18}H_{18}N_2O_5Na$: 365.1113; found: 365.1121.

Compounds 15a–d

To a perfectly dry two neck round-bottom flask was added compound **14a** (200 mg, 0.554 mmol) dissolved in dry CH_2Cl_2 (8 mL). The mixture was allowed to stir for 5 min at 0 °C, followed by the addition of anisole (0.23 mL, 2.33 mmol) and $AlCl_3$ (233.6 mg, 1.75 mmol) at the same temperature. The mixture was allowed to stir overnight at 0 °C. The reaction was worked up by adding H_2O (20 mL) to the mixture, followed by the addition of 1 M HCl at 0 °C until the pH was 2–3. The mixture was further stirred at 0 °C for 5 min and then extracted with EtOAc. The organic layer was washed with H_2O, dried over $MgSO_4$ and evaporated to give the crude product as a diastereomeric mixture of **15a** and **15b**. Both diastereomers were separated/purified by column chromatography (MeOH and CH_2Cl_2). Compounds **15c** and **15d** were synthesized by following the same sequence of steps starting from compound **14b** (on 200 mg scale).

(–)-(2*R*,4*R*)-1-[2-Ethynyl-2-(hydroxymethyl)-1,3-dioxolan-4-yl]-thymine (15a)

Yield: 53 mg (36%); white solid; mp 247–248 °C; R_f = 0.57 (EtOAc–hexane, 20:80); $[\alpha]_D^{20}$ –16.95 (*c* 1.0, MeOD); purity >99% [determined by HPLC on a 5 µM Fortis C18 column (150 × 4.6 mm), MeCN–H_2O, 20:80; flow rate = 0.6 mL/min, λ = 254 nm]; t_R = 6.12 min.

^{1}H NMR (500 MHz, MeOD): δ = 1.83 (s, 3 H), 3.17 (s, 1 H), 3.88 (dd, *J* = 13.5, 7.5 Hz, 2 H), 4.27 (d, *J* = 10.5 Hz, 1 H), 4.42–4.45 (m, 1 H), 6.42 (d, *J* = 6 Hz, 1 H), 7.79 (s, 1 H).

^{13}C NMR (125 MHz, MeOD): δ = 10.9, 63.7, 69.4, 74.8, 78.2, 80.9, 103.9, 110.5, 136.8, 151.2, 164.9.

HRMS (Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{11}H_{12}N_2O_5Na$: 275.0644; found: 275.0646.

(+)-(2*R*,4*S*)-1-[2-Ethynyl-2-(hydroxymethyl)-1,3-dioxolan-4-yl]-thymine (15b)

Yield: 30.3 mg (21%); white solid; mp 205–206 °C; R_f = 0.42 (EtOAc–hexane, 20:80); $[\alpha]_D^{20}$ +9.67 (*c* 1.0, MeOD); purity >99% [determined by HPLC on a 5 μM Fortis C18 column (150 × 4.6 mm), MeCN–H_2O, 20:80; flow rate = 0.6 mL/min, λ = 254 nm]; t_R = 9.93 min.

^{1}H NMR (500 MHz, MeOD): δ = 1.78 (s, 3 H), 3.19 (s, 1 H), 3.60 (dd, *J* = 12.5, 8 Hz, 2 H), 4.09 (dd, *J* = 9.5, 4 Hz, 1 H), 4.41–4.45 (m, 1 H), 6.22 (t, *J* = 5 Hz, 1 H), 7.60 (s, 1 H).

^{13}C NMR (125 MHz, MeOD): δ = 11.2, 65.8, 70.7, 76.4, 79.0, 83.1, 104.7, 110.3, 136.1, 151.1, 164.8.

HRMS (Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{11}H_{12}N_2O_5Na$: 275.0644; found: 275.0647.

(+)-(2*S*,4*S*)-1-[2-Ethynyl-2-(hydroxymethyl)-1,3-dioxolan-4-yl]-thymine (15c)

Yield: 50 mg (34%); white solid; mp 247–248 °C; R_f = 0.57 (EtOAc–hexane, 20:80); $[\alpha]_D^{20}$ +16.21 (*c* 1.0, MeOD); purity >99% (determined by HPLC on a 5 μM Fortis C18 column (150 × 4.6 mm), MeCN–H_2O, 20:80; flow rate = 0.6 mL/min, λ = 254 nm]; t_R = 5.89 min.

^{1}H NMR (500 MHz, MeOD): δ = 1.74 (s, 3 H), 3.10 (s, 1 H), 3.74 (dd, *J* = 13.0, 8.5 Hz, 2 H), 4.18 (d, *J* = 10 Hz, 1 H), 4.32–4.35 (m, 1 H), 6.33 (d, *J* = 6.0 Hz, 1 H), 7.70 (s, 1 H).

^{13}C NMR (125 MHz, MeOD): δ = 10.7, 63.3, 69.1, 74.6, 77.9, 80.5, 103.6, 110.2, 136.5, 150.9, 164.6.

HRMS (Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{11}H_{12}N_2O_5Na$: 275.0644; found: 275.0647.

(–)-(2*S*,4*R*)-1-[2-Ethynyl-2-(hydroxymethyl)-1,3-dioxolan-4-yl]-thymine (15d)

Yield: 26.6 mg (18%); white solid; mp 205–206 °C; R_f = 0.42 (EtOAc–hexane, 20:80); $[\alpha]_D^{20}$ –9.33 (*c* 1.0, MeOD); purity >99% [determined by HPLC on a 5 μM Fortis C18 column (150 × 4.6 mm), MeCN–H_2O, 20:80; flow rate = 0.6 mL/min, λ = 254 nm]; t_R = 9.87 min.

^{1}H NMR (500 MHz, MeOD): δ = 1.77 (s, 3 H), 3.18 (s, 1 H), 3.59 (dd, *J* = 13.0, 7.5 Hz, 2 H), 4.07 (d, *J* = 9.5 Hz, 1 H), 4.42 (t, *J* = 8 Hz, 1 H), 6.21 (s, 1 H), 7.59 (s, 1 H).

^{13}C NMR (125 MHz, MeOD): δ = 11.2, 65.8, 70.7, 76.4, 79.0, 83.1, 104.7, 110.3, 136.0, 151.1, 164.9.

HRMS (Q-TOF): *m*/*z* [M + Na]$^+$ calcd for $C_{11}H_{12}N_2O_5Na$: 275.0644; found: 275.0645.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1561637.

References

(1) (a) Jung, M.; Lee, S.; Kim, H. *Curr. Med. Chem.* **2000**, *7*, 649. (b) Muenchhoff, M.; Prendergast, A. J.; Goulder, P. J. R. *Front. Immunol.* **2014**, *5*, 1. (c) De Clerq, E. *J. Med. Chem.* **2005**, *48*, 1297.
(2) WHO report **2014** on HIV, see: http://www.who.int/gho/hiv/en/.
(3) For details, see: https://aidsinfo.nih.gov/education-materials/fact-sheets/21/58/fda-approved-hiv-medicines.
(4) Zhan, P.; Pannecouque, C.; De Clercq, E.; Liu, X. *J. Med. Chem.* **2016**, *59*, 2849.
(5) Chung, J.; DiGiusto, D. L.; Rossi, J. J. *Expert Opin. Biol. Ther.* **2013**, *13*, 437.
(6) Huang, B.; Kang, D.; Zhan, P.; Liu, X. *Expert Opin. Drug Discovery* **2015**, *10*, 1271.
(7) (a) Sari, O.; Roy, V.; Balzarini, J.; Snoeck, R.; Andrei, G.; Agrofoglio, L. A. *Eur. J. Med. Chem.* **2012**, *53*, 220. (b) Ohrui, H. *Proc. Jpn. Acad., Ser. B* **2011**, *87*, 53. (c) Haraguchi, K.; Shimada, H.; Kimura, K.; Akutsu, G.; Tanaka, H.; Abe, H.; Hamasaki, T.; Baba, M.; Gullen, E. A.; Dutschman, G. E.; Cheng, Y.-C.; Balzarin, J. *ACS Med. Chem. Lett.* **2011**, *2*, 692. (d) Wanga, Q.; Li, Y.; Song, C.; Qian, K.; Chen, C.-H.; Lee, K.-H.; Chang, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4053. (e) Haraguchi, K.; Takeda, S.; Tanaka, H.; Nitanda, T.; Baba, M.; Dutschman, G. E.; Cheng, Y.-C. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3775. (f) Ohrui, H.; Kohgo, S.; Kitano, K.; Sakata, S.; Kodama, E.; Yoshimura, K.; Matsuoka, M.; Shigeta, S.; Mitsuya, H. *J. Med. Chem.* **2000**, *43*, 4516.
(8) (a) Bondada, L.; Detorio, M.; Bassit, L.; Tao, S.; Montero, C. M.; Singletary, T. M.; Zhang, H.; Zhou, L.; Cho, J.-H.; Coats, S. J.; Schinazi, R. F. *ACS Med. Chem. Lett.* **2013**, *4*, 747. (b) Kim, E.; Liu, L. J.; Lee, W.; Hong, J. H. *Nucleosides, Nucleotides Nucleic Acids* **2012**, *31*, 85. (c) Bera, S.; Malik, L.; Bhat, B.; Carroll, S. S.; MacCoss, M.; Olsen, D. B.; Tomassini, J. E.; Eldrup, A. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4455. (d) Luo, M.-Z.; Liu, M.-C.; Mozdziesz, D. E.; Lin, T.-S.; Dutschman, G. E.; Gullen, E. A.; Cheng, Y.-C.; Sartorelli, A. C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2145. (e) Kim, H. O.; Ahn, S. K.; Alves, A. J.; Beach, J. W.; Jeong, L. S.; Choi, B. G.; Roey, P. V.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **1992**, *35*, 1987.
(9) (a) Caso, M. F.; D'Alonzo, D.; D'Errico, S.; Palumbo, G.; Guaragna, A. *Org. Lett.* **2015**, *17*, 2626. (b) Marcuccio, S. M.; Epa, R.; White, J. M.; Deadman, J. J. *Org. Process Res. Dev.* **2011**, *15*, 763. (c) Nguyen-Ba, N.; Lee, N.; Chan, L.; Zacharie, B. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2223. (d) Romeo, G.; Chiacchio, U.; Corsaro, A.; Merino, P. *Chem. Rev.* **2010**, *110*, 3337.
(10) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
(11) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijin, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1998**, *121*, 309.
(12) (a) Zhang, Q.; Qu, Y.; Chen, W.; Wang, Y.; Wu, Q. *Lett. Org. Chem.* **2006**, *3*, 271. (b) Leftheris, K.; Goodman, M. *J. Med. Chem* **1990**, *33*, 216.