Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

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Satoru KOHGO[a], Hiroaki MITSUYA[bc] & Hiroshi OHRUI[a]

[a] Division of Life Science, Graduate School of Agricultural Science, Tohoku University

[b] Department of Immunopathophysiology and Internal Medicine II, Kumamoto University School of Medicine

[c] Experimental Retrovirology Section, Medicine Branch, Division of Clinical Sciences, National Cancer Institute

Published online: 22 May 2014.

To cite this article: Satoru KOHGO, Hiroaki MITSUYA & Hiroshi OHRUI (2001) Synthesis of the L-Enantiomer of 4'-C-Ethynyl-2'-deoxycytidine, Bioscience, Biotechnology, and Biochemistry, 65:8, 1879-1882, DOI: 10.1271/bbb.65.1879

To link to this article: http://dx.doi.org/10.1271/bbb.65.1879

Biosci. Biotechnol. Biochem., **65** (8), 1879-1882, 2001

Note

Synthesis of the L-Enantiomer of 4′-*C*-Ethynyl-2′-deoxycytidine

Satoru KOHGO,[1] Hiroaki MITSUYA,[2,3] and Hiroshi OHRUI[1,†]

[1]*Division of Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan*

[2]*Department of Immunopathophysiology and Internal Medicine II, Kumamoto University School of Medicine, Honjo, Kumamoto 860-8556, Japan*

[3]*Experimental Retrovirology Section, Medicine Branch, Division of Clinical Sciences, National Cancer Institute, Bethesda, Maryland 20892, USA*

Received January 9, 2001; Accepted March 2, 2001

The L-enantiomer of 4′-*C*-ethynyl-2′-deoxycytidine (2) was synthesized, but did not show any activity against HIV-1 up to 100 μM.

Key words: 4′-*C*-ethynyl-2′-deoxycytidine; L-configuration nucleosides; anti-HIV activity

A number of nucleosides with unusual L-configuration sugar that exhibited antiviral activity and no cytotoxicity[1] have recently been noticed, and these L-nucleosides are expected to pave the way for the development of more potent and less toxic antiviral nucleoside drugs.

We have synthesized 4′-substituted several nucleosides.[2-5] Among these compounds, 4′-*C*-ethynyl-2′ deoxycytidine (**1**)[5] had very potent anti-HIV activity with an EC_{50} value of 0.0048 μM; however, this compound also showed potent cytotoxicity (IC_{50}, 0.92 μM). These results prompted us to synthesize the L-enantiomer of 4′-*C*-ethynyl-2′-deoxycytidine (**2**), which is reported here.

4-*C*-Hydroxymethyl-D-*xylo*-pentofuranose (**3**)[2] which was obtained from D-glucose, was treated with sodium hydride and *p*-methoxybenzyl chloride to afford an inseparable mixture of **4a** and **4b** (**4a**:**4b** = ca. 3:7). This mixture **4** was converted to **8** by Swern oxidation of the alcohol and conversion of the resulting aldehyde to a triethylsilylethynyl group.[6] Treatment of mixture **8** with DDQ[7] gave D-*xylo* 3,5-*O*-*p*-methoxybenzylidene acetal **9a** and debenzylated L-*arabino* derivative **9b**. **9a** and **9b** were separated by silica-gel column chromatography. L-Arabinose derivative **9b** was benzoylated to give **10** and methanolysis of **10** gave methyl glycoside **11**. Thionocarbonylation of the hydroxyl group of **11** and subsequent radical reduction[8] with tri-*n*-butyltin hydride gave 2-deoxy-L-ribose derivative **13**, which was then converted to acetate **15**. Acetate **15** was condensed with N^4-acetylcytosine[9] to give an L-cytosine nucleoside as anomeric mixture **16** ($\alpha:\beta = 1.1:0.9$). After separating the anomers by silica-gel column chromatography, less-polar fraction **17** was obtained and then deprotected under alkaline conditions to give **2**. The stereochemical assignment of **2** was achieved by comparing both the ^{1}H-NMR spectrum and magnitude of the optical rotation of **2** with these of D-enantiomer **1**.[5]

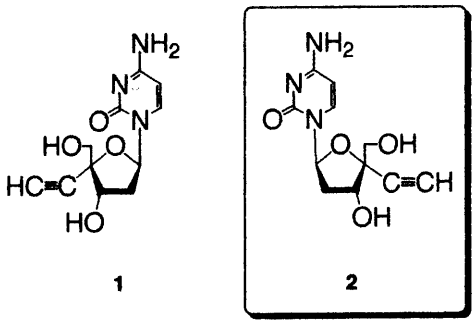


Fig. 1. Structures of 4′-*C*-Ethynyl-2′-deoxycytidine **1** and Its Enantiomer **2**.

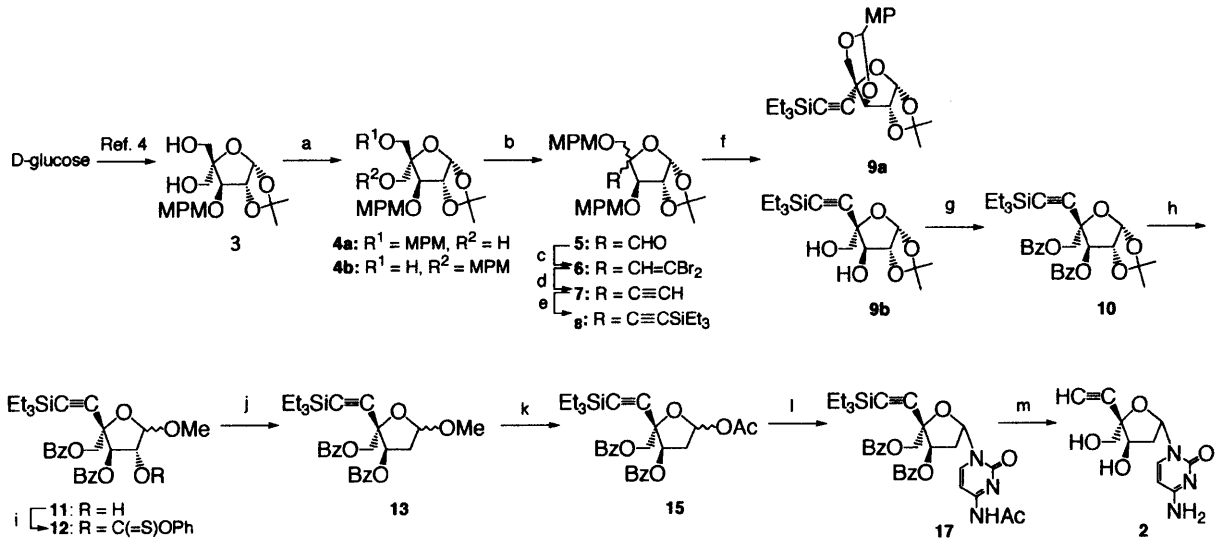
The L-enantiomer of 4′-*C*-ethynylcytidine (**2**) was evaluated for its anti-HIV activity toward MT-2 cells by an MTT assay. However, **2** was inactive against HIV-1 at concentrations up to 100 μM.

Experimental

1,2-O-Isopropylidene-4-C-triethylsilylethynyl-β-L-arabino-pentofuranose (9b). To a solution of **3** (3.00 g, 8.81 mmol) in DMF (30 ml) was added sodium hydride (60% in oil, 0.39 g, 9.8 mmol) at —20°C and the mixture was stirred for 30 min. *p*-Methoxybenzyl chloride (1.31 ml, 9.66 mmol) was added to the solution, and the mixture was stirred at —20°C

† To whom correspondence should be addressed. Tel: +81-22-717-8803; Fax: +81-22-8806; E-mail: ohrui@biochem.tohoku.ac.jp

Abbreviations: HIV, human immunodeficiency virus; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; AIBN, 2,2′-azobis(isobutyronitrile); BSA, *N*,*O*-bis(trimethylsilyl)acetamide; TMSOTf, trimethylsilyl trifluoromethanesulfonate



Scheme 1. Reagents and Conditions.

(a) PMBCl, NaH, DMF, −20°C, 12 h, 49%; (b) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N, −60°C, 30 min; (c) CBr_4, PPh_3, CH_2Cl_2, 0°C, 1 h, 83% from **4**; (d) *n*-BuLi, THF, −78°C, 10 min; (e) *n*-BuLi, THF, −78°C, 5 min, then Et_3SiCl, −78°C, 10 min, 88% from **6**; (f) DDQ, MeOH, CH_2Cl_2, r.t., overnight, 75%; (g) BzCl, pyridine, r.t., 3 h, 81%; (h) 1% HCl, $HC(OMe)_3$, MeOH, r.t., overnight, 89%; (i) PhOC(=S)Cl, DMAP, MeCN, r.t., 1 h, 88%; (j) *n*-Bu_3SnH, AIBN, toluene, 85°C, 2 h; (k) 70% AcOH containing CF_3CO_2H, r.t., overnight, 70% from **12**; (l) Ac_2O, pyridine, r.t., 5 h, 100%; (m) trimethylsilylated N^4-acetylcytosine, TMSOTf, $Cl(CH_2)_2Cl$, 50°C, overnight; (n) separation by silica-gel column chromatography, 43%; (o) 1N NaOH aq., MeOH, r.t., 3 h, 80%.

for 12 h. The reaction mixture was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was passed through a silica-gel column chromatography (*n*-hexane:AcOEt = 1:1) to give a mixture of **4a** and **4b** (2.00 g, 4.34 mmol, 49.3%). This mixture of **4a** and **4b** (4.86 g, 10.6 mmol) was converted to **8** (4.37 g, 7.68 mmol, 72.5% from **4**) by the method previously described.[4-6] To the solution of **8** (4.37 g, 7.68 mmol) in CH_2Cl_2 (90 ml) were added DDQ (5.23 g, 23.0 mmol) and MeOH (10 ml), and the mixture was stirred for 12 h at room temperature. The reaction mixture was filtered, and the filtrate was washed with 5% $Na_2S_2O_3$. The organic layer was dried over $MgSO_4$ and evaporated. The resulting residue was purified by silica-gel column chromatography (*n*-hexane:AcOEt = 6:1–2:1) to give **9b** (1.88 g, 5.72 mmol, 74.5%). $[\alpha]_D$ +25° (c = 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ for **9b**: 5.96 (1H, d, H-1, J = 3.9), 4.64 (1H, dd, H-2, J = 3.9, 1.5), 4.23 (1H, br.s, H-3), 3.81 (2H, s, H-5), 2.37, 2.10 (each 1H, br.s, 3-OH, 5-OH), 1.54, 1.35 (each 3H, s, acetonide), 1.00 (9H, t, Et-Si-, J = 8.1), 0.64 (6H, q, Et-Si-, J = 8.1). EIMS m/z: 328 (M^+). HRMS m/z: 328.1708 (M^+); calcd. for $C_{16}H_{28}O_5Si$: 328.1706.

9a could not be completely separated from *p*-methoxybenzaldehyde which was produced by DDQ oxidation of *p*-methoxybenzyl ether. Thus, the precise isolated yield of **9a** could not be determined. The yield of **9a** determined by a ^{1}H-NMR analysis of the mixture of **9a** and *p*-methoxybenzaldehyde was about 22%. ^{1}H-NMR and mass analyses of **9a** were performed by using pure fraction which had been obtained by repurifying crude **9a**. ^{1}H-NMR ($CDCl_3$) δ for **9a**: 7.35, 6.88 (each 2H, d, aromatic, J = 8.70), 6.08 (1H, d, H-1, J = 3.90), 5.47 (1H, s, MeOPh-C*H*-), 4.66 (1H, d, H-2, J = 3.90), 4.60 (1H, s, H-3), 4.40, 4.10 (each 1H, d, H-5, J = 12.9), 3.80 (3H, s, -OMe), 1.67, 1.31 (each 3H, s, acetonide), 0.99 (9H, t, Et-Si-, J = 8.10), 0.61 (6H, q, Et-Si-, J = 8.10). EIMS m/z: 446 (M^+). HRMS m/z: 446.2126 (M^+); calcd. for $C_{24}H_{34}O_6Si$: 446.2125.

3,5-Di-O-benzoyl-1,2-O-isopropylidene-4-C-triethylsilylethynyl-β-L-arabino-pentofuranose (10). To a solution of **9b** (1.88 g, 5.72 mmol) in pyridine (40 ml) was added BzCl (1.46 ml, 12.6 mmol), and the mixture stirred for 3 h at room temperature. The reaction mixture was evaporated, and the resulting residue was partitioned between AcOEt and sat. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (*n*-hexane:AcOEt = 2:1) to give **10** (2.50 g, 4.66 mmol, 81.5%). $[\alpha]_D$ −7.5° (c = 1.1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.13–7.40 (10H, m, aromatic), 6.12 (1H, H-1, J = 4.00), 5.70 (1H, d, H-3, J = 1.50), 4.87 (1H, dd, H-2, J = 4.00, 1.50), 4.74, 4.63 (each 1H, d, H-5, J = 11.0), 1.61, 1.39 (each 3H, s, acetonide), 0.81 (9H, t, Et-Si-, J = 8.00), 0.40 (6H, q, Et-Si-, J = 8.00). EIMS m/z: 536 (M^+). HRMS m/z: 536.2226 (M^+); calcd. for $C_{30}H_{36}O_7Si$: 536.2230.

1-O-Acetyl-3,5-di-O-benzoyl-2-deoxy-4-C-triethylsilylethynyl-L-ribo-pentofuranose (15). The mixture

of **10** (1.30 g, 2.42 mmol), trimethyl orthoformate (5 ml) and MeOH containing 1% HCl (50 ml) was stirred for 12 h at room temperature. To this reaction mixture was added pyridine (5 ml), and the mixture was evaporated. The resulting residue was partitioned between AcOEt and sat. $NaHCO_3$, and was then dried over $MgSO_4$. The organic layer was evaporated, and the residue was purified by silica-gel column chromatography (*n*-hexane:AcOEt = 3:1) to give **11** (1.10 g, 2.15 mmol, 88.8%).

To a solution of **11** (1.00 g, 1.96 mmol, $\alpha:\beta = 4:3$) in MeCN were added 4-dimethylamiopyridine (1.20 g, 9.82 mmol) and phenyl chlorothionoformate (0.68 ml, 4.9 mmol), and the mixture was stirred for 1 h at room temperature. The reaction mixture was evaporated and the resulting residue was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (*n*-hexane:AcOEt = 5:1) to give **12** (1.11 g, 1.72 mmol, 87.8%). To a solution of **12** in toluene (25 ml) were added tri-*n*-butyltin hydride (0.93 ml, 3.5 mmol) and AIBN (10 mg) at 85°C, and the mixture was stirred for 2 h. The reaction mixture was evaporated, and the resulting residue was passed through a silica-gel column (*n*-hexane:AcOEt = 6:1) to give crude **13**. Crude **13** was dissolved in 70% AcOH (30 ml) and CF_3CO_2H (3 ml), and the solution was stirred for 12 h at room temperature. The reaction mixture was evaporated, and the resulting residue was purified by silica-gel column chromatography (*n*-hexane:AcOEt = 4:1) to give **14** (0.58 g, 1.2 mmol, 70% from **12**). To a solution of **14** (0.50 g, 1.0 mmol) in pyridine (10 ml) was added acetic anhydride (0.49 ml, 5.2 mmol), and the mixture was stirred for 5 h at room temperature. The reaction mixture was evaporated, and the resulting residue was purified by silica-gel column chromatography (*n*-hexane:AcOEt = 3:1) to give **15** (0.53 g, 1.0 mmol, quantitative yield). ^{1}H-NMR ($CDCl_3$) δ for the 1st eluted compound. 8.12–7.39 (10H, m, aromatic), 6.50 (1H, t, H-1, $J = 3.50$), 5.85 (1H, d, H-3, $J = 7.50$), 4.75, 4.55 (each 1H, d, H-5, $J = 12.0$), 2.66 (2H, dd, H-2, $J = 3.50, 7.50$), 1.90 (3H, s, Ac), 0.86 (9H, t, Et-Si-, $J = 8.00$), 0.47 (6H, q, Et-Si-, $J = 8.00$); δ for the 2nd elutied compound: 8.15–7.43 (10H, m, aromatic), 6.48 (1H, dd, H-1, $J = 1.00, 5.00$), 5.74 (1H, dd, H-3, $J = 2.50, 7.50$), 4.61, 4.58 (each 1H, d, H-5, $J = 11.5$), 2.68, 2.42 (each 1H, m, H-2), 2.14 (3H, s, Ac), 0.82 (9H, t, Et-Si-, $J = 8.00$), 0.37 (6H, q, Et-Si-, $J = 8.00$). EiMS m/z: 545 (MNa^+). HRMS m/z: 545.1976 (MNa^+); calcd. for $C_{29}H_{34}O_7SiNa$: 545.1972.

1-(3,5-Di-O-benzoyl-2-deoxy-4-C-triethylsilylethynyl-β-L-ribo-pentofuranosyl)-N⁴-acetylcytosine (17). A mixture of **15** (0.50 g, 0.96 mmol), N^4-acetylcytosine (0.29 g, 1.9 mmol) and BSA (1.42 ml, 5.74 mmol) in 1,2-dichloroethane (25 ml) was stirred for 1 h at 85°C and then cooled to room temperature. To this solution was added TMSOTf (0.23 ml, 1.3 mmol), and the mixture was stirred for 12 h at 50°C. The reaction mixture was partitioned with sat. $NaHCO_3$, the organic layer being dried over $MgSO_4$ and evaporated to give crude **16**. Anomeric mixture **16** was separated by silica-gel column chromatography (*n*-hexane:AcOEt = 1:2) to give β anomer **17** as the 1st-eluted compound (0.25 g, 0.41 mmol, 43%) and the α anomer as the 2nd-eluted compound (0.28 g, 0.45 mmol, 47%). ^{1}H-NMR ($CDCl_3$) δ for β anomer **17**: 8.11–7.27 (12H, m, aromatic), 6.42 (1H, t, H-1′, $J = 6.00$), 5.75 (1H, dd, H-3′, $J = 5.50, 7.50$), 4.84, 4.72 (each 1H, d, H-5′, $J = 12.5$), 3.06, 2.44 (each 1H, m, H-2′), 2.25 (3H, s, Ac), 0.88 (9H, t, Et-Si-, $J = 8.00$), 0.48 (6H, q, Et-Si-, $J = 8.00$). FABMS m/z: 616 (MH^+). HRMS m/z: 616.2473 (MH^+); calcd. for $C_{33}H_{38}N_3O_7Si$: 616.2479. ^{1}H-NMR ($CDCl_3$) δ for the α anomer: 8.55–7.37 (12H, m, aromatic), 6.32 (1H, dd, H-1′, $J = 1.50, 7.00$), 5.90 (1H, dd, H-3′, $J = 2.00, 6.50$), 4.65 (2H, s, H-5′), 3.16, 2.65 (each 1H, m, H-2′), 2.31 (3H, s, Ac), 0.82 (9H, t, Et-Si-, $J = 8.00$), 0.38 (6H, q, Et-Si-, $J = 8.00$). FABMS m/z: 616 (MH^+); HRMS m/z: 616.2474 (MH^+), calcd. for $C_{33}H_{38}N_3O_7Si$: 616.2479.

1-(2-Deoxy-4-C-ethynyl-β-L-ribo-pentofuranosyl) cytosine (2). A solution of **17** (0.23 g, 0.37 mmol), 1 N NaOH (3 ml) and MeOH (27 ml) was stirred for 3 h at room temperature. The reaction mixture was neutralized by additig AcOH and then evaporated. The resulting residue was purified by ODS reverse-phase chromatography to give **2** (0.12 g, 0.48 mmol, 72%). $[\alpha]_D$ —76.0° ($c = 0.265$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 7.76 (1H, d, H-6, $J = 7.50$), 7.18, 7.13 (each 1H, br.s, NH_2), 6.13 (1H, dd, H-1′, $J = 4.75, 7.25$), 5.70 (1H, d, H-5, $J = 7.50$), 5.49 (1H, d, 3′-OH), 5.41 (1H, t, 5′-OH), 4.30 (1H, br.t, H-3′), 3.64, 3.58 (each 1H, m, H-5′), 3.49 (1H, s, ethynyl), 2.24, 2.06 (each 1H, m, H-2′). FABMS m/z: 252 (MH^+). HRMS m/z: 252.0983 (MH^+); calcd for $C_{11}H_{14}N_3O_4$: 252.0984.

Acknowledgment

This work was supported in part by a Sasagawa Scientific Research Grant.

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