Synthesis of novel L-*N*-MCd4T as a potent anti-HIV agent†

Ah-Young Park,[a] Hyung Ryong Moon,*[a] Kyung Ran Kim,[a] Moon Woo Chun[b] and Lak Shin Jeong*[c]

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L-*N*-MCd4T (1) has been synthesized as a potent anti-HIV agent starting from (*R*)-epichlorohydrin using tandem alkylation, chemoselective reduction of ester in the presence of lactone functional group, RCM reaction and Mitsunobu reaction as key steps and was found to be a very potent anti-HIV-1 (EC_{50} = 6.76 μg mL^{-1}) agent without cytotoxicity up to 100 μg mL^{-1}, indicating that the anti-HIV-1 activity found is similar to that of ddI (EC_{50} = 4.95 μg mL^{-1}), which is used clinically for the treatment of AIDS patients.

Human immunodeficiency virus (HIV) is a pathogenic retrovirus and the causative agent of AIDS.[1] In the search for an effective chemotherapeutic agent of HIV infections, initial attempts were focused on the development of 2′,3′-dideoxynucleosides (ddNs).[2] Among them, AZT, ddI, ddC and d4T[3] have been clinically used for the treatment of AIDS patients. Peculiarly, d4T has a double bond at its pseudosugar ring, which renders the pseudosugar ring to be nearly planar and highly rigid. 3TC[4] has been also used as anti-HIV and anti-HBV agents. Because it does not have hydroxyl substituents at both the 2′- and 3′-position, 3TC might be considered as a member of ddNs family. Therefore, ddN analogs still seem to be the most promising candidates for AIDS treatment, although most of them have the property of easy cleavage of their glycosidic bond, compared with normal nucleosides under acidic conditions.

Carbocyclic nucleosides[5] have been synthesized mainly with the purpose of overcoming cleavage of the glycosidic bond, which readily occurs in conventional nucleosides by chemical or enzymatic means.[5a,6] On the other hand, conformationally rigid methanocarba (MC) nucleosides built on a bicyclo[3.1.0]hexane template have a south (S, $_3$E)[7] or north (N, $_2$E)[8] conformation as in normal nucleosides. These rigid MC nucleosides have been synthesized to study the conformational preferences of various enzymes involved in nucleoside and nucleotide metabolism. Recently, D-*N*-MCd4T,[9] bearing a locked North conformation and a 2′,3′-double bond in a single structure has been synthesized and its anti-HIV activity was compared with that of d4T (Fig. 1). Although D-*N*-MCd4T was somewhat less potent than d4T, it showed significant antiviral activity against HIV-1 and HIV-2 with less cytotoxicity and was stable under conditions that would cleave d4T. On the other hand, a number of L-nucleosides such as 3TC,[4] FTC,[10] L-d4FC[11] and L-5-FddC[12] have been also reported to show highly potent anti-HIV activity.

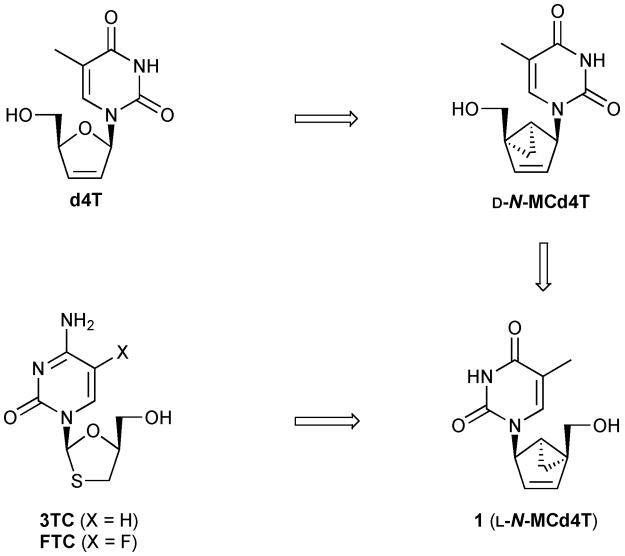

Fig. 1 The rationale for the design of the desired nucleoside, L-*N*-MCd4T (**1**).

As a part of our ongoing efforts to search for novel antiviral agents with better potency and less cytotoxicity, it was of interest to synthesize L-*N*-MCd4T (**1**), an enantiomer of D-*N*-MCd4T as a potent anti-HIV agent on the basis of these findings. Here, we wish to report a new synthetic approach to conformationally locked L-*N*-MCd4T (**1**) using cyclopropanation *via* tandem alkylation, ring-closing metathesis (RCM) and Mitsunobu reaction as the key steps from (*R*)-epichlorohydrin and its anti-HIV activity.

A new strategy for the synthesis of L-*N*-MCd4T (**1**) was highly required because the method used for the synthesis of D-*N*-MCd4T,[9] the enantiomer of L-*N*-MCd4T (**1**) was somewhat lengthy.

First, synthesis of a glycosyl donor, bicyclo[3.1.0]hexenol template (2*S*)-**10** for the synthesis of L-*N*-MCd4T (**1**) is described in Scheme 1. Compound **2** was synthesized in two steps according to the procedure reported by Tsuji and his co-workers.[13,14] Reaction of (*R*)-epichlorohydrin with diethyl malonate and sodium metal gave cyclopropane-fused lactone **2** *via* tandem alkylation followed by lactonization. The lactone moiety of **2** was more susceptible to hydrolysis than the ester, maybe due to the structural constraint caused by a fused cyclopropane ring. Treatment of **2** with 1 equiv. of NaOH in ethanol afforded monocarboxylate sodium salt **2a**, the ester of which was chemoselectively reduced by $NaBH_4$ under reflux and recyclized back to lactone **3** under acidic conditions. Protection of hydroxyl group of **3** with *tert*-butyldiphenylsilyl

[a]*Laboratory of Medicinal Chemistry, College of Pharmacy and Research Institute for Drug Development, Pusan National University, Busan, 609-735, Korea. E-mail: mhr108@pusan.ac.kr; Fax: +82 51 513 6754; Tel: +82 51 510 2815*
[b]*College of Pharmacy, Seoul National University, Seoul, 151-742, Korea*
[c]*Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul, 120-750, Korea*

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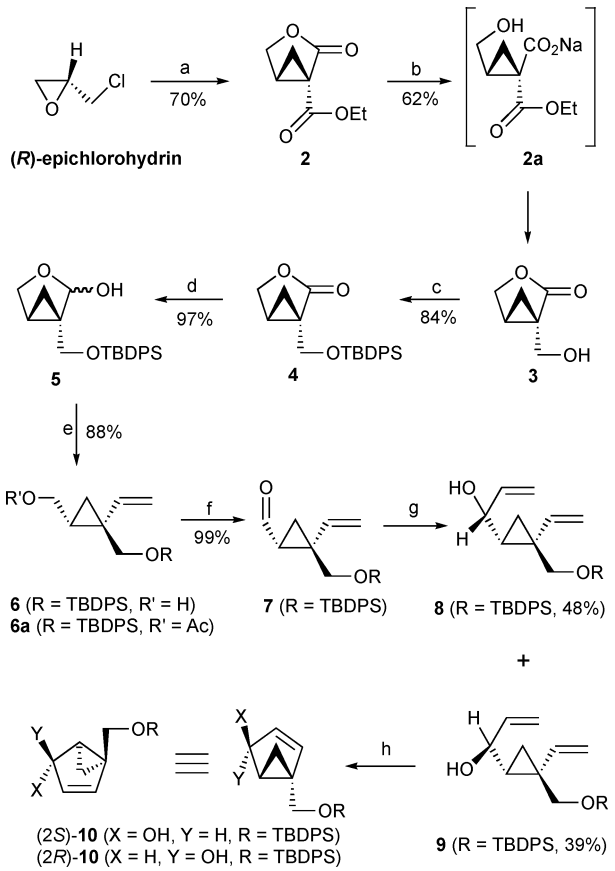
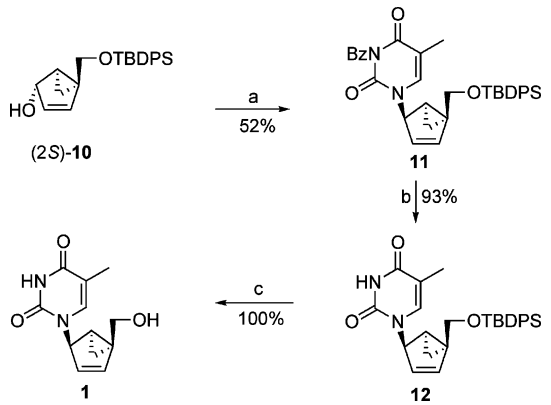


Scheme 1 *Reagents and conditions*: (a) $(EtO_2C)_2CH_2$, Na, EtOH, 80 °C, 20 h; (b) i) 1 eq. NaOH, EtOH, rt, 16 h; ii) $NaBH_4$, reflux, 3 h, then 2 M HCl, rt, 18 h; (c) TBDPSCl, imidazole, CH_2Cl_2, rt, overnight; (d) Dibal-H, CH_2Cl_2, −78 °C, 30 min; (e) CH_3PPh_3Br, *t*-BuOK, THF, rt, 3 h; (f) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, 1 h, then Et_3N, rt, 1 h; (g) vinylmagnesium bromide, THF, −78 °C, 1 h; (h) 2nd generation Grubbs catalyst, CH_2Cl_2, rt, 1.5 h, 85% for (2*S*)-**10**, 84% for (2*R*)-**10**.

chloride produced silyl ether **4**, which was reduced with Dibal-H at −78 °C to give the corresponding lactol **5** in 97% yield. Wittig reaction of lactol **5** with methyltriphenylphosphonium bromide in the presence of potassium *tert*-butoxide afforded hydroxy olefin **6** in 88% yield after quenching with aqueous NH_4Cl solution. However, when after the Wittig reaction the reaction mixture was extracted with EtOAc and water without adding aqueous ammonium chloride solution, a new by-product appeared at a higher R_f value than that of the desired compound **6** in a 10–20% yield. It turned out to be acetylated compound **6a** by ^1H, ^{13}C and COSY NMR experiments. Swern oxidation of **6** with oxalyl chloride and DMSO at −78 °C smoothly gave aldehyde **7**, which was subjected to a Grignard reaction with vinylmagnesium bromide to afford allylic alcohols **8**‡ (48%) and **9**§ (39%), easily separated by normal silica gel column chromatography. The stereochemistry of **8** and **9** couldn't be determined until the bicyclo[3.1.0]hexenol template was formed *via* the RCM reaction. RCM reaction[15] of diene **9** and **8** with a second generation Grubbs catalyst produced the bicyclo[3.1.0]hex-3-en-2-ol templates (2*S*)-**10**¶ and (2*R*)-**10**‖, respectively. The stereochemistry of these was confirmed by comparing their ^1H NMR spectra with that of the authentic enantio-counterpart, an enantiomer of (2*S*)-**10**, prepared from a chiral bicyclo[3.1.0]hexane template,[16] indicating that the compound derived from **9** has the desired (2*S*)-stereochemistry. (2*S*)-**10** was ready for the coupling of nucleobases to yield various nucleosides.

Synthesis of L-*N*-MCd4T (**1**) from bicyclo[3.1.0]hexenol (2*S*)-**10** *via* a coupling reaction with N^3-benzoylthymine is described in Scheme 2. Condensation of (2*S*)-**10** with N^3-benzoylthymine under Mitsunobu conditions afforded *N*-alkylated product **11** in 52% yield. The desired final product, L-*N*-MCd4T (**1**) was obtained after removal of benzoyl and TBDPS groups by successively treating with ammonium hydroxide in methanol and tetrabutylammonium fluoride (TBAF) in THF.



Scheme 2 *Reagents and conditions*: (a) N^3-benzoylthymine, DEAD, PPh_3, THF, 0 °C, 6 h; (b) 28% NH_4OH–MeOH (1 : 10), rt, 7 h; (c) TBAF, THF, rt, 1 h.

L-*N*-MCd4T (**1**) exactly matched with all spectral properties of the enantio-counterpart, D-*N*-MCd4T prepared from chiral bicyclo[3.1.0]hexane template except the sign of optical rotation value.[9]

Antiviral activity of the synthesized L-*N*-MCd4T (**1**) was measured against a HIV (human immunodeficiency virus) type 1 and 2. In MT4 (HTLV-1-infected human T lymphocyte) cells, L-*N*-MCd4T (**1**) exhibited significant anti-HIV-1 activity (EC_{50} = 6.76 μg mL^{-1}), which was about 1.3-fold less potent than ddI being clinically used, without cytotoxicity up to 100 μg mL^{-1}. Interestingly, L-*N*-MCd4T (**1**) was inactive against HIV-2 whereas ddI showed moderate activity (EC_{50} = 16.00 μg mL^{-1}).

In conclusion, we have accomplished the synthesis of novel L-*N*-MCd4T (**1**), an enantiomer of D-*N*-MCd4T as a potent anti-HIV agent employing a novel synthetic strategy starting from (*R*)-epichlorohydrin. Tandem alkylation, lactonization, chemoselective reduction of ester in the presence of lactone functional group, Grignard reaction, RCM reaction, and Mitsunobu reaction were the key reactions to achieve the synthesis of L-*N*-MCd4T (**1**). L-*N*-MCd4T (**1**) was found to show potent anti-HIV-1 activity similar to that of ddI, which is a clinically useful anti-AIDS drug, in MT-4 cells without appreciable cytotoxicity up to 100 μg mL^{-1}. This observation shows that L-type bicyclo[3.1.0]hexene (2*S*)-**10** might be regarded as a new template for the development of anti-HIV agents.

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Notes and references

‡ **(−)-(1*R*)-1-[(1*R*,2*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-vinyl-cyclopropyl]prop-2-en-1-ol** (**8**). $[\alpha]_D^{25}$ −22.8 (*c* 1.06, in $CHCl_3$) (Found: C, 76.51; H, 8.39. Calc. for $C_{25}H_{32}O_2Si$: C, 76.48; H, 8.22%); δ_H (200 MHz; $CDCl_3$) 7.69–7.35 (10 H, m, 2 × Ph), 6.06–5.87 (2 H, m, 2 × -C*H*=CH_2), 5.37–5.09 (4 H, m, 2 × -CH=CH_2), 3.75 (1 H, d, *J* 10.4, TBDPSC*Ha*), 3.75–3.66 (1 H, m, C*H*OH), 3.50 (1 H, d, *J* 10.4, TBDPSC*Hb*), 1.25–1.13 (1 H, m, methine H), 1.07 (9 H, s, *t*-Bu), 0.89 (1 H, s, methylene Ha), 0.85 (1 H, s, methylene Hb); δ_C (50 MHz; $CDCl_3$) 140.5, 137.3, 136.0, 134.0, 130.0, 130.0, 128.0, 116.3, 114.5, 73.5, 68.9, 30.1, 29.2, 27.2, 19.7, 13.6; *m*/*z* (ESI) 415 (83, M + Na)$^+$.

§ **(−)-(1*S*)-1-[(1*R*,2*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-vinyl-cyclopropyl]prop-2-en-1-ol** (**9**). $[\alpha]_D^{25}$ −55.9 (*c* 1.84, in $CHCl_3$) (Found: C, 76.20; H, 8.58. Calc. for $C_{25}H_{32}O_2Si$: C, 76.48; H, 8.22%); δ_H (200 MHz; $CDCl_3$) 7.71–7.36 (10 H, m, 2 × Ph), 6.13–5.90 (2 H, m, 2 × -C*H*=CH_2), 5.33–5.09 (4 H, m, 2 × -CH=CH_2), 3.76 (1 H, d, *J* 10.4, TBDPSC*Ha*), 3.67–3.65 (1 H, m, C*H*OH), 3.58 (1 H, d, *J* 10.4, TBDPSC*Hb*), 1.23–1.11 (1 H, m, methine H), 1.09 (9 H, s, *t*-Bu), 0.86 (1 H, dd, *J* 5.2, 8.8, methylene Ha), 0.72 (1 H, t, *J* 5.2, methylene Hb); δ_C (50 MHz; $CDCl_3$) 140.1, 137.3, 136.1, 136.1, 134.0, 134.0, 130.1, 130.0, 128.0, 128.0, 116.6, 114.7, 73.5, 69.0, 30.4, 30.2, 27.3, 19.7, 14.2; *m*/*z* (ESI) 415 (60, M + Na)$^+$.

¶ **(+)-(1*R*,2*S*,5*S*)-5-(*tert*-Butyl-diphenyl-silanyloxymethyl)-bicyclo[3.1.0]-hex-3-en-2-ol** ((2*S*)-**10**). To a stirred solution of **9** (748 mg, 1.91 mmol) in CH_2Cl_2 (45 mL) was added Grubbs catalyst 2nd generation (56 mg, 0.07 mmol) at 0 °C, and the reaction mixture was stirred for 1.5 h at room temperature. After the volatiles were removed, the resulting residue was purified by flash column chromatography using hexane and ethyl acetate (4 : 1) as the eluent to give bicyclo[3.1.0]hexenol (2*S*)-**10** (590 mg, 85%) as a colorless oil. ^{1}H NMR data were identical to those of the authentic sample, the enantio-counterpart[9] synthesized by Marquez and his co-workers $[\alpha]_D^{25}$ +10.3 (*c* 1.13, $CHCl_3$) (Found: C, 75.81; H, 7.56. Calc. for $C_{23}H_{28}O_2Si$: C, 75.78; H, 7.74%); δ_C (50 MHz; $CDCl_3$) 138.3, 135.8, 135.7, 134.0, 133.9, 131.0, 129.8, 127.8, 78.0, 64.9, 38.8, 27.0, 22.9, 20.3, 19.4; *m*/*z* (EI) 346 (7, M − H_2O)$^+$.

‖ **(−)-(1*R*,2*R*,5*S*)-5-(*tert*-Butyl-diphenyl-silanyloxymethyl)-bicyclo[3.1.0]-hex-3-en-2-ol** ((2*R*)-**10**). (2*R*)-**10** was synthesized in 84% yield in the similar procedure to the synthesis of (2*S*)-**10** $[\alpha]_D^{25}$ −30.9 (*c* 1.05, in $CHCl_3$) (Found: C, 76.03; H, 7.80. Calc. for $C_{23}H_{28}O_2Si$: C, 75.78; H, 7.74%); δ_H (500 MHz; $CDCl_3$) 7.70–7.39 (10 H, m, 2 × Ph), 6.21 (1 H, d, *J* 5.5, vinylic H), 5.58–5.57 (1 H, br d, *J* 5.5, vinylic H), 4.39 (1 H, d, *J* 1.5, 2-H), 3.97 (1 H, d, *J* 11.0, TBDPSOC*Ha*), 3.74 (1 H, d, *J* 11.5, TBDPSOC*Hb*), 1.64 (1 H, dd, *J* 4.0, 8.5, 1-H), 1.08 (9 H, s, *t*-Bu), 1.00 (1 H, dd, *J* 4.0, 8.0, 7-Ha), 0.20 (1 H, t, *J* 4.0, 7-Hb); δ_C (50 MHz; $CDCl_3$) 140.4, 135.8, 135.8, 134.0, 133.9, 130.1, 129.8, 127.8, 127.8, 77.6, 65.1, 37.2, 30.1, 27.0, 25.5, 19.5; *m*/*z* (EI) 346 (12, M − H_2O)$^+$.

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