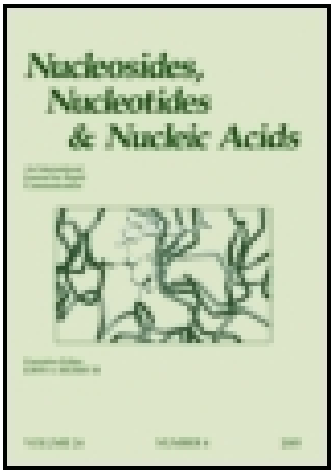

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Hyung Ryong Moon [a], Ah-Young Park [a], Kyung Ran Kim [a], Moon Woo Chun [b] & Lak Shin Jeong [c]

[a] College of Pharmacy and Research Institute for Drug Development, Pusan National University, Busan, Korea

[b] College of Pharmacy, Seoul National University, Seoul, Korea

[c] College of Pharmacy, Ewha Womans University, Seoul, Korea

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ASYMMETRIC SYNTHESIS AND BIOLOGICAL ACTIVITY OF L-BICYCLOCARBA-d4T

Hyung Ryong Moon, Ah-Young Park, and Kyung Ran Kim □ *College of Pharmacy and Research Institute for Drug Development, Pusan National University, Busan, Korea*

Moon Woo Chun □ *College of Pharmacy, Seoul National University, Seoul, Korea*

Lak Shin Jeong □ *College of Pharmacy, Ewha Womans University, Seoul, Korea*

□ *Novel L-bicyclocarba-d4T (**1**), an enantiomer of D-N-MCd4T has been enantiopurely 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, Grignard reaction, RCM reaction, and Mitsunobu reaction as key steps. L-N-MCd4T (**1**) was found to be very potent anti-HIV-1 ($EC_{50} = 6.76\ \mu g/mL$) agent with no cytotoxicity.*

Keywords Anti-HIV agent; novel L-bicyclocarba-d4T; tandem alkylation; (R)-epichlorohydrin

INTRODUCTION

HIV (human immunodeficiency virus) is a pathogenic retrovirus and the causative agent of AIDS.[1] Since AZT has been known as a potent anti-HIV agent, initial attempts were focused on the development of 2′,3′-dideoxynucleosides (ddNs).[2] Among them, ddI, ddC, and d4T[3] have been clinically used for the treatment of AIDS patients. Particulary, d4T has a double bond at its pseudosugar ring, which renders the pseudosugar ring to be nearly planar. 3TC[4] also has been used as anti-HIV and anti-HBV agents and might be considered as a member of ddNs family because it does not have hydroxyl substituents at both 2′- and 3′-position. Therefore, ddN analogs still seem to be the most promising candidate for the AIDS drug, although most of them undergo an easy glycosidic

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Address correspondence to Hyung Ryong Moon, College of Pharmacy and Research Institute for Drug Development, Pusan National University, San 30 Jangjeon-dong, Geumjeong-gu, Busan 609-735, Korea. E-mail: mhr108@pusan.ac.kr

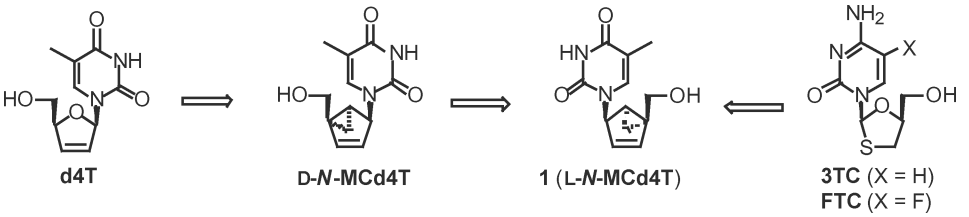


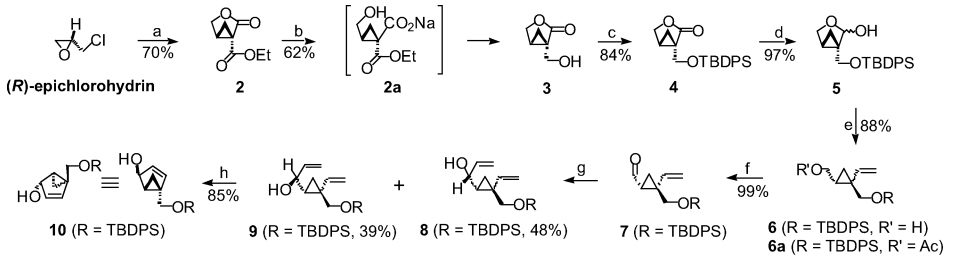
FIGURE 1 The rationale for the design of the desired nucleoside, **L-*N*-MCd4T (1)**.

bond cleavage, comparing to normal nucleosides under acidic conditions. Carbocyclic nucleosides[5] have been synthesized mainly with the purpose of overcoming cleavage of the glycosidic bond.[5,6] 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)[8,9] conformation as in normal nucleosides. Marquez and co-workers have synthesized these rigid MC nucleosides to study the conformational preferences of various enzymes involved in nucleoside and nucleotide metabolism. D-*N*-MCd4T,[10] bearing a locked North conformation and a 2′,3′-double bond in a single structure has been recently synthesized (Figure 1). Although D-*N*-MCd4T was somewhat less potent than d4T, it still showed significant antiviral activity against HIV-1 and -2 with a less cytotoxicity and was more stable under acidic conditions. On the other hand, a number of L-nucleosides such as 3TC,[4] FTC, L-d4FC, and L-5-FddC have been also reported to show highly potent anti-HIV activity.

On the basis of these findings, it was interesting to synthesize L-*N*-MCd4T (**1**, L-bicyclocarba-d4T), an enantiomer of D-*N*-MCd4T as a potent anti-HIV agent. Here, we report a new synthetic methodology of L-*N*-MCd4T (**1**) using cyclopropanation via tandem alkylation and ring-closing metathesis (RCM) as key steps from (*R*)-epichlorohydrin and its anti-HIV activity.

CHEMISTRY

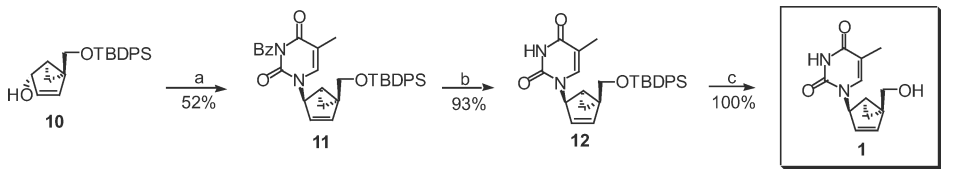
Synthesis of a glycosyl donor, bicyclo[3.1.0]hexenol template **10** for the synthesis of L-*N*-MCd4T (**1**) is described in Scheme 1. Compound **2** was synthesized starting from (*R*)-epichlorohydrin according to the procedure reported by Tsuji and his coworkers.[11,12] The reaction was accomplished by tandem alkylation followed by lactonization in 62% yield. The lactone moiety of **2** was more susceptible to hydrolysis than the ester, probably due to ring strain derived from fused cyclopropane ring. Therefore, it is possible to chemoselectively reduce the ester in the presence of the lactone group. Treatment of **2** with 1 equiv. of NaOH in ethanol afforded monocarboxylate sodium salt **2a** and the ester was reduced by $NaBH_4$ under reflux and recyclized back to lactone **3** under acidic conditions. Protection of hydroxyl group of **3** with *tert*-butyldiphenylsilyl chloride produced silyl ether **4**, which



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.

was reduced with Dibal-H to give the corresponding lactol **5**. Wittig reaction of lactol **5** with methylidenephosphorane afforded hydroxy olefin **6** in 88% yield after quenched with aqueous NH_4Cl solution. 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%) as an easily separable diastereoisomeric mixture. The stereochemistry of **8** and **9** could be determined after a RCM reaction. RCM reaction[13,14] of diene **9** and **8** with second generation Grubbs catalyst produced the bicyclo[3.1.0]hex-3-en-2-ol template **10** and its (2*R*)-diastereomer, respectively. The stereochemistry of **10** and its diastereomer was confirmed by comparing their ^1H NMR spectra with that of the authentic enantio-counterpart, an enantiomer of **10**, prepared from chiral bicyclo[3.1.0]hexane template,[15] indicating compound **10** has the desired (2*S*)-stereochemistry.

Synthesis of L-*N*-MCd4T (**1**) from glycosyl donor, bicyclo[3.1.0]hexenol **10** via a coupling reaction with N^3-benzoylthymine is described in Scheme 2. Condensation of **10** with N^3-benzoylthymine under Mitsunobu conditions afforded N^1-alkylated product **11** in 52% yield. 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. L-*N*-MCd4T (**1**) exactly matched with all spectral properties of the authentic D-*N*-MCd4T except the sign of optical rotation value.[10]



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.

BIOLOGICAL RESULTS

Anti-HIV type 1 and 2 activities of L-*N*-MCd4T (**1**) were measured in cell culture assays with HIV-1_{IIIb} and HIV-2_{ROD} infected MT-4 cells. MTT assay was performed for its IC_{50} and EC_{50}. In MT4 (HTLV-1-infected human T lymphocyte) cells, L-*N*-MCd4T (**1**) showed potent anti-HIV-1 activity ($EC_{50} = 6.76\ \mu g/mL$), which was about 1.3-fold less potent than ddI being clinically used for the treatment of AIDS patient, without cytotoxicity up to 100 $\mu g/mL$. However, L-*N*-MCd4T (**1**) was inactive against HIV-2 whereas ddI showed moderate activity ($EC_{50} = 16.00\ \mu g/mL$).

CONCLUSION

We have accomplished the enantiopure synthesis of novel L-*N*-MCd4T (**1**) as a potent anti-HIV agent employing a novel synthetic strategy starting from (*R*)-epichlorohydrin. For the synthesis of L-*N*-MCd4T (**1**), tandem alkylation, lactonization, chemoselective reduction of ester in the presence of lactone functional group, Grignard reaction and RCM reaction were used as the key reactions. L-*N*-MCd4T (**1**) was found to exhibit very potent anti-HIV-1 activity in MT-4 cells with no cytotoxicity. This observation indicates that L-type bicyclo[3.1.0]hexene **10** might be regarded as one of novel templates for the development of anti-HIV agents.

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