Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 2979–2982

Synthesis of 4′,4′-*C*-diaminomethyl nucleoside derivative as a building block for constructing libraries via amide bond formation

Chung-Shan Yu,* Ren-Tsong Wang, Li-Wu Chiang and Ming-Hsun Lee

Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, No. 101 sec. 2, Guang-Fu Road, Hsinchu 300, Taiwan

Received 13 January 2007; revised 15 February 2007; accepted 1 March 2007
Available online 3 March 2007

Abstract—Preparation of the 4′,4′-*C*-diaminomethyl uridine analog starting from the commercial uridine via 4′,4′-*C*-dihydroxymethyl uridine, 4′,4′-*C*-bis-trifluoromethanesulfonyloxymethyl uridine, and 4′,4′-*C*-diazidomethyl uridine in total eight steps was achieved in 8% yield. Steric hindrance between 3′-*O*-TBDMS and 5′-*O*-triflate prevented the undesired ring closure which made the synthesis of target compound feasible.

1. Introduction

Nucleoside analogs have become potential compounds for both therapeutic and diagnostic purposes.[1] For example, 5-bromovinyl arabinosyl uridine (BVaraU) and 5-fluorouracil (5-FU) have been widely used as anti-viral[2] and anti-cancer[3] chemotherapeutic agents. Advances of the in vivo imaging of cancer cells transfacted by foreign gene have been greatly contributed by positron-emitter labeling nucleosides in the past decades. In this approach, cancer cells were first transfected by herpes simplex virus thymidine kinase gene (HSV TK), followed by the introduction of nontoxic prodrugs such as nucleoside analogs.[4] Upon phosphorylation at 5′-OH by this HSV TK, the prodrugs could transform into toxic metabolites, leading to the cytotoxic effect. Such in vivo images could be also obtained when nucleosides were tagged by positron emitters including ^{18}F or ^{124}I.[5]

With the important roles of nucleosides in medical application, the development of efficient approaches to build nucleoside libraries was thus crucial in discovering more potential inhibitors. Recently, Wong and co-workers[6] and Bertozzi and co-workers[7] have independently established a methodology combining an in situ synthesis of compound libraries and an enzymatic bioassay. These libraries are produced via amide-bond formation followed by a bioassay performing on microtiter plates.[6,7] The nucleoside scaffolds are established by extension at 5′-amino group of a core pyrimidine nucleoside via coupling with various carboxylic acids. Indeed, some novel nucleoside analogs are discovered to be cytotoxic against bacteria through this approach.

To our aim, we wish to establish a substrate library for HSV TK for which a free 5′-hydroxy group or a 5′-amino group is available for phosphorylation. A nucleoside 4′-*C*-aminomethyl uridine bearing both the 5′-hydroxy and an amino group or 4′,4′-*C*-diaminomethyl uridine bearing two amino groups could be rational core compounds (Fig. 1). To this end, 4′-*C*-hydroxymethyl nucleoside **2** can serve as an adequate intermediate (Scheme 1).

Synthesis of compound **2** was first reported by Wengel's group.[8] Starting from commercial uridine **1**, compound **2** was obtained via 5 steps in 30% overall yield. Selective introduction of mono tosyl group under mild condition was achieved. The site of tosylation has been proved impossible to be verified by NMR techniques.[9] Nevertheless, the assignment was established indirectly from the cyclized LNA derivative (locked nucleic acid) **6**,[10] which could be prepared from the corresponding triflate **5** as discussed below (Scheme 2). Whereas steric bulk of tosyl group might be greater than triflate group, the

Keywords: Library; Diamine; Nucleoside; Ditriflate; HSV TK.
* Corresponding author. Tel.: +886 3 5751922; fax: +886 3 5718649; e-mail: csyu@mx.nthu.edu.tw

0040-4039/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2007.03.002

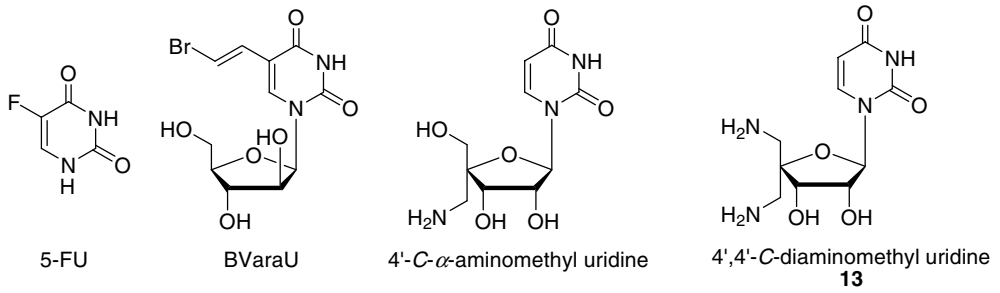
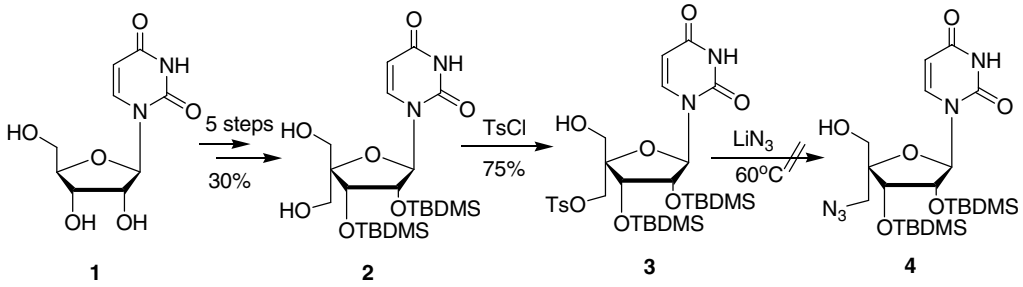
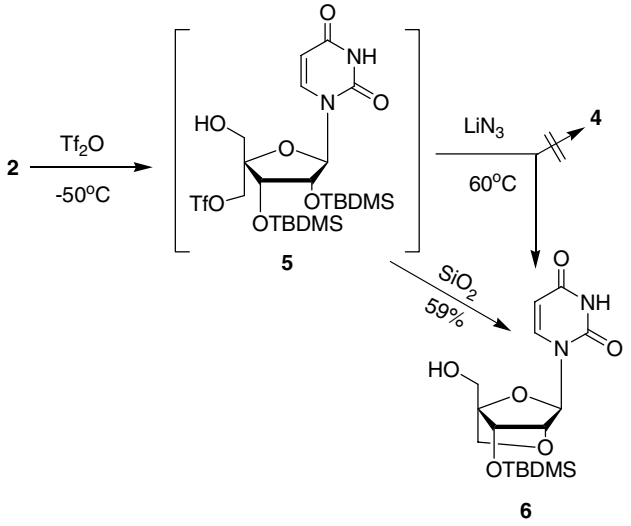


Figure 1. Chemotherapeutic reagents derived from uracil derivatives.



Scheme 1. Attempt to prepare 4′-*C*-azidomethyl uridine **4**.



Scheme 2. Unexpected ring-closure affected by silica.

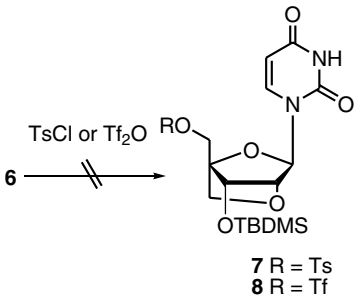
same substitution site was suggested. Subsequent nucleophilic attack by azido group under various conditions failed to afford even a trace amount of the desired compound **4**.

Triflate **5** served as an alternative preparation method owning to the excellent leaving capacity of triflic group. However, the labile triflate can easily form the cyclized LNA analog **6** during column chromatography. Attempts to substitute the triflic group with azido group only lead to the complete formation of **6**. Configurations of **6** were confirmed from the coupling patterns of H-1′, H-2′, and H-3′ in ^{1}H NMR with respect of the three singlets equivalent to the reported data.[11]

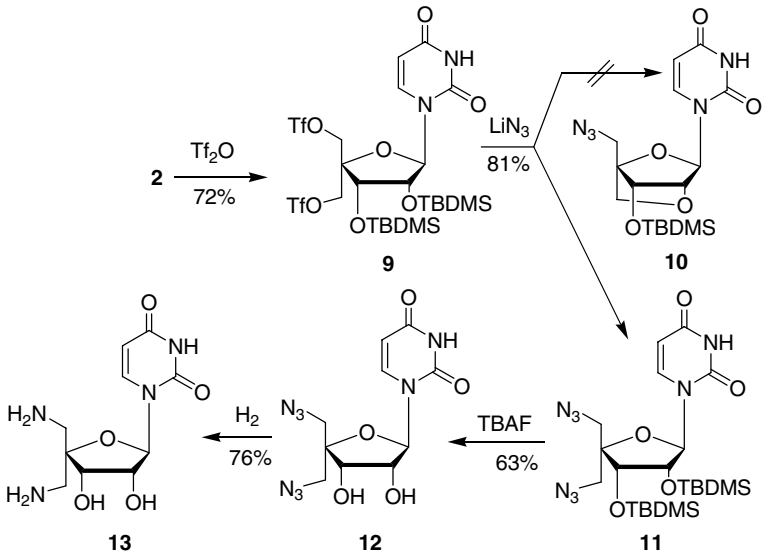
Attempt to substitute the remaining free hydroxyl group with either tosyl or triflic groups under various conditions failed to afford the desired products **7** and **8**; probably due to the rigid dioxabicyclo[2,2,1] heptane skeleton prohibiting the nucleophilic attack by hydroxyl group (Scheme 3).

To prepare the other target compound: diamino nucleoside **13**, diazido compound **11** and its precursor **9** need to be synthesized in advance in spite of the possible formation of the undesired ring closure as discussed above (Scheme 4).

Upon substituting **9** with lithium azide, unexpectedly, instead of the cyclized nucleoside **10**, diazido nucleoside **11** was obtained. The conformational difference between compound **5** and **9** could be seen from a 3-D molecular modeling coupled with the MM2 program (Fig. 2).[12] The up-triflate group of ditriflate **9** forced 3′-OTBDMS close to 2′-OTBDMS thereby blocking its nucleophilic attack onto the down-triflate group. Contradicting to this result, monotriflate **5** with a less repulsion allowed



Scheme 3. Inertness due to the rigid bicyclo[2,2,1] configuration.



Scheme 4. Preparation of diamino nucleoside **13** via diazido nucleoside **11**.

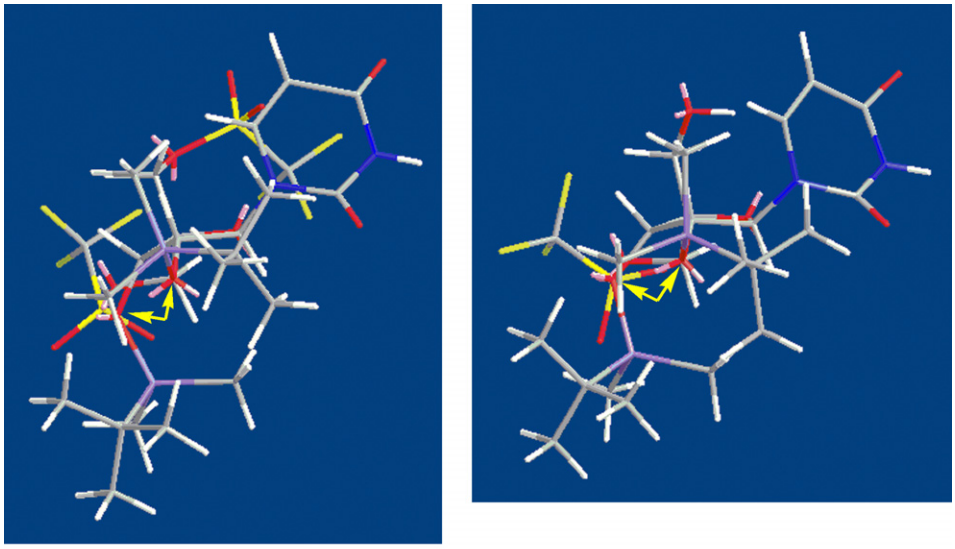


Figure 2. Steric hindrance between the groups of 2′ and 3′ prevented 6′-C from nucleophilic attack by 2′-silyl group.

the attack of 2′-OTBDMS on triflic group. Subsequent deprotection with HCl failed to produce any desired product **12**, unless TBAF was used. Reduction of the azido groups of **12** under Pd-catalyzed hydrogen condition yields diamino nucleoside **13**. Whereas the current synthetic route did not lead to one of our target compounds: the single amino nucleoside, diamino moieties **13**, may fulfill the requirement of a core compound for probing HSV TK. Both the mono- and di-amide compounds will be prepared through the coupling of core compound **13** with more than 100 carboxylic acids without purification. After transferring to the microtiter-plates containing cells, the cytotoxicities will be evaluated and the role of the free amino group will be better clarified. These relevant coupling experiments and bioassays are in progress.

2. Typical synthetic procedures

2.1. 1-(2,3-Bis-*O*-*tert*-butyldimethylsilyl-4-*C*-trifluoromethanesulfonyloxymethyl-5-*O*-trifluoromethanesulfonyl-β-D-erythro-pentofuranosyl)uracil (9)

To a dried round-bottomed flask (50 mL), compound **2** (0.68 g, 1.4 mmol), pyridine (1.1 mL, 13.5 mmol, 10 equiv), CH_2Cl_2 (14 mL) and Tf_2O (930 μL, 5.5 mmol, 4 equiv) were added sequentially. The mixture was stirred at −50 °C for 20 min, until product **9** ($R_f = 0.43$, EtOAc/*n*-hexane 3:7) was detected on TLC along with the disappearance of **2**. After the removal of the volatile solvents, the residue was partitioned between CH_2Cl_2 (60 mL) and satd solution of NH_4Cl (50 mL × 1) and water (50 mL × 2) sequentially. The organic layer was

then dried with Na_2SO_4 and filtered. After concentration, the residue was purified by column chromatography on silica gel with EtOAc/*n*-hexane (1:4→3:7) containing 3% Et_3N to give **9** in 72% yield (767 mg) as a colorless glassy solid. Anal. $C_{24}H_{40}F_6N_2O_{11}S_2Si_2$, calcd. mass: 776.9 amu, ESI + Q-TOF MS, M = 776.2 (*m*/*z*), $[M+H]^+ = 767.3$, $[2M+H]^+ = 1533.6$; ^1H NMR (500 MHz, C_6D_6) δ −0.05 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H), 0.14 (s, 3H), 0.90 (s, 9H), 0.94 (s, 9H), 4.46 (d, $J = 12.0$ Hz, 1H), 4.49 (d, $J = 12.0$ Hz, 1H), 4.73 (dd, $J = 5.5$ Hz, $J = 2.0$ Hz, 1H), 4.76 (d, $J = 12.0$ Hz, 1H), 4.98 (d, $J = 5.5$ Hz, 1H), 5.13 (d, $J = 2.0$ Hz, 1H), 5.26 (dd, $J = 8.5$ Hz, $J = 1.5$ Hz, 1H), 5.45 (d, $J = 12.0$ Hz, 1H), 6.19 (dd, $J = 8.5$ Hz, $J = 3.5$ Hz, 1H), 9.59 (br s, 1H, N–H); ^{13}C NMR (125 MHz, C_6D_6) δ −5.3 (CH or CH_3), −4.9 (CH or CH_3), −4.3 (CH or CH_3), −4.2 (CH or CH_3), 17.8 (quart. C), 17.9 (quart. C), 25.9 (CH or CH_3), 72.8 (CH_2), 73.0 (CH or CH_3), 75.0 (CH or CH_3), 74.8 (CH_2), 83.3 (quart. C), 98.1 (CH or CH_3), 103.3 (CH or CH_3), 119.0 (ddd, $J_{C,F} = 320$ Hz, –CF_3), 119.2 (ddd, $J_{C,F} = 319.9$ Hz, –CF_3), 143.6 (CH or CH_3), 150.6 (quart. C), 162.5 (quart. C).

2.2. 1-4-*C*-Aminomethyl-5-amino-β-D-*erythro*-pentofuranosyl uracil (13)

Compound **12** (54 mg, 0.16 mmol), MeOH (6 mL), water (2 mL) and Pd/C (5 mg) were added sequentially to a round-bottomed flask. Under the atmosphere of H_2, the mixture was stirred vigorously at rt for 20 min. Compound **13** ($R_f = 0$) was detected on TLC (MeOH/$CHCl_3$ 1:1). Upon completion, the reaction suspension was filtered twice through filter paper and the filtrate was concentrated to provide the crude white product **13**. The purity could not be further improved by recrystalization from water and MeOH. The white solid obtained was very hygroscopic and easily formed a gel-like solid. Yield: 76 % (34 mg). Anal. $C_{10}H_{16}N_4O_5$, calcd mass: 272.3 amu, ESI + Q-TOF MS, M = 272.1 (*m*/*z*), $[M+H]^+ = 273.1$; ^1H NMR (500 MHz, D_2O) δ 2.83 (d, $J = 14.5$ Hz, 1H), 2.87 (d, $J = 14.5$ Hz, 1H), 3.01 (d, $J = 13.7$ Hz, 1H), 3.11 (d, $J = 13.7$ Hz, 1H), 4.25 (d, $J = 6.0$ Hz, 1H), 4.52 (dd, $J = 6.0$ Hz, $J = 6.0$ Hz, 1H), 5.78 (d, $J = 8.0$ Hz, 1H, H-5), 5.82 (d, $J = 6.0$ Hz, 1H, H-1′), 7.52 (d, $J = 8.0$ Hz, 1H, H-6); ^{13}C NMR (125 MHz, D_2O) δ 41.39 (CH_2), 45.11 (CH_2), 72.41 (CH or CH_3), 72.77 (CH or CH_3), 85.73 (quart. C), 90.83 (CH or CH_3), 102.80 (CH or CH_3), 142.15 (CH or CH_3), 155.46 (quart. C), 171.21 (quart. C).

Acknowledgments

We thank the National Science Council of Taiwan and the National Tsing-Hua University for providing financial support (NSC-95-2113-M-007-039) and the fund of WCRC.

Supplementary data

Experimental procedures and tabulated spectroscopic data for **3**, **6** and **11**–**12**; ^1H NMR and ^{13}C NMR spectra of compounds **3**, **6**, **9** and **11**–**13**; COSY spectrum of compound **6**; and ESI-MS spectrum of **13**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.03.002.

References and notes

1. (a) Kumar, R.; Semaine, W.; Johar, M.; Tyrrell, D. L. J.; Agrawal, B. *J. Med. Chem.* **2006**, *49*, 3693–3700; (b) Beadle, J. R.; Wan, W. B.; Ciesla, S. L.; Keith, K. A.; Hartline, C.; Kern, E. R.; Hostetler, K. Y. *J. Med. Chem.* **2006**, *49*, 2010–2015; (c) Somu, R. V.; Boshoff, H.; Qiao, C. H.; Bennett, E. M.; Barry, C. E.; Aldrich, C. C. *J. Med. Chem.* **2006**, *49*, 31–34; (d) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewiez, K. W. *J. Med. Chem.* **2005**, *48*, 5504–5508.
2. Onishi, T.; Mukai, C.; Nakagawa, R.; Sekiyama, T.; Aoki, M.; Suzuki, K.; Nakazawa, H.; Ono, N.; Ohmura, Y.; Iwayama, S.; Okunishi, M.; Tsuji, T. *J. Med Chem.* **2000**, *43*, 278–282.
3. Wagner, A. D.; Grothe, W.; Haerting, J.; Kleber, G.; Grothey, A.; Fleig, W. E. *J. Clin. Oncol.* **2006**, *24*, 2903–2909.
4. (a) deVries, E. F. J.; Buursma, A. R.; Hospers, G. A. P.; Mulder, N. H.; Vaalburg, W. *Curr. Pharm. Des.* **2002**, *8*, 1435–1450; (b) Gambhir, S. S.; Herschman, H. R.; Cherry, S. R. *Neoplasia* **2000**, *2*, 118–138; (c) Nichol, C.; Kim, E. E. *J. Nucl. Med.* **2001**, *42*, 1368–1374; (d) Gibson, R. E.; Burns, H. D.; Hamill, T. G.; Eng, W. S.; Francis, B. E.; Ryan, C. *Curr. Pharm. Des.* **2000**, *6*, 973–989.
5. For [^{18}F]-FHBG: (a) Yaghoubi, S. S.; Barrio, J. R.; Namavari, M.; Satyamurthy, N.; Phelps, M. E.; Herschman, H. R.; Gambhir, S. S. *Cancer Gene Ther.* **2005**, *12*, 329–339; For [^{124}I]-FIAU analogs: (b) Simoes, M. V.; Miyagawa, M.; Reder, S.; Stadele, C.; Haubner, R.; Linke, W.; Lehner, T.; Epple, P.; Anton, M.; Schwaiger, M.; Beng, F. M. *J. Nucl. Med.* **2005**, *46*, 98–105; (c) Yu, C.-S.; Chiang, L.-W.; Wu, C.-H.; Wang, R.-T.; Chen, S.-W.; Wang, H.-Y.; Yeh, C.-H. *Nucl. Med. Biol.* **2006**, *33*, 367–370.
6. (a) Brik, A.; Wu, C. Y.; Wong, C. H. *Org. Biomol. Chem.* **2006**, *4*, 1446–1457; (b) Brik, A.; Wu, C. Y.; Best, M. D.; Wong, C. H. *Bioorg. Med. Chem.* **2005**, *13*, 4622–4626; (c) Wu, C. Y.; Brik, A.; Wang, S. K.; Chen, Y. H.; Wong, C. H. *Chembiochem* **2005**, *6*, 2176–2180; (d) Wu, C. Y.; Chang, C. F.; Chen, J. S. Y. *Angew. Chem., Int. Ed.* **2003**, *42*, 4661–4664.
7. Hang, H. C.; Yu, C.; Ten Hagen, K. G.; Tian, E.; Winans, K. A.; Tabak, L. A.; Bertozzi, C. R. *Chem. Biol.* **2004**, *11*, 337–345.
8. Nielsen, K. D.; Kirpekar, F.; Roepstorff; Wengel, J. *Bioorg. Med. Chem.* **1995**, *3*, 1493–1502.
9. Meldgaard, M.; Hansen, F. G.; Wengel, J. *J. Org. Chem.* **2004**, *69*, 6310–6322.
10. Obika, S.; Morio, K.-I.; Nanbu, D.; Hari, Y.; Itoh, H.; Imanishi, T. *Tetrahedron* **2002**, *58*, 3039–3049.
11. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607–3630.
12. ChemDraw3D Pro 8.0: MM2 with energy minimization.