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

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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.

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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-viral2 and anti-cancer3 chemotherapeutic agents. Advances of the in vivo imaging of cancer cells transfected 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 18F or 124I.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-workers6 and Bertozzi and co-workers7 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
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 1H 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

Attempt to substitute the following nucleosides with azido group under various conditions led to the complete formation of 6 in all cases.
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.

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

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

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), CH₂Cl₂ (14 mL) and Tf₂O (930 μL, 5.5 mmol, 4 equiv) were added sequentially. The mixture was stirred at −50 °C for 20 min, until product 9 (R₁ = 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 CH₂Cl₂ (60 mL) and satd solution of NH₄Cl (50 mL × 1) and water (50 mL × 2) sequentially. The organic layer was
then dried with Na$_2$SO$_4$ 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$_3$N to give 9 in 72% yield (767 mg) as a colorless glassy solid. Anal. C$_{29}$H$_{40}$F$_3$N$_2$O$_{11}$S$_4$Si$_2$, calcd. mass: 776.9 amu, ESI + Q-TOF MS, M = 776.2 (m/z), [M+H]$^+$ = 767.3, [2M+H]$^{2+}$ = 1533.6. $^1$H NMR (500 MHz, CD$_3$OD), $\delta$ = 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 (dd, 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 (dd, J = 5.5 Hz, J = 1.5 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, CD$_3$OD), $\delta$ = 5.5 Hz, 1H), 5.13 (d, J = 2.0 Hz, 1H), 4.76 (d, J = 8.0 Hz, 1H, H-5), 5.82 (d, J = 12.0 Hz, 1H), 4.73 (dd, J = 5.5 Hz, J = 1.5 Hz, 1H), 150.6 (quart. C), 162.5 (quart. C). $^1$H NMR (600 MHz, D$_2$O), $\delta$ = 6.0 Hz, 1H), 5.78 (d, J = 0.6 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$_2$O), $\delta$ = 41.39 (CH$_3$), 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

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Supplementary data

Experimental procedures and tabulated spectroscopic data for 3, 6, 9 and 11–12; $^1$H 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


11. ChemDraw3D Pro 8.0: MM2 with energy minimization.