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An Easy Access of 2′,3′-Dideoxy-3′-α-*C*-Formyl -Adenosine and -Guanosine Analogs via Stereoselective *C-C* Bond Forming Radical Reaction

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Abstract: A large-scale, facile and stereoselective synthesis of 1-[5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-3-α-*C*-formyl-β-D-erythro-pentofuranosyl] -adenine (**7b**) and -N^2-isobutyrylguanine (**7c**) using an intermolecular radical *C-C* bond formation reaction is reported. The utility of these nucleosides (**7b** and **7c**) as building blocks for antisense oligonucleosides is discussed.

Novel, neutral, and achiral linkages that replace the native phosphate linkage of a deoxyoligonucleotide have attracted a great deal of attention recently because of their usefulness in the design of antisense oligonucleosides (AO).[1] Among the various surrogates of the phosphate backbone studied in our group,[2] we have selected MMI[3] [**8**, methylene (methylimino)] and MDH[4] [**9**, methylene (dimethylhydrazo)] as the best linkages for further studies and incorporations into AO. Retrosynthetic analyses of MMI **8** and MDH **9** linkages indicated that 3′-deoxy-3′-α-*C*-formyl nucleosides **7a-c** could serve as the common building-blocks (upper-half) for such molecules (Scheme 1). In our last reports,[3,4] we had prepared only pyrimidine dimers (T*T; * = novel linker) containing MMI and MDH linkages. Other groups[5a-c] have prepared various backbone modified dimers utilizing thymidine as their starting material. Therefore, the database on AO containing backbone modifications is limited to pyrimidine nucleosides only. To broaden and strengthen the database on AO, we wish to incorporate purine nucleoside building-blocks (X*T; X = dA or dG) into AO sequences for the first time.[5d]

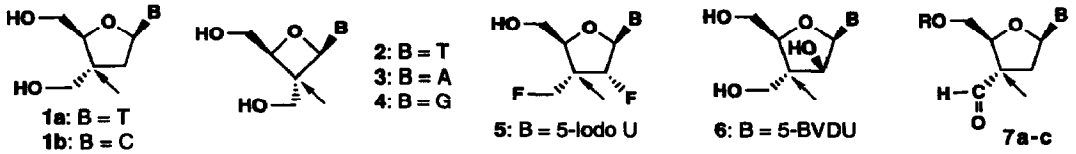


Figure 1. Antiviral and antitumor nucleoside analogs **1-6** and desired **7** containing α-*C-C* bond (shown by an arrow)

Interestingly, 3′-α-*C*-branched nucleoside **7a** may also serve as a precursor for the potent antiviral and antitumor agents [3′-α-*C*-hydroxymethyl -thymidine **1a** and -cytidine **1b** (Figure 1)].[6] Some of the synthetic and naturally occurring *C*-branched nucleosides [A-73209 (**2**),[7] oxetanocin **3**,[8] oxetanocin G **4**,[8] 3′-α-*C*-fluoromethyl **5**,[9] and arabinoside **6**[9]] have exhibited significant therapeutic value. Such reports and our need for the nucleoside building-blocks **7b** and **7c** prompted us to develop a new, shorter and simpler synthesis of α-*C*-branched nucleosides. This letter describes a direct and efficient method of introducing an α-*C-C* unit onto 2′-deoxynucleosides on a large-scale (>100 mmol) using an intermolecular radical reaction.

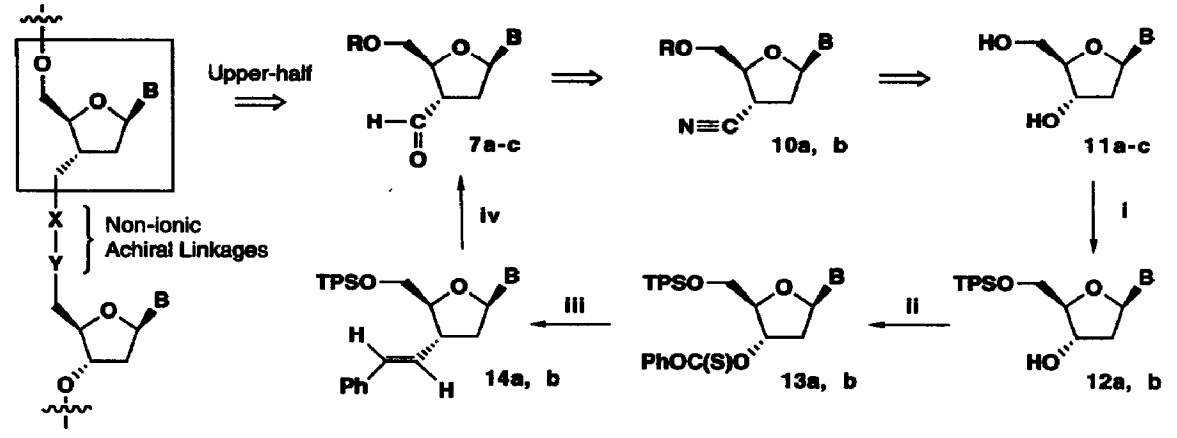
In an earlier paper,[3] we reported the synthesis of 3′-α-*C*-formyl thymidine **7a** via DIBAL-H reduction of 3′-α-*C*-cyanothymidine **10a**. A similar synthesis of 3′-α-*C*-formyl adenine **7b** from **10b** has been reported on a much smaller scale (0.013 mmol of **7b**).[10] In both cases, the key step of *C*-*C* bond formation was found to be non–stereoselective. The reaction also required the use of excessive *tert*-butylisonitrile, an expensive and toxic reagent. Accepting the non stereoselectivity of this reaction, the expense, toxicity and large molar excess (~25 fold) of *tert*-butylisonitrile precludes the scale-up of such a reaction. Therefore, we sought an alternative method of *C*-*C* bond formation, which could provide complete stereocontrol with the possibility of scale-up of **7b** and **7c**.

We chose a mild radical reaction[11] which involved addition of a *C*-centered radical to β-tri-n-butylstannyl-styrene (TBS)[12] followed by β-elimination of a tri-*n*-butylstannyl radical species. The 5′-*O*-TPS protected **12a** was prepared on a large-scale (0.5 mol) from 2′-deoxyadenosine (**11a**) in 80% isolated yield.[13] Acylation[14] of **12a** with phenyl chlorothionoformate in the presence of 4-dimethylaminopyridine provided a good yield (79%) of **13a** as crystalline material (Scheme 1). The radical reaction was carried out with **13a**, TBS, and AIBN in degassed benzene at 80° C for 75 h to furnish **14a** in ~43% isolated yield.[15] The stereochemistry at the 3′-carbon and structure of **14a** was established by COSY and NOESY NMR studies. One of the reasons for high stereoselectivity was attributed to the sterically bulky 5′-*O*-TPS group which forces the entry of the styryl species from the less hindered α-face of the 3'-*C*-center radical. The observed prolonged reaction time may be due to the slower rate of attack of the 3′-*C*-radical on the TBS double bond. This may be due to a less electron-withdrawing phenyl group compared to traditional activated alkenes,[16] and our use of only 1 molar equiv. excess of the TBS reagent compared to 4 equiv. reported in the literature.[11]

A one pot reaction consisting of *cis*-hydroxylation of **14a** with OsO_4 and oxidative cleavage of the product with $NaIO_4$ provided **7b**[17] in 80% yield. The synthesis of 5′-*O*-and N^2-protected **7c** was achieved in four steps from readily available N^2-isobutyryl deoxyguanosine[18] **11c**. A regioselective 5′-*O*-silylation[13] of **11c** with TPS-Cl furnished 81% of **12b**. Phenoxythiocarbonylation[14] of **12b** in a standard manner provided **13b**. The radical reaction of **13b** was tried with 2.7 molar equiv. excess of TBS reagent with an expectation to reduce the reaction time and improve the yield (Scheme 1). Indeed, we were able to speed-up the reaction and found it to be complete in 16 h. However, the yields of **14b** remained the same as that of **14a**, i.e. 45%.[15] Subsequently, the desired building-block **7c** was obtained in a two-step (OsO_4 / $NaIO_4$) one pot reaction from **14b** in 66% overall yield. The structural proof of **14b** was obtained in a manner described for **14a**, confirming the α-configuration at the 3′-position.[17]

In summary, the intermolecular radical reaction described herein represents a promising method to introduce *C*-formyl or *C*-hydroxymethyl equivalents into nucleosides. The methodology appears to be versatile and should be applicable for the scale-up of biologically interesting nucleosides (**1** - **6**) and complex carbohydrates bearing a *C*-*C* bond. Particularly, the *C*-styryl group generated by this method may serve as a 'protected' form of a formyl, hydroxymethyl, or carboxy group during multistep synthetic transformations of natural products.[19] We are in the process of utilizing the described building-blocks for the construction of the mixed base MMI and MDH dimers and their evaluation as phosphate surrogates for AO.[20]

Scheme 1. Synthesis of 3'-α-*C*-formyl nucleosides **7b** and **7c**



	X	Y	B
8	NMe	O	T
9	NMe	NMe	T

7	R	B
a	Tr	T
b	TPS	A
c	TPS	G.ibu

10	R	B
a	Tr	T
b	TBS	A

11	B
a	T
b	A
c	G.ibu

12	B
a	A
b	G.ibu

13	B
a	A
b	G.ibu

14	B
a	A
b	G.ibu

Abbreviations, Reagents and Conditions: T = thymine; A = adenine; G.ibu = N^2-isobutyrylguanine; TPS = *tert*-butyldiphenylsilyl; TBS = *tert*-butyldimethylsilyl; Tr = trityl; **(i) 11b –> 12a**: **11b** (0.5 mol), pyridine (2.0 l), Et_3N (0.51 l), DMAP (0.085 mol), *tert*-$BuPh_2SiCl$ (0.5 mol), r.t., 48 h, 80% of **12a**; **11c –> 12b**: **11c** (0.27 mol), pyridine (1.0 l), *tert*-$BuPh_2SiCl$ (0.30 mol), r.t., 20 h, 81% of **12b**; **(ii) 12a –> 13a**: **12a** (0.25 mol), CH_3CN (1.0 l), DMAP (1.26 mol), PhOC(S)Cl (0.30 mol), r.t., 18 h, 79% of **13a**; **12b –> 13b**: **12b** (0.22 mol), CH_3CN (1.0 l), DMAP (1.12 mol), PhOC(S)Cl (0.26 mol), r.t., 20 h, 44% of **13b**; **(iii) 13a –> 14a**: **13a** (0.18 mol), C_6H_6 (1.5 l), AIBN (10 x 0.067 mol), PhCH=CHSnBu$_3$ (0.36 mol), 80° C, 75 h, 43% of **14a**; **13b –> 14b**: **13b** (0.09 mol), C_6H_6 (0.7 l), AIBN (10 x 0.022 mol), PhCH=CHSnBu$_3$ (0.34 mol), 80° C, 16 h, 45% of **14b**; **(iv) 14a –> 7b, 14b** (0.00521 mol), 1,4-dioxane (115 ml), water (20 ml), OsO_4 (0.0003 mol, 3% in *tert*-BuOH), $NaIO_4$ (0.00024 mol), r.t., 24 h, 80% of **7b**; **14b –> 7c, 14b** (0.019 mol), 1,4-dioxane (0.4 l), water (0.1 l), OsO_4 (1% aq. soln., 0.00117 mol), $NaIO_4$ (0.086 mol), r.t., 26 h, 66% of **7c**.

General procedure for the radical reactions: A stirred solution of xanthate **13a** or **13b** (1 equiv. in benzene; 0.2 M concentration) and TBS (2-4 equiv.) was degassed (argon 40° C). The stirred solution was refluxed gently (80° C) under an argon atmosphere. AIBN (2-4 equiv. added in portions) was added to the reaction mixture in small portions (~10) over a period of 16-75 h until all of the starting material was consumed (TLC, CH_2Cl_2:MeOH; 95:5; v/v; 3-developments). The reaction mixture was cooled to room temperature and the products were purified by silica gel column chromatography [CH_2Cl_2 → CH_2Cl_2:MeOH; 97:3, v/v; excess TBS (detected as the most non-polar spot on TLC) from the radical reaction can be recovered from the column chromatography and redistilled for further use]. Appropriate fractions were pooled and concentrated to furnish **14a** (43%) or **14b** (45%) as a white foam.

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17. Spectral data: **7b** ^{1}H NMR (CD_3CN) δ 9.72 (d, 1, C*H*O, J=2.0 Hz), 8.17 (s, 1, C8*H*), 8.07 (s, 1, C2*H*), 7.30-7.63 (m, 10, arom.), 6.28 (pseudo t, 1, *H*1', J1',2'=5.4 Hz, J1',2" = 5.6 Hz), 5.94 (br s, 2 N*H*2), 4.42 (m, 1, *H*4'), 3.70-3.95 (m, 2, *H*5'), 3.55 (m,1, *H*3'), 2.87 (d, 1, *H*2', J1',2' = 5.5 Hz), 2.83 (d, 1, *H*2", J1',2' = 5.6 Hz), 0.98 (s, 6, C*H*3); ^{13}C NMR ($CDCl_3$) δ 198.96, 155.69, 152.98, 152.83, 138.93, 135.56, 85.24, 81.23, 64.68, 52.16, 32.84, 26.93, 19.24; FAB MS: M/z 502 $(M+H)^+$; **7c**. ^{1}H NMR ($CDCl_3$) δ 12.20 (br s, 1, C2N*H*), 9.77 (br s, 1, N1*H*), 9.65 (s, 1, C*H*O), 7.83 (s, 1, C8*H*), 7.29-7.59 (m, 10, arom.), 6.01 (dd, 1, *H*1', J1',2' = 4.3 Hz, J1',2" = 6.7 Hz), 4.32 (m, 1, *H*4'), 3.83 (m, 2, *H*5'), 3.51 (m, 1, *H*3'). 2.48-2.80 (m, 3, *H*2',2", C*H*-CH3), 1.24 (d, 6, CH-C*H*3, J=6.7 Hz), 1.04 (s, 6, C-C*H*3); ^{13}C NMR ($CDCl_3$) δ 199.00, 179.39, 155.79, 147.85, 137.01, 135.48, 132.53, 130.09, 129.86, 127.91, 127.75, 84.76, 81.07, 64.79, 51.99, 36.25, 32.87, 29.30, 26.87, 19.02; FAB MS: M/z 588 $(M+H)^+$.
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