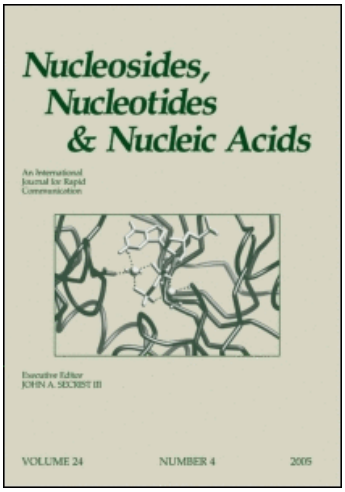

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BIOPHYSICS/BIOCHEMISTRY

SYNTHESIS OF FOUR STEREOISOMERS OF CARBOCYCLIC 5'-NOR D4A AND EVALUATION OF THEIR TRIPHOSPHATES AS SUBSTRATES FOR DNA POLYMERASES

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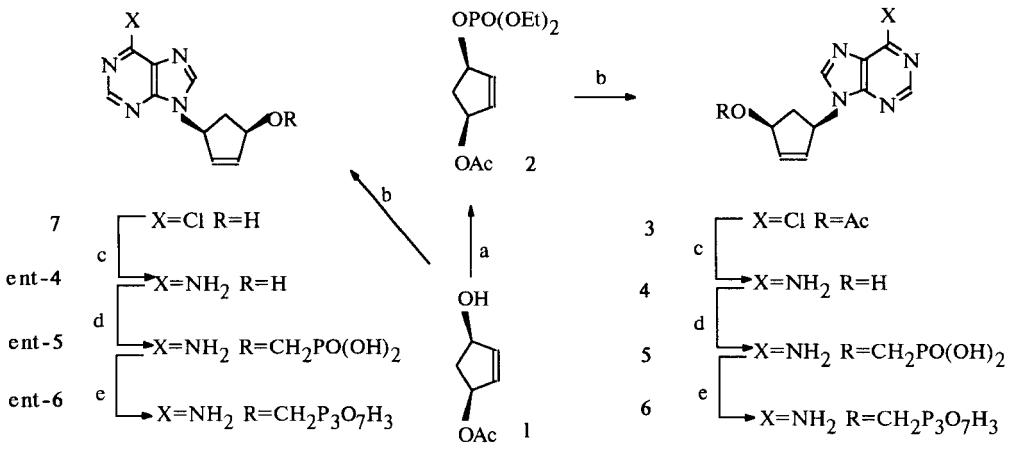
Abstract A method was developed for synthesis of the four stereoisomeric enantiomerically pure 5'-nor carbocyclic nucleosides **4b**, **ent-4b**, **10** and **ent-10** starting from the common enantiomerically pure allylic monoacetate **1**. Nucleoside analogues were converted to the corresponding triphosphate derivatives **6**, **ent-6**, **12**, and **ent-12**. The substrate properties of the latters towards different DNA polymerases were evaluated.

Some carbocyclic analogues of 2',3'-dideoxy-2',3'-didehydro-nucleoside 5'-triphosphates[1] as well as phosphonyldiphosphates of the corresponding 5'-nor derivatives[2] have been recently shown to be substrates for retroviral reverse transcriptases (RTs) and some DNA polymerases. We synthesized all four possible stereoisomers of carbocyclic analogues of 5'-nor D4A (two enantiomeric pairs of diastereomers), as well as their phosphonate and pyrophosphorylphosphonate derivatives to study the chiral recognition by DNA polymerases.

Enantiomerically pure allylic acetate **1**[3] was used as the starting material for synthesis of carbocyclic nucleoside analogues[4]. Reaction of cyclopentene **1** with the sodium salt of 6-chloropurine under Pd(0)-catalysis (Scheme 1) gave **7**. The configuration at the stereocenter adjacent to the acetate group was retained[5]. Compound **7** yielded the adenine derivative **ent-4** by reaction with NH_4OH (yield 42% rel. to **1**, m.p. 196-197^0, acetone). Transformation of **1** into corresponding phosphonate **2** switches the reactivity in the subsequent Pd-catalyzed substitution to give **3** due to the higher reactivity of an allylic phosphate compared with the



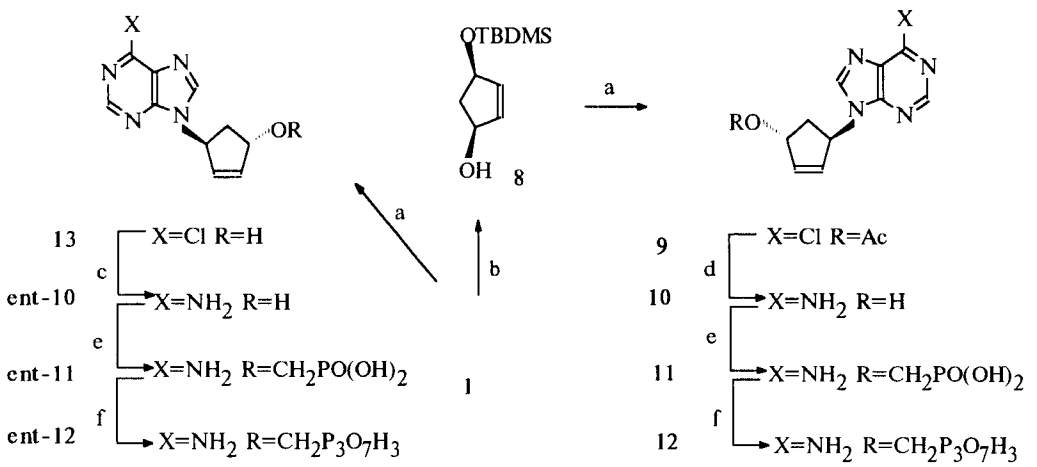
a: $(EtO)_2POCl$, imidazole, MeCN; b: 6-chloropurine, NaH, $Pd(PPh_3)_4$, PPh_3
c: NH_3/MeOH; d: 1.(EtO)(HO)$POCH_2OTs$, NaH, DMF; 2.Me_3SiBr, DMF
e: $P_2O_7H_4 \cdot 2Bu_3N$, CDI, DMF

Scheme 1

corresponding acetate[6]. Compound 3 afforded after subsequent amination **4** (yield 25% rel. to **1**, m.p. 192-193^0, acetone). Treatment of unprotected **4** and **ent-4** with monoethyl p-tolylsulfonyloxymethane phosphonate[7] gave a mixture of 4'-phosphonate and 4',^{1}N -*bis*-phosphonate (25%). Deetherification led to **5** and **ent-5** in a yield of 35%. Pyrophosphorylation *via* imidazole derivatives afforded **6** and **ent-6**, correspondingly.

Mitsunobu reaction[8] of **1** with 6-chloropurine (Scheme 2) proceeded with inversion of the configuration at the carbon atom attached to the hydroxyl group and afforded **13**. Silylation of **1** followed by deacetylation gave **8**, which yielded **9** in Mitsunobu reaction with 6-chloropurine. Amination of **9** and **13** led to adenine derivatives **10** (yield 25% relative to **1**, m.p.207-209^0, ethanol) and **ent-10** (yield 41% rel. to **1**, m.p.208-209^0, ethanol). Further phosphonylation and pyrophosphorylation was performed as described above for *cis* derivatives, but no *bis*-phosphonate was detected. Phosphonate derivatives **11** and **ent-11** were isolated in a yield of 37% relative to corresponding nucleosides.

We evaluated four carbocyclic D4A analogues as substrates for HIV RT and avian myeloblastosis virus (AMV) RT and terminal deoxynucleotidyltransferase



a: 1. t-BuMe$_2$SiCl, imidazole, MeCN; 2. NaOMe/MeOH;
b: 6-chloropurine, PPh$_3$, DEAD;, c: NH$_3$/MeOH; d:1. TBAF, 2.NH$_3$/MeOH;
e:1.(EtO)(HO)POCH$_2$OTs, NaH, DMF; 2.Me$_3$SiBr, DMF
f: P$_2$O$_7$H$_4$·2Bu$_3$N, CDI, DMF

Scheme 2

Table 1. Kinetic constants for **6**, **ent-6**, **12** and **ent-12** in primer extension catalyzed by TdT

	6	**ent-6**	**12**	**ent-12**
K_M, μM	3.1±0.3	5.0±0.2	10.1±0.4	5.2±0.2
V_{max}/V_{max}(**6**)	1.00	0.88	0.45	0.76

(TdT), using DNA and RNA templates. Triphosphates **6** and **ent-6** were efficiently incorporated into the DNA chain by HIV and AMV RTs, whereas **ent-12** displayed weaker substrate properties, and **12** was not recognized by the enzymes. All four tested compounds were recognized by TdT and extended the primer chain, **6** and **ent-6** being better substrates than **ent-12** and, especially, **12**. Kinetic assays were carried out as described in [9]. The kinetic constants in TdT-catalyzed primer extension are summarized in Table 1.

Thus, RTs recognize only *cis* isomers (**6** and **ent-6**), while template-independent TdT incorporates both *cis* and *trans* (**12** and **ent-12**) into the primer chain with a similar efficiency. These data suggest that for the template-independent

enzyme, tight fixation of the nucleic base with respect to the triphosphate residue is not required.

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