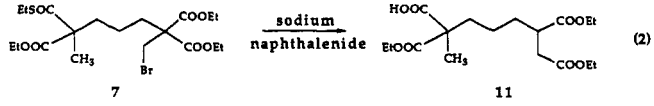
This model makes it possible to compare the outcome of reactions originating from the carbon–cobalt bonded intermediate with those from free radical initiated ones. Moreover, free radicals in this series can be generated by two independent means. In one approach, the carbon–cobalt bonded adduct **3** was photolyzed at 27 °C in water under Ar yielding mainly the direct reduction product **10** (90%), but the rearrangement product **8** was detected and is present to the extent of 2–4%. In the second method, the starting bromide was treated under high-dilution conditions with tri-*n*-butyltin hydride. Reduction of the bromide to **10** was the major reaction course, but rearrangement product **8** was detected in 11% yield. If a further dilution was effected using syringe pump addition, the yield of rearrangement product **8** was 23%. From these experiments, after generation of the presumed free radical intermediate by two independent means and observation of rearrangement product in both instances, it is safe to suggest that the free radical pathway is a competent mechanism for the thermal rearrangement in Scheme III.

We also explored a carbanion pathway by treating the bromide **7** with sodium naphthalenide (eq 2). The product, formed in about 80% yield, was the ester-migrated product **11**;[7a,b] none of the thioester rearrangement product was detected.



7 11 (2)

If the rearrangement of **3** to **8** is carried out in CH_3OD, deuterium is incorporated into the products **8-d** (100% d_1), **10-d** (80% d_1), and **10-d'** (80% d_1) as shown in Scheme IV. The presence of deuterium in **10-d** suggests that a carbanion could play a role in the latter stages of the rearrangement. Thus, in addition to the radical path, a combination of radical and electron transfer steps leading to a carbanion as the penultimate intermediate can also be considered for the mechanism of the rearrangement in Scheme III.

Acknowledgment. This research was generously supported by the Institute for General Medical Sciences of the National Institutes of Health under Grant GM 19906.

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(7) (a) The structure of this substance was established by the full range of spectroscopic tools including exact mass determination. (b) The structure of this product was established by direct comparison with an independently synthesized sample.

Isodideoxynucleosides: A Conceptually New Class of Nucleoside Antiviral Agents

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Among active site directed inhibitors of HIV reverse transcriptase are the "natural" dideoxynucleosides: 2′,3′-dideoxyadenosine (ddA) and its metabolite, 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxyguanosine (ddG), and 2′,3′-dideoxycytidine (ddC).[1-5] However, dideoxynucleosides, particularly those of the purine family, are inherently unstable with respect to cleavage of the glycosidic bond,[6,7] because of the absence of the –I effect of the OH groups and the involvement in hydrolysis of the proximal ring oxygen. The discovery of therapeutically useful antiviral compounds that would be stable both with respect to glycosidic bond cleavage and enzymatic deamination, would be of considerable value in this area. Although numerous analogs of dideoxynucleosides (Scheme I, structure A) are known (see, for example, references 8–14), the synthesis of optically active regioisomeric structures has received much less attention.[15,16] One such class of compounds involves transposition of the base moiety from the natural 1′- to the 2′-position (using normal nucleoside numbering), while maintaining its cis relationship with the CH_2OH (Scheme I, structure B, *S,S* absolute configuration). We wish to report on the design and synthesis of this new family of stable, optically active compounds as antiviral agents.[17]

The chemistry will be illustrated with the case of 4(*S*)-(6-amino-9*H*-purin-9-yl)tetrahydro-2(*S*)-furanmethanol (**5**). A key step of the synthesis involved "glycosylation" of adenine with an appropriately tailored carbohydrate **4**, which was prepared in 17% overall yield from natural D-xylose, through preparation of **2**[18,19] and its conversion in several steps to **3** (Scheme II) involving deoxygenation (of imidazole thiocarbonyl ester of **2** with AIBN/Bu_3SnH), methyl acetal formation (MeOH, HCl), and demethoxylation (HMDS, TMSCl followed by Et_3SiH, TMS trilfate[20]). Condensation of the tosylate **4** with adenine (K_2CO_3,

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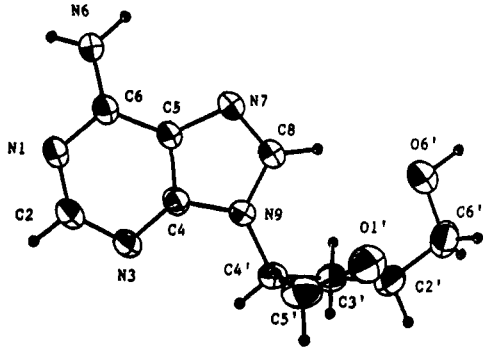
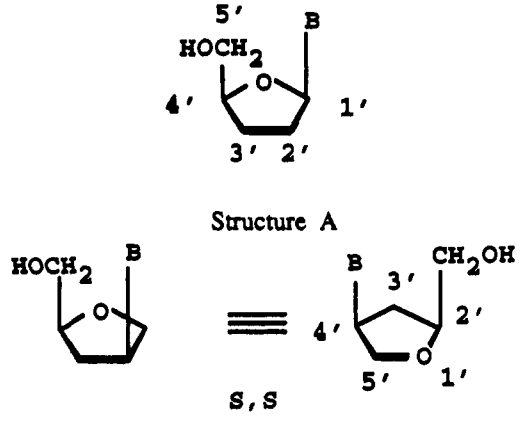


Figure 1. ORTEP plot showing the structure, configuration, and conformation of compound **5**.

Scheme I



18-crown-6, DMF, Δ, 64% purified yield) and deprotection resulted in target molecule **5** (see footnote 21 for physical data). Compound **5** was levorotatory, and the magnitude and sign of this rotation ($[\alpha]_D$ −26.6°) was the same as that for ddA. Final confirmation of structure and stereochemistry came from single-crystal X-ray data (Figure 1), which also showed the base in the preferred anti conformation and the carbohydrate moiety in the C-5′-exo/O-1′-endo conformation.[22,23]

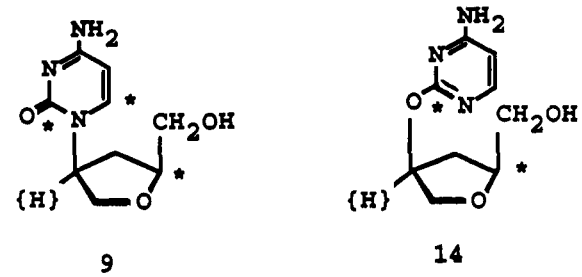
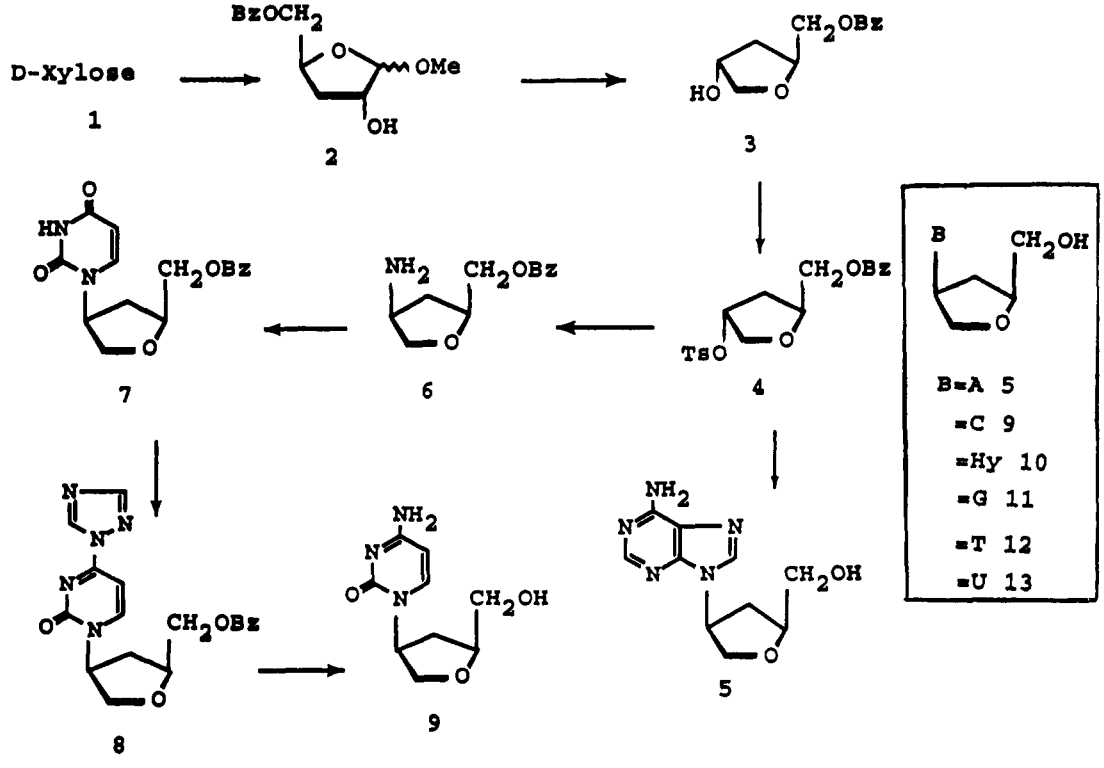


Figure 2. Representative results of 1D *J*-modulated selective INEPT ^1H–^{13}C NMR correlations (irradiations are depicted as {H} and three bond carbon correlations as *).

The isoddI and isoddG compounds, **10** and **11**, were synthesized by coupling **4** with 6-chloropurine and 2-amino-6-chloropurine, respectively, subsequently deprotecting the 5′-benzoate group, and introducing the lactam functionality with aqueous NaOH. The thymine analog **12** was synthesized by direct coupling procedures, although the yields were low (30%) and the conversion was accompanied by bis-O-alkylated and bis-N-alkylated products. Uracil gave similar results (compound **13**). Unequivocal synthesis of isoddU (**13**) was realized through the conversion of **4** to the β-amine **6** via the azide and construction of the uracil ring on **6** by reaction with 3-ethoxy-2-propenoyl isocyanate[24] and subsequent acid-catalyzed cyclization. The cis stereochemical relationship of the base and 2′-CH_2OH was confirmed through the formation of the corresponding cyclonucleoside. Utilization of the direct coupling methodology for the preparation of isoddC **9** led only to the undesired O-alkylated compound **14**. However, **9** could be prepared from **7** in good yields via the triazole derivative **8** followed by ammonolysis[25] and deprotection. Unequivocal differentiation between the N^1- and O^2-alkylated products, **9** and **14**, respectively, came from selective INEPT ^1H-^{13}C correlations[26] which clearly established the position of the "glycosidic bond" in each case (Figure 2).[27]

The stability of the isodideoxynucleosides was also studied. For example, isodideoxynucleoside **5** was found to be very stable with respect to hydrolysis of the glycosidic bond; its $t_{1/2}$ was greater than 16 days at pH 1 (cf. ddA, $t_{1/2} < {}^1/_2$ h at pH 3). It was almost

Scheme II



totally resistant to hydrolytic deamination by mammalian adenosine deaminase (0.0017% of the rate of ddA), and it was a moderate to weak competitive inhibitor of this enzyme ($K_i < 10^{-4}$). It exhibited potent in vitro anti-HIV activity in the low micromolar range in MT-4 cells with no apparent toxicity.

In summary, a conceptually new class of optically active isomeric dideoxynucleosides with *S,S* absolute stereochemistry has been designed, and representative members have been regiospecifically and stereospecifically synthesized. These compounds are stable with respect to glycosidic bond cleavage, and enzymatic deamination and preliminary biological results show that they have significant antiviral potential.

Acknowledgment. We thank the National Institutes of Health for support of this research through AI32851 and the Burroughs Wellcome Company for the collaborative antiviral studies. The high-field NMR spectrometers (Bruker AC-300 and AMX-600) used in this work were purchased in part by funds provided by the NSF and NIH. V.N. thanks the University of Iowa for a Faculty Scholar Award.

Registry No. 1, 58-86-6; **2**, 143191-74-6; **3**, 143191-75-7; **4**, 143191-76-8; **5**, 143191-77-9; **6**, 143191-78-0; **7**, 143191-79-1; **8**, 143191-80-4; **9**, 143288-99-7; **10**, 143191-81-5; **11**, 143191-82-6; **12**, 143191-83-7; **13**, 143191-84-8; **14**, 143191-85-9; 6-chloropurine, 87-42-3; 2-amino-6-chloropurine, 10310-21-1; thymine, 65-71-4; uracil, 66-22-8; adenine, 73-24-5; 3-ethoxy-2-propenoyl isocyanate, 57796-78-8.

(21) Data for compound **5**: mp 152-154 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.09 (m, 1 H), 2.58 (m, 1 H), 3.55 (m, 2 H), 3.99 (m, 3 H), 4.95 (m, 1 H), 5.17 (m, 1 H), 7.25 (s, 2 H), 8.15 (s, 1 H), 8.26 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 33.9, 53.9, 62.4, 71.8, 79.6, 118.7, 138.9, 149.3, 152.3, 155.9; UV (H_2O) λ_{max} 260 nm (13 788); $[\alpha]_D$ = (-)26.6 (c = 0.27, MeOH); mass spectrum, m/z 235 (M^+). Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.77. Found: C, 51.40; H, 5.56; N, 29.66.

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(27) Regiochemistry of glycosylation and identification of specific regioisomers are recurring problems in nucleoside chemistry.

Stereochemistry of Carbon-Phosphorus Cleavage in Ethylphosphonate Catalyzed by C-P Lyase from *Escherichia coli*

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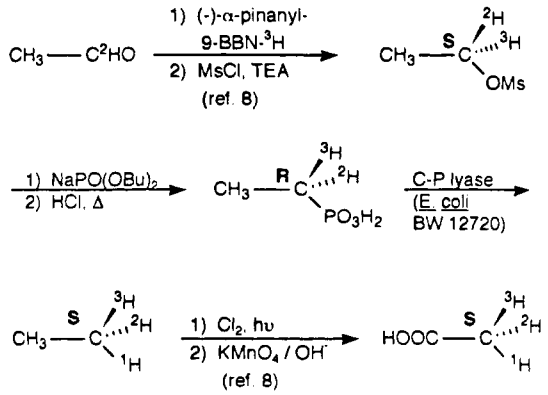
Various microorganisms contain an enzyme, called C-P lyase, which enables them to cleave unactivated alkylphosphonates, such as ethylphosphonate, into the corresponding alkane and inorganic phosphate.[1] Unlike phosphonatase,[2] which cleaves functionalized phosphonates, such as phosphonoacetaldehyde, by a Schiff's base mechanism,[3] the mechanism of action of C-P lyase is poorly understood. Although genes which confer upon *Escherichia coli* the ability to grow on alkylphosphonates have been cloned and sequenced,[4] only one group has so far claimed purification of an enzyme system as two proteins of molecular weights 560 000 and 110 000 Da.[5] The scant information on the mechanism of C-P lyase is derived from in vivo experiments[6] and from model studies.[6a,b,7] All of the hydrogens of deuterated methylphosphonate are retained in the resulting methane,[6b] and the model studies seem to point to a mechanism of bond cleavage leading to an alkyl radical.[6a,b,7] However, attempts to demonstrate a radical mechanism by use of diagnostic substrates (e.g., (cyclopropylcarbinyl)- and *cis*-(1,2-dideuterio-1-propenyl)phosphonate) have not given unequivocal results.[6b,c] The results reported here support the intermediacy of an ethyl radical and establish the steric course of the replacement of phosphorus by hydrogen in the cleavage of ethylphosphonate.

Scheme I



The steric course of the C-P lyase reaction was examined with the substrates, (*R*)- and (*S*)-[1-^{2}H$_1$,1-^{3}H]ethylphosphonate, which were synthesized from previously prepared (*S*)- and (*R*)-[1-^{2}H$_1$,1-^{3}H]ethyl mesylate[8] by reaction with sodium dibutyl phosphite and subsequent acid hydrolysis (Scheme I).[9] Samples of the *R* and *S* isomers (5 μCi, 0.78 μCi/μmol and 6.6 μCi, 0.69 μCi/μmol, respectively) were incubated with *E. coli* BW12720[4] in 5 mL of MOPS medium[10] in crimp-sealed 10-mL vials for 30 h at 37 °C with shaking. GC analysis of the head space[6c] revealed the formation of 85 nmol of ethane per vial from the *R* and 125 nmol from the *S* isomer. The ethane samples were then diluted with unlabeled carrier and converted into acetic acid by halogenation and subsequent hydrolysis and permanganate oxidation as previously described.[8] Configurational analysis by the method of Cornforth et al. and Arigoni and co-workers[11] indicated that

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‡ Harvard University Medical School.

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(12) The *F* value, the percentage of tritium retention in the fumarase reaction of the configurational analysis of chiral acetate,[13] is related to the enantiomeric purity of the methyl group as follows:[14]

$$\text{enantiomeric excess [\%]} = \frac{|F - 50|}{29}100$$

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