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.

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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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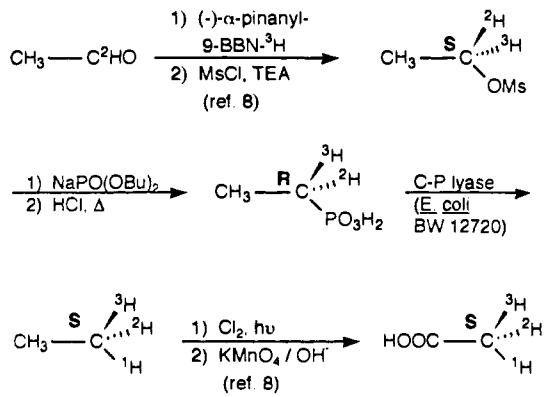
Various microorganisms contain an enzyme, called C-P lyase, which enables them to cleave unactivated alkylphosphonates, such as ethylphosphonate, into the corresponding alkane and inorganic phosphate.[1] Unlike phosphonatase,[2] which cleaves functionalized phosphonates, such as phosphonoacetaldehyde, 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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$$\text{enantiomeric excess [\%]} = \frac{|F - 50|}{29}100$$

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the acetate samples derived from the *R* isomer of ethylphosphonate had predominantly the *S* configuration (*F* value[12] 39.7 = 36% ee *S*), whereas the acetate from the *S* ethylphosphonate contained an excess of *R* isomer (*F* value 59.1 = 32% ee *R*). Hence, the replacement of the phosphonate group by a hydrogen occurs in a retention mode.

The enantiomeric purity of the methyl groups in the product is substantially lower than that of the starting material. Although the enantiomeric excess of the substrate itself was not determined, a reference value was obtained by reacting an aliquot of the intermediate ethyl mesylate of *S* configuration with $LiEt_3BH$ to give ethane which was subjected to the same degradation as the enzymatically generated samples. The *F* value of the resulting acetic acid was 28.1, corresponding to 75% ee *S*.[8] Since the two steps of the transformation of ethyl mesylate to ethylphosphonate are not likely to involve significant racemization, it follows that the C-P lyase reaction must be accompanied by more than 50% racemization at the reacting carbon atom. This provides support for a mechanism involving a carbon-based radical intermediate, i.e., an ethyl radical.[15] The lifetime of the radical intermediate must be long enough for an apppreciable fraction of this species to undergo configurational inversion by rotation around the carbon–carbon bond prior to its acquisition of a hydrogen atom from a source in close proximity to the departed phosphonate group.

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Regiochemistry of the Bisosmylation of C_{60}: "Ortho, Meta, and Para" in Three Dimensions

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The polyfunctionalization of C_{60} through multiple addition reactions can potentially lead to a vast array of novel compounds and materials. To obtain pure compounds from these reactions, both stoichiometry and regiochemistry must be controlled. With the osmylation of C_{60}, we learned how to control stoichiometry with the ligands on osmium.[1,2] Here we report an analysis of the regiochemistry of the bisosmylation of C_{60}, including the first characterization of difunctionalized C_{60} frameworks.[3] Coupling

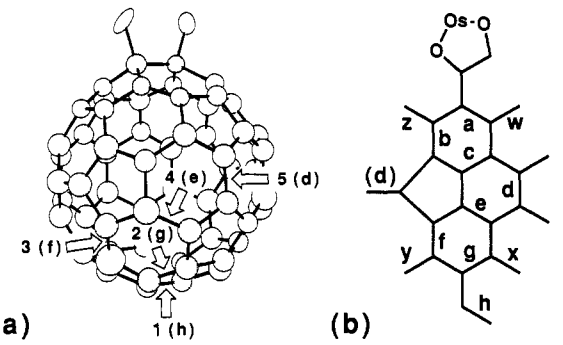

Figure 1. (a) Positions for the second osmyl group in the five regioisomers of $C_{60}(OsO_4L_2)_2$: isomers **3** and **5** are rigorously assigned; isomers **1**, **2**, and **4** are consistent with the observed symmetries and elution orders. (b) Unique cluster bonds in $C_{60}(OsO_4L_2)$ showing the possible second osmylation sites indicated in Table I. Bonds a–h are 6,6 ring fusions; bonds w–z are 6,5 fusions corresponding to C_S isomers.

constants show that the bond alternation character of C_{60} is maintained in these derivatives with band-shaped π-systems.

If totally random, the bisosmylation of C_{60} would yield 54 regioisomers. We know from crystallography[1] and NMR[4] that the first OsO_4 adds to the fusion of two six-membered rings (6,6), reducing the number of possibilities to 24. We observe five regioisomers,[1,5] and to account for this selectivity, we propose that the second OsO_4 also adds to a 6,6 ring fusion (reducing the number of possibilities to eight) and that the hemisphere containing the first osmyl group is sterically inaccessible (reducing the number of possibilities to five) (Figure 1).

The five regioisomers of $C_{60}[OsO_4(py)_2]_2$ were separated by preparative HPLC, converted to their 4-*tert*-butylpyridine analogs, and analyzed by 1D NMR (Table I). The first (least retained) isomer is quite insoluble and could not be analyzed, but the other four isomers gave completely resolved ^{13}C spectra and almost completely resolved ^1H spectra. Either 30 or 32 cluster carbon peaks were observed, corresponding to C_2 symmetry (where none of the carbons lie on the axis of symmetry) or C_s symmetry (where four of the carbons lie on the plane of symmetry), respectively. In agreement with these symmetries, either two or three types of O-bonded carbons and two or three sets of 4-*tert*-butylpyridine resonances were observed. The three sets of peaks found for isomer **5** correspond to the positioning of one of the osmyl groups across the mirror plane. On the basis of these considerations, possible positions for the second osmyl group can be assigned for isomers **2**–**5** (Table I). All of the 6,5 ring fusions can be ruled out except those with C_S symmetry (w, x, y, and z).

To uniquely define two of the 2:1 adducts, we enriched the C_{60} with ^{13}C for 2D NMR analyses. To increase the ^{13}C content beyond the 5% level which we had previously obtained from cored, ^{13}C-packed rods,[4,6] we baked the packed rods at 900 °C at 0.1 mmHg for 24 h before vaporization in the simple contact-arc apparatus at Berkeley and routinely obtained a maximum of 12–14% ^{13}C. With its highly controlled plasma, the NEC instrument can give much higher levels of enrichment.[7] An average ^{13}C content of 11% was used for the 2D NMR experiments.

Isomers **3** and **5** were identified by 2D NMR (Figure 1, Table I). While the INADEQUATE pulse sequence was sufficient to assign the five types of carbons in C_{70}[8] and the 17 types of carbons

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