The filtrate was concentrated, the residue dissolved in methanol, and the solvent again removed. The residue was chromatographed on a silica gel G column (80 g, 4 cm × 14 cm) and eluted with the system C_6H_6–EtOAc (8:2). Fractions (20 ml each) containing a uv absorbing component with R_f's 0.7 (C_6H_6–EtOAc–MeOH, 8:1:1) or 2.5 (C_6H_6–EtOAc, 8:2) were pooled and concentrated, and the crystalline residue was washed with ether and dried (250 mg). It was recrystallized from methanol, and the obtained pure **49** was washed with ether and dried *in vacuo:* mp 191–192°; pmr (DMSO-d_6) τ 8.8 (t, 3, J = 7 Hz, OCH_2CH_3), 8.3 (s, 3, CH_3C), 7.1 (d, sharpens upon addition of D_2O, 2, J = 6 Hz, CH_2C), 5.86 (q, 2, J = 7 Hz, OCH_2CH_3), 5.35 (m, sharpens upon addition of D_2O to a triplet, J = 6 Hz, ArCH), 2.65 (s, 5, ArH). A signal of an exchangeable proton appears at τ 2.25 and that of a second is masked by that of the protons of the phenyl group: uv (H_2O), for the neutral species, λ_{max} 277 nm (ϵ 15.9 × 10^3); λ_{min} 230 nm (ϵ 2.17 × 10^3).

Uv Absorption Properties. The uv spectrum (neutral species) of the various 5-carbalkoxy-3,4-dihydro-2(1*H*)-pyrimidinones showed a major symmetrical absorption peak at 280–310 nm (Table I) with no shift in weak acid except for **7** and **8**. For those unsubstituted at N-1, only a slight shift was observed in weak base. However, with the latter 3,4-dihydro-2(1*H*)-pyrimidinones in stronger basic solutions (~0.5 *N* NaOH), a second peak appeared and rapidly decayed (half-life ~10 min) while the principal absorption underwent ultimately a small hypsochromic and hypochromic (loss of ~15% of o.d.) shift. The fleeting band observed is most likely due to extension of the conjugated system resulting from ionization at N-1 position. Its disappearance is the result of the concomitant hydrolysis of the ester function at C-5. Since the resulting carboxylic acid function is expected to be more acidic than the pyrimidinone, the original conjugated system is reestablished. In strongly alkaline solutions in which both the pyrimidinone ring and the C-5 carboxylic group are ionized, the absorption at 330–345 nm is stable.

Acknowledgments. The authors are indebted to Dr. George Bosworth Brown for his encouragement and continued interest and Dr. Anthony Playtis for many helpful discussions. They also express their appreciation to Ms. Pamela Strotmeyer for excellent technical assistance and Mr. Marvin Olsen for recording the nmr spectra.

Structure and Synthesis of Dihydroxypentyluracil from Bacteriophage SP-15 Deoxyribonucleic Acid

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Abstract: The structural elucidation and synthesis of (*S*)-(+)-5-(4′,5′-dihydroxypentyl)uracil, a base which replaces thymine in bacteriophage SP-15 DNA, is described. The synthesis starting from (*S*)-(−)-malic acid establishes the 4′ configuration as being *S*, which is opposite to that at C-4′ in D-ribose. The optical purity (100%) of a synthetic intermediate has been checked from the nmr of a di-MTPA ester. The synthesis involves a C_1-homologation which can be carried out under the presence of acid-sensitive groups, and which also allows further modifications of functionalities during the course of homologation.

In 1948, 5-methylcytosine was detected in DNA;[2] since then a large number of modified bases have been isolated and identified.[3] About 40 modified bases from tRNA have been identified; structural modifications range from simple methylated bases to the highly modified Y series[4–6] in which there is a third ring fused to a guanine moiety. In contrast, only seven modified bases have been identified in DNA including (*S*)-(+)-5-(4′,5′-dihydroxypentyl)uracil (DHPU).[7–9]

The structural variety of modified bases in DNA decreases as one proceeds up the evolutionary scale from bacteriophage to mammalian tissue. The only modified base detected in the DNA of animals and plants is 5-methylcytosine, while in bacteria 5-methylcytosine and N^6-methyladenine have been identified. Bacteriophages can be divided into two classes depending on the modified base content of their DNA. In one class the DNA contains only trace amounts of modified bases, most commonly 5-methylcytosine and N^6-methyladenine, while in the other class the modified base completely replaces one of the major bases.[3] The bacteriophages SP-15 from which DHPU was recently isolated and φW-14 from which *N*-thyminylputrescine[10] was isolated appear to form a third class on the basis of DNA base composition in that the relatively highly modified bases replace almost half of the thymine.[11]

Bacteriophage SP-15 is a large, generalized transducing phage of *Bacillus subtilis* and *Bacillus licheniformis*. Its DNA was first isolated in 1963[12] and has unusual

(1) (a) Columbia University; (b) Albert Einstein College of Medicine.
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(11) Thymine, however, may be an artefact resulting from the partial degradation of these modified bases by the vigorous acid hydrolysis used during their isolation.
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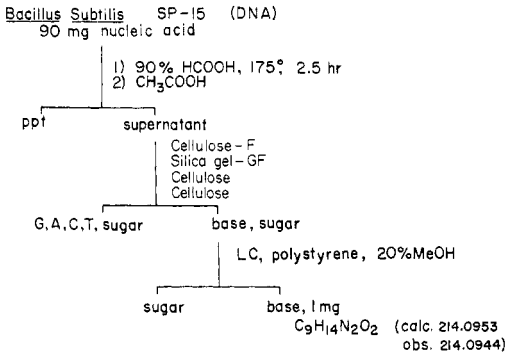


Figure 1. Isolation of DHPU.

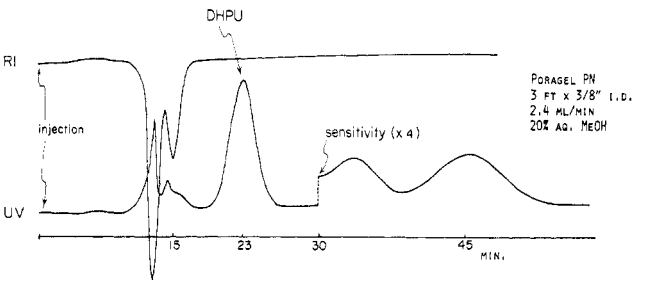
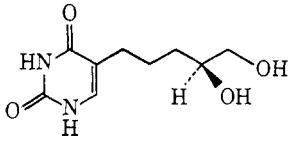


Figure 2. Final purification of DHPU by high speed lc. The two bands around 15 min on the RI (refractive index) trace are due to sugars; the two weak bands at 35–45 min on the uv scale are as yet unidentified constituents.

physical[13] and chemical[7] properties: very low melting temperature (61.5°), very high buoyant density (1.761 g/ml), presence of orcinol-reactive sugar, alkali-sensitive phosphodiester bonds, and an unusual base. Other phage nucleic acids that have shown a discrepancy in base composition as estimated from their denaturation temperature (T_m) and buoyant density measurements have been found to contain modified bases; therefore the existence of a modified base in SP-15 DNA was predicted. The base partially replaces the thymine and represents 12 mol % of all the bases and it is responsible for the low T_m of the nucleic acid, since after alkaline hydrolysis to remove side-chain sugar, the remaining material melts at the same temperature as the native nucleic acid. Upon alkaline hydrolysis the buoyant density decreases to the value expected for its base composition.[7]

We have recently reported in short communications the planar structure[8] and synthesis[9] of the optically active base obtained by formic acid hydrolysis of *B. subtilis* phage SP-15 DNA. In this paper we present details on purification, and structural and synthetic studies of this new pyrimidine, which is the sole DNA base known so far to contain a chiral side chain. The planar structure was elucidated by analysis of spectral data, and the structure and absolute configuration of the new pyrimidine **1** was established as being (*S*)-(+)-5-(4′,5′-dihydroxypentyl)uracil by synthesis from (*S*)-(−)-malic acid.



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Isolation

Perchloric acid hydrolysis[14] of SP-15 DNA completely destroyed the modified base, but 90% formic acid[15] gave five major uv absorbing spots on cellulose tlc. The R_f values of DHPU in solvent systems I [1-butanol–glacial acetic acid–water (4:1:1, v/v)] and II [2-propanol–concentrated hydrochloric acid–water (65:16.6:18.4, v/v)] were, respectively, 0.47 and 0.81.[7] However, as it was subsequently found that system II led to partial decomposition of DHPU when tlc separations were carried out on a preparative scale, the use of mineral acids was avoided in the following procedure.

SP-15 DNA (90 mg) was hydrolyzed in 45 ml of 90% formic acid for 2.5 hr at 175° in three sealed tubes (Figure 1), and the residue was submitted to four tlc separations in solvent I using water–dioxane (1:1, v/v) for recovery by elution. The material eluted from the band corresponding to the new base was finally purified by high-speed reverse phase liquid chromatography (lc) to afford 1 mg of the pure base, amorphous powder.

High-speed lc was crucial for the purification since it allowed removal of non-uv-absorbing material such as sugars and degraded sugars, which constituted the bulk of the tlc eluate (the ratio of base **1** to sugars, etc. was *ca.* 1:10). Moreover, it is impossible to elute small quantities of sample from tlc plates with aqueous systems without eluting traces of exotic tlc material. The lc was run in 20% aqueous methanol on a 3 ft × $^3/_8$ in. i.d. Poragel PN (neutral cross-linked polystyrene resin) column at a flow rate of 2.4 ml/min. The eluates were monitored by a differential refractometer and a 254 nm uv detector (Figure 2).

Spectral Measurements and Planar Structure

High-resolution mass spectroscopy established its molecular formula to be $C_9H_{14}N_2O_2$ (obsd, 214.0944, calcd, 214.0953). The similarity of the uv of **1** to that of thymine rather than isothymine (6-methyluracil)[16] suggested it to be a 5-alkylated uracil (Table I). Moreover, the wavelength and small amplitude of the CD Cotton effect, $\Delta\epsilon_{264}$ +0.6 (in H_2O), indicated that the chiral center was at some distance from the aromatic chromophore. A comparison of the ir (KBr) of **1** and thymine showed the absence of a band at 1380 cm^{-1}

Table I. Uv Data

	Acid and neutral, λ (ε) nm	Base, λ (ε), nm	pK_a
Natural			
(*S*)-(+)-DHPU I	206 (9550), 264 (7050)	291 (5900)	9.7[a]
Synthetic			
(*S*)-(+)-DHPU	207 (9500), 265 (7700)		
Thymine	207 (9550), 265 (7940)	291 (5250)	9.9
Isothymine	261 (10,000)	277 (6760)	9.7

[a] Apparent pK_a.

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(15) G. R. Wyatt and S. S. Cohen, *J. Biochem.*, **55**, 774 (1953).
(16) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).

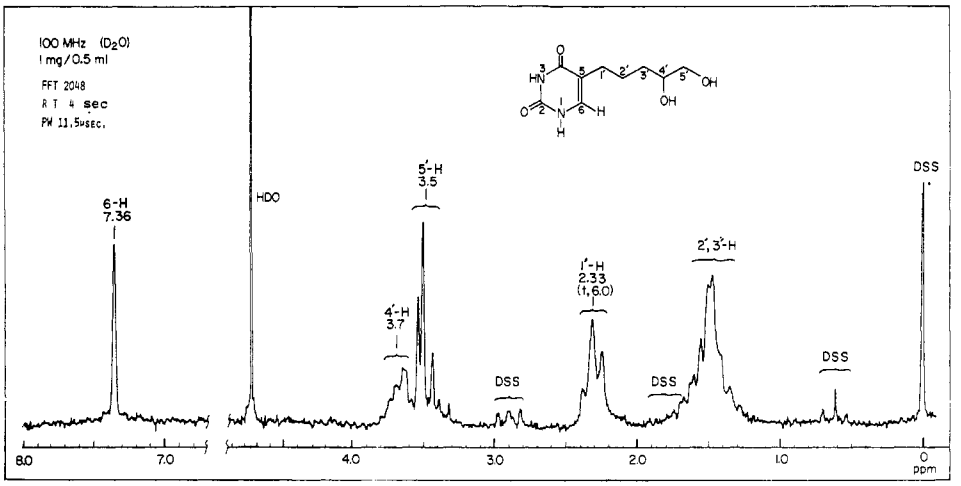

Figure 3. Nmr of DHPU in D_2O (100% D atom): JEOL JNM-PS-100, 100 MHz, Fourier transform system.

Table II. Nmr Data

	6-H (1 H)	1′-H (1 H)	2′-H/3′-H (4 H)	4′-H (1 H)	5′-H (2 H)	Labile H (4 H)
DMSO-d_6	7.20 (br, d, J = 6 Hz)	2.16 (br, t, J = 6 Hz)[a]	1.4 (m)	4.1	3.3[a]	N^1–H, 10.72 N^3–H, 11.05 OH, 6.38 OH, 6.73
100% D_2O	7.36 (s)	2.33 (t, J = 6 Hz)	1.5 (m)	3.7 (m)	3.5 (m)	

[a] Temperature increased to 53°.

(methyl group in thymine) and presence of two C–O stretching absorptions (1090 cm^{-1}, secondary hydroxyl; 1068 cm^{-1}, primary hydroxyl) in **1**, both absent in thymine.

The 220-MHz spectrum of DHPU in DMSO-d_6, which in spite of careful manipulation contained a considerable amount of H_2O ($W_{1/2}$ = *ca.* 0.5 ppm at 3.5 ppm), showed the presence of four labile protons (Table II). The two broad signals at lower field were in close agreement with the N^1 and N^3 protons of thymine at 10.55 and 10.95 ppm (DMSO-d_6), respectively. The remaining two labile protons could be ascribed to the two extra oxygens (as compared to thymine) shown by the molecular formula, and hence to two hydroxyl groups. The 7.20-ppm broad doublet ($J_{6\text{–H}/N^1\text{–H}}$ = 6 Hz) became a singlet upon addition of D_2O, a behavior closely resembling that of the 6-H of thymine at 7.23 ppm (dq, $J_{6\text{–H}/N^1\text{–H}}$ = 6 Hz, $J_{6\text{–H}/CH_3}$ = 2 Hz). This provides corroborative evidence for the 5-substituted uracil nucleus.

It was not possible to carry out a clear analysis of the aliphatic proton region in the DMSO-d_6 nmr discussed above due to: (i) the strong water band as compared to the weak peaks of the sample (less than 1 mg), and (ii) high noise level which prevented double irradiation. Accordingly, special precautions were taken by means of the tube assembly shown in Figure 7 (Experimental Section) to obtain a spectrum in D_2O containing the least amount of H_2O.

The "100% D_2O" solution thus prepared gave a satisfactory spectrum following 2048 FFT scans (Figure 3). The molecular formula requires a $C_5H_{11}O_2$ side chain, *i.e.*, a C_5 chain with two hydroxyl groups. It is clear that the nmr peak pattern is consistent only with a straight C_5 chain with two terminal hydroxyl groups. Namely, the slanted triplet shape of the 2.33 ppm allylic methylene (1′-H) shows that the adjacent C-2′ carries two protons and that these are contained in the high-field four proton signal at 1.5 ppm. The 3.7- (one proton) and 3.5-ppm (two proton) signals are clearly due to carbinyl protons, and therefore the presence of secondary and primary hydroxyl groups is evident. Moreover, the shape of the 3.5-ppm signal shows that it is coupled to the 3.7-ppm signal, *i.e.*, ABC system (where C is further coupled), and not to the distant 1.5-ppm signal (CH_2 groups).

Structure **1** is fully supported by high-resolution mass spectral data (Figures 4 and 5). Formation of the base peak (h) at *m/e* 138 is rationalized by the six-membered transition state involving a hydrogen γ to the hydroxyl;[17a] in the present case this fragmentation is particularly favored because it leads to a styrenoid cation (h).[17b] Retro-Diels–Alder peaks j and k are also well documented for uracil derivatives.[17c,18]

Synthesis

The synthesis of optically active DHPU was first attempted by linking a 2,4-dihydroxypyrimidine moiety to a five-carbon side chain carrying oxygen functions at C-1, -2, and -5, where the absolute configuration at C-2 was known. The starting materials chosen for the C_5 unit were glutamic acid, its γ-methyl ester, and pentahomoserine (γ-hydroxynorvaline). Retention of configuration at C-2 upon replacement of the amino group with a hydroxyl group in α-amino acid is well established;[19,20] in the present case the course of the

(17) (a) H. Budzikiewicz, C. Djerassi, and D. H. Williams in "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p 84; (b) *ibid.*, p 99.

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(19) D. Brewster, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, *Nature (London)*, **166**, 178 (1950).

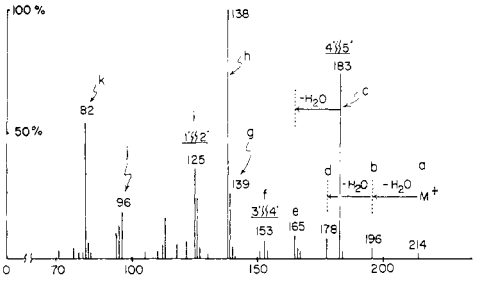


Figure 4. Mass spectrum of DHPU. All compositions are based on exact mass measurements determined on an MS-9, 70 eV, PFK standard, $m/\Delta m$ = 10,000; all observed values are within 3 nm of calculated values.

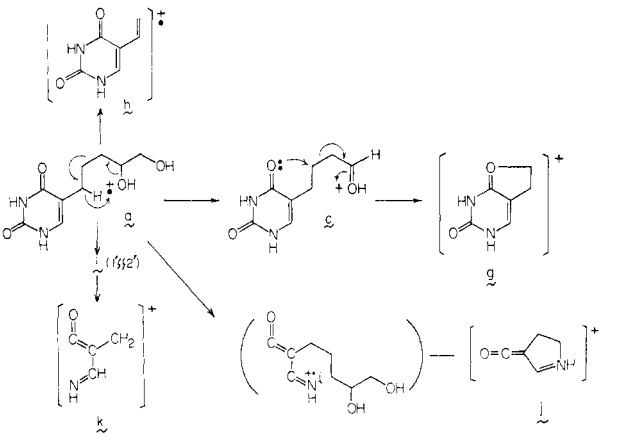


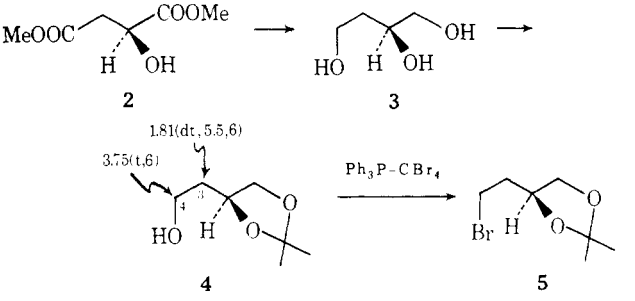
Figure 5. Postulated structures of several ions observed in the mass spectrum of DHPU.

reaction could be complicated by neighboring participation of the terminal function. Nevertheless, as it was conceivable that the absolute configuration of the terminal α-glycol unit could be deduced by a method now being developed,[21] the synthesis of 1,2,5-pentanetriol from each of the three compounds described above was attempted. However, the deaminations of glutamic acid and its α-methyl ester were unsuccessful due to formation of complex mixtures. The conversion of pentahomoserine to 1,2,5-pentanetriol by nitrosation also failed due to ready cyclization to give a five-membered ether, tetrahydro-2-furancarboxylic acid.[22]

In view of the difficulty involved in starting from a C_5 unit the scheme was changed to start with a C_4 chain and to carry out a homologation at a later stage. In addition, as there is evidence that a sugar is attached to the terminal α-glycol group in native DNA,[23] it would also be desirable to devise a method which would enable homologation to be carried out in the presence of a sugar unit. Thus it was planned to start from optically active malic acid, extend the chain by one carbon, and link this to the aromatic nucleus. Although several efficient C_1-homologation reactions are known,[24-27] it was felt that they might not be suitable if modification of the α-glycol group to incorporate a sugar was desired.

In the present scheme, bromide **5** was condensed with phenylacetaldehyde through a Wittig reaction to give the homoconjugated **6**, which was isomerized to **7**. The stability of styrenoid **7**, a potential C_5 aldehyde, would allow facile attachment of sugars to the α-dioxygenated moiety. Ozonolysis of **7** yielded aldehyde **8**; the C_4-bromide **5** was converted into C_5-aldehyde **8** in 50% overall yield.

(*S*)-(−)-Malic acid was esterified to the known dimethyl ester **2** to facilitate reduction with lithium aluminum hydride. The triol **3** thus obtained upon reduction (50% yield after distillation) was converted to its acetonide (95%), which according to nmr studies consisted only of the five-membered species **4**. The absence of the six-membered isomer was not unexpected in view of the favored formation of five-membered isopropylidene derivatives (but six-membered benzylidene derivatives) in sugar chemistry, and indeed there was no detectable amount of a second product as checked by nmr, and nmr after addition of trisdipivalomethanatoeuropium(III) [Eu(dpm)$_3$]. The coupling pattern of the signals at 3.7 ppm (t, J = 6 Hz, 4-H) and 1.81 ppm (dt, J = 5.5 and 6 Hz, 3-H) requires a freely rotating $-CH_2CH_2CH-$ moiety which is clearly consistent only with the structure **4** shown.



The triol acetonide **4** was brominated to **5** in satisfactory yield (77%) under mild neutral conditions by addition of triphenylphosphine (1 molar equiv) in dichloromethane to a mixture of acetonide **4** and carbon tetrabromide (1 :1.5 equiv) in the same solvent. It was essential to add triphenylphosphine at a very slow rate in order to achieve a satisfactory yield. Otherwise evidence from tlc indicated that the reaction was incomplete and accompanied by considerable hydrolysis.

Chlorination with carbon tetrachloride and triphenylphosphine is well suited for application to acid-sensitive compounds,[28] but the chlorides thus formed are not suited for further Wittig reactions. On the other hand, there have been only a limited number of transformations using carbon tetrabromide,[29-31] and the yields are low unless 2 molar equiv of both reagents are em-

(20) D. Brewster, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, *Nature (London)*, **166**, 179 (1950).
(21) CD measurement with addition of Ni(AcAc)$_2$: J. Dillon and K. Nakanishi, unpublished results.
(22) Steric course of deamination of pentahomoserine will be published elsewhere.
(23) A nucleotide can be isolated from SP-15 DNA following digestion with exonuclease I from *E. coli;* this nucleotide, upon acid hydrolysis, yields deoxyribose, DHPU, and a sugar of unknown structure.
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(27) A. I. Meyers, A. Nabega, H. W. Adickes, I. R. Politzer, G. R. Malone, A. C. Kenelesky, R. L. Nolen, and R. C. Portnoy, *J. Org. Chem.*, **38**, 36 (1973).
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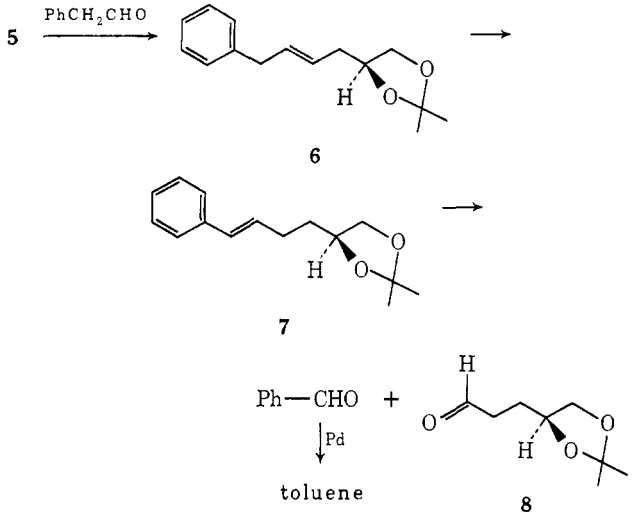
(30) R. G. Weiss and E. I. Snyder, *J. Org. Chem.*, **36**, 403 (1971).
(31) L. Brener, J. S. Ward, and R. Pettit, *J. Amer. Chem. Soc.*, **93**, 4957 (1971).

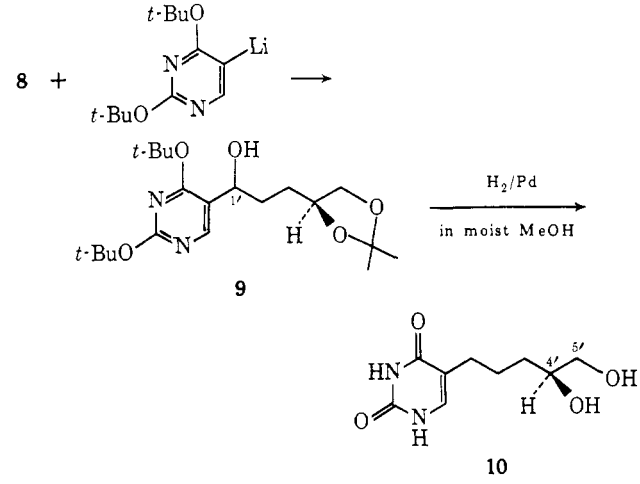
ployed.[32] Two moles are required since triphenylphosphine and carbon tetrabromide react rapidly to give triphenylphosphine dibromide and triphenyldibromomethylenephosphorane (a nonbrominating agent);[33] the former then reacts with alcohols to yield the bromide, hydrogen bromide, and triphenylphosphine oxide. In order to avoid formation of triphenylphosphine dibromide and hence hydrogen bromide from the intermediary complex $Ph_3P^+(Br^-)CBr_3$, it is necessary to let the salt react with the alcohol before it reacts with a further mole of triphenylphosphine. Indeed, a reaction of acetonide **4** with triphenylphosphine dibromide gave preponderantly the cleaved glycol bromide.

Heating (100°, 2 days) of the bromide **5** and triphenylphosphine in ether gave a white precipitate of the triphenylphosphine salt, which was washed with ether. The hygroscopic salt could not be induced to crystallize. Without further purification it was converted into the phosphorane with *n*-butyllithium in tetrahydrofuran under nitrogen. To this wine-colored solution, 1 molar equiv of phenylacetaldehyde was added dropwise at −70°; this low temperature was necessary to prevent proton abstraction from the aldehyde by the phosphorane.[34] After the color had faded the mixture was warmed to room temperature for 2 hr to complete the reaction. This gave the homoconjugated *E* compound **6** containing a trace of *Z* isomer in a combined 60% yield. The formation of the *E* and *Z* mixture is immaterial as the double bonds are destroyed later. The mixture was treated with potassium *tert*-butoxide in dimethyl sulfoxide[35] at room temperature for 2 hr to give the thermodynamically more stable isomer **7** exclusively (95% yield, all *E* isomer). Evidence for exclusive formation of the *E* isomer was gained from the nmr spectrum which showed only signals due to trans vinyl protons at 6.21 (dt, J = 6 and 15.5 Hz, β proton) and 6.38 ppm (d, J = 15.5 Hz, α proton).



Ozonization of **7** in dry methanol followed by reduction of the ozonide with excess dimethyl sulfide[35] gave a mixture of aldehyde **8**, benzaldehyde, and dimethyl sulfoxide. Difficulties were encountered in isolating the aldehyde because of its decomposition on the tlc plate, which presumably was enhanced by the presence of benzoic acid resulting from air oxidation of benzaldehyde; moreover, the two aldehydes could not be separated by distillation because of close boiling points. However, **8** could be readily isolated by submitting the mixture to hydrogenolysis with a large excess of palladium black in dry methanol, which selectively converted benzaldehyde into toluene. A large excess of palladium black was necessary in order to counteract the dimethyl sulfoxide, a weak catalyst poison. Evaporation of the hydrogenolysis solution after removal of dimethyl sulfoxide gave pure aldehyde **8** in 95% yield.[36]

Condensation of aldehyde **8** and 1.1 equiv of 5-lithio-2,4-di-*tert*-butoxypyrimidine[37] in tetrahydrofuran at −70° under argon yielded product **9** (95%) as a *ca.* 1:1 epimeric mixture at C-1. This was deduced from the observation that the aromatic 6-H nmr signal appeared as two singlets (*ca.* 1:1 ratio) at 8.18 and 8.20 ppm, both of which were sharpened upon irradiation of the 1′-H at 4.70 ppm.



Finally, stirring acetonide **9** with palladium black under hydrogen in moist methanol for 18 hr at room temperature resulted in hydrogenolysis of the allylic hydroxyl, accompanied by hydrolysis of the *tert*-butyl and the acetonide groups (quantitative yield). Removal of acetonide groups in compounds such as **4**–**7** requires acid conditions. The contrasting facile hydrolysis of the acetonide **9** in moist methanol is presumably caused by intramolecular catalytic action of the 2,4-dihydroxypyrimidine proton generated by fast cleavage of the more labile *tert*-butyl groups. The physical constants of **10** thus obtained were as follows: mp 225–226°; uv (in H_2O) 207 nm (ϵ 9500), 265 (7700); CD (in H_2O) $\Delta\epsilon_{265}$ +0.5. The properties of synthetic and natural DHPU were in agreement in every respect. The transformation of aldehyde **8** into DHPU **10** was similarly carried out on a tenfold scale on *dl*-aldehyde **8** (Scheme I) with correspondingly high yields.

The optical purity of base **10** was checked at the stage of styrenoid **7** since the insolubility of base **10** in nonpolar solvents precluded usage of the chiral nmr shift reagents; in addition, it was expected that preparation of diastereomeric derivatives at C-4′ in **10** would be

(32) J. Hooz and S. S. H. Gilani, *Can. J. Chem.*, **46**, 86 (1968).
(33) F. Ramirez, N. B. Desai, and N. McKelvie, *J. Amer. Chem. Soc.*, **84**, 1745 (1962).
(34) A. Maercker, *Org. React.*, **14**, 350 (1965).
(35) J. J. Pappas, W. P. Keaveney, E. Gaucher, and M. Berger, *Tetrahedron Lett.*, 4273 (1966).
(36) In a medium scale experiment, preferably the dimethyl sulfoxide should be removed prior to hydrogenolysis.
(37) D. M. Brown, M. G. Burdon, and R. P. Slatcher, *J. Chem. Soc. C*, 1051 (1968).

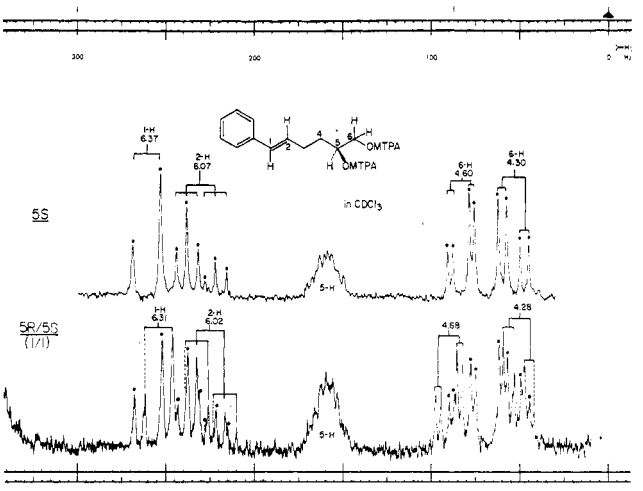
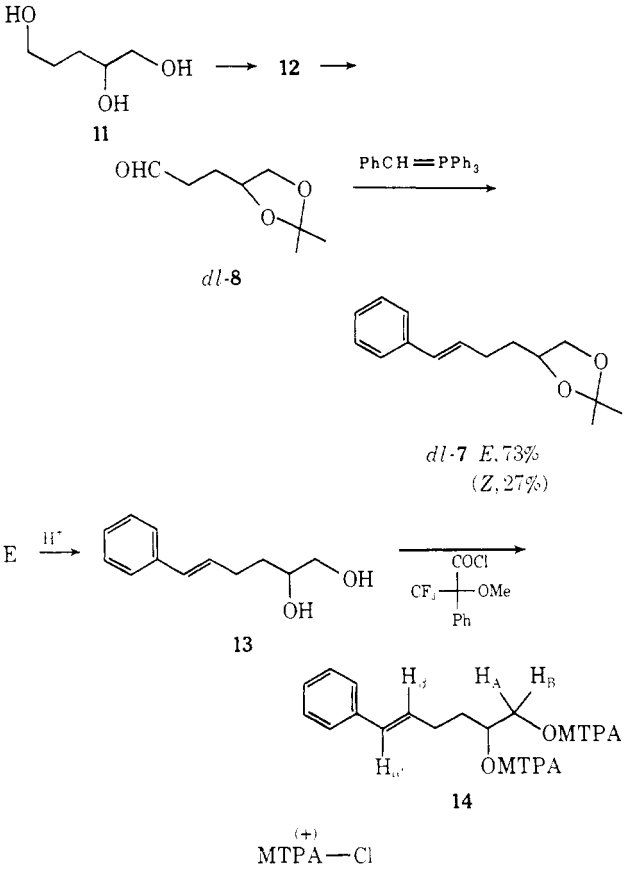


Figure 6. Partial nmr (100 MHz, Varian HA-100) of 5-(*R*)-/5-(*S*)- and 5-(*S*)-**14**.

Scheme I

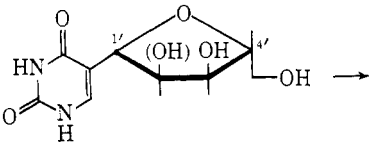


complicated by the reactivity of the ring nitrogens. Racemic **7** was prepared according to Scheme I. The triol **11** derived from tetrahydrofurfuryl alcohol[38] was converted to acetonide **12**, followed by Collins oxidation to give *dl*-acetonide aldehyde **8** (62% overall yield). The aldehyde was condensed with triphenylbenzylidenephosphorane to give 73% *dl*-**7** and 27% of its *Z* isomer in a combined 78% yield which could be readily separated by gas–liquid chromatography.

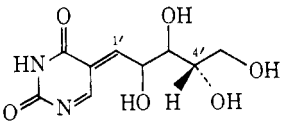
Addition of nmr chiral shift reagents to *dl*-**7**[39a,b] resulted in hardly any change in shifts, presumably because of the steric bulk of the *gem*-dimethyl group of the acetonide. The *dl*-*E* isomer **7** was therefore hydrolyzed to the α-glycol **13** but the two antipodal glycols also could not be distinguished by nmr (^{1}H and ^{13}C) after addition of the chiral shift reagents used above.

The *dl*-glycol **13** was therefore acylated with (+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride[40a,b] to give a (1 :1) mixture of the two diastereomers **14** which were distinguishable by two sets of nmr peaks for the vinylic and terminal methylene protons (Figure 6). The ^{19}F nmr, however, showed only a single peak.

The optically active specimen **7** from (*S*)-(−)-malic acid was similarly hydrolyzed and converted to the di-



A



B

MTPA ester corresponding to **14**. The ester showed only one set of nmr peaks corresponding to one of the diastereomers (Figure 6). Partial racemization during the conversion of **7** to **10** can be discounted so that the final product **10** can also be regarded as optically pure.

The correspondence in sign of the Cotton effect and the other chemical and physical properties of synthetic (+)-**10** with those of the natural base establish the full structure of this unique DNA base, including the *S* configuration of the chiral center.

It should be noted that this configuration is opposite to that of the C-4′ of ribose. A possible biogenetic precursor is pseudouridine A (or 2′-deoxypseudouridine) which could be converted to DHPU by cleavage of the ether ring followed by reduction. It is known that the sugar moiety of pseudouridine undergoes facile epimerization at C-1′ and interconversion to the pyranose form, presumably through the intermediate B;[41] however, as the C-4′ configuration in B is opposite to that of DHPU **1**, cleavage of pseudouridine must occur with inversion by nucleophilic attack at C-4′ in order to give DHPU of natural *S* configuration. Another possible biogenetic precursor for the side chain is glutamic acid.

Experimental Section

Molecular formulas were obtained by microanalyses (Schwarzkopf Microanalytical Laboratory, Inc.) or high resolution mass spectrometry (CEC 21-110 B). Melting points were determined on a hot-stage microscope and are uncorrected, as are boiling points. Infrared (ir) spectra were measured on a Perkin-Elmer 621 spectrophotometer. Ultraviolet (uv) spectra were recorded on a Cary 15 instrument. Circular dichroism (CD) spectra were obtained using a Cary 60 instrument. Nuclear magnetic resonance (nmr) spectra were obtained with a Varian HA-100 spectrometer. A Varian Aerograph 90-P instrument was utilized for vapor

(38) C. L. Wilson, *J. Chem. Soc.*, 49 (1945).

(39) (a) [Tris(3-trifluoromethylhydroxymethylene)-*d*-camphoratojeuropium(III): H. L. Georing, J. N. Eikenberry, and G. S. Kermer, *J. Amer. Chem. Soc.*, **93**, 5913 (1971). (b) [Tris(3-heptafluoropropylhydroxymethylene)-*d*-camphoratojeuropium(III): R. R. Fraser, M. A. Petit, and J. K. Saunders, *Chem. Commun.*, 1450 (1971).

(40) (a) J. A. Dale, D. L. Dull, and H. S. Mosher, *J. Org. Chem.*, **34**, 2543 (1969); (b) J. A. Dale and H. S. Mosher, *J. Amer. Chem. Soc.*, **95**, 512 (1973).

(41) R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, N. Y., 1971, p 395.

phase chromatography. Column chromatography was carried out on silica gel (Baker Chemical Co. 3405). Thin-layer separations were performed on silica gel or cellulose precoated plates (Analtech Co.).

Spectra and separations were obtained using the instruments and materials described above unless otherwise specified.

Preparation of SP-15 DNA. Purification of *B. subtilis* phage SP-15 and DNA was essentially carried out as described by Szybalski.[42]

The DNA was extracted from the purified phage with phenol saturated with TMK buffer at pH 8. Further purification was carried out with chloroform–isoamyl alcohol (24:1) three times, followed by ethanol precipitation.

Hydrolysis of the DNA and Purification of Modified Base. All of the tlc plates for the following purifications were washed with acetone, chloroform–methanol (10 : 1, v/v), and *n*-BuOH–AcOH–H_2O (4:1:1, v/v) consecutively before use.

Ninety milligrams of the DNA was dissolved in 45 ml of 90% formic acid and hydrolyzed in three 30-ml sealed tubes at 175° for 2.5 hr. After the solution was cooled, the formic acid was evaporated. The residue was dissolved in 20 ml of acetic acid, the solution was filtered, and the filtrate was spotted onto four tlc cellulose F plates (Analtech precoated plates, 500 μ, 20 × 20 cm). Developing solvent was *n*-BuOH–AcOH–H_2O (4:1:1, v/v). Several uv absorbing bands were visible after development; the top two were combined and eluted with 80 ml of dioxane–water (1:1, v/v), and rotary evaporated. The residue was respotted onto two plates of silica gel GF (Analtech precoated plate, 250 μ, 20 × 20 cm) and developed in *n*-BuOH–AcOH–H_2O (4:1:1, v/v). Although the modified base and thymine could not be separated, cytosine, adenine, and guanine were readily separated by this method. The mixture of DHPU and thymine was spotted onto six cellulose plates (Analtech precoated plate, 100 μ, 20 × 20 cm) and developed with *n*-BuOH–AcOH–H_2O (4:1:1, v/v), and the two uv absorbing bands of DHPU (R_f 0.52) and thymine (R_f 0.70) were scraped and eluted with dioxane–water (1:1, v/v). The final tlc purification was carried out on a 250-μ cellulose plate (Analtech precoated plate, 20 × 20 cm) using the developing agent mentioned above. In order to remove the cellulose particles eluted from the tlc plates, and as a further check on purity, the eluate was chromatographed in 40% aqueous methanol on a column of poragel-PN (3 ft × $^3/_8$ in. i.d.) with a Waters ALC-100 high-speed liquid–liquid chromatograph. Removal of the solvent *in vacuo* gave approximately 10 mg of colorless oil. Although the oil had a uv absorption at 265 nm, the nmr (in DMSO-d_6) showed that the bulk of material was sugars and their degradation products. Consequently the separation by high-speed reverse phase liquid–liquid chromatography was repeated, this time using 20% aqueous methanol, flow rate 2.4 ml/min, and differential refractometer and uv for monitoring the sugars and DHPU, respectively (Figure 2). Evaporation of the main uv active band afforded 1 mg of pure modified base as an amorphous powder.

Spectroscopic Measurement of the Modified Base (DHPU). All spectroscopic measurements were carried out on 1 mg of the pure base; high-speed chromatography (reverse phase) was routinely used to recover the base after each spectroscopic measurement.

Nmr Measurements. In Pyridine-d_5. The proton nmr spectrum of the base was obtained at 36° on a Varian HA-100 spectrometer using pyridine-d_5 solvent and tetramethylsilane (TMS) as internal standard. The sampling was carried out under nitrogen atmosphere using a disposable plastic drybox. A micro tube (0.1 ml) and 99 atom % D pyridine was used for the measurement. The obtained nmr spectrum showed peaks at 1.85 (4 H, m, 2′-H and 3′-H), 2.54 (2 H, br s, 1′-H), and 3.56–4.56 ppm (3 H, 4′-H and 5′-H). A big water peak appeared at 4.9 ppm and the aromatic region was obscured by pyridine peaks.

In DMSO-d_6. The 220-MHz spectra were obtained at 18–53° on a Varian HR-220 spectrometer using 100 atom % D DMSO (Diaprep Co.) as solvent and TMS as internal standard. The sampling method and nmr tube were the same as above. The spectrum obtained at 18° showed signals at 1.4 (4 H, m, 2′-H and 3′-H), 2.16 (2 H, br s, 1′-H), 3.50 (strong water peak), 4.1 (1 H, m, 4′-H, partially obscured by water peak), 6.38 (OH), 6.73 (OH), 7.20 (1 H, d, J = 6 Hz, 6-H), 10.72 (1 H, br s, NH), 11.05 ppm (1 H, s, NH). The 5′-methylene protons were covered by the strong water peak. After addition of D_2O, signals at 6.38, 6.73, 10.72, and 11.05 ppm (labile protons) disappeared and the 6-H signal at 7.20 ppm was converted to a singlet. The water peak sharpened and shifted to 3.7 ppm upon raising the temperature to 53°, and this made the 5′-methylene protons appear at 3.28 ppm as an apparent doublet (J = 5 Hz); the broad signal at 2.16 ppm (1′-H) also became better defined to give a broad triplet (J = 6 Hz).

Decoupling experiments were unsuccessful because all measurements had to be carried out under a high gain due to the small amount of sample.

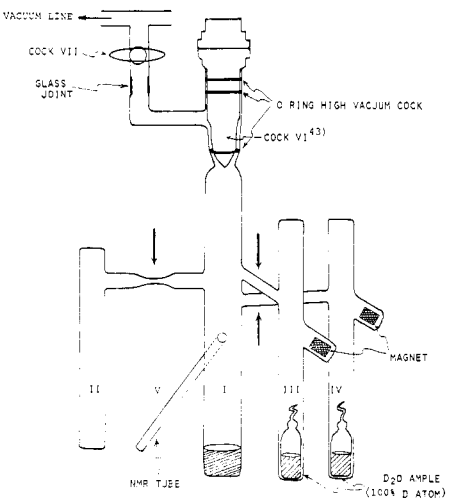


Figure 7. Sealed system for nmr sampling.

In D_2O (Fourier Transform). A JEOL JNM-PS-100 spectrometer equipped with a fast Fourier transform (FFT) unit was employed for recording the proton nmr shown in Figure 3 (2048 scans, RT 4/sec, PW 11 μsec), solvent 100 atom % D D_2O (SIC Co.), internal standard sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

The following precautions were taken in order to obtain a FFT nmr spectrum devoid of H_2O peaks. The sample and *ca.* 0.1 mg of DSS were dissolved in 1 ml of D_2O and transferred to tube I in the assembly shown in Figure 7. The assembly was connected to a mercury diffusion pump, tube I was frozen in Dry Ice–acetone, and the Teflon high vacuum cock VI[43] was opened and the pressure was lowered to 10^{-6} mm. Cock VI was closed and tube I was degassed by gradual warming. After several repetitions of degassing, the solution was transferred to tube II by immersing tube II in liquid nitrogen. In order to avoid loss of sample through freeze-drying, tube I was kept warm so that it did not solidify.

Tube II was disconnected by fusing at the point shown by the arrow in Figure 7, cocks VI and VII were opened and moisture was thoroughly removed by flaming the system. Cock VI was closed, the ampoule in tube III was cracked with the magnet, and the D_2O was transferred to tube I after degassing. The D_2O was retransferred to tube III and the latter was disconnected by fusion similarly, half of the ampoule in tube IV (0.5 ml) was transferred to tube I, tube IV was disconnected, cock VI was closed, the system was disconnected from the vacuum line, and the D_2O solution was mechanically transferred into the nmr tube V. The nmr tube was disconnected from the assembly by fusion.

(*S*)-(−)-Dimethyl Malate. (*S*)-(−)-Dimethyl malate was prepared from (*S*)-(−)-malic acid by a Fisher esterification.

(*S*)-1,2,4-Butanetriol. (*S*)-(−)-Dimethyl malate (25.9 g) dissolved in 30 ml of dry tetrahydrofuran was added dropwise to 21 g of lithium aluminum hydride in 1 l. of dry tetrahydrofuran and refluxed overnight. Addition of water (160 ml) gave a white precipitate, which was filtered and washed with four 130-ml portions of dry ethanol. The combined solution was evaporated to near dryness *in vacuo*. The inorganic material contained in the residual oil was removed by short column chromatography over 50 g of silica gel [elution with 560 ml (3:1 v/v) and 670 ml (2:1 v/v) of chloroform–ethanol]. Removal of solvent gave 12 g (60%) of a slightly yellow oil, indicated to be practically pure by nmr. This oil was submitted to fractional distillation to give a colorless oil (9.35 g, 50%): bp 145–148° (1.4 mm); M^+ 106.0646 (calcd for $C_4H_{10}O_3$, 106.0629); ir (neat) 3337 cm^{-1} (OH); nmr (pyridine) 2.14 (2 H, m), 3.97 (2 H, dd, J = 5 Hz), 4.17 (2 H, dt, J = 5, J = 6 Hz), 5.38 (1 H, m), 6.00 ppm (3 H, hydroxyls).

(42) W. Szybalski in "Methods in Enzymology," L. Grossman and K. Moldave, Ed., Academic Press, New York, N. Y., 1971, p 350.

(43) The Teflon high vacuum cock is supplied by Eck and Krebs, Inc.

(*S*)-1,2-*O*-Isopropylidenebutane-1,2,4-triol. (*S*)-1,2,4-Butanetriol (9 g) was stirred in acetone (500 ml) with 400 mg of *p*-toluenesulfonic acid at room temperature for 1.5 hr, sodium bicarbonate was suspended in the solution, and the stirring was continued for an additional 10 min. The acetone was evaporated to dryness; the residue was taken up in ethyl acetate and washed with aqueous solutions of sodium bicarbonate and sodium chloride, and dried over $MgSO_4$. After removal of the solvent, distillation of the residue gave 11.6 g (95%) of a colorless oil: bp 87° (22 mm); M^+ 146.0929 (calcd for $C_7H_{14}O_3$, 146.0928); ir (neat) 3430 cm^{-1} (OH); nmr ($CDCl_3$) 1.36 (3 H, q, J = 0.75 Hz), 1.39 (3 H, q, J = 0.75 Hz), 1.81 (2 H, dt, J = 5.5, J = 6 Hz), 3.10 (1 H, br s), 3.58 (1 H, dd, J = 7, J = 7.5 Hz), 3.75 (2 H, t, J = 6 Hz), 4.07 (1 H, dd, J = 6, J = 7 Hz), 4.26 ppm (1 H, diffuse heptet, J = 6.5 Hz).

(*S*)-1-Bromo-*O*-isopropylidenebutane-3,4-diol. Triphenylphosphine (5.23 g, 20 mmol) in 5.7 ml of dry dichloromethane (kept in molecular sieves 4A) was added dropwise through a capillary during a period of 4 hr to a well-stirred solution of (*S*)-1,2-*O*-isopropylidenebutane-1,2,4-triol (2.92 g, 20 mmol) and carbon tetrabromide (9.95 g, 30 mmol) in 4 ml of dry dichloromethane at room temperature. After an additional 1 hr of stirring, the reaction mixture was treated with 150 ml of *n*-pentane and the resulting precipitate (triphenylphosphine oxide) was removed by filtration and washed several times with *n*-pentane. The combined *n*-pentane solution was washed with 5% sodium bicarbonate, water, and sodium chloride solution and dried ($MgSO_4$). After removal of the solvent, the residue was submitted to distillation, and the volatile compound was trapped with Dry Ice–acetone. (*S*)-1-Bromo-*O*-isopropylidenebutane-3,4-diol was easily separated from carbon tetrabromide by passage through a short column of silica gel. Elution with *n*-pentane gave 3.2 g (77%) of a slightly yellow oil: $M^+ - CH_3$, 194.98682 (calcd for $C_7H_{13}O_2Br$, 194.98449); nmr ($CDCl_3$) 1.35 (3 H, s), 1.40 (3 H, s), 2.11 (2 H, m, $W_{1/2}$ = 15 Hz), 3.50 (2 H, t, J = 8 Hz), 3.58 (1 H, dd, J = 7.8, J = 8.5 Hz), 4.09 (1 H, dd, J = 6, J = 7.8 Hz), 4.26 ppm (1 H, m, $W_{1/2}$ = 12 Hz).

(*E*)-(*S*)-*O*-Isopropylidene-1-phenyl-1-hexene-5,6-diol. (*S*)-1-Bromo-*O*-isopropylidenebutane-3,4-diol (5 mmol) and triphenylphosphine (7.5 mmol) in 10 ml of ether were heated at 100° in a sealed tube for 2 days. The precipitate of the triphenylphosphine salt was collected by filtration and washed several times with ether and dried in a vacuum oven at 80°. The yield of the amorphous salt was 2.07 g (87%): nmr ($CDCl_3$) 1.28 (6 H, s), 1.48 (2 H, m, $W_{1/2}$ = 30 Hz), 3.6 (1 H, m, $W_{1/2}$ = 10 Hz), 3.62 (1 H, dd, J = 3, J = 4.2 Hz), 4.14 (1 H, dd, J = 3, J = 4.5 Hz), 4.5 (2 H, m, methine and one of the methylene groups adjacent to phosphorus), 7.74 ppm (15 H, m).

Two grams of this salt in 70 ml of dry ether were converted, without further purification, into the phosphorane by addition of 1 molar equiv of *n*-butyllithium (1.3 *M* solution in pentane) under nitrogen. This mixture was stirred for 2 hr at room temperature, cooled to −70 to −80°, and treated dropwise with 1 mol equiv of phenylacetaldehyde in 5 ml of dry ether for a period of 20 min. The mixture was then stirred for 2 hr at room temperature. Filtration of the white precipitate and removal of solvent gave the crude product. Chromatography over 50 g of silica gel [elution with hexane–acetone (20:1 v/v)] gave 560 mg (60%) of the homoconjugated *E* isomer mixed with a trace of the *Z* isomer. Its nmr ($CDCl_3$) showed signals at 1.38 (3 H, s), 1.45 (3 H, s), 2.46 (2 H, m, $W_{1/2}$ = 25 Hz), 3.41 (2 H, bd, J = 7 Hz), 3.58 (1 H, dd, J = 6.5, J = 7 Hz), 4.08 (2 H, m, methine and one of the methylenes adjacent to oxygen), 5.61 (2 H, m, vinyl), 7.23 ppm (5 H, m).

The double bond mixture (420 mg) was shaken with 10 ml of dimethyl sulfoxide and 262 mg of freshly sublimed potassium *tert*-butoxide for 2 hr at room temperature, and the mixture was poured into 60 ml of water and extracted with 100 ml of ether (five times) and 100 ml of chloroform (three times). The combined solution was washed with water and aqueous sodium chloride and dried ($MgSO_4$). Removal of the solvent gave a slightly yellow oil. The small amount of dimethyl sulfoxide and yellow impurity were readily removed by a short silica gel column [elution with hexane–acetone (10:1, v/v)]. Drying up of the eluate gave pure conjugated *E* compound (403 mg, 96%): M^+ 232.1451 (calcd for $C_{15}H_{20}O_2$, 232.1463); ir (neat) 3107–3027 (four peaks), 2987, 2940, 2870, 1649, 1599, 1499, 1447, 1382, 1373, 1243, 1213, 1156, 1068, 964, 855, 740, 691 cm^{-1}; nmr ($CDCl_3$) 1.38 (3 H, q, J = 0.75 Hz), 1.42 (3 H, q, J = 0.75 Hz), 1.74 (2 H, m, $W_{1/2}$ = 24 Hz), 2.28 (2 H, m, $W_{1/2}$ = 18 Hz), 3.52 (1 H, m, $W_{1/2}$ = 11 Hz), 4.02 (1 H, m, $W_{1/2}$ = 6.5 Hz), 4.12 (1 H, m, $W_{1/2}$ = 14 Hz), 6.21 (1 H, dt, J = 6, J = 15.5 Hz), 6.38 (1 H, d, J = 15.5 Hz), 7.24 ppm (5 H, m); uv (hexane) 251 nm (ε 18,500); CD (hexane) $\Delta\epsilon_{230}$ +1.87. The yellow impurity could also be removed by bulb-to-bulb distillation (0.6 mm, bath 135–140°).

(*S*)-*O*-Isopropylidenepentane-3,4-diol-1-al. Ozone was bubbled through a solution of (*E*)-(*S*)-*O*-isopropylidene-1-phenyl-1-hexene-5,6-diol (278 mg, 1.2 mmol) in 10 ml of dry methanol for 2 min (1 mmol/min) at −70°, and excess dimethyl sulfide was added to the reaction mixture. The reaction flask was tightly stoppered and kept overnight at −70°, and then for 30 min at room temperature. The solvent was evaporated *in vacuo*, and the trace of dimethyl sulfide was removed completely by three additions of dry methanol to the residual oil and evaporation. The residue was dissolved again in 10 ml of dry methanol and submitted to catalytic hydrogenation with palladium black (200 mg) to convert the benzaldehyde into toluene. The reaction mixture was filtered and the solvent evaporated. The residue was evaporated three times with dry benzene *in vacuo* to remove traces of methanol which would be deleterious in the next reaction. Finally the residue was dissolved in benzene, washed with water and saturated salt solution, and dried ($MgSO_4$). Removal of solvent gave 180 mg (95%) of colorless oil: M^+ 158.0926 (calcd for $C_8H_{14}O_3$, 158.0942); ir (neat) 2826, 2724, 1725, 1382, 1373 cm^{-1}; nmr ($CDCl_3$) 1.32 (3 H, q, J = 0.75 Hz), 1.38 (3 H, q, J = 0.75 Hz), 3.53 (1 H, m), 4.11 (1 H, m, $W_{1/2}$ = 13 Hz), 4.20 (1 H, dd, J = 6.5, J = 6.5 Hz), 9.78 ppm (1 H, t, J = 1.2 Hz). Application of preparative tlc to separate the pentanal and benzaldehyde resulted in decomposition.

5-Bromo-2,4-di-*tert*-butoxypyrimidine[37] was prepared from uracil *via* 5-bromouracil[44] and 2,4-dichloro-5-bromopyrimidine[45] by a known method. The overall yield was 48%.

(1′*S*- and 1′*R*,4′*S*)-5-(*O*-Isopropylidene-1′,4′,5′-trihydroxypentyl)-2,4-di-*tert*-butoxypyrimidine. Freshly sublimed 5-bromo-2,4-di-*tert*-butyoxypyrimidine (334 mg, 1.1 mmol) was dissolved in 7 ml of dry tetrahydrofuran and the solution was cooled to −70 to −80° and stirred under argon atmosphere. *n*-Butyllithium (1.1 mmol, 1.21 *M* solution in *n*-pentane) was added and the solution was stirred for 10 min. (*S*)-*O*-Isopropylidenepentane-4,5-diol-1-al (158 mg, 1 mmol) in 2 ml of dry tetrahydrofuran was added dropwise during 20 min and kept at −70° for 2 hr with stirring. The cooling bath was removed and the solution was stirred at room temperature for 13 hr. After evaporation of the solvent to dryness, 30 ml of water was poured into the residue and the product was extracted with ether (40, 20, 3 × 15 ml). The combined ether extracts were washed with saturated salt solution and dried ($MgSO_4$), and the solvent was removed to leave a slightly yellow oil (394 mg). Chromatography over 8 g of Merck extra pure neutral silica gel (elution with dichloromethane–tetrahydrofuran, 30:1, v/v) to remove 2,4-di-*tert*-butoxypyrimidine gave 365 mg (95.5%) of sticky colorless oil (1′-diastereomiric mixture, 1:1); R_f 0.16, chloroform–tetrahydrofuran, 10:1: M^+ 382.2484 (calcd for $C_{20}H_{34}O_5N_2$, 382.2467); ir ($CHCl_3$), 3406, 3005, 2986, 2936, 2975, 1593, 1554, 1480, 1456, 1417, 1372, 1367, 1152, 1049, 1015, 931, 903, 843 cm^{-1}; nmr ($CDCl_3$) 1.35 (3 H, s), 1.40 (3 H, s), 1.60 (9 H, s), 1.63 (9 H, s), 1.77 (4 H, m), 3.28 (1 H, bs), 3.50 (1 H, m), 4.02 (1 H, dd, J = 6, J = 6 Hz), 4.08 (1 H, m, $W_{1/2}$ = 15 Hz), 4.70 (1 H, br t, J = 6.5 Hz), 8.18 (0.5 H, s), 8.20 ppm (0.5 H, s). The presence of nonequivalent diastereotopic groups were shown by the splitting of the 6-H aromatic signal at 8.19 ppm into a doublet, which sharpened upon irradiation of the peak at 4.70 ppm (1′-H). Attempted separation of the two 1′-epimers through tlc or high pressure liquid chromatography was not successful.

(*S*)-5-(4′,5′-Dihydroxypentyl)uracil. The epimeric mixture (at C-1′) of (*S*)-5-(*O*-isopropylidene-1′,4′,5′-trihydroxypentyl)-2,4-*tert*-butoxypyrimidine (230 mg) was dissolved in 15 ml of moist methanol and stirred with 20 mg of palladium black under hydrogen at room temperature for 18 hr. The reaction mixture was filtered and evaporation of methanol *in vacuo* gave pulverous (*S*)-5-(4′,5′-dihydroxypentyl)uracil (quantitative yield), the tlc and high-pressure lc of which showed it to be a single compound. Recrystallization of this material from water gave an analytically pure sample. It had superimposable ir and nmr, in addition to an identical mass spectrum, tlc, R_f value (*n*-BuOH–AcOH–H_2O, 4:1:1, v/v), and retention volume in high pressure lc (MeOH–H_2O, 1:4 v/v; poragel-PN column, 3 ft × 3/8″ i.d.) with natural 5-(4′,5′-dihydroxypentyl)uracil: mp 225–226°; uv (in H_2O) 207 nm (ε 9500), 265 (7700); CD (in H_2O) $\Delta\epsilon_{265}$ + 0.5.

Anal. Calcd for $C_9H_{14}O_4N_2$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.41; H, 6.67; N, 12.87.

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***dl*-1,2-*O*-Isopropylidene-1,2,5-pentanetriol.** *dl*-1,2,5-Pentanetriol[38] was prepared from tetrahydrofurfuryl alcohol *via* the triacetate. The triol (12 g) was stirred in acetone (150 ml) with 500 mg of *p*-toluenesulfonic acid at room temperature for 2 hr, sodium bicarbonate was suspended in the solution, and the stirring was continued for an additional 10 min. The acetone was evaporated *in vacuo* and the residue was taken up with ethyl acetate (300 ml) and washed with water (50 ml) and saturated salt solution and dried (Na_2SO_4). The solvent was removed and the residue was distilled to give 15 g (93%) of colorless oil: bp 125° (15 mm); $M^+ - CH_3$ 145.0877 (calcd for $C_8H_{16}O_3$, 145.0864); ir (neat) 3390 (OH); nmr ($CDCl_3$) 1.35 (3 H, q, J = 0.75 Hz), 1.41 (3 H, q, J = 0.75 Hz), 1.64 (4 H, m, $W_{1/2}$ = 8.5 Hz), 3.22 (1 H, bs), 3.49 (1 H, m), 3.61 (2 H, m, $W_{1/2}$ = 10 Hz), 4.20 (1 H, dd, J = 6, J = 6 Hz), 4.10 ppm (1 H, m, $W_{1/2}$ = 14 Hz).

***dl*-*O*-Isopropylidenepentane-4,5-diol-1-al.** Chromium trioxide (36 g, 0.36 mol) was added to a mechanically stirred solution of 58.2 ml (0.72 mol) of pyridine in 900 ml of methylene chloride[46] under dry nitrogen at 0°, and the stirring was continued for 15 min. The alcohol (9.6 g, 0.06 mol) in 30 ml of methylene chloride was added in one portion to the solution of chromium trioxide–pyridine complex and the mixture was stirred for 15 min at room temperature. The solution was decanted from precipitates and the solvent was evaporated. The residue was taken up with 1.4 l. of ether and the insoluble chromium salt was removed by filtration. The filtrate was washed with 5% sodium bicarbonate solution, cupric sulfate solution, and saturated brine and dried over anhydrous sodium sulfate, and the solvent was removed to give 6.2 g (65.5%) of practically pure aldehyde. The aldehyde could be purified further by distillation if necessary; bp 95–97° (30 mm). The ir and nmr spectra were identical with authentic (*S*)-*O*-isopropylidenepentane-4,5-diol-1-al.

(*Z*)- and (*E*)-*dl*-*O*-Isopropylidene-1-phenyl-1-hexene-5,6-diol. Triphenylbenzylphosphine chloride (857 mg, 2 mmol) was dissolved in 70 ml of dry ether, and 1.8 ml (2.2 mmol) of *n*-butyllithium (1.21 *M* solution in pentane) was added under nitrogen. The resulting phosphorane solution was cooled to −70 to −80° and the aldehyde (316 mg, 2 mmol) in 30 ml of ether was added dropwise. After removing the cooling bath, the solution was allowed to stir for 2 days at room temperature. White precipitates were filtered off and the solvent was evaporated. A bulb-to-bulb distillation of the residue gave a colorless oil (360 mg, 78%) of the *E* and *Z* mixture. Analysis by both nmr and glpc showed it consisted of 78% *E* isomer and 23% *Z* isomer. The mixture was separated by glpc; the double bond isomers had retention times of 20 min (*Z* compound) and 36 min (*E* compound) on a 10 ft × 0.25 in. 5% SE-30 column at 170°. Physical constants of *Z* isomer: M^+ 232.1462 (calcd for $C_{15}H_{20}O_2$, 232.1463); ir (neat) 3114–3019 (five peaks), 2998, 2945, 2879, 1607, 1580, 1453, 1386, 1376, 1233, 1163, 1088, 1053, 988, 973, 923, 863, 803, 773, 705 cm^{-1}; nmr ($CDCl_3$) 1.33 (3 H, s), 1.39 (3 H, s), 1.66 (2 H, m, $W_{1/2}$ = 22 Hz), 2.41 (2 H, m, $W_{1/2}$ = 20 Hz), 3.48 (1 H, m, $W_{1/2}$ = 12.5 Hz), 4.04 (2 H, m, methyne proton and one of the protons in terminal methylene adjacent to oxygen), 5.63 (1 H, dt, J = 12, J = 8 Hz), 6.43 (1 H, dt, J = 12, J = 2 Hz), 7.28 (5 H, m, aromatic proton). The ir and nmr of *E* isomer were identical with authentic optically active compound which was synthesized from (*S*)-(−)-malic acid.

(+)-MTPA Diester of (*E*)-*dl*-1-Phenyl-1-hexene-5,6-diol. (*E*)-*dl*-*O*-Isopropylidene-1-phenyl-1-hexene-5,6-diol (50 mg) was dissolved in 8 ml of methanol and 0.8 ml of 0.1 *N* hydrochloric acid solution and stirred for 1 day at room temperature. After evaporation of methanol, the residue was taken up by 40 ml of ether and washed with 5% sodium bicarbonate and saturated salt solution and dried ($MgSO_4$). Removal of the solvent gave 20 mg (50%) of the glycol. The low yield of the glycol was due to the poor solubility in ether. The reaction itself was almost quantitative, which was indicated by tlc. The nmr signals of the glycol were at 1.61 (2 H, dt, J = 7, J = 7 Hz), 2.35 (2 H, dt, allylic methylene, J = 6, J = 7 Hz, and 2 H, br s, hydroxyl groups), 3.64 (3 H, m, methine proton and methylene protons adjacent to oxygen), 6.22 (1 H, dt, J = 16, J = 6 Hz), 6.41 (1 H, d, J = J = 16 Hz), 7.29 ppm (5 H, m, aromatic protons).

The glycol (20 mg) was reacted with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (100 mg) in 1 ml of dry pyridine at room temperature overnight. Ether (40 ml) was added to the reaction mixture, which was then washed with water, 5% cupric sulfate solution, and brine, and passed through anhydrous magnesium sulfate. Evaporation of the solvent and purification by preparative tlc gave the diastereomeric mixture (1:1) of the (+)-MTPA diesters, 90% yield. Although the fluorine nmr spectrum of this diastereomeric mixture showed only a single peak, the proton spectrum clearly showed two sets of signals corresponding to two vinyl protons and methylene protons adjacent to the ester group: methylene protons, 4.30 (1 H, dd, J = 12.5, J = 5 Hz), 4.60 (1 H, dd, J = 12.5, J = 3 Hz), 4.28 (1 H, dd, J = 12, J = 5 Hz), 4.68 (1 H, dd, J = 12, J = 3 Hz); vinyl protons, 6.07 (1 H, dt, J = 16, J = 6.5 Hz), 6.37 (1 H, d, J = 16 Hz), 6.02 (1 H, dt, J = 16, J = 6.5 Hz), 6.31 ppm (1 H, d, J = 16 Hz).

(+)-MTPA Diester of (*E*)-(*S*)-1-Phenyl-1-hexene-5,6-diol. (*E*)-(*S*)-*O*-Isopropylidene-1-phenyl-1-hexene-5,6-diol (25 mg) was converted to the (+)-MTPA diester in the manner as described for the *dl* compound: M^+ 624.1964 (calcd for $C_{32}H_{30}O_6F_6$, 624.1946); ir ($CHCl_3$) 3100, 3030 (several peaks), 2947, 2847, 1754, 1660, 1492, 1450, 1269, 1237, 1171, 1123, 1082, 1014, 964, 914 cm^{-1}; nmr ($CDCl_3$) 1.86 (2 H, m, $W_{1/2}$ = 12 Hz), 2.21 (2 H, m, $W_{1/2}$ = 13 Hz), 3.34 (6 H, q, J = 1 Hz), 4.30 (1 H, dd, J = 12.6, J = 5 Hz), 4.60 (1 H, dd, J = 12.5, J = 3 Hz), 5.38 (1 H, m, $W_{1/2}$ = 13 Hz), 6.07 (1 H, dt, J = 16, J = 6.5 Hz), 6.37 (1 H, d, J = 16 Hz), 7.32 ppm (15 H, m, aromatic protons).

Determination of Optical Purity by Nmr. The proton nmr spectra were obtained on a Varian Associates HA-100 spectrometer using deuteriochloroform solvent and tetramethylsilane (TMS) as an internal standard. The ^{13}C and fluorine nmr spectra were obtained with a JEOL JNM-PS-100 spectrometer, 25 and 94 MHz.

(i) The proton nmr of (*E*)-*dl*-*O*-isopropylidene-1-phenyl-1-hexene-5,6-diol was measured after addition of the chiral shift reagents, [tris-(3-trifluoromethylhydroxymethylene)-*d*-camphorato]europium-(III). The observed shifts were very poor and also diastereomeric pairs were not split.

(ii) The proton and carbon-13 nmr of (*E*)-*dl*-1-phenyl-1-hexene-5,6-diol were measured after addition of the chiral shift reagents mentioned above. The proton nmr showed considerable shifts, but the signals broadened quickly as the amount of the reagents was increased. The two antipodal glycols could not be distinquished either by proton or carbon-13 nmr spectra.

(iii) As the above-mentioned methods failed, the optical purity was assessed by comparing the proton nmr spectra of two MTPA esters, *i.e.*, **14** from *dl*-**7**, and **14** from optically active **7** (Figure 6).

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