Pseudonucleoside Analogs. Synthesis and Spectral Properties of 5-(*cis*-3-Hydroxymethylcyclopentane)uracil, a Carbocyclic Analog of 2′,3′-Dideoxypseudouridine[1]

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In our search for the specific structural features necessary for the observed differences in certain physicochemical properties of α- and β-pseudouridines, we have synthesized the title compound, **1**. Two alternative syntheses of the key *cis*-3-hydroxymethylcyclopentaneacetic acid lactone (**16**) from norbornylene were investigated. In each instance the cis stereochemistry of **16** and thus that of 5-(*cis*-3-hydroxymethylcyclopentane)uracil (**1**) was assured by the cyclic nature of the intermediates. The lactone **16** was obtained by Baeyer–Villiger oxidation of the bicyclo[3.2.1]octan-3-one **15**, and it could be formylated to the sodium enolate derivative **19**. Base-catalyzed condensation with thiourea yielded 5-(*cis*-3-hydroxymethylcyclopentane)-2-thiouracil (**20**), which was converted to **1** by treatment with aqueous chloroacetic acid. From ir and uv spectral studies of **1**, it can be inferred that there is an interaction between the hydroxy group on the side chain and the pyrimidine ring, but it appears that such an interaction cannot alone be the cause of the differences in the uv spectral properties and equilibrium between the tautomeric monoanionic species in the anomeric pseudouridines. A specific role for the "glycosyl" ring oxygen in the pseudouridines is proposed.

Certain physicochemical properties of pseudouridine, a nucleoside from transfer RNA's, have been the subject of studies in a number of laboratories during the past several years. Since the early reports[2,3] on the different ratio of the two tautomers A and B (Figure 1) in the α- and β-pseudouridine monoanion, reflected in the uv spectral shifts of these compounds at pH ~11–12, several explanations have been offered, and promptly challenged, in an attempt to correlate this phenomenon with specific structural features and intramolecular interactions.

Chambers[2] originally suggested that an intramolecular H bond from the C-5′ hydroxyl to the C-4 carbonyl group (D, Figure 1) was stabilizing the N-1 over the N-3 anion (A and B respectively, Figure 1) in the β isomer. Implicitly this proposal postulates the predominance of the syn conformer in aqueous solution.

In earlier reports on the synthesis[4,5] and ir spectra[5] of various 5-(hydroxycyclopentane)pyrimidines that bear structural similarities to pseudonucleosides, we suggested that a weak intramolecular hydrogen bond to the π-electron orbitals of the pyrimidine ring could explain the difference in the equilibrium of monoanionic species observed in α- and β-pseudouridines. Corollary to these interpretations is the predominance of the anti rotamer population of β-pseudouridine, since the C-5′ hydroxy group is stereochemically the most likely intramolecular H-bonding donor to the ring π system (C, Figure 1).

More recently the uv absorption properties of β-pseudouridine were carefully reexamined by Dugaiczyk.[6] The only observed difference between the spectra of the respective ionic species of β-pseudouridine and its 2′,3′-isopropylidene and 5′-*O*-acetyl-2′,3′-*O*-isopropylidene derivatives was a small (1 and 0.5 nm respectively) hypsochromic shift in the latter two. The spectrum of the uracil monoanion represents the combined contribution of the two tautomeric species each of which is characterized by a different pK_a. Paradoxically the $\epsilon_{280/260}$ ratio at pH 11.2–12.2, which is taken as a quantitative measure of the relative proportions of the two monoanionic forms, varied from 2.22 for the parent compound to 2.40 for the 2′,3′-*O*-isopropylidene and 2.58 for the 5′-*O*-acetyl-2′,3′-isopropylidene derivatives, but the pK_a's of all three compounds were reported to be the same, i.e., 9.10.[6] Of course, the possibility cannot be excluded that with each progressive substitution on the "sugar" ring, the individual pK_a's at N-1 and N-3 of the pyrimidine are proportionally modified so that there is no net change in the observed pK_a. Also the conformational change(s) affecting the interrelationship(s) of the various structural entities of the pseudonucleoside, induced by the additional substitution, have not been considered. It has been experimentally confirmed, for example, that in uridine derivatives a 2′,3′-*O*-isopropylidene group facilitates interaction between the 5′ position and the aglycon.[7] In addition, a 5′-acetoxy group should be expected to have an effect upon the conformation of the exocyclic CH_2OH group and upon the sugar–base torsion angle. Extrapolation of these effects to the pseudouridine series is reasonable because it has been shown (NMR) that the ribose conformations of pseudouridine and uridine are very similar.[8]

Elaborate discussions of ^{1}H NMR (proton magnetic resonance), NOE (nuclear Overhauser effect) data,[8–10] and molecular orbital calculations[10] of pseudouridine led to the contradicting conclusions that in the β isomer an intramolecular bond between C-5′ hydroxyl and C-4 carbonyl is unlikely because even though the gauche–gauche rotamer of the 5′-hydroxymethyl group is favored a substantial fraction of the molecules exist in the anti conformation,[9] that both syn and anti conformations coexist in rapid equilibrium in roughly equal populations, and that the syn conformation is stabilized by the existence of a hydrogen bond between the C-5′ hydroxyl and C-4 carbonyl groups.[10] Whatever conclusions are made, however, are dependent upon the somewhat arbitrary selection of the factors employed in the theoretical calculations. For example, the assumption that the 2′ and 3′ hydroxyl groups do not come in proximity to either the base or the 5′-hydroxymethyl group led to calculations in which the 2′ and 3′ hydroxyl groups were replaced by hydrogen atoms. That assumption and the corollary calculations may not be valid. By analogy to the molecule of uridine, intramolecular interaction between C-2′ hydroxyl and the C-4 carbonyl group in the β-pseudouridine is to be considered.[11a] Also the range of concentration (0.1 *M*) at which the NOE measurements were conducted are not unequivocally regarded as precluding intermolecular associations.[12] Indeed, ir studies indicate that 5′-*O*-acetyl-2′-3′-*O*-isopropylidene β-pseudouridine at concentrations 10^{-2} *M* or below ($CDCl_3$) self-associates to a considerable extent.[13] It should be noted that different types of self-associations are probably involved, depending upon the nature of the solvent; i.e., in water the mode of as-

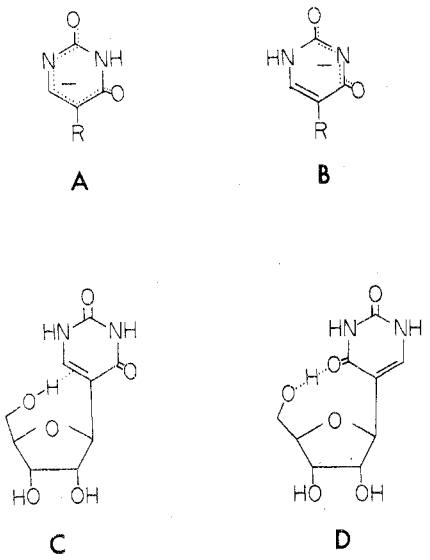


Figure 1.

sociation would be by vertical stacking of the bases while in apolar solvents it would be by horizontal hydrogen bonding.[11b]

Clearly the factors involved in the equilibrium of the monoanionic species of the "anomeric" pseudouridines merit further study. An inspection shows that various uracils carrying a substituent at position 5 fall into two distinct spectral groups based on the relative predominance of the N-1 over the N-3 monoanion (Figure 2). While the balance between the mesomeric and inductive effects among the side groups may vary,[22] those derivatives in which the C-5 substituents are joined to C-5 by carbon–carbon linkages are directly comparable. Among these, it is apparent that the presence of an allylic oxygen on the side–side chain favors the formation of the N-1 monoanion.

Intramolecular hydrogen bonding potentials, inductive, mesomeric, and electrostatic through-space effects, as well as interactions with the solvent, are all pertinent factors. It is recognized that more than one of these factors may be operative at any one time on the total molecule and may contribute to the apparent net effect.

In the α- and β-pseudouridines the C-2′, C-3′, and C-5′ hydroxyl groups are potential donors to an intramolecular hydrogen bond linking the substituent on C-5 to the pyrimidine. In addition, the inductive, mesomeric, and electrostatic effects of the tetrahydrofuran ring of the furanose must be taken into account. Substitution of the cyclopentane ring for the furanose ring of pseudouridines retains the stereochemical arrangement of the individual groups, essentially unchanged, with the exception of the allylic ether oxygen. In both ring systems (tetrahydrofuran and cyclopentane) the angle of maximum puckering rotates without substantial change in potential energy, but the presence of one or more endocyclic or exocyclic substituents gives rise to induced potential energy barriers opposing pseudorotation.[23] It is reasonable to assume that particular substituents impose analogous restrictions upon either the cyclopentane pseudonucleoside analogs or the parent tetrahydrofuran compounds. Thus they allow the formation of analogous conformational species for either of the ring systems in the C-5 substituent.

In our systematic attempt to develop model systems which would help to elucidate this problem, we have synthesized some additional appropriately substituted cyclopentane analogs of pseudouridine that will hopefully provide additional insight into the character and influence of the intramolecular hydrogen bonding. Moreover, the comparison of their physicochemical properties to those of analogous tetrahydrofuran derivatives, such as the ones depicted in Figure 2, should also allow definitive conclusions regarding the chemical reactivity and its effect on these properties of the "allylic" ether oxygen of the "sugar" ring in the pseudonucleosides. One illustration of the importance of the ether function is its reversible cleavage on either acid or base solutions,[2] leading in either case to intermediates susceptible to the addition of nucleophiles.

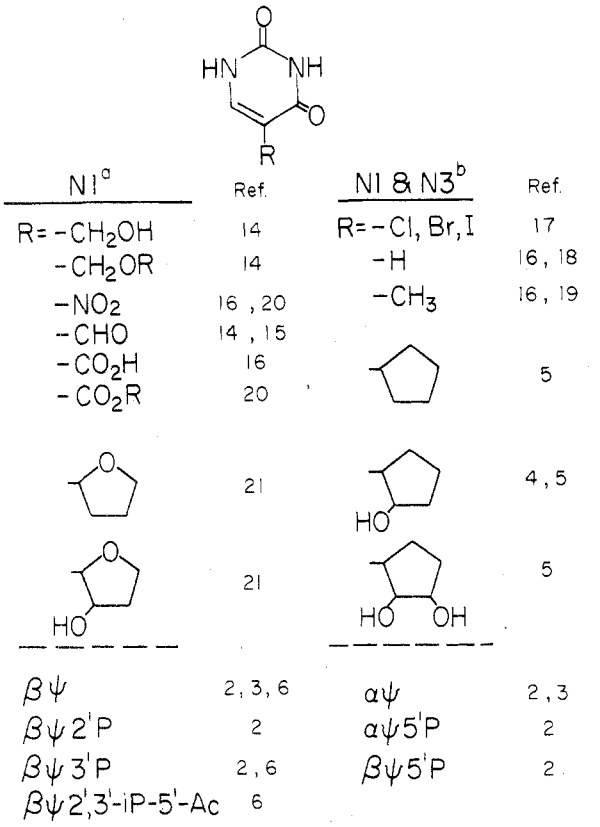


Figure 2. (a) The N-1 monoanion predominates in this series. (b) The N-1 and N-3 monoanions exist in approximately equal concentrations.

In this report we discuss the synthesis of 5-(*cis*-3-hydroxymethylcyclopentane)uracil (**1**, Figure 3) and its uv and ir spectral properties. Some conclusions are reached regarding the possible role of the C-5′ hydroxyl group and the "glycosyl" ring oxygen in effecting the equilibrium of the tautomeric monoanions of the pseudouridines. This model compound, **1**, incorporates the aglycon moiety and the stereochemical features at the C-5′ of β-pseudouridine (**3**), but it lacks the allylic cyclic ether oxygen and hydroxyls at the C-2′ and C-3′ positions. Molecules of **1** should possess greater freedom of rotation around the C-5 and C-1′ bond. This reduced restriction upon the range of the torsion angle should enhance the intramolecular hydrogen bonding potential of the C-5′ hydroxyl group, and any effect that this may have upon the spectral and ionization properties of the pyrimidine moiety.

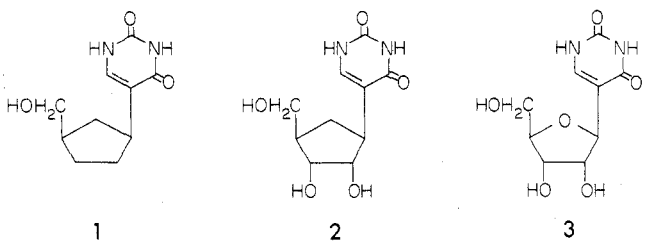
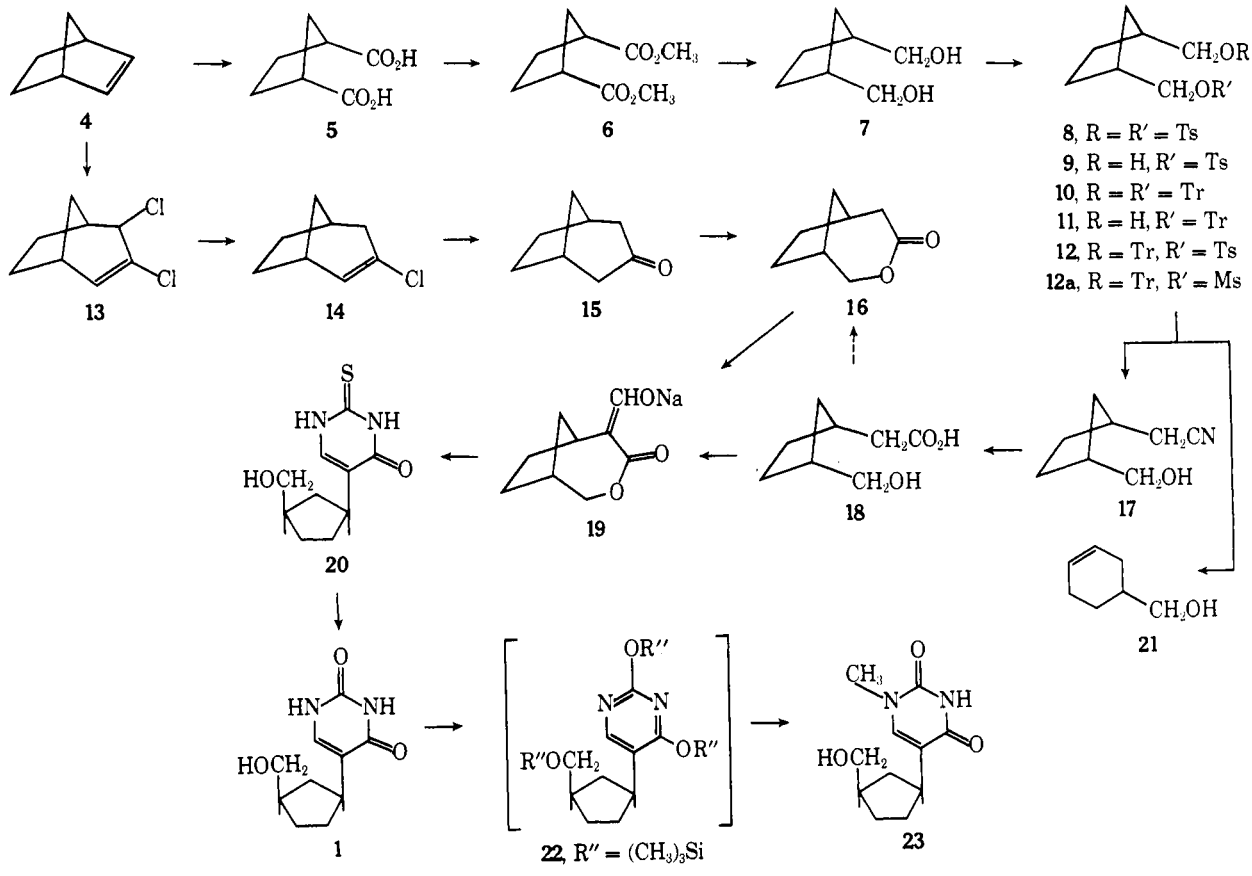


Figure 3.

Scheme I



The pathways investigated for the synthesis of the cyclopentane analog, **1**, are shown in Scheme I. Two alternative syntheses of the key lactone, **16**, from norbornylene were investigated. In each instance the cis stereochemistry of **16**, and thus that of **1**, was assured by the cyclic nature of the intermediates.

The preparation of the *cis*-1,3-cyclopentanedicarboxylic acid (**5**) by oxidation of bicyclo[2.2.1]heptene-2 (norbornylene) with sodium permanganate,[25] and more recently with $NaIO_4$ in the presence of RuO_2,[26] has been reported. In our hands the oxidation of norbornylene in 100-g quantity with $NaMnO_4$, as described,[25] gave inconsistent results. When heptane and $KMnO_4$ were substituted for 2,2,4-trimethylpentane and $NaMnO_4$,[27] the yield of the crude dicarboxylic acid, **5**, was 59%. The modified method of Shealy and Clayton[28] for the oxidation of *exo-cis*-norbornylene-2,3-diol consistently gave high yields, 90%. The published method[29] for the preparation of the diethyl ester of **5** was considered too cumbersome. The simpler general method of Clinton and Laskowski[30] also gave comparable yields, 78%, but the procedure of choice that gave high average yields, 96%, was based on that of Radin et al., employing 2,2-dimethoxypropane (DNP).[31]

Reaction of the diol, **7**, with 1 equiv of *p*-toluenesulfonyl chloride in pyridine produced a mixture of the di- and monotosylates, **8** and **9**, in a ratio of ~1:4. These tosylated derivatives were readily resolved on a silica gel column.

Substitution of the sulfonyloxy group of **9** by CN^- led to the *cis*-3-hydroxymethylcyclopentaneacetonitrile (**17**), which was hydrolyzed to *cis*-3-hydroxymethylcyclopentaneacetic acid (**18**). In order to improve the overall yield of **17** from the diol **7**, the latter was tritylated. It was hoped that the first trityl group to be introduced would sterically inhibit further reaction at that stage. Yet a mixture of the di- and monotrityl derivatives, **10** and **11**, was obtained. The combined yield of these products was somewhat higher than that of the tosyl derivatives, **8** and **9**, but the ratio between the mono- and disubstituted trityl derivatives was 1:1.75. Although the sulfonates, **12** and **12a**, were obtained in almost quantitative yield, 95%, from **11**, the use of the trityl derivatives does not offer any particular advantage over the direct tosylation of **7**. The *cis*-3-hydroxymethylcyclopentaneacetonitrile (**17**) was obtained from the monotosylate **9** with KCN in DMF.[32] Hydrolysis of that nitrile gave the corresponding carboxylic acid, **18**. A small analytical sample was obtained by high vacuum distillation in a molecular still, but that operation was accompanied by extensive polymerization. A product was isolated from the residue of that distillation in crystalline form. Its NMR spectrum was very similar to that of the acid **18**, except for the absence of the OH signal. Also, in its ir spectrum the OH band was greatly attenuated and the carbonyl band was broadened and shifted (25 cm^{-1}) to higher frequency. Unfortunately, an accurate molecular formula could not be derived from elemental analysis for C and H only. It was presumed to be an oligomer of **18**.

Various attempts to effect the cyclization of the hydroxy acid **18** to the lactone **16** resulted in only traces of the desired product. Recent investigations on the kinetics of cyclization of ω-halogenocarboxylic acids to medium-ring (8–11 membered) lactones and on the stability of these products offer an explanation for the failure of the acid **18** to cyclize. Cyclization to **16** would involve the formation of a bicyclic ring structure with a newly formed eight-membered lactone ring, but measurements of the tendency for lactone formation compared with ring size reveal a reactivity minimum lying at the eight- and nine-membered rings.[33] Another factor is the stability of the lactone structure. It was found, for example, that eight-membered rings with a cis ester function hydrolyze 10^3–10^5 times as fast as the

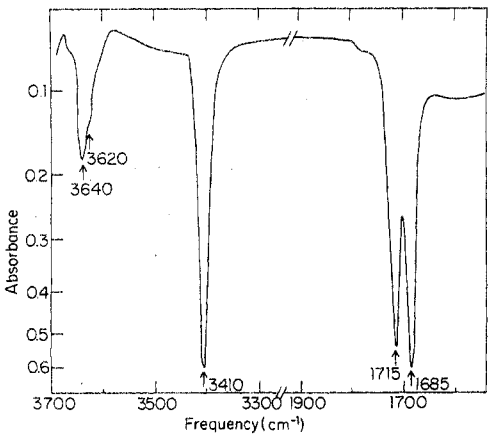


Figure 4. The bands of the O–H and N–H are depicted at 5 × and those of the C=O at 1 × scale.

higher homologs.[34] This property has been related to the transition state intermediate involved in lactone formation, which has been presumed to be ring shaped and to possess the same less stable cis conformation of the ester function.[33,35]

The desired lactone, **16**, was prepared satisfactorily by Baeyer–Villiger oxidation of the bicyclo[3.2.1]octan-3-one **15**, which in turn was obtained by known methods from norbornylene (**4**) as illustrated in Scheme I.[36] The ketone **15** was oxidized to *cis*-3-hydroxymethylcyclopentaneacetic acid lactone (**16**). As anticipated,[37] that lactone proved to be unstable in both acid and basic media, and when heated was converted to polymeric products.

The formylation of the lactone **16** with methyl formate in the presence of CH_3ONa gave a preparation of the sodium derivative, **19**. From the condensation of that material with thiourea in sodium ethoxide–ethanol, 5-(*cis*-3-hydroxymethylcyclopentane)-2-thiouracil (**20**) was obtained and was converted quantitatively to the corresponding uracil, **1**.

Spectral Studies. Intramolecular interaction between an OH group and the π electrons in aromatic or olefinic systems has been extensively investigated by ir spectroscopy. Such studies have shown clearly that the frequency separation between the free and the bonded OH bands ($\Delta\nu$) is a measure of the enthalpy of the interaction.[38,39] In the neutral species of the 5-(*cis*-3-hydroxymethylcyclopentane)uracil **1**, there are two potential acceptor sites for a hydrogen bond, i.e., the region of the C-4 carbonyl group, including both the n and π orbitals, and the C-5–C-6 double bond. Of these, the double bond is the weaker base. Because of the extremely low solubility of the pyrimidine **1** in carbon tetrachloride, it was necessary to carry out the present hydrogen-bonding spectral study with the corresponding N-1 methyl derivative, **23**. That methylation does not impair the extrapolation of the results obtained to the parent compound. A comparison of the frequencies of some principal ir bands in the anion of N-1 and N-3 monomethyl thymines with the corresponding bands in the equilibrium mixture of the two tautomeric anions of thymine itself has shown that the introduction of a methyl group at either N-1 or N-3 causes only a small ~5-cm^{-1} shift.[20] The methyl group exerts both a mesomeric and an inductive effect,[40] but the $\Delta\nu$ values attributable to its electronic effects should be of the order of only ~10 cm^{-1} based on the increased hydrogen bond basicity upon alkylation of aromatic hydrocarbons.[41]

Part of the ir spectrum of **23** is illustrated in Figure 4. The band at ~3640 cm^{-1} can be assigned to the ν_{OH} of the free hydroxyl group. However, it appears to be unsymmetrical with a shoulder at ~3620 cm^{-1}. This second unresolved band at the lower frequency must be derived from the hydroxyl group interacting with the pyrimidine ring. Thus it seems that in solution in an inert solvent and sufficiently dilute ($<5 \times 10^{-4}$ *M*) to prevent intermolecular associations, **1** displays two partially unresolved, concentration-independent bands in the O–H stretching region. The data indicate that, in carbon tetrachloride at least, a significant proportion of the population of **1** exists as an intramolecularly associated species. The small $\Delta\nu$ (~20 cm^{-1}) value between the band of the nonbonded OH group at 3640 cm^{-1} and that at 3620 cm^{-1} must be attributed to a weak interaction analogous to an ordinary hydrogen bond, but one that depends on the overlap between the occupied π orbitals in the electron donor (i.e., C-5–C-6 double bond of the pyrimidine ring) and the vacant O–H antibonding orbital.[42] The band at 3410 cm^{-1} is assigned to the ring N–H. The absence of any other band in this region that theoretically could be attributed to a strong hydrogen bond of the side chain hydroxyl to the C-4 carbonyl group is noteworthy. The lack of an interaction involving a carbonyl group is also substantiated by the high frequency, $\nu_{C=O}$, of the two carbonyl bands (1685 and 1715 cm^{-1}), as well as by the narrowness and symmetry of these bands.[43] These ir spectra in carbon tetrachloride demonstrate that the intramolecular interaction is, at the very least, stereochemically permissible, but direct examination of such interactions in an aqueous environment remains impractical. Some information regarding that problem can be derived from a comparison of the pK_a's and of the uv spectra of the compounds studied.

In the uracil[18] and thymine[19] series, introduction of a methyl group at N-1 raises the pK_a by ~0.2 to 0.4 p*K* units. In some other uracil derivatives where the C-5 substituent exerts a resonance effect upon the N-1 of the pyrimidine,[44] methylation at that center causes an even greater increase of about 0.7 p*K* units. The 5-nitrouracil is an extreme case where methylation at N-1 causes an increase in the pK_a from 5.55 ± 0.03[45] to 7.20 ± 0.02.[46] From this and the examples shown in Figure 2 which illustrate the results of the inductive effect of various C-5 substituents on the ionization of N-1, it appears reasonable to assume that the greater the increase in the pK_a upon methylation at N-1 in a particular uracil derivative the lower the proportion of the N-3 monoanion (B, Figure 1) in the equilibrium mixture of the tautomeric species A and B of the unmethylated pyrimidine. As there is virtually no difference between the pK_a's of **1** (10.22 ± 0.05) and its N-1 monomethyl derivative, **23** (10.34 ± 0.05), it is probable that in the equilibrium mixture of the tautomeric monoanions of **1** species B predominates over species A. Certainly the prominent shoulder at 270 nm in the uv absorption curve of the "monoanion(s)" of **1** at pH ~12 is strong support for the relative abundance of the anionic species B.[47] This could be the result of a sup-

Table I
Uv Spectral Properties

Compd	Charge	λmax, nm ($\epsilon \times 10^{-3}$)	λmin, nm ($\epsilon \times 10^{-3}$)	Apparent pK_a's (±)
20	0	279 (18.75)	242 (2.25)	
	−1	310 (8.70)	292 (7.4)	
		262 (13.85)	245 (11.00)	
		237 (12.15)		
1	0	266 (7.65)	236 (2.10)	10.22 (0.05)
	−1	290 (5.6)	245 (2.10)	
		265–270 sh		
23	0	273 (4.5)	239 (0.9)	10.34 (0.06)
	−1	270 (3.25)	246 (1.45)	

pression of the ionization from N-1, as might be expected from the inductive effect of the 5 substituent. There is no obvious reason to support the alternative of an enhancement of the ionization from N-3.

Other evidence on the issue of intramolecular interactions in aqueous solutions of **23** is obtained from a detailed study of its uv spectra in the strongly alkaline pH region. It shows a minor but distinct shift in the spectrum between pH 12.4, at which there must be complete conversion of the pyrimidine to the corresponding monoanion, and pH 15, a pH range over which the ionization of the hydroxy function at the 5 substituent should occur. This evidence of an intramolecular interaction between the hydroxy group in the side chain and a region of the pyrimidine is in analogy to the spectral differences between the ribosyl and the deoxyribosyl nucleosides at high alkaline pH, which are related to the ionization of the 2′-OH group.[48,49] It is confirmatory of the conclusions from the ir studies of **23.**

In summary, with regard to the uv spectral properties and ratio of monoanionic species at equilibrium of the "anomeric" uracil pseudonucleosides and some analogous cyclopentane isosteres, the following points emerge: The hydroxy group at the 5′ terminal of the pseudonucleosides alone does not exert a substantial influence. Similar conclusions can now be drawn for the 2′- and 3′-hydroxyl groups from our previous studies.[5] On the other hand, the allylic oxygen function in the "sugar" moiety of the pseudonucleosides, or in simpler tetrahydrofuran analogs, such as the ones illustrated in Figure 2, is sufficient for the modification of the equilibrium of the monoanionic species. This may be attributed to the field effect of that lone pair of oxygen electrons which possesses π character.[50] Thus the problem related to the differences in spectra and equilibrium of monoanionic tautomeric species in the α- and β-pseudouridines can be now focused on the identification and mode of interaction of the single or the combination of structural features that, particularly in the α anomer, interfere with the effect of the allylic oxygen of the "sugar".

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. The ^{1}H NMR spectra were obtained using a Varian A-60 spectrometer with tetramethylsilane as an internal reference. Routine uv (H_2O) and infrared spectra were determined with Unicam SP800 and Perkin-Elmer Infracord spectrophotometers. Accurate ϵ values in the uv were measured with a Beckman DU. For the ir studies of hydrogen bonding a long-path (25 mm) cell with NaCl windows and spectrograde CCl_4 freshly distilled from P_2O_5 were used. The concentration of the examined material in solution was less than 5×10^{-3} *M*. A matched cell filled with the same solvent served as a reference, and the spectra were recorded with a Perkin-Elmer 221 spectrophotometer. All solvents were removed in a Buchler flask evaporator under reduced pressure unless otherwise indicated. All solids were dried under reduced pressure over P_2O_5 at suitable temperature. An Eastman chromagram silica gel sheet was used for TLC and developed as indicated. Composition and homogeneity of liquid samples were monitored by a Varian Aerograph 2440 gas chromatograph using a column (5 ft × ⅛ in.) packed with 1.5% OV-101 on 100/120 Chromosorb H/P. Silica gel for column chromatography was grade 923, 100–200 mesh from Grace-Davison Chemical, unless otherwise specified.

***cis*-1,3-Cyclopentanedicarboxylic Acid (5).** A solution of 66.6 g (0.34 mol) of $NaMnO_4 \cdot 3H_2O$ in 500 ml of H_2O was added dropwise and with continuing vigorous stirring over a period of ~3 hr to a mixture of 10.34 g (0.11 mol) of norbornylene in 580 ml of 2,2,4-trimethylpentane and 730 ml of H_2O into which was bubbled a constant stream of CO_2. Throughout the addition of the permanganate solution, the temperature of the mixture was maintained at 10–15° and vigorous CO_2 sparging was continued. Immediately after the addition was completed the resulting mixture was decolorized with SO_2 at <20°, then concentrated to about 400 ml, filtered, and the filtrate was cooled in an ice bath and acidified with 30 ml of concentrated HCl. The resulting salts were removed by filtration, washed well with ether, and the washings were saved. The aqueous filtrate was extracted with ether (3 × 200 ml) and the combined ether extract and washings were dried with Na_2SO_4. Evaporation of the solvent gave a white solid which, without further purification, was used in the next step. The average yield was 90% and the melting point of each batch varied over a 2° range between 116 and 121°. A sample purified by recrystallization from ether melted at 123–124° (lit. mp 119.9–120.6°);[25] NMR ($CDCl_3$) τ 6.8–8.5 (envelope, 2, CH_2), 7.5–8.5 (envelope, 4, CH_2CH_2); ir λ_{max} (KBr) 1680 cm^{-1} (ν_{CO}).

Dimethyl *cis*-Cyclopentane-1,3-dicarboxylate (6). A. The dicarboxylic acid **5** (15.8 g, 0.1 mol) was combined with 60 ml of ethylene dichloride, 25 ml of CH_3OH, and 3 ml of concentrated H_2SO_4, and the solution was heated at reflux overnight. The mixture was then washed successively with 100 ml each of water, a saturated solution of $NaHCO_3$, and water again, and then dried with Na_2SO_4. Concentration of the dry solution and distillation of the residue gave 14.6 g (78%) of the dimethyl ester: bp 78–80° (1–2 Torr); NMR ($CDCl_3$) τ 6.32 (s, 6, 2 OCH_3), 6.9–7.6 (envelope, 2, CH_2), 7.6–8.3 (envelope, 4, CH_2CH_2); ir λ_{max} (neat) 3000, 1740 (ν_{CO}), 1435 (δ_{as} OCH_3), 1365 (δ_s OCH_3), four partially resolved bands at 1265, 1250, 1200, and 1183, and broad bands centered at 1040, 1008, and 920 cm^{-1}.

Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.57. Found: C, 58.27; H, 7.60.

B. The same quantity of the acid was dissolved in 117 ml of CH_3OH and to the solution were added 11.76 ml of concentrated HCl and 294 ml of 2,2-dimethoxypropane. The container was stoppered, and the reaction mixture was stirred at room temperature for 1 hr, then concentrated to ~200 ml and added (with the aid of a small volume of benzene for rinsing) to 400 ml of CH_2Cl_2. The resulting solution was washed with saturated $NaHCO_3$ solution twice, then water, and dried over Na_2SO_4. After evaporation of the solvent the residue was distilled, and the product (17.9 g, 96%) was collected at 77–79° (1–2 Torr). Combined preparations were distilled on a spinning band column. The pure product distilled at 79° (1.2 Torr).

***cis*-1,3-Bis(hydroxymethyl)cyclopentane (7)** was prepared by the reduction of the dimethyl ester **6** with $LiAlH_4$, using a modification of the procedure of Birch and Dean.[29] To a suspension of 2.8 g of $LiAlH_4$ in 400 ml of dry ether was added dropwise a solution of 13.7 g (0.074 mol) in 100 ml of ether. The mixture was allowed to stir overnight at room temperature under nitrogen, then was cooled in an ice bath, and the reaction was quenched by the successive addition of 2.8 ml of H_2O, 2.8 ml of NaOH (15%), and 9 ml of H_2O. The salts were removed by filtration and continuously extracted (Soxhlet) with ether. The combined filtrate and extract was dried over Na_2SO_4, then concentrated, and the residue was distilled to give 9.27 g (96.5%) of the product, boiling at 78–79° (0.040 Torr) [lit. bp 118° (0.5 Torr)]:[29] NMR (Me_2SO-d_6) τ 5.62 (t, 2, 2 OH, J = 5.5 Hz), 6.71 (pair of d, 4, $J_1 = J_2$ = 5.5 Hz, 2 CH_2O), 7.65–9.5 (envelope, 8); ir λ_{max} (neat) 3400 (broad, ν_{OH}), 3900, 3800, 1450, 1380, 1060, 1020, 950 cm^{-1}.

***cis*-1,3-Bis(hydroxymethyl)cyclopentane Ditosylate (8) and Monotosylate (9).** *cis*-1,3-Bis(hydroxymethyl)cyclopentane (**7**, 17.20 g, 0.132 mol) was dissolved in 75 ml of dry pyridine, and the solution was cooled in an ice bath. A solution of 27.7 g (0.145 mol) of *p*-toluenesulfonyl chloride in ~100 ml of dry ether was added dropwise with mechanical stirring. Upon completion of the addition, the mixture was allowed to stand at 0–5° for 3 days and then poured with efficient stirring into 150 ml of ice water. Stirring was continued until the ice had melted, and then the mixture was extracted with benzene (6 × 80 ml). The extract was then washed successively with 2 × 100 ml of 3 *N* HCl and 100 ml of saturated $NaHCO_3$ solution, then dried over Na_2SO_4 and concentrated to a viscous oil which was charged directly onto a silica gel column (500 g, 7 × 23 cm). The two tosylated derivatives were eluted with a 4:1 mixture of benzene–ether (ditosylate, **8**, 8.67 g, 0.0198 mol, 15%) followed by ether (monotosylate, **9**, 19.64 g, 0.069 mol, 52.5%). The elution was monitored by TLC (benzene).

Analytically pure material of the monotosylate **9** could be obtained by dissolving the crude product in petroleum ether (bp 40–60°), treating the resulting solution with Norit, filtering, evaporating the solvent, and holding the clear viscous residue under high vacuum for at least 12 hr:[51] NMR (Me_2SO-d_6) τ 5.58 (s, 1, OH), 6.08 (d, 2, J = 6.5 Hz, CH_2OTs), 6.72 (d, 2, J = 6 Hz, CH_2OH), 7.58 (s, 3, CH_3Ph); ir λ_{max} (neat) 3400 (broad, ν_{OH}), 1350 (ν_{as} SO_2), 1170 cm^{-1} (ν_s SO_2).

Anal. Calcd for $C_{14}H_{20}O_4S$ (9): C, 59.13; H, 7.08; S, 11.27. Found: C, 59.09; H, 7.19; S, 11.20.

The crude ditosylate 8 was dissolved in ~200 ml of hot ether by gradually adding a little methanol. Several crops of pure material were collected as white needles, mp 105–107° (lit. mp 108.5–109°),[29] by adding petroleum ether and cooling; NMR (Me_2SO-d_6) τ 6.09 (d, 4, J = 6.5 Hz, 2 CH_2O), 7.57 (s, 3, CH_3Ph); ir λ_{max} (KBr) 3000, 1600, 1350, 1195, 1175, 1100, 1020, 960, 943, 930, 880, 835, 825, 798, 710, 675 cm^{-1}.

Anal. Calcd for $C_{21}H_{26}O_6S_2$ (8): C, 57.51; H, 5.97; S, 14.62. Found: C, 57.58; H, 6.01; S, 14.68.

***cis*-1,3-Bis(hydroxymethyl)cyclopentane Ditrityl (10) and Monotrityl (11) Derivatives.** A solution of the dimethanol 7 (3.9 g, 0.03 mol) in ~50 ml of dry pyridine was cooled to about 7° and trityl chloride (9.17 g, 0.033 mol) was added in one portion. The reaction mixture was stirred in the cold for 72 hr, then the bulk of the solvent was removed in vacuo at below 30°. The viscous residue was treated with 500 ml of crushed ice, and, when the ice melted, the supernatant solution was discarded and the gummy residue was dissolved in benzene. The resulting solution was washed twice with aqueous saturated NaCl solution, dried over Na_2SO_4, and concentrated to a small volume which was charged on a silica gel column (4 × 50 cm). The products were eluted with benzene–ether (8:2). Homogeneous fractions [TLC, C_6H_6–EtOEt (8:2)] were pooled and evaporated to dryness. The ditrityl derivative **10** was recrystallized from petroleum ether (bp 30–60°) to yield 4.98 g (0.00811 mol) of product: mp 129–131°; NMR ($CDCl_3$) τ 2.32–2.90 (envelope, 30), 7.03 (d, 4, J = 6.5 Hz, 2 CH_2O); ir λ_{max} (KBr) 3040, 2910, 2850, 1590, 1480, 1440, 1205, 1070, 1055, 900, 775, 763, 748, 705 cm^{-1} (broad).

Anal. Calcd for $C_{45}H_{42}O_2$ (10): C, 87.90; H, 6.88. Found: C, 87.95; H, 6.85.

The monotrityl derivative 11 was recrystallized from ether–petroleum ether (bp 30–60°). Three crops were obtained, totaling 5.30 g (0.0142 mol) of product: mp 127–128°; NMR ($CDCl_3$) τ 2.45–3.0 (envelope, 15), 6.63 (d, 2, J = 6 Hz, CH_2OH), 7.03 (d, 2, J = 6 Hz, CH_2OTr); ir λ_{max} (KBr) 3400 (broad), 3050, 2910, 2880, 1590, 1480, 1440, 1210, 1030, 900, 776, 768, 754, 710, 698 cm^{-1}.

Anal. Calcd for $C_{26}H_{28}O_2$ (11): C, 83.83; H, 7.57. Found: C, 83.90; H, 7.63. The combined yield of the two trityl derivatives **10** and **11** was 74%.

***O*-Trityl-*cis*-1,3-bis(hydroxymethyl)cyclopentane *p*-Toluenesulfonate (12) and Methanesulfonate (12a).** The monotrityl derivative 11 (1 g, 0.0027 mol) was dissolved in 5 ml of dry pyridine, and to that solution was added an excess of *p*-toluenesulfonyl or methanesulfonyl chloride. The mixture was stirred at room temperature overnight, then poured into ice water with stirring, and the product was extracted with chloroform. The extracts were washed with aqueous saturated NaCl solution and dried over Na_2SO_4. Evaporation of the solvent gave a gummy or oily residue which was chromatographed on a silica gel column (*l* 25 cm). The product was eluted with benzene–ether (4:1). Removal of the solvent and vacuum drying gave a quantitative yield of a pale yellow oil.

***p*-Toluenesulfonate 12:** NMR ($CDCl_3$) τ 6.22 (d, 2, J = 6.5 Hz, CH_2OTs), 7.13 (d, 2, J = 6.5 Hz, CH_2OTr), 7.69 (1, 3, CH_3Ph).

Anal. Calcd for $C_{33}H_{34}O_4S$: C, 75.25; H, 6.51; S, 6.09. Found: C, 75.41; H, 6.56; S, 6.12.

Methanesulfonate 12a. The oil solidified to a waxy material after several weeks: NMR ($CDCl_3$) τ 6.0 (d, 2, J = 6.5 Hz, CH_2OMs), 7.05 (d, 2, J = 5.0 Hz, CH_2OTr), 7.12 (s, 3, CH_3SO_2); ir λ_{max} (neat) 3030, 2930, 2850, 1590, 1480, 1440, 1350, 1170, 1070, 900 (broad), 825 (broad), 765, 750, 710 cm^{-1}.

Anal. Calcd for $C_{27}H_{30}O_4S$: C, 71.91; H, 6.71; S, 7.11. Found: C, 71.94; H, 6.71; S, 7.00.

***cis*-3-Hydroxymethylcyclopentaneacetonitrile (17).** A stirred solution of 16.90 g (0.0595 mol) of *cis*-1,3-bis(hydroxymethyl)cyclopentane monotosylate (9), 0.18 g (1.43 mmol) of iodine, and 5.65 g (0.087 mol) of potassium cyanide in a mixture of 43.5 ml of dimethylformamide and 25.5 ml of water was kept in a stoppered flask at 60° for 3 days. It was poured with stirring into 120 ml of ice water, and, after the ice had melted, the mixture was extracted with CH_2Cl_2 (5 × 90 ml). The combined extracts were dried with Na_2SO_4 and concentrated, and the viscous residue was first fractionated in vacuo (5 Torr) through a short Vigreux column with an oil bath at 50°. The distillate, collected between 32 and 43°, consisted of the bulk of DMF and a trace of the product. When this distillation ceased, the remaining liquid was transferred into a smaller flask, and the product (5.0 g, 60%) was distilled at 86–90° (0.015–0.017 Torr):[32] NMR ($CDCl_3$) τ 6.46 (s, 1, OH), 6.56 (s, 2, CH_2O), 7.6 (s, 2, CH_2CN); ir λ_{max} (neat) 3400, 2960, 2890, 2250 (ν_{CN}), 1450, 1420, 1050, 1009 cm^{-1}.

Anal. Calcd for $C_8H_{13}NO$: C, 69.03; H, 9.41; N, 10.06. Found: C, 69.27; H, 9.47; N, 10.00.

***cis*-3-Hydroxymethylcyclopentaneacetic Acid (18).** A solution of the nitrile 17 (4.70 g, 0.0338 mol) in a mixture of 50 ml of ethanol, 12.5 ml of water, and 6.0 g of KOH was heated to reflux under nitrogen for 6 days. The ethanol was removed by distillation, while the volume of the reaction mixture was maintained at ~50 ml by successive addition of small volumes of water. The resulting mixture of the aqueous solution and a small amount of solid was continuously extracted with ether for 24 hr, then cooled, acidified with 9 ml of concentrated HCl, and again continuously extracted with a fresh volume of ether for an additional 24 hr. The latter extract was dried with Na_2SO_4 and concentrated to a yellowish oil. The bulk of the product was purified (as indicated by GLC) by filtration through a pad (*d* 1 cm, *l* 1 cm) of Norit, yield 4.2 g (78%). An analytical sample was obtained by high vacuum (0.013 Torr) distillation in a molecular still:[52] NMR ($CDCl_3$) τ 3.04 (s, 2, 2 OH), 6.42 (d, 2, J = 6 Hz, CH_2O), 7.6 (s, 2, CH_2CO); ir λ_{max} (neat) 3400 (sh), 2950, 2600 (sh), 1700, 1400, 1020, 1005 cm^{-1}.

Anal. Calcd for $C_8H_{14}O_3$: C, 60.73, H, 8.92. Found: C, 60.76; H, 8.88.

***cis*-3-Hydroxymethylcyclopentaneacetic Acid Lactone (16).** A mixture of 15 g (0.121 mol) of the ketone **15**, 35 g of purified *m*-chloroperbenzoic acid,[53] and 21.0 g (0.25 mol) of sodium bicarbonate in 500 ml of $CHCl_3$ (freed of ethanol by passing over basic alumina) was mechanically stirred in a sealed flask and in the dark for 1 week. During that time, the built-up pressure was periodically released. The mixture was filtered and the solids were washed well with $CHCl_3$. The combined filtrates were washed several times with small volumes of cold 10% sodium sulfite solution until it gave a negative test with starch–iodide paper (~350 ml of the sulfite solution is required), then with cold $NaHCO_3$ solution and dried over Na_2SO_4. After the solvent was removed the remaining oil was chromatographed on a silica gel column (250 g) developed with a mixture of petroleum ether (bp 30–60°)–$CHCl_3$ (4:1). Two components identified (ir and ^1H NMR) as *m*-chlorobenzoic acid [recrystallized from ether–petroleum ether (bp 30–60°), mp 156–157°] and starting material (purified by sublimation) were eluted first. The composition of the eluent was then changed to 1:1, and fractions containing the lactone **16** were pooled and concentrated, leaving an oil that on drying in vacuo became a waxy solid. Recrystallization[54] from petroleum ether (bp 30–60°), including treatment with Norit, gave a total of 10.4 g (61%) of product: mp 125–129°; NMR ($CDCl_3$) τ 5.81 (d, 2, J = 3 Hz, CH_2O), 6.9–7.8 (envelope), 7.8–8.6 (envelope); ir λ_{max} (KBr) 2980, 1725 (ν_{CO}), 1460, 1420, 1390, 1340, 1320, 1260, 1215, 1160, 1090, 1040, 990, 970, 935, 875, 852, 780, 700 cm^{-1}.

Anal. Calcd for $C_8H_{12}O_2$: C, 68.54; H, 8.63. Found: C, 68.35; H, 8.52.

α-Hydroxymethylene-(*cis*-3-hydroxymethyl)cyclopentaneacetic Acid Sodium Salt (19). To a cooled (ice bath) stirred suspension of 1.62 g (0.03 mol) of sodium methylate in 60 ml of dry ether, a mixture of 4.20 g (0.03 mol) of the lactone **16** and 3.6 g (3.85 ml, 0.06 mol) of methyl formate was added dropwise. The ice was allowed to melt and the mixture was stirred for 4 days. The solvent was decanted from the precipitated gum, which was repeatedly triturated with dry ether to give a granular, brownish solid, collected and dried in vacuo, yield 3.150 g. In aqueous solution the solid showed a strong absorption at 277 nm which was eliminated upon acidification.

5-(*cis*-3-Hydroxymethylcyclopentane)-2-thiouracil (20). In a representative experiment, the sodium enolate **19** (3.04 g) was added to a solution of thiourea (2.43 g, 0.032 mol) in 250 ml of dry EtOH containing 736 mg (0.032 mol) of sodium, and the mixture was heated at reflux for 6 hr. It was acidified with an excess of glacial acetic acid and the solvent was removed. The residue was chromatographed on a Dowex 50 (H^+, 200–400 mesh) column (*l* 50 cm) which was washed with water. The fraction containing the product was evaporated to dryness, the residue was dissolved in methanol, and the solution was treated with Norit and filtered. Water (~20 ml) was added to the filtrate, which was then concentrated. Upon removal of the methanol, an aqueous suspension of the product was obtained. After overnight cooling the product was collected, washed once with water, and dried in vacuo at 80° (365 mg, mp 211–212°): NMR (Me_2SO-d_6) τ −0.90 (broad, 2, 2 NH), 2.87 (s, 1, HC=), 5.56 (poorly resolved triplet, OH), 6.69 (t, 2, J =

5 Hz, CH_2O), 7.28 (broad, 1), 6.58–8.65 (envelope, 7); ir λ_{max} (KBr) 3400, 3200, 2980, 2900, 1660, 1560, 1470, 1210 (broad), 1120, 1050, 1000, 915 cm^{-1} (broad).

Anal. Calcd for $C_{10}H_{14}N_2O_2S$: C, 53.07; H, 6.23; N, 12.38; S, 14.17. Found: C, 53.05; H, 6.24; N, 12.27; S, 14.11.

5-(*cis*-3-Hydroxymethylcyclopentane)uracil (1). A mixture of 362 mg (0.0016 mol) of the thiouracil **20** and ~15 ml of water containing 198 mg (0.002 ml) of $ClCH_2COOH$ was heated at gentle reflux. Within 30 min a clear yellow solution resulted. After ~18 hr, 0.7 ml of concentrated HCl was added to the solution, and the refluxing was continued for a total of 30 hr. Upon cooling a solid separated and was collected by filtration, washed with a small volume of cold water, and dried. The combined filtrates were chromatographed on a Dowex 50 (H^+, 200–400 mesh) column (*l* 50 cm) and washed with water. After evaporation of the appropriate fraction, an additional small amount of material was recovered. The crude product was dissolved in 40 ml of methanol, the solution was decolorized with Norit and filtered, and the filtrate was concentrated on the steam bath until it became turbid. After cooling (5°) overnight the product was collected, washed once with a small volume of cold methanol and then with ether, and dried in vacuo (280 mg, mp 274–275°). An additional 37 mg was recovered from the mother liquors, total yield 94%: NMR (Me_2SO-d_6) τ −0.96 (s, 1, N-3 H), −0.61 (d, 1, J = 3 Hz, N-1 H), 2.88 (d, 1, J = 3 Hz, HC=), 5.55 (t, 1, J = 5 Hz, OH), 6.70 (t, 2, J = 5 Hz, collapses to a doublet with D_2O, J = 6 Hz, CH_2O), 7.26 (multiplet, 1), 7.69–9.08 (envelope, 7); ir λ_{max} (KBr) 3500 (sh), 3250, 3100, 2950, 1740, 1670, 1460, 1420, 1245, 1208, 1060, 1015, 945, 920, 850 (broad), 780, 775 cm^{-1}.

Anal. Calcd for $C_{10}H_{14}N_2O_3$: C, 57.13; H, 6.71; N, 13.32. Found: C, 57.13; H, 6.65; N, 13.29.

5-(*cis*-3-Hydroxymethylcyclopentane)-1-methyluracil (23). A mixture of the uracil derivative 1 (210 mg, 0.001 mol), 7 ml of hexamethyldisilazane, and 0.4 ml of trimethylchlorosilane was heated in a bath at 150–160° for ~20 hr. The solvents were removed by distillation in vacuo by maintaining the temperature of the bath at 50° while gradually reducing the pressure to 0.050 Torr. To the viscous residue, **22**, was added 10 ml of CH_3I and the solution was heated under reflux overnight. The solvent was then evaporated by boiling, the residue was dissolved in ~10 ml of EtOH, and the solution was heated under reflux for 12 hr. After removal of the solvent the residue was chromatographed on a silica gel 60 (E. Merck) column (4 × 16 cm) which was washed with C_6H_6–CH_3OH (8:2). Fractions containing the product (TLC) were pooled, the solvent was removed, and the viscous residue was dried in vacuo and then dissolved in EtOH. The solution was treated with Norit and again the solvent was removed. Upon the addition of a few milliliters of dry ether and standing at room temperature for several hours, the residue solidified. The solvent was then decanted, and the solid was dissolved in 2–3 drops of ethanol. Addition of an excess of dry ether induced crystallization (rosettes). After standing overnight at room temperature the product was collected, washed with dry ether, and recrystallized once more, as above, with Norit treatment. The pure product was collected, washed with ether, and dried under high vacuum at room temperature: yield 150 mg (67%); mp, shrinks at 127° and melts at 147–148°, NMR (Me_2SO-d_6) τ −1.12 (s, 1, N-3 H), 2.59 (s, 1, HC=), 5.54 (t, 1, J = 5 Hz, OH), 6.68 (t, 2, J = 6 Hz, CH_2O), 6.78 (s, 3, CH_3N), 7.25 (broad, 1), 7.77–9.11 (envelope, 7); ir λ_{max} (KBr) 3400 (sh), 3200, 3050, 2920, 2850, 1715, 1685, 1450, 1320, 1180–1210 (three partially resolved bands), 1100, 1045, 1000, 930, 900, 840, 760 cm^{-1}.

Anal. Calcd for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: C, 58.91; H, 7.15; N, 12.47.

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Registry No.—**1**, 55606-06-9; **4**, 498-66-8; **5**, 876-05-1; **6**, 39590-04-0; **7**, 3965-56-8; **8**, 55606-07-0; **9**, 55606-08-1; **10**, 55606-09-2; **11**, 55606-10-5; **12**, 55606-11-6; **12a**, 55606-12-7; **15**, 14252-05-2; **16**, 55606-13-8; **17**, 55606-14-9; **18**, 55606-15-0; **19**, 55606-16-1; **20**, 55606-17-2; **22**, 55606-18-3; **23**, 55606-19-4; *p*-toluenesulfonyl chloride, 98-59-9; trityl chloride, 76-83-5; methanesulfonyl chloride, 124-63-0; potassium cyanide, 151-50-8; thiourea, 62-56-6; hexamethyldisilazane, 999-97-3; trimethylchlorosilane, 75-77-4.

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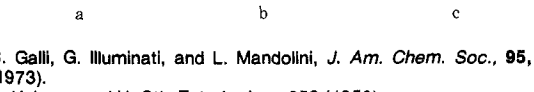

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(51) Several attempts to crystallize the monotosylate derivative failed. It decomposes during sublimination in vacuo, and it separates from methanol, petroleum ether, or various solvent mixtures as an oil.
(52) The distillation leaves a waxy residue from which a crystalline material (mp 65–67°) was isolated by recrystallization from ether containing a trace of chloroform. The ir spectrum of this material is similar to that of **18** but shows a greatly attenuated OH band and a small shift of the carbonyl absorption to 1725 cm^{-1}.
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(54) The material can also be purified by sublimation in vacuo at <50°.

Side Reactions in Peptide Synthesis. II.[1] Formation of Succinimide Derivatives from Aspartyl Residues

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Acylation of peptides in which the side chain of an aspartyl residue is unprotected can result in the formation of succinimide derivatives. The presence of base enhances the formation of such by-products: the carboxylate attacks the carbonyl of active esters and forms a reactive intermediate, probably a mixed anhydride, that in turn acylates the adjacent amide. This is a particularly serious side reaction in acylation with activated derivatives of glycine. The observation that substituted phenols liberated in active ester reactions can combine with tertiary bases and thus liberate the free side-chain carboxyl groups from their salts is also reported.

In our continued effort toward the total synthesis of the gastrointestinal hormone cholecystokinin,[2] synthesis of the N-terminal octapeptide was reported earlier.[3] Preparation of peptides corresponding to C-terminal sequences of this hormone was also undertaken and involved several intermediates that were used in synthesis of gastrin[4,5] and caerulein,[6] peptides which have C-terminal sequences closely related to that of cholecystokinin, and also in the synthesis of a biologically active dodecapeptide portion of cholecystokinin.[7] The initial steps of our synthesis are summarized in Scheme I. The aspartyl residue in the penultimate position was incorporated as the β-benzyl ester, but the side-chain protection was removed from the resulting dipeptide amide. Thus, from there on the synthesis was continued with amino components in which the carboxyl group of the aspartyl residue remained unprotected. This approach, applied also in other laboratories,[4,7] seemed to offer certain advantages. Protection of a side-chain carboxyl in the form of *tert*-butyl ester requires prolonged acidolysis at the deprotection stage; this was undesirable because of the presence of several acid-sensitive residues (tryptophan, tyrosine *O*-sulfate) in cholecystokinin. Benzyl ester protection was also considered but the presence of methionines in the sequence made it questionable whether or not hydrogenolysis can be applied for deblocking.[8] Last, but not least, a free carboxyl was thought to be less likely to lead to aminosuccinimide derivatives[9] (Chart I) than β esters of aspartic acid. Early removal of the benzyl ester protection, prior to the incorporation of the first methionine residue, was expected to circumvent these difficulties.

Scheme I
Synthesis of the Protected Pentapeptide VI

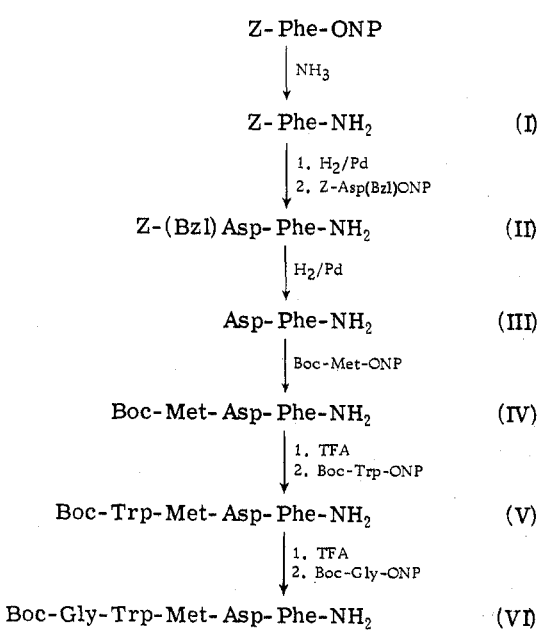
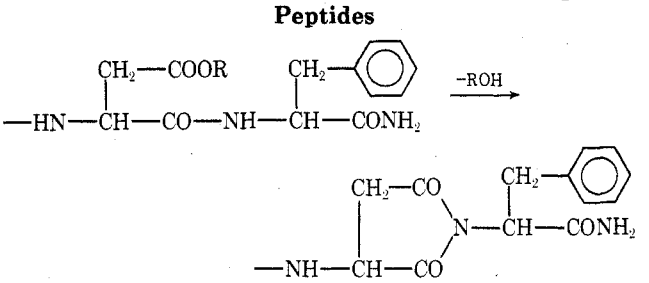


Chart I
Formation of Succinimide Derivatives from Aspartyl Peptides



In the synthesis of the protected tetrapeptide amide V and the protected pentapeptide amide VI, the formation of several by-products was observed. Most of these by-products represented only minor impurities. The presence of slow moving, polar materials on thin layer plates was attributed to air oxidation, producing peptides in which the methionine residues were oxidized to sulfoxides or sulfones. These impurities could be readily removed by recrystallization of the crude products. On the other hand, some less readily explained by-products could also be detected in the protected tetra- and pentapeptide derivatives. These products seemed to be less polar, since they moved faster on the chromatograms than the desired intermediates. In the step leading from the tetrapeptide derivatives to the protected