

solutions were determined with an Orion Research Model 701A digital pH meter. The CD spectra were recorded on a Jobin Yvon Dichrograph II using 0.1- and 1.0-cm cells, a scan rate of 12 nm/min, and a 10-s time constant.

Racemic warfarin and 1-octanol were purchased from Sigma Biochemicals. All other chemicals were of reagent grade.

(S)-(-)-Warfarin [3-(1-Phenyl-3-oxobutyl)-4-hydroxycoumarin (1)]. Racemic warfarin was resolved by the method of West:⁹ $[\alpha]_D^{25} = -148.8$ (0.4) $^\circ$ (c 1.2, 0.5 N NaOH); CD ($\times 10^3$) (0.0967 mg/mL of 50 mM phosphate buffer, pH 7.38) $[\theta]_{332}$ 0, $[\theta]_{290}$ -4.0, $[\theta]_{260}$ 0, $[\theta]_{240}$ +10.7, $[\theta]_{250}$ 0, $[\theta]_{210}$ -11.8; CD ($\times 10^3$) (0.1127 mg/mL of octanol) $[\theta]_{340}$ 0, $[\theta]_{305}$ -3.6, $[\theta]_{286}$ 0, $[\theta]_{260}$ +9.0, $[\theta]_{235}$ 0, $[\theta]_{225}$ -42.4.

(S)-(-)-Phenprocoumon [3-(1-Phenylpropyl)-4-hydroxycoumarin (2)]. Phenprocoumon was prepared by the method of Link¹⁰ and resolved by the method of West:¹¹ $[\alpha]_D^{25} = -120.0$ (1.0) $^\circ$ (c 1.5, 95% EtOH); CD ($\times 10^3$) (0.1084 mg/mL of 50 mM phosphate buffer, pH 7.35) $[\theta]_{330}$ 0, $[\theta]_{310}$ +4.6, $[\theta]_{290}$ 0, $[\theta]_{270}$

-7.2, $[\theta]_{240}$ 0, $[\theta]_{227}$ +5.6, $[\theta]_{220}$ 0, $[\theta]_{210}$ -29.1; (0.0995 mg/mL of octanol) $[\theta]_{340}$ 0, $[\theta]_{280}$ -6.0, $[\theta]_{255}$ 0, $[\theta]_{240}$ +11.5, $[\theta]_{225}$ 0, $[\theta]_{210}$ -25.6.

(S)-(-)-Warfarin 4-Methyl Ether [3-(1-Phenyl-3-oxobutyl)-4-methoxycoumarin (3)]. This compound was prepared from (-)-1 as previously described:¹² $[\alpha]_D^{25} = -9.1$ (2.0) $^\circ$ (c 1.8 MeOH); CD ($\times 10^3$) (0.1070 mg/mL of octanol) $[\theta]_{340}$ 0, $[\theta]_{310}$ +18.9, $[\theta]_{290}$ 0, $[\theta]_{275}$ -15.9, $[\theta]_{240}$ 0, $[\theta]_{222}$ +21.8, $[\theta]_{215}$ 0, $[\theta]_{210}$ -35.8.

(S,S)-(+)-Cyclocoumarol [2(3H)-2-Methyl-2-methoxy-4-phenyl-5-oxobenzopyrano[3,4-b]dihydropyran (4)]. This compound was prepared from (-)-1 as previously described:¹² $[\alpha]_D^{25} = +33.1$ (0.6) $^\circ$ (c 1.9, CHCl₃); CD ($\times 10^3$) (0.1044 mg/mL of octanol) $[\theta]_{325}$ 0, $[\theta]_{305}$ -7.1, $[\theta]_{280}$ 0, $[\theta]_{265}$ +21.4, $[\theta]_{235}$ 0, $[\theta]_{220}$ -91.6.

Partition Experiments. Saturated solutions of (S)-(-)-1 and (S)-(-)-2 in 50 mM phosphate buffer, pH 7.4, were agitated with equal volumes of 1-octanol on a shaker bath at 25 $^\circ$ C for 16 h. Aliquots were removed from each phase and the CD spectra promptly recorded.

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2,5-Anhydro-1-deoxy-1-phosphono-D-altritol, an Isosteric Analogue of α -D-Ribofuranose 1-Phosphate¹

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Condensation of tetramethyl methylenephosphonate with 2,3-O-isopropylidene-5-O-trityl-D-ribose gave a mixture of 2,5-anhydro-1-deoxy-1-(diethoxyphosphinyl)-2,3-O-isopropylidene-5-O-trityl-D-altritol and -allitol. Separation of the isomers and deprotection gave 2,5-anhydro-1-deoxy-1-phosphono-D-altritol and -allitol. The former is the stable isosteric methylenephosphonate analogue of α -D-ribose 1-phosphate, the ribose donor in nucleoside phosphorylase catalyzed nucleoside biosynthetic reactions. It did not, however, inhibit purine nucleoside phosphorylase at concentrations of 6 mM.

Investigations into the mechanism of inborn immunodeficiency diseases have pointed out the role of the purine salvage pathways in the viability of T and B lymphocytes. A deficiency in purine nucleoside phosphorylase has been identified as the cause of loss of immunocompetence in T lymphocytes.³ The loss of this enzymic activity is responsible for the accumulation of 2'-deoxyguanosine, which apparently is selectively toxic to T lymphocytes.⁴ It seems reasonable, therefore, that a compound that inhibits purine nucleoside phosphorylase might be a highly selective antineoplastic agent for leukemias and lymphomas derived from T lymphocytes.

Purine nucleoside phosphorylase catalyzes the reversible phosphorolysis of inosine (1) or guanosine (2) to give the corresponding purine, hypoxanthine (3) or guanine (4), and α -D-ribofuranose 1-phosphate (R-1-P, 5). Our initial approach to the design of inhibitors of this enzyme was to prepare an unreactive isosteric analogue of R-1-P. The

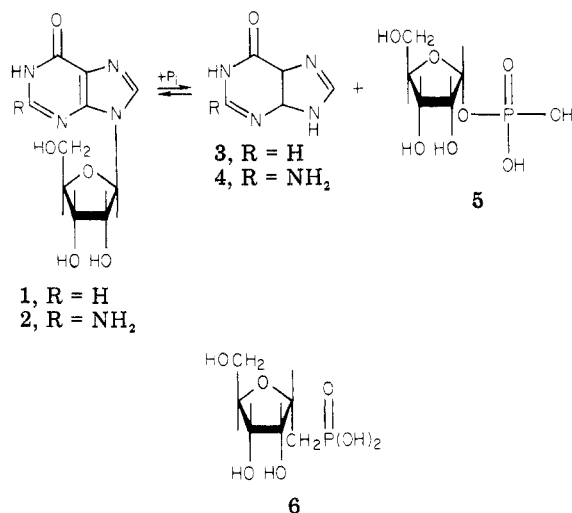
substitution of the phosphate ester oxygen with a methylene has given a number of biologically active compounds in the past,⁵ and we undertook the preparation of 2,5-anhydro-1-deoxy-1-phosphono-D-altritol⁶ (6) to see if this strategy would yield an inhibitor of purine nucleoside phosphorylase.

The synthesis of this class of phosphonate analogue of glycosyl phosphates has very few literature precedents. Very recently, Russo and co-workers have prepared the methylenephosphonate analogues of α - and β -D-glucosyl phosphate via the Michaelis-Arbuzov reaction from blocked 1-bromo-1-deoxy-2,6-anhydroheptitols.⁷ We have found a method, however, that gives blocked ribosyl methanephosphonates in one step from readily prepared blocked ribose derivatives.

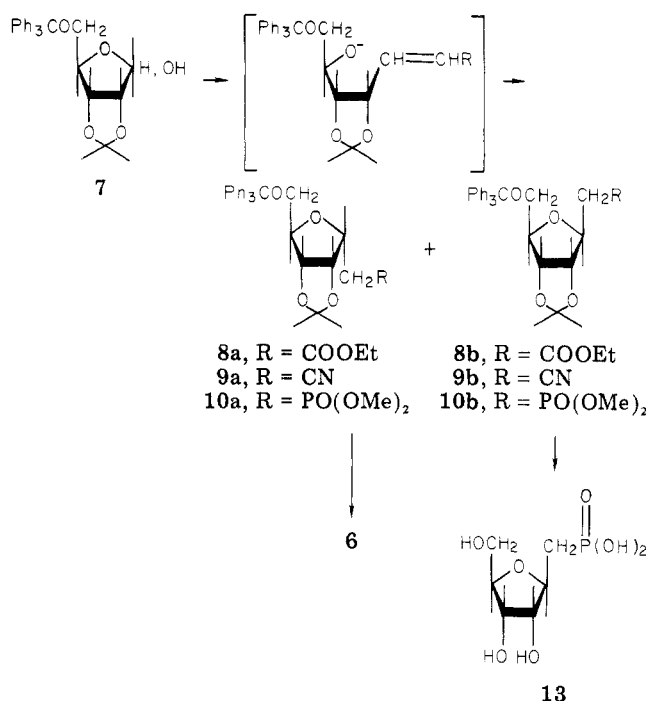
The synthetic plan was based on the recently successful preparation of derivatives of 3,6-anhydro-D-altra- and -allo-heptonic acids 8 and 9 from 2,3-O-isopropylidene-5-

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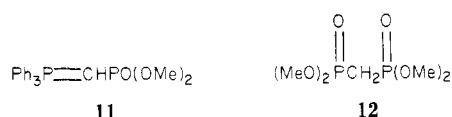
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O-trityl-D-ribose (**7**) and [(ethoxycarbonylmethylene]- or (cyanomethylene)triphenylphosphorane, respectively.⁸⁻¹⁰ These Wittig reactions were reported to proceed in nearly quantitative yield. In our hands, however, [(dimethoxyphosphinyl)methylene]triphenylphosphorane¹¹ (**11**) and **7** did not give the desired phosphonate **10**.



The Wadsworth-Emmons modification of the Wittig reaction is known to greatly enhance the reactivity of otherwise unreactive phosphoranes.¹¹ The requisite Wadsworth-Emmons reagent, tetramethyl methylenebisphosphonate (**12**) was prepared by modifications of liter-



ature procedures.¹² The reaction of **12** with **7** proceeded readily at room temperature to give **10a** and **10b**. The most convenient conditions were found to be a two-phase mixture of methylene chloride and 50% aqueous NaOH, without the necessity of a phase-transfer catalyst.¹³ The two isomers could be separated by preparative TLC at this point.

Deblocking was readily accomplished by treatment with bromotrimethylsilane¹⁴ to exchange the methyl esters for trimethylsilyl esters, followed by removal of all protecting groups with 1:1 trifluoroacetic acid/water. The free acid of **6** thus obtained could not be crystallized and was purified by precipitation of the barium salt, which in turn was converted to the dilithium salt for analysis by passage through IR-120 (Li⁺) cation-exchange resin. Alternatively, dissolution of the free acid in dilute ammonia, followed by lyophilization, gave a slightly hygroscopic form of **6** suitable for biological testing. The ammonium salt of *allo* isomer **13** was obtained similarly from **10b**.

The definitive structural evidence for the structures of **10a** and **10b**, **6**, and **13** came from analysis of the ¹³C NMR spectra of the respective compounds, which are shown in Table I. The assignments of resonances C-1 and C-2 were based primarily on their ¹³C-³¹P coupling constants. C-5 and C-6 were readily distinguished by their chemical shifts (most downfield and most upfield, respectively, in the sugar region 60–85 ppm), and C-3 and C-4 were tentatively assigned by analogy to the ¹³C spectra of **8** and **9** where it was shown that for *allo* derivatives the resonance for C-3 was upfield from that of C-4, whereas in the *altro* derivatives the reverse was true.⁸

The assignment of the *allo* and *altro* configurations to our compounds was based in the fact that the ¹³C chemical shifts of the individual carbons in compounds that are somewhat conformationally rigid are very sensitive to steric interaction.¹⁵ In particular, Roberts and co-workers have shown that the C-1, C-2, and methyl resonances in *cis*-2-methylcyclopentanol lie at higher field than those of the corresponding *trans* isomer.¹⁵ This effect has been observed for furanoses in general¹⁶ and for **8a,b** and **9a,b** in particular.⁸ The two pairs of compounds show ¹³C shifts for C-2 (comparable to C-1 or 10) of the *altro* isomer at 3.5–4 ppm upfield of the corresponding peak of the *allo* isomer. We therefore feel confident in assigning the *altro* configuration (**10a**) to our isomer that was faster moving on TLC. The magnitude of the shift difference (2.5 ppm for the blocked compounds and 3.8 ppm when deblocked) of C-1 between our two isomers would seem to leave little doubt as to the validity of this assignment. Recently, the methylenephosphonate isomers of α- and β-glucose 1-phosphate have been prepared by quite different routes.⁷ Although hexitols have different conformations available than pentitols, those workers also found the ¹³C resonance of the C-1 to be at about 3 ppm higher field in the *cis* isomer than the *trans* isomer.

After publications of our initial report of this work,¹ a report of the synthesis of the diphenyl esters of **6** and **13** appeared.¹⁷ Our assignments of the *allo* and *altro* isomers

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Table I. ^{13}C NMR Spectral Data^a

compd	C-1	C-2	C-3	C-4	C-5	C-6	isopropylidene		
							Me	Me	C
10a	25.2 (141.6)	81.6 (9.0)	76.2	83.0	86.8	64.1	24.7	25.9	111.9
10b	28.0 (141.8)	84.6 (11.8)	79.2	79.0 (3.5)	86.3	64.1	25.2	27.0	113.9
6	28.1 (136.7)	73.4 (8.5)	72.9	76.5	82.0	62.5			
11	31.9 (135.5)	76.01 (12.2)	71.4	78.6 (3.5)	84.5	62.4			

^a Chemical shifts are in ppm downfield from Me_4Si , computed with use of shifts of the internal standards as 77.2 ppm (CHCl_3) or 67.4 (dioxane). Numbers in parentheses are coupling constants to phosphorus in hertz. Compounds 10a and 10b were taken in CDCl_3 , and compounds 6 and 11 were taken as the free acid in D_2O .

are in agreement with that report.

Purine Nucleoside Phosphorylase Inhibition. Both the pure isomers 6 and 13 were tested as inhibitors of bovine spleen purine nucleoside phosphorylase. The reversible enzymic reaction was assayed in the biosynthetic direction with ribose 1-phosphate (0.4 mM) and guanine (0.2 mM) as substrates (pH 7.4) and followed by the change in absorption at 252 nm.¹⁸ At concentrations as high as 6 mM, neither 6 or 13 gave any inhibition of purine nucleoside phosphorylase. It would seem that either the O-1 in ribose 1-phosphate must be a critical binding point or that the C-C-P bond differs too greatly from the C-O-P bond.

Additionally, it has been found that 6 did not inhibit uridine phosphorylase at 4 mM when 5-fluorouracil and ribose 1-phosphate were used as substrates.¹⁹

Experimental Section

Analytical thin-layer chromatography was run on Bakerflex silica gel plates, and preparative TLC was performed with Analtech Uniplates. Proton and ^{13}C NMR spectra were obtained on a JEOL FX-90Q at 90 and 22.5 MHz, respectively. Elemental analyses were obtained either from the microanalytical lab, University of California, Berkeley, or from Galbraith Labs, Knoxville, TN. Exact mass measurement, using fast atom bombardment (FAB) and detecting positive ions, were performed on a VG7070H instrument.

TLC plates were visualized with UV light or with an ethanol/*p*-anisaldehyde/sulfuric acid (10:1:1) spray. After heating, red spots are obtained for all of the 2,5-anhydro-1-deoxy-1-phosphono sugars, whereas green spots are obtained for other sugars.

2,5-Anhydro-1-deoxy-2,3-O-isopropylidene-1-(dimethoxyphosphinyl)-5-O-trityl-D-altritol (10a) and -allitol (10b). A solution of 7²⁰ (2.1 g, 5 mmol) in 7.5 mL of CH_2Cl_2 was mixed with tetramethyl methylenebisphosphonate¹² (12; 1.4 g, 6 mmol) in 7.5 mL of 50% aqueous NaOH, and this two-phase mixture was stirred vigorously for 18 h. The aqueous phase was separated and washed with 10 mL of CH_2Cl_2 , and the combined organic phases were dried with MgSO_4 , and the solvent was removed by evaporation. The residue was chromatographed on four preparative TLC plates (2000 μm silica gel coating, 20 \times 20 cm) developed with EtOAc/hexane (1:4), and the two closely spaced components were isolated together. The compounds were eluted from the silica with MeOH, and the residue remaining after evaporation of the solvent crystallized on standing, giving 3.1 g (61%) of 10a and 10b in a 3:2 ratio (estimated by NMR), mp 61–64 °C. Anal. $\text{C}_{30}\text{H}_{35}\text{O}_7\text{P}$: H; C: calcd, 66.90; found, 66.34.

This mixture was rechromatographed on five preparative TLC plates (1000 μm , 20 \times 20 cm), using the same solvent system as above. The fast-moving fraction corresponded to *altro* isomer

10a and the slow-moving fraction to *allo* isomer 10b. Neither could be induced to crystallize, but they were found to be at least 95% pure by ^1H and ^{13}C NMR.

10a: ^1H NMR (CDCl_3) δ 1.33, 1.49 (s, 3 H each, CH_3), 2.30 (dq, 2 H, H-1), 3.21 (m, 2 H, H-6), 3.74 (dd, 6 H, ester CH_2), 4.21 (t, 2 H, H-5), 4.69 (m, 3 H, H-2,3,4), 7.39 (m, 15 H, trityl).

10b: ^1H NMR (CDCl_3) δ 1.32, 1.51 (s, 3 H each, CH_3), 2.30 (m, 2 H, H-1), 3.23 (m, 2 H, H-6), 3.72 (dd, 6 H, ester CH_2), 4.20 (t, 1 H, H-5), 4.61 (m, 3 H, H-2,3,4), 7.36 (m, 15 H, trityl).

2,5-Anhydro-1-deoxy-1-phosphono-D-altritol (6). To a solution of 372 mg (0.69 mmol) of 10a (obtained as above) in 2 mL of CDCl_3 was added 264 mg (2.5 mmol) of bromotrimethylsilane. The ^{13}C spectra taken after 20 min (see Table 1) indicated, by loss of signals corresponding to the phosphonate methyl esters, that the reaction has gone to completion. This mixture was then added to 2 mL of a 1:1 mixture of trifluoroacetic acid/water. After 30 min 20 mL of water was added, the triphenylmethanol was removed by filtration, and the solvents were removed in vacuo. To this residue was added 20 mL of water and the solvent was removed in vacuo; this was repeated twice, or until all trace of trifluoroacetic acid was gone. The residue was dissolved in 20 mL of water, and this was neutralized with 1 N barium hydroxide. Methanol (50 mL) was slowly added, and the barium salt of the product was collected by filtration was washed with 50 mL of methanol. The barium salt was then dissolved in a minimum of water and eluted through a 50-mL column or IR-120 (Li^+) with water. Lyophilization of the eluent yielded 110 mg (63.9%) of the dilithium salt of 6 (95% *altro* by NMR) as a hygroscopic solid. 6 free acid: NMR (D_2O) δ 2.03 (dd, 2 H, H-1, $J = 18.5, 6.5$ Hz), 3.5–4.2 (unresolved m, 6 H); exact mass calcd for $\text{C}_6\text{H}_{14}\text{O}_7\text{P}$ ($\text{M} + \text{H}^+$) 229.0672, found 229.0636. Anal. $\text{C}_6\text{H}_{11}\text{Li}_2\text{O}_7\text{P} \cdot 1/2\text{H}_2\text{O}$: C, H.

For preparation of the *allo* isomer 13, or for preparation of larger samples of 6 for biological testing from either 10a or 10b, the residue after evaporation of the trifluoroacetic acid (no detectable impurities by NMR) was dissolved in dilute ammonia and lyophilized; hygroscopic ammonium salts were obtained. 13 free acid: NMR (D_2O) δ 2.06 (dd, 2 H, H-1, $J = 19, 4.5$ Hz), 3.4–4.2 (unresolved m, 6 H); exact mass calcd for $\text{C}_6\text{H}_{14}\text{O}_7\text{P}$ ($\text{M} + \text{H}^+$) 229.0672, found 229.0606.

Purine Nucleoside Phosphorylase Assay. The method used was essentially that of Lewis and Glantz.¹⁸ Bovine spleen purine nucleoside phosphorylase was purchased from Sigma Biochemicals, St. Louis, MO. The reaction mixture, in a final volume of 1 mL, contained 0.02 unit of enzyme preparation, 4×10^{-4} M ribose 1-phosphate (obtained from Aldrich Chemical Co., Milwaukee) as the dicyclohexylammonium salt, 2×10^{-4} M guanine, and various concentrations of inhibitor in 0.2 M citrate buffer, pH 7, at 25 °C. The reaction was followed by measuring the increase in absorbance at 252 nm on a Gilford 2600 spectrophotometer.

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Registry No. 6, 86762-89-2; 6·2Li, 90693-28-0; 7, 55726-19-7; 10a, 90481-99-5; 10b, 90482-00-1; 12, 1984-15-2; 13, 90580-54-4; 13·NH₃, 90638-60-1; α-D-ribofuranose 1-phosphate, 18646-11-2; purine nucleoside phosphorylase, 9030-21-1; uridine phosphorylase, 9030-22-2.