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] $[\alpha]^{25}_D = -148.8$ (0.4)° (c 1.2, 0.5 N NaOH); CD (×10³) (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 (×10³) (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 Link[10] and resolved by the method of West:[11] $[\alpha]^{25}_D$ = −120.0(1.0)° (c 1.5, 95% EtOH); CD (×10³) (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, $[\theta]_{290}$ −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] $[\alpha]^{25}_D = -9.1$ (2.0)° (c 1.8 MeOH); CD (×10³ (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(3*H*)-2-Methyl-2-methoxy-4-phenyl-5-oxobenzopyrano[3,4-*b*]dihydropyran (4)]. This compound was prepared from (−)-1 as previously described:[12] $[\alpha]^{25}_D$ = +33.1 (0.6)° (c 1.9, $CHCl_3$); CD (×10³) (0.1044 mg/mL of octanol) $[\theta]_{325}$ 0, $[\theta]_{305}$ −7.1, $[\theta]_{260}$ 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 °C for 16 h. Aliquots were removed from each phase and the CD spectra promptly recorded.

Acknowledgment. This investigation was supported by a Grant GM 25136 from the National Institutes of Health.

(9) B. D. West, S. Preis, C. H. Schroeder, and K. P. Link, *J. Am. Chem. Soc.*, **83**, 2676 (1961).
(10) C. H. Schroeder, E. D. Titus, and K. P. Link, *J. Am. Chem. Soc.*, **79**, 3291 (1957).
(11) B. D. West and K. P. Link, *J. Heterocycl. Chem.*, **2**, 93 (1965).
(12) J. Ikawa, M. Stahrmann, and K. P. Link, *J. Am. Chem. Soc.*, **66**, 902 (1944).

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

Rich B. Meyer, Jr.,* Thomas E. Stone,[2] and Pradipta K. Jesthi

College of Pharmacy, Washington State University, Pullman, Washington 99163. *Received September 8, 1983*

Condensation of tetramethyl methylenebisphosphonate 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.

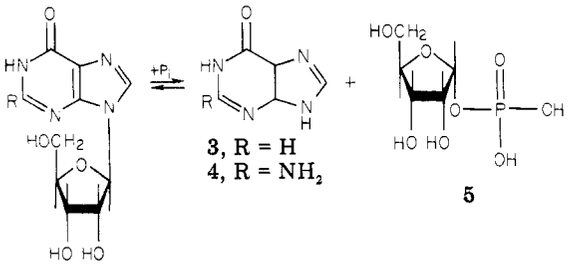
Purine nucleoside phosphorylase catalyzes the reversible phosphorolysis of inosine (**1**) or guanosine (**2**) to give the corresponding purine, hypoxanthine (**3**) or guanine (**4**), and α-D-ribofuranose 1-phosphate (R-1-P, **5**). Our initial approach to the design of inhibitors of this enzyme was 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-altritol[6] (**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-*altro*- and -*allo*-heptonic acids **8** and **9** from 2,3-*O*-isopropylidene-5-

(1) A preliminary account of this work has appeared: Jesthi, P. K.; Stone, T. E.; Meyer, R. B., Jr. "Abstracts of Papers", 185th National Meeting of the American Chemical Society, Seattle, WA, March 1983; American Chemical Society: MEDI 28.
(2) Present address: Millipore Corp., Bedford, MA.
(3) Giblett, E. L., Amman, A.J.; Wara, D. W.; Sandman, R.; Diamond, L. K. *Lancet* **1975**, 1010.
(4) Gudas, L. J.; Ullman, B.; Cohen, A.; Martin, D. W., Jr. *Cell* **1978**, *14*, 201.
(5) Engel, R. *Chem. Rev.* **1977**, *77*, 349.
(6) The nomenclature of **6** is the preferred name by both IUPAC and current Chemcial Abstracts practice. Another descriptive, if trivial, name might be α-D-ribofuranosylmethanephosphonic acid. A similar name was used in ref 7 for the glucosyl analogue. McClard (see ref 17) named compound **6** as D-*altro*-2,5-anhydrodeoxyhexanophosphonate.
(7) Nicotra, F.; Ronchetti, F.; Russo, G. *J. Org. Chem.* **1982**, *47*, 4459.

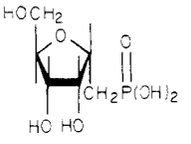
0022-2623/84/1827-1095$01.50/0



1, R = H
2, R = NH_2

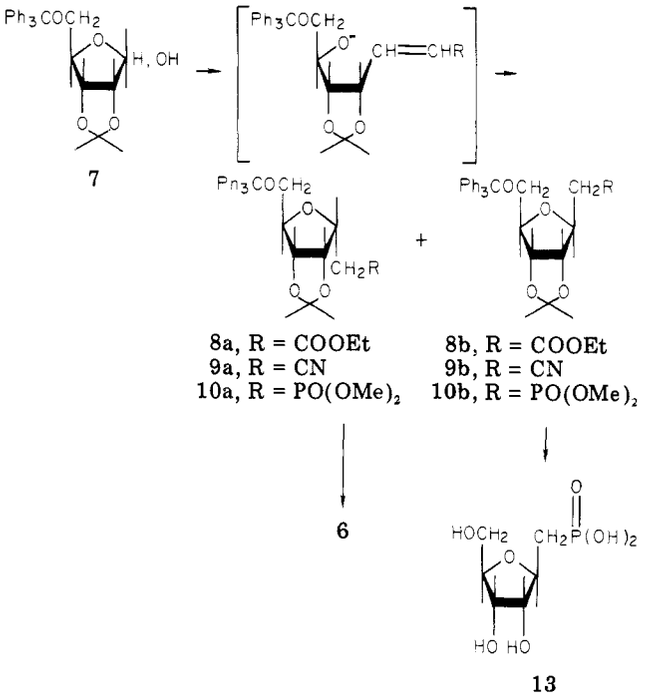
3, R = H
4, R = NH_2

5



6

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



7

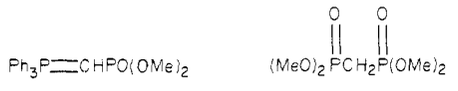
8a, R = COOEt
9a, R = CN
10a, R = $PO(OMe)_2$

8b, R = COOEt
9b, R = CN
10b, R = $PO(OMe)_2$

6

13

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 literature 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.



11

12

Deblocking was readily accomplished by treatment with bromotrimethylsilane[14] 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 ^{13}C 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 ^{13}C–^{31}P 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 ^{13}C 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 ^{13}C 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 general[16] and for **8a,b** and **9a,b** in particular.[8] The two pairs of compounds show ^{13}C 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 isosters 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 ^{13}C 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

(8) Ohrui, H.; Jones, G. H.; Mofatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byram, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 4602.
(9) Cousineau, T. J.; Secrist, J. A., III *J. Carbohydr., Nucleosides, Nucleotides* **1976**, *3*, 185.
(10) Wadsworth, W. S., Jr.; Emmons, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 1733.
(11) De Bernardo, S.; Weigele, M. *J. Org. Chem.* **1977**, *42*, 109.
(12) Jones, G. H.; Hammura, E. K.; Moffatt, J. G. *Tetrahedron Lett.* **1968**, 5731.
(13) Mikolajczyk, M.; Grzejszczak, S.; Midura, W.; Zatorski, A. *Synthesis* **1976**, *6*, 396.
(14) McKenna, C. E.; Higa, M. T.; Cheug, N. H.; McKenna, M.-C. *Tetrahedron Lett.* **1977**, 155.
(15) Christl, M.; Reich, H. J.; Roberts, J. D. *J. Chem. Soc.* **1971**, *93*, 3463.
(16) Perlin, A. S.; Cyr, N.; Koch, H. J.; Korsch, B. *Ann. N.Y. Acad. Sci.* **1973**, *222*, 935.

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

compd	C-1	C-2	C-3	C-4	C-5	C-6	isopropylidine Me	isopropylidine Me	isopropylidine 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 **7**[20] (2.1 g, 5 mmol) in 7.5 mL of CH_2Cl_2 was mixed with tetramethyl methylenebisphosphonate[12] (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 × 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. $C_{30}H_{35}O_7P$: H; C: calcd, 66.90; found, 66.34.

This mixture was rechromatographed on five preparative TLC plates (1000 μm, 20 × 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_3), 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_3), 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 $C_6H_{14}O_7P$ ($M + H^+$) 229.0672, found 229.0636. Anal. $C_6H_{11}Li_2O_7P \cdot {}^1/_2H_2O$: 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 $C_6H_{14}O_7P$ ($M + H^+$) 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.

Acknowledgment. The expert assistance of Carla Robertson in performing the enzyme assays is appreciated. We also thank Dr. Corrine G. Wong for some of the enzyme assays. This work was supported by Grant CA 30157 from the National Cancer Institute, USPHS, and by GM 29291 from NIGMS, USPHS. A portion of this work was conducted at the Department of Pharmaceutical Chemistry, University of California, San Francisco, where it was supported by a grant from the University of California

(17) McClard, R. W. *Tetrahedron Lett.* **1983**, *24*, 2631.
(18) Lewis, A. S.; Glantz, M. D. *Biochemistry* **1976**, *15*, 4451.
(19) Handschumacher, R. E., private communication.
(20) (a) Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1976**, *52*, 95. (b) Klein, R. S.; Ohrui, H.; Fox, J. J. *J. Carbohydr., Nucleosides, Nucleotides* **1974**, *1*, 265.

Cancer Research Coordinating Committee. We are indebted to Dr. David W. Martin, Jr., for many stimulating discussions. We thank Kurt L. Loening of the Chemical Abstracts Serivice for assistance with nomenclature, Dr. Fritz Knorr for obtaining the mass spectra, and Dr. Robert Handschumacher for communicating to us his uridine phosphorylase results.

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**·NH_3, 90638-60-1; α-D-ribofuranose 1-phosphate, 18646-11-2; purine nucleoside phosphorylase, 9030-21-1; uridine phosphorylase, 9030-22-2.