Anal. Calcd for $C_{22}H_{16}N_2OS$: C, 74.13; H, 4.52; N, 7.86. Found: C, 74.15; H, 4.64; N, 8.03.

Reaction of 3-Methyl-2-phenyl-1-azirine (4b) with Benzoyl Isothiocyanate (3). The cycloaddition was carried out as described above to give **12b** in 65% conversion as white needles: mp 138–139.5°; ir ν_{max} (Nujol) 3170 (NH), 1680 (C=O), 1545 (amide II) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 1.97 (s, 3 H), 7.24–8.10 (m, 10 H), 12.01 (s, br, 1 H, rapid exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 15.27, 126.28, 127.52, 128.17, 128.81, 129.03, 132.27, 132.80, 141.87, 157.62, 165.98; mass spectrum *m/e* 294 (M^+), 266, 191, 148, 121, 116.

Anal. Calcd for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.35; H, 5.08; N, 9.34.

Reaction of 2-phenyl-1-azirine (4c) with benzoyl isothiocyanate (3) was carried out as described above but for 48 hr at 25°. The adduct **12c** was obtained in 15% yield as pale yellow needles: mp 212–213°; ir ν_{max} (Nujol) 3165 (NH), 1685 (C=O), 1570 (amide II) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 7.08 (s, 1 H), 7.21–8.15 (m, 10 H), 12.75 (s, br, 1 H, rapid exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 126.12, 127.90, 128.33, 128.87, 129.14, 136.67, 132.80, 133.02, 143.60, 159.51, 166.03; mass spectrum *m/e* 280 (M^+), 252, 134, 121, 105.

Anal. Calcd for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.32; N, 9.99. Found: C, 68.64; H, 4.63; N, 9.78.

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Registry No.—**1,** 4461-33-0; **2,** 3553-61-5; **3,** 532-55-8; **4a,** 16483-98-0; **4b,** 16205-14-4; **4c,** 7654-06-0; **5a,** 52920-29-3; **5b,** 52977-07-8; **5c,** 52920-30-6; **6a,** 52920-31-7; **6b,** 52920-32-8; **6c,** 52920-33-9; **7a,** 52920-34-0; **8a,** 52920-35-1; **9a,** 52920-36-2; **9b,** 52920-37-3; **10b,** 52920-38-4; **10c,** 52920-39-5; **12a,** 52920-40-8; **12b,** 52920-41-9; **12c,** 52920-42-0.

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Di(2-*tert*-butylphenyl) Phosphorochloridate. A New Selective Phosphorylating Agent[1]

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A procedure for the preparation of 5′-nucleotides is described. 5′-Phosphates of adenosine, cytidine, uridine, guanosine, and thymidine were prepared directly from unprotected nucleosides in good yields by two-step synthesis using a new selective phosphorylating agent, di(2-*tert*-butylphenyl) phosphorochloridate (**1d**). The agent is stable, versatile, and highly selective for a primary hydroxyl in the presence of unprotected secondary hydroxy groups. The *tert*-butylphenyl protective groups are quite resistant toward dilute base and acid hydrolysis and are easily removed by hydrogenolysis in a nearly quantitative yield.

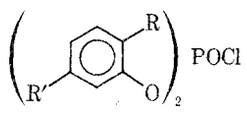
The polyfunctional nature and unique properties of nucleosides and carbohydrates present a considerable problem as to the choice of protective groups to achieve selectivity in phosphorylations. Recently, attention has been focused on the preparation of 5′-phosphates of various natural and synthetic compounds using selective phosphorylating agents.[2] A new phosphorylating agent has been explored, possessing the following properties: (a) ease of preparation, (b) relatively stable, (c) selective for the primary hydroxyl in the presence of unprotected secondary hydroxyls, (d) protective groups are stable under dilute base or acid conditions, and (e) protective groups are easily removable by hydrogenolysis.

This reagent does not offer any advantage over the phosphoryl chloride–triethyl phosphate procedure for the direct synthesis of 5′-mononucleotides.[2b] However, the presence of an acid and base stable protected 5′-phosphate group allows for further chemical modification of a nucleotide derivative prior to removal of the protective groups by hydrogenolysis.

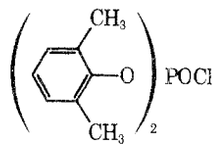
Results and Discussion

Considering properties of phosphorylating agents studied previously,[2] and the spatial arrangement of various furanosides and pyranosides, it is reasonable to assume that steric hindrance can facilitate selective phosphorylation of the nucleoside primary hydroxy group in the presence of unprotected secondary hydroxy groups. In an effort to investigate this premise we synthesized phenolic esters of phosphate (**1a–f**) as potentially selective phosphorylating agents.

Phosphorochloridates **1a**, **1b**, **1c**, and **1d** were synthe-



1a, R = H; R′ = H
b, R = ethyl; R′ = H
c, R = isopropyl; R′ = H
d, R = *tert*-butyl; R′ = H
e, R = *tert*-butyl; R′ = CH_3



1f

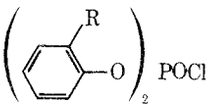
sized by a slightly modified procedure for preparation of diphenyl phosphorochloridate.[3] Although the reaction time was increased the yields were similar to that of diphenyl phosphorochloridate, ranging between 40 and 60%. The isolation of products was based upon removal of the low boiling fraction (unreacted alkylphenol and $POCl_3$) and alkylphenyl phosphorodichloridate. The residue was usually analytically pure dialkylphenyl phosphorochloridate. No further purification was performed because of the high hygroscopicity of product; the corresponding amides were prepared for analytical purposes. Structure assignments were based on nmr and mass spectrometry. Loss of the *tert*-butyl group was observed during the attempted preparation of **1e**, most probably by a retro Friedel–Crafts reaction. A significant amount of *tert*-butyl chloride from reaction of isobutylene with hydrogen chloride generated in the reaction was isolated from the carrier gas (N_2). An attempt to prepare **1f** was unsuccessful; the presence of two methyl groups adjacent to phenolic OH apparently presents a severe steric problem. Loss of the *tert*-butyl group also was observed from di(2-*tert*-butylphenyl) phosphorochloridate (**1d**) after prolonged heating at 160°.

The di(alkylphenyl) phosphorochloridates **1a, 1b, 1c,** and **1d** were found to phosphorylate thymidine in pyridine in reasonable yields, ranging between 50 and 75%. The phosphorylation was found to be relatively insensitive to temperature. Although yields of phosphorylated thymidine were highest when the reaction was performed at 0° followed by overnight stirring at room temperature, the yields of reactions at 40 and 70° were only slightly lower. The isolation and purification of products was limited to evaporation and extraction of a chloroform solution of the residue with water, saturated aqueous $NaHCO_3$ removal of solvent, and solidifying the resulting semisolid by washing with ether. No further purification was performed and the products were hydrogenated as such. The alkylphenyl phosphates appear to be quite resistant toward mild alkaline and acidic hydrolysis; however, treatment under rather vigorous conditions (6 *N* NaOH, 4 *N* HCl) gave partial decomposition.

The hydrogenolysis failed to proceed in commonly used solvents such as methanol, ethanol, tetrahydrofuran, or ethyl acetate. However, reduction was rapid in glacial acetic acid, the product forming in nearly quantitative yields.

For analysis of the procedure using **1a–d** (Table I) the overall yields of thymidine phosphates were determined by the following procedure. After evaporation of pyridine, the entire residue was dissolved in glacial acetic acid and hydrogenated in the presence of catalyst. The catalyst was filtered, the solvent evaporated, and the residue washed several times with ether and lyophilyzed from a 2% ammonia water solution. This residue was analyzed by chromatographic resolution, elution of the ultraviolet absorbing spots, and ultraviolet analysis at 262 nm to determine the yield from the ratio thymidine phosphates: thymidine. The percentage of 5′-phosphate with respect to diphosphate and 3′-phosphate respectively was determined by incubation with snake venom 5′-nucleotidase according to standard procedures.[4] The selectivity of 5′-phosphate formation essentially decreased with decreasing bulk of substituent (Table I). Di(2-*tert*-butylphenyl) phosphorochloridate **1d** was found to phosphorylate solely at the 5′ position when used in equimolar quantities. Loss of specificity was observed when an excess of the reagent was used. The phosphates of uridine, cytidine, adenosine, and guanosine were prepared and examined in the manner similar to that of thymidine. Dimethylformamide was used as a cosolvent in order to solubilize guanosine, cytidine, and adenosine.

Table I
Yield and Selectivity of Phosphorylation Using Di(*o*-alkylphenyl) Phosphorochloridates



	R	Ratio[a]	Yield[b]	5′ product[c]
1a	H	1	57	89
1b	Ethyl	1	50	83
1c	Isopropyl	1	62	91
1d	*tert*-Butyl	1	75	100
1d	*tert*-Butyl	1.2	98	96
1d	*tert*-Butyl	1.5	> 100[d]	93[e]

[a] Molar ratio of phosphorochloridate to thymidine. [b] Yield of thymidine di(2-alkylphenyl) phosphates. [c] Calculated as per cent of total thymidine phosphate found. [d] Some diphosphorylated product was found. [e] The remaining 7% is probably a mixture of 3′-mono- and 3′,5′-disubstituted thymidine.

The hydrogenation of purine nucleotides proceeded well without serious side reactions. The situation is more complex in the case of pyrimidines. Hydrogenation of pyrimidines has been studied extensively. Brown and Johnson[5] observed that reduction of the 5,6 double bond of uracil can be effected by colloidal platinum or palladium and 5,6-dihydrouracil can be prepared by direct hydrogenation of uracil in acidic media. Similarly the nuclear reduction of pyrimidines is effected by palladium on charcoal or barium sulfate as well.[6] These authors also note that Adams catalyst seems to be less effective in mediating the nuclear reductions than corresponding palladium catalysts.[6] Cytosine can undergo extensive hydrogenolysis of the 4-amino group in addition to ring reduction reported for uracil.[7] The exocyclic reductions of pyrimidines can be performed with Adams catalyst under controlled reduction conditions. Using Adams catalyst, very little if any side reactions were reported using the preparation of uridine and cytidine phosphates.[8] This observation was confirmed in the hydrogenolysis of *tert*-butylphenyl protective groups which proceeded in preference to nuclear reduction during preparation of 5′-phosphates of uridine and cytidine. For example, quantitative chromatographic resolution on paper and ultraviolet analysis of the eluted uridine and uridine phosphate accounted for all of the starting material.

The yields of 5′-phosphates of adenosine, guanosine, cytidine, and uridine (54–63%) were determined by spectrophotometric comparison of absorbance of eluted spots corresponding to the nucleosides and nucleotides after electrophoresis of the reduction products. The eluted nucleotides were incubated with 5′-nucleotidase and aliquots examined by electrophoresis (Na_2HPO_4–NaOH buffer pH 12) and spectrophotometrically. No phosphates other than 5′ were detected. All synthetic phosphates were compared with an authentic sample of corresponding nucleotide on electrophoresis and paper chromatography.

Experimental Section

Nucleosides and nucleotides used as reagents or standards and 5′-nucleotidase from *Crotalus adamanteus* venon Grade II were purchased from Sigma Chemical Co., St. Louis, Mo. The former were checked for purity by paper chromatography before use. Descending paper chromatography was performed on Whatman No. 1 or 3MM paper using the solvent system 2-propanol:concentrated ammonia:water = 7:1:2. Paper electrophoresis was performed on a Brinkmann Pherograph type Mini 68 apparatus using Whatman No. 1 paper and 0.05 *M* phosphate buffer at pH 8 or 12. Ultraviolet spectra were recorded on Cary 14 spectrometer, absorbance values on a Beckmann DB spectrophotometer, and nmr on a Varian T60 spectrometer.

Di(alkylphenyl) Phosphorochloridates. The alkylphenol (1 mol) was mixed with $POCl_3$ (78 g, 0.5 mol) and heated at 150–160° for 16 hr under nitrogen. The mixture was distilled and forerun to 84° (0.4 mm) consisting of unreacted $POCl_3$, starting material, and alkylphenyl phosphorodichloridate discarded. The residue was used as such.

Di(2-ethylphenyl) phosphorochloridate (**1b**) was used as the distillation residue boiling over 66° (0.03 mm) yielding 45% of relatively pure product as judged from nmr data.

Di(2-isopropylphenyl) phosphorochloridate (**1c**) was obtained in 46% yield: mass spectrum, calcd mol wt 353.8, found 354.

Di(2-*tert*-butylphenyl) phosphorochloridate (**1d**) as the distillation residue (58% yield) was analytically pure: mass spectrum, calcd mol wt 380.8, found 381.

Anal. Calcd for $C_{20}H_{26}O_3PCl$: C, 63.15; H, 6.84. Found: C, 62.84; H, 6.99.

Di(alkylphenyl) Phosphoamidates. Dialkylphenyl phosphorochloridate (1.5 mmol) was dissolved in 40 ml of anhydrous ether and dry ammonia was bubbled through the solution. The white precipitate (NH_4Cl) was filtered. The ether solution was extracted with water and dried ($MgSO_4$). The white solid resulting from evaporation of solvent was recrystallized from ether and petroleum ether (1:4).

Diphenyl phosphoramidate was prepared in 94% yield (recrystallized from ether:petroleum ether), mp 144–145, long white needles.

Anal. Calcd for $C_{12}H_{12}PO_3N$: C, 57.83; H, 4.85; N, 5.62. Found: C, 57.92; H, 5.09; N. 5.62.

Di(2-ethylphenyl) phosphoramidate was prepared in 91% yield (recrystallized from ether:petroleum ether), mp 81.5–82°, long white needles.

Anal. Calcd for $C_{16}H_{20}PO_3N$: C, 62.94; H, 6.60; N, 4.95. Found: C, 62.68; H, 6.62; N. 4.84.

Di(2-isopropylphenyl) phosphoramidate was prepared in 96% yield mp 69.5°.

Anal. Calcd for $C_{18}H_{24}O_3PN$: C, 64.85; H, 7.26; N, 4.20. Found: C, 64.91; H, 7.24; N, 4.08.

Synthesis of Thymidine 5′-Phosphate. Di(2-*tert*-butylphenyl) phosphorochloridate (**1d**, 4 mmol) was dissolved in 25 ml of dry pyridine and 0.9 g of thymidine was (3.75 mmol) added. After stirring at 25° (0, 40, 70°, respectively) for 1 day the pyridine was removed *in vacuo* at 40°. The semisolid residue was dissolved in 70 ml of chloroform, washed with 30 ml of water, 20 ml of a saturated solution of sodium bicarbonate, and again with 30 ml of water, and the chloroform layer was dried. After evaporation, the residue (75% of theoretical) was dissolved in 40 ml of acetic acid and added to 100 ml of acetic acid containing prereduced platinum oxide (0.6 g). The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed (8 equiv of H_2). The catalyst was filtered and the solvent evaporated *in vacuo* at 25°. The semisolid residue was washed several times with ether, dissolved in 30 ml of 2% ammonia solution, filtered, and lyophylized to afford the ammonium salt of thymidine 5′-phosphate in 70% overall yield; the product was identical with an authentic sample by paper chromatography and electrophoresis.

Anal. Calcd for $C_{10}H_{21}N_4O_8P \cdot 4H_2O$: C, 28.04; H, 6.82; N, 13.08. Found: C, 27.76; H, 6.40; N, 13.16.

Confirmation of the 5′-phosphate as the only isomer was by hydrolysis with snake venom 5′-nucleotidase.

This procedure was also used in the synthesis of thymidine 5′-phosphate using the other phosphorochloridates, and with similar yields; however, concurrent formation of some 3′-phosphate was observed (Table I).

Uridine 5′-phosphate was prepared by this procedure in 63% yield.

Guanosine 5′-Phosphate. Guanosine (250 mg, 0.85 mmol) was dissolved in 20 ml of dry pyridine, 12 ml of dry dimethylformamide was added, and the solution was acidified with acetic acid. After cooling to 0°, 380 mg of di(2-*tert*-butylphenyl) phosphorochloridate (1 mmol) was added and the mixture was stirred for a day at 25°. The sequence from this point was identical with that used in the synthesis of thymidine 5′-phosphate. A 55% yield of product was observed; it was identical with an authentic sample of guanosine 5′-phosphate in paper chromatography, electrophoresis, and to snake venom 5′-nucleotidase action.

Similarly, adenosine 5′-phosphate and cytidine 5′-phosphate were prepared in 62 and 53% yield, respectively.

Enzyme Hydrolysis. The enzyme used was the 5′-nucleotidase Grade II from *Crotalus adamanteus* venom with activity of 15–20 μmol of adenosine 5′-phosphate hydrolyzed per minute per milligram of enzyme. The assay, at pH 8.5, contained 0.2 ml of 1 *M* glycine–NaOH buffer, 0.2 ml of 0.1 *M* $MgCl_2$, 0.16 mg of enzyme, and about 15 μ*M* of nucleotide in a total volume of 0.5 ml. After 30 min at 37° the mixture was resolved by electrophoresis and the spots corresponding to the nucleoside and remaining nucleotide were eluted with 10 ml of water and the ratio calculated from the ultraviolet absorbance.

Registry No.—**1a,** 2524,64-3; **1b,** 52555-40-5; **1c,** 52555-41-6; **1d,** 52555-42-7; $POCl_3$, 10025-87-3; 2-ethylphenol, 90-00-6; 2-isopropylphenol, 88-69-7; 2-*tert*-butylphenol, 88-18-6; diphenyl phosphoramidate, 2015-56-7; di(2-ethylphenyl) phosphoramidate, 52555-43-8; di(2-isopropylphenyj)phosphoramidate, 52555-44-9; thymidine, 50-89-5; thymidine 5′-phosphate ammonium salt, 20706-32-5.

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