A ^{31}P NMR Stereochemical and Kinetic Study of the Alkaline Hydrolysis of *cis*-Nucleoside 3',5'-Cyclic Aryl [^{18}O]Monophosphates and Unlabeled Analogs

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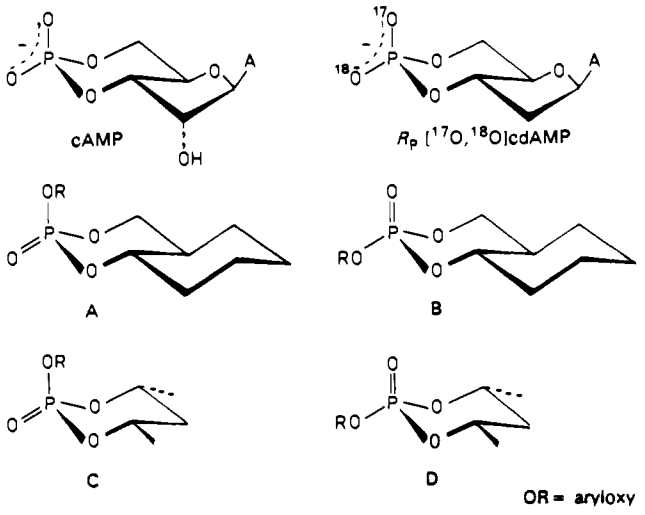
Abstract: The alkaline hydrolysis of the P-chiral *cis*-nucleoside 3',5'-cyclic aryl [^{18}O]monophosphates **4a–c** and of the unlabeled analogs **3a–c** was studied. Hydrolysis of the ^{18}O-labeled phosphate triesters **4a–c** yielded three products: 3',5'-cyclic [^{18}O]phosphate diester, 5'-acyclic aryl [^{18}O]phosphate diester, and 3'-acyclic aryl [^{18}O]phosphate diester. The stereochemistry of the formation of the 3',5'-cyclic [^{18}O]phosphate diester was determined by means of methylating the hydrolysis products with methyl iodide. The formation of the 3',5'-cyclic [^{18}O]phosphate diester during hydrolysis of compounds **4a** and **4c** proceeds with 17% inversion of configuration at phosphorus, whereas 40% inversion is found during hydrolysis of **4b**. Inversion of configuration indicates the existence of a P^V-TBP with a *diequatorially* located dioxaphosphorinane ring. Retention of configuration (83% for **4a** and **4c**, and 60% for **4b**) can be explained in terms of Berry pseudorotation. The formation of the 5'-acyclic aryl [^{18}O]phosphate diester during hydrolysis of compounds **4a** and **4c** proceeds with about 50% inversion of configuration at phosphorus, whereas formation of the 3'-acyclic aryl [^{18}O]phosphate diester proceeds with an inversion/retention ratio of 88:12 or 12:88 for **4a** and 79:21 or 21:79 for **4c**. It is clear that Berry pseudorotation takes place during hydrolysis of the 3',5'-cyclic phosphate triesters **4a–c**. This is in contrast with earlier hydrolysis studies on 3',5'-cyclic phosphate diesters proceeding without Berry pseudorotation, leading to complete inversion of configuration at phosphorus. Because of the very small amounts of 3'- and 5'-acyclic aryl [^{18}O]phosphate diesters formed during the hydrolysis reaction of compound **4b**, the stereochemistry could not be determined. The hydrolysis reactions, which have been studied on the unlabeled compounds **3a–c**, obey second-order kinetics. Changing the ribose ring to a deoxyribose ring or changing the adenine base to thymine in the 3',5'-cyclic phosphate triester does not dramatically influence the second-order reaction rate constant. However, the nature of the P–OR substituent significantly influences the reaction rate. The 3',5'-cyclic phosphate triester with *p*-nitrophenoxy as substituent hydrolyzes approximately 18 times (T = 294 K) faster than the corresponding triester with phenoxy as substituent.

Introduction

Cyclic adenosine 3',5'-monophosphate (cAMP; Chart I) is known to be an important molecule which regulates a wide variety of biochemical processes.[1] For example, cAMP acts as a mediator of hormone action and as a modulator of enzymatic activity. cAMP is synthesized from adenosine triphosphate by the action of adenylate cyclase. Breakdown of cAMP is catalyzed by phosphodiesterase, which hydrolyzes cAMP into 5'-adenosine monophosphate (5'-AMP). Both processes result in maintaining a steady-state intracellular concentration of cAMP. The structural requirements for the binding of cAMP to the regulatory subunit of protein kinases as well as to phosphodiesterases have been investigated in detail.[2]

Besides structure–activity studies, numerous studies have been performed in order to investigate the stereochemistry of the enzymatic reactions. It was found that hydrolysis of the R_P diastereoisomer of 2'-deoxyadenosine 3',5'-cyclic [^{17}O,^{18}O]monophosphate (R_P [^{17}O,^{18}O]cdAMP; Chart I) by cAMP phosphodiesterases takes place with inversion of configuration at phosphorus.[3] Nonenzymatic hydrolysis of R_P [^{17}O,^{18}O]cdAMP at 100 °C and in a barium hydroxide solution (0.2 M), yields a 4:1 mixture of the S_P diastereoisomer of 3'-[^{16}O,^{17}O,^{18}O]dAMP and the R_P diastereoisomer of 5'-[^{16}O,^{17}O,^{18}O]dAMP.[4] Both products are formed with inversion of configuration at phosphorus. Under enzymatic conditions, cAMP is converted exclusively to 5'-AMP by 3',5'-cyclic nucleotide phosphodiesterase. It has been suggested that during the interaction between the enzyme and cAMP, the electrostatic negative charge of the phosphoryl oxygens of cAMP is shielded by positively charged amino acid residues or bivalent metal ions.[5] In order to mimic this shielding effect, the nonenzymatic hydrolysis of cyclic aryl phosphate triesters has been studied in $H_2{}^{18}O$ (e.g., A and B; Chart I) or $H_2{}^{17}O$ (e.g., C and D; Chart I). These compounds can be regarded as models for

Chart I



the interaction of cAMP with the enzyme. The main product formed during hydrolysis of the cyclic aryl phosphate triesters A–D is a cyclic phosphate diester, which is formed both with inversion

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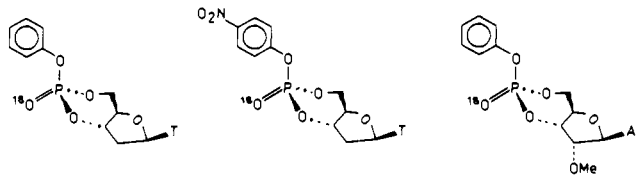
and retention of configuration at phosphorus.[6,7] However, the nature of the aryl substituent determines the retention/inversion ratio.

In this paper we report the results on the alkaline nonenzymatic hydrolysis of the chiral ^{18}O-labeled 3',5'-cyclic nucleoside phosphate triesters **4a–c** and the unlabeled (^{16}O) counterparts **3a–c**.



4a **4b** **4c**

These compounds are more realistic model systems for cAMP than compounds A–D. Some structural changes with respect to cAMP are made. In compounds **3a**, **4a**, **3b**, and **4b** a thymine base is used, while a β-D-2'-deoxyribose ring is used instead of the β-D-ribose ring. Compounds **3c** and **4c** may be regarded as much more realistic models since they contain the normal adenine base, as well as an oxygen substituent on $C_{2'}$. The 2'-OH group of cAMP is blocked by a methyl group in order to prevent possible side reactions.

Experimental Section

Materials and Chromatography. For all column separations, we used Merck silica gel 60 (particle size 0.040–0.063 mm or 0.063–0.200 mm). Dowex-H^+ 50X8-100 was purchased from Janssen Chimica. Dry diethyl ether was obtained by storing diethyl ether, predried on calcium chloride, on sodium wire. Ethyl acetate refluxed on calcium hydride prior to atmospheric distillation. Pyridine was distilled from KOH pellets and stored on 4-Å molecular sieves. Acetonitrile (DNA grade) was purchased from Merck and used as received. Tetrahydrofuran was refluxed on calcium hydride and distilled from lithium aluminum hydride. Tetrahydrofuran and acetonitrile, used as solvents for ^{18}O labeling reactions, were deoxygenized by subjecting the solvents to three freeze–pump–thaw cycles. [^{18}O]Water (97 atom %) was obtained from Sigma. Hexamethylphosphorous triamide ($(Me_2N)_3P$) (97%) was purchased from Janssen Chimica and used as received. *tert*-Butyl hydroperoxide was used as an 80% (8.0 M) solution in di-*tert*-butyl peroxide, which was obtained from Merck. 1*H*-Tetrazole was purified through sublimation. *p*-Nitrophenol was recrystallized from a mixture of petroleum ether and ethanol. Reactions were routinely run in an inert atmosphere of dry nitrogen or argon and were run at ambient temperature, unless otherwise noted.

NMR Measurements. ^1H NMR spectra were recorded at 400.13 MHz on a Bruker AM 400 spectrometer. Tetramethylsilane (TMS) was used as the internal standard in ^1H and ^{13}C NMR. ^{13}C and ^{31}P NMR were recorded at 100.62 and 161.98 MHz, respectively, on the same instrument. The ^{31}P NMR spectra were referenced against 85% H_3PO_4 as the external standard.

UV/Visible Spectroscopy. The hydrolysis of compound **3b** was monitored at 405 nm by means of a Hitachi 150-20 spectrophotometer.

***trans*-Thymidine 3',5'-Cyclic *N,N*-Dimethylphosphoramidite (1a).**[8,9] According to the method described by Bentrude et al.[8,9] we obtained *trans*-thymidine 3',5'-cyclic *N,N*-dimethylphosphoramidite as a white foam in 47% yield: cis/trans ratio 2:98; mp 89 °C; ^{31}P NMR[9] ($CDCl_3$) δ 146.3 (trans), 140.3 (cis); ^1H NMR[9] ($CDCl_3$) (**1a**) δ 9.51 (1 H, b s, NH of T), 7.09 (1 H, q, H_6 of T), 6.27 (1 H, dd, $H_{1'}$), 4.46 (1 H, m, $H_{5''}$), 4.22–4.08 (2 H, m, $H_{5'}$ and $H_{3'}$), 3.58 (1 H, m, $H_{4'}$), 2.72 (6 H, d, $N(CH_3)_2$, $^3J_{PNCH}$ = 9.2 Hz), 2.56 (1 H, m, $H_{2''}$), 2.32 (1 H, m, $H_{2'}$), 1.97 (3 H, d, CH_3 of T); ^{13}C NMR[10] ($CDCl_3$) (**1a**) δ 163.7 (C_2 or C_4 of T), 150.3 (C_4 or C_2 of T), 134.9 (C_6 of T), 111.7 (C_5 of T), 83.6 ($C_{1'}$), 75.1 ($C_{4'}$, $^2J_{POC}$ = 12.3 Hz), 74.8 ($C_{3'}$, $^2J_{POC}$ = 9.4 Hz), 66.8 ($C_{5'}$), 36.5 ($C_{2'}$, $^3J_{POCC}$ = 5.7 Hz), 34.9 (2 C, $N(CH_3)_2$, $^2J_{PNC}$ = 21.4 Hz), 12.6 (CH_3 of T).

2'-*O*-Methyl-*trans*-adenosine 3',5'-Cyclic *N,N*-Dimethylphosphoramidite (1b). Hexamethylphosphorous triamide ($(Me_2N)_3P$) (0.65 g, 4.00 mmol) was added to a solution of 2'-*O*-methyladenosine[11] (1.13 g, 4.00 mmol) in 60 mL of dry acetonitrile, and the mixture was stirred for 4 h at 50 °C. Afterwards, the ^{31}P NMR spectrum showed complete conversion of hexamethylphosphorous triamide and the formation of **1b** and its epimer (δ(CH_3CN/CD_3CN) cis 137.2; trans 147.3; cis/trans ratio 2:8). After being stirred for 15 h at room temperature (time is needed for the cis → trans epimerization), the mixture was filtered and 3 mL of the filtrate (approximately 5%) was evaporated in vacuo and subjected to ^{31}P NMR analysis. This indicated additional signals at 19 and 4 ppm, probably due to phosphonates (<5%). The filtrate, predominantly containing compound **1b**, was stored at −20 °C in order to prevent degradation. After evaporation of the volatiles of the 3-mL filtrate the ^1H NMR spectrum indicated some dimethylamine (released during cyclization) and some impurities (approximately 5%). Estimated yield 1.1 g (77%): cis/trans ratio 1:9; ^{31}P NMR (CD_3CN) δ 147.3 (trans), 137.2 (cis); ^1H NMR (CD_3CN) (**1b**) δ 8.26 (1 H, s, H_8 or H_2 of A), 8.01 (1 H, s, H_2 or H_8 of A), 6.32 (2 H, b s, NH_2 of A), 6.03 (1 H, b s, $H_{1'}$), 4.59 (1 H, m, $H_{3'}$), 4.41 (1 H, m, $H_{5''}$), 4.29 (1 H, d, $H_{2'}$), 4.12 (1 H, t, $H_{5'}$), 3.95 (1 H, m, $H_{4'}$), 3.54 (3 H, s, 2'-OMe), 2.69 (6 H, d, $N(CH_3)_2$, $^3J_{PNCH}$ = 9.6 Hz).

***cis*-Thymidine 3',5'-Cyclic Phenyl Monophosphite (2a).**[9] *trans*-Thymidine 3',5'-cyclic *N,N*-dimethylphosphoramidite (**1a**) (1.97 g, 6.25 mmol) was added to a solution of phenol (0.60 g, 6.38 mmol) in a mixture of acetonitrile (25 mL) and dichloromethane (15 mL). During this reaction two 3',5'-cyclic phenyl monophosphates were formed (cis and trans), as revealed by ^{31}P NMR (δ($CDCl_3$) *cis*-**2a** 115.0 ppm; *trans*-**2a** 121.1 ppm). After the mixture was stirred for 5 h at room temperature, the cis/trans ratio was 5:1, whereas complete formation of **2a** was achieved after 70 h, which was evident from the ^{31}P NMR data (δ-($CDCl_3$) 115.1 ppm). After evaporating the volatiles, the residue was dissolved in 30 mL of dichloromethane, washed five times with 5-mL portions of a sodium carbonate solution (0.5 M) (to remove the phenol and the amine) and the organic layer was dried on magnesium sulfate. After filtration, the solution was concentrated in vacuo, affording a white solid containing phenol, which was evident from ^1H NMR data. Addition of dry diethyl ether afforded a white precipitate of **2a**. Isolation of the solid was accomplished by decanting the clear solution, yielding 0.69 g of **2a** as a white solid. The decanted clear solution was concentrated in vacuo, and dry diethyl ether was added, resulting in a second crop of **2a** as a white precipitate (0.30 g): total yield 0.99 g (44%); mp 140 °C; ^{31}P NMR[9] ($CDCl_3$) δ 114.9; ^1H NMR[9] ($CDCl_3$) δ 8.77 (1 H, b s, NH of T), 7.35 (2 H, m, 2H's of PhO), 7.13 (3 H, m, 3H's of PhO), 7.07 (1 H, q, H_6 of T), 6.20 (1 H, dd, $H_{1'}$), 4.67 (1 H, m, $H_{3'}$), 4.62 (1 H, m, $H_{5'}$), 4.44 (1 H, m, $H_{5''}$), 3.75 (1 H, m, $H_{4'}$), 2.54 (1 H, m, $H_{2''}$), 2.42 (1 H, m, $H_{2'}$), 1.96 (3 H, d, CH_3 of T); ^{13}C NMR ($CDCl_3$) δ 163.2 (C_2 or C_4 of T), 156.6 (C_{ipso} of PhO), 150.0 (C_4 or C_2 of T), 134.9 (C_6 of T), 129.9 (2 meta C's of PhO), 124.0 (1 para C of PhO), 119.8 (2 ortho C's of PhO, $^3J_{POCC}$ = 7.8 Hz), 111.9 (C_5 of T), 82.1 ($C_{1'}$), 74.9 ($C_{4'}$, $^3J_{POCC}$ = 7.4 Hz), 69.2 ($C_{3'}$), 66.6 ($C_{5'}$), 36.1 ($C_{2'}$), 12.7 (CH_3 of T). Anal. Calcd for $C_{16}H_{17}N_2O_6P$: C, 52.7; H, 4.7; N, 7.7. Found: C, 52.1; H, 4.8; N, 7.5.

***cis*-Thymidine 3',5'-Cyclic *p*-Nitrophenyl Monophosphite (2b).** *p*-Nitrophenol (0.49 g, 3.53 mmol) was dissolved in 25 mL of dichloromethane, and *trans*-thymidine 3',5'-cyclic *N,N*-dimethylphosphoramidite (**1a**) (1.1 g, 3.49 mmol) was added. After being stirred for 70 h, complete formation of **2b** was evident from ^{31}P NMR data (δ ($CDCl_3$) 114.5 ppm). To remove the *p*-nitrophenol and the amine, the mixture was washed three times with 8-mL portions of a sodium carbonate solution (0.07 M) and the organic layer was dried on magnesium sulfate. After filtration, the solution was concentrated in vacuo, affording a yellowish solid. The ^1H NMR spectrum indicated a purity of >98%. Yield 0.3 g (21%): ^{31}P NMR ($CDCl_3$) δ 114.5; ^1H NMR ($CDCl_3$) δ 8.57 (1 H, b s, NH of T), 8.25 (2 H, m, 2H's of *p*-NO_2PhO), 7.22 (2 H, m, 2H's of *p*-NO_2PhO), 7.03 (1 H, q, H_6 of T), 6.11 (1 H, dd, $H_{1'}$), 4.69 (1 H, m, $H_{3'}$), 4.61 (1 H, m, $H_{5'}$), 4.48 (1 H, m, $H_{5''}$), 3.77 (1 H, m, $H_{4'}$), 2.60–2.45 (2 H, m, $H_{2'}$ and $H_{2''}$), 1.95 (3 H, d, CH_3 of T).

2'-*O*-Methyl-*cis*-adenosine 3',5'-Cyclic Phenyl Monophosphite (2c). Phenol (188 mg, 2.0 mmol) was added to 20 mL of the filtered acetonitrile solution, mainly containing **1b** (approximately 1.1 mmol). 1*H*-Tetrazole (8.5 mg, 0.12 mmol) was added and after 1 1/2 h the ^{31}P NMR spectrum showed 25% conversion of **1b** into the *cis*- and *trans*-phenyl phosphite triester (δ(CH_3CN/CD_3CN) cis 116.1; trans 121.7; cis/trans ratio 1:1). After 60 h, **1b** was completely converted into the *cis*-phenyl phosphite triester (**2c**) (^{31}P NMR δ 116.1 ppm). The solution was fil-

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tered and evaporated until approximately 5 mL of solvent remained. Then, the solution was diluted with diethyl ether and washed two times with a 0.1 M sodium hydroxide solution, in order to remove the phenol and the 1*H*-tetrazole. After evaporation of all volatiles, a white solid was obtained, with a purity of 90%, according to ^{1}H- and ^{31}P NMR data. Yield 210 mg (47%): ^{31}P NMR (CD_3CN) δ 116.2; ^{1}H NMR (CD_3CN) δ 8.25 (1 H, s, H_8 or H_2 of A), 8.02 (1 H, s, H_2 or H_8 of A), 7.40 (2 H, m, 2H's of PhO), 7.20 (3 H, m, 3H's of PhO), 6.20 (2 H, b s, NH_2 of A), 5.99 (1 H, b s, $H_{1'}$), 5.32 (1 H, m, $H_{3'}$), 4.68 (1 H, m, $H_{5'}$), 4.47 (1 H, m, $H_{5''}$), 4.42 (1 H, d, $H_{2'}$), 4.12 (1 H, m, $H_{4'}$), 3.56 (3 H, s, 2'-OMe); ^{13}C NMR (CD_3CN) δ 157.0 (C_4 or C_6 of A), 154.0 (C_8 of A), 152.9 (C_{ipso} of PhO), 150.2 (C_6 or C_4 of A), 140.9 (C_2 of A), 131.0 (2 meta C's of PhO), 125.1 (para C of PhO), 121.1 (C_5 of A and 2 ortho C's of PhO), 88.5 ($C_{1'}$), 82.3 ($C_{2'}$), 72.4 ($C_{4'}$, $^3J_{POCC}$ = 7.3 Hz), 71.6 ($C_{3'}$), 68.0 ($C_{5'}$, $^2J_{POC}$ = 4.1 Hz), 59.1 (2'-OMe).

***cis*-Thymidine 3',5'-Cyclic Phenyl Monophosphate (3a).**[9] According to the procedure described by Bentrude et al.[9] we obtained *cis*-thymidine 3',5'-cyclic phenyl monophosphate. After recrystallization from ethyl acetate, the white solid still contained ethyl acetate (±10% w/w), which could not be removed even at 50 °C and under vacuo (20 mmHg). Yield 34%: mp 135–138 °C (lit.[9] mp 134–136 °C); ^{31}P NMR[9] ($CDCl_3$) δ −12.3; ^{1}H NMR[9] ($CDCl_3$) δ 8.83 (1 H, b s, NH of T), 7.39 (2 H, m, 2H's of PhO), 7.28 (3 H, m, 3H's of PhO), 7.01 (1 H, q, H_6 of T), 6.05 (1 H, dd, $H_{1'}$), 5.00 (1 H, m, $H_{3'}$), 4.68 (1 H, m, $H_{5''}$), 4.54 (1 H, m, $H_{5'}$), 4.00 (1 H, m, $H_{4'}$), 2.7–2.6 (2 H, m, $H_{2'}$ and $H_{2''}$), 1.96 (3 H, d, CH_3 of T); ^{13}C NMR ($CDCl_3$) δ 163.8 (C_2 or C_4 of T), 150.4 (C_{ipso} of PhO), 150.0 (C_4 or C_2 of T), 137.0 (C_6 of T), 130.0 (2 meta C's of PhO), 125.5 (1 para C of PhO), 119.6 (2 ortho C's of PhO, $^3J_{POCC}$ = 4.7 Hz), 111.8 (C_5 of T), 87.3 ($C_{1'}$), 78.4 ($C_{3'}$), 74.0 ($C_{4'}$), 69.9 ($C_{5'}$, $^2J_{POC}$ = 9.2 Hz), 34.8 ($C_{2'}$), 12.4 (CH_3 of T).

***cis*-Thymidine 3',5'-Cyclic *p*-Nitrophenyl Monophosphate (3b).** Compound **2b** (0.3 g, 0.73 mmol) was dissolved in 10 mL of dichloromethane, and NO_2/N_2O_4 gas was bubbled through the solution until a greenish color appeared. During this oxidation, a precipitate of **3b** was observed. After evaporation of the solvent, a light yellow-brown solid was obtained. Yield 0.27 g (87%): mp 155–158 °C; according to ^{31}P and ^{1}H NMR, the solid showed a purity of >95%; ^{31}P NMR (1,4-dioxane-d_8/D_2O 4:1 v/v) δ −11.3; ^{1}H NMR (1,4-dioxane-d_8/D_2O 4:1 v/v) δ 8.28 (2 H, m, 2H's of *p*-NO_2PhO), 7.51 (2 H, m, 2H's of *p*-NO_2PhO), 7.35 (1 H, q, H_6 of T), 6.12 (1 H, dd, $H_{1'}$), 5.09 (1 H, m, $H_{3'}$), 4.71 (1 H, m, $H_{5''}$), 4.60 (1 H, m, $H_{5'}$), 4.02 (1 H, m, $H_{4'}$), 2.65–2.50 (2 H, m, $H_{2'}$ and $H_{2''}$), 1.91 (3 H, d, CH_3 of T); ^{13}C NMR (1,4-dioxane-d_8/D_2O 4:1 v/v) δ 165.1 (C_2 or C_4 of T), 155.5 (C_{ipso} of *p*-NO_2PhO, $^2J_{POC}$ = 6.3 Hz), 151.1 (C_4 or C_2 of T), 146.0 (para C of *p*-NO_2PhO), 138.7 (C_6 of T), 126.7 (2 meta C's of *p*-NO_2PhO), 121.4 (2 ortho C's of *p*-NO_2PhO, $^3J_{POCC}$ = 4.7 Hz), 111.8 (C_5 of T), 87.5 ($C_{1'}$), 79.9 ($C_{3'}$, $^2J_{POC}$ = 5.7 Hz), 74.1 ($C_{4'}$, $^3J_{POCC}$ = 6.2 Hz), 71.7 ($C_{5'}$, $^2J_{POC}$ = 9.3 Hz), 34.7 ($C_{2'}$, $^3J_{POCC}$ = 9.0 Hz), 12.3 (CH_3 of T).

2'-*O*-Methyl-*cis*-adenosine 3',5'-Cyclic Phenyl Monophosphate (3c). To a stirred solution of **2c** (50 mg, 0.12 mmol) in 2 mL of acetonitrile was added *tert*-butyl hydroperoxide. After 4 h, the solvent was evaporated in vacuo. Pure **3c** was obtained as a white solid after column chromatography, using a gradient of methanol (0% → 5% v/v) in acetonitrile as eluent. Yield 40 mg (80%): according to ^{31}P and ^{1}H NMR the solid was pure for more than 95%; R_f 0.24 (methanol/acetonitrile 5:95 v/v); ^{31}P NMR (CD_3OD) δ −10.3; ^{1}H NMR (CD_3OD) δ 8.28 (1 H, s, H_8 or H_2 of A), 8.22 (1 H, s, H_2 or H_8 of A), 7.50–7.30 (5 H, m, 5H's of PhO), 6.20 (1 H, b s, $H_{1'}$), 5.82 (1 H, m, $H_{3'}$), 4.77 (1 H, m, $H_{5''}$), 4.62 (1 H, m, $H_{5'}$), 4.58 (1 H, d, $H_{2'}$), 4.42 (1 H, m, $H_{4'}$), 3.59 (3 H, s, 2'-OMe); ^{13}C NMR (CD_3OD) δ 157.5 (C_4 or C_6 of A), 154.1 (C_8 of A), 151.5 (C_{ipso} of PhO), 150.2 (C_6 or C_4 of A), 142.1 (C_2 of A), 131.3 (2 meta C's of PhO), 127.0 (para C of PhO), 120.9 (C_5 of A and 2 ortho C's of PhO), 92.3 ($C_{1'}$), 81.7 ($C_{2'}$, $^3J_{POCC}$ = 7.6 Hz), 80.9 ($C_{3'}$, $^2J_{POC}$ = 6.1 Hz), 72.1 ($C_{4'}$ and $C_{5'}$), 59.6 (2'-OMe).

***cis*-Thymidine 3',5'-Cyclic Phenyl [^{18}O]Monophosphate (4a).** Compound **2a** (106 mg, 0.29 mmol) and pyridine[12] (2.4 equiv, 0.70 mmol, 56 μL) were dissolved in 3 mL of dry acetonitrile. To this solution was added slowly 1.75 mL (6 equiv of $H_2{}^{18}O/H_2{}^{16}O$ and 1.2 equiv of I_2) of a stock solution.[13] Complete formation of **4a** was realized after 70 h, which was evident from ^{31}P NMR data (δ(CD_3CN/CH_3CN) −10.95 (^{16}O) and −10.99 ppm (^{18}O), ratio $^{18}O/^{16}O$ 91:9). Acetonitrile and diethyl ether were evaporated in vacuo. After addition of 120 μL (0.2 equiv) of a 5% sodium bisulfite solution, the solution was extracted with 10 mL of dichloromethane. After evaporation of the dichloromethane, **4a** was obtained. ^{1}H NMR indicated that pyridine (9% w/w) was present; ^{31}P NMR ($CDCl_3$) δ −12.24 (^{16}O) and −12.29 ppm (^{18}O) ($\Delta\delta$ = 6.8 Hz), ratio $^{18}O/^{16}O$ 92:8). The ^{1}H and ^{13}C NMR spectra were identical to those for **3a**.

***cis*-Thymidine 3',5'-Cyclic *p*-Nitrophenyl [^{18}O]Monophosphate (4b).** Compound **2b** (312.5 mg, 0.76 mmol) and pyridine[12] (2 equiv, 120 μL) were dissolved in 4 mL of dry tetrahydrofuran. To this solution was added slowly a mixture of I_2 (271.9 mg, 1.4 equiv) and $H_2{}^{18}O$ (2 equiv, 33 μL, 97 atom %) in 1 mL of dry tetrahydrofuran. After 12 h, compound **2b** was completely converted into **4b** and the excess of I_2 was removed in a reaction with 3 mL of 5% sodium bisulfite solution. After addition of 5 mL of chloroform, two layers were formed. The organic layer was evaporated in vacuo, affording a light yellow foam. Yield 0.18 g (55%): ^{31}P NMR (CD_3CN) δ −11.85 (^{16}O) and −11.90 ppm (^{18}O) ($\Delta\delta$ = 6.8 Hz), ratio $^{18}O/^{16}O$ 93:7). The ^{1}H and ^{13}C NMR spectra were identical to those for **3b**.

2'-*O*-Methyl-*cis*-adenosine 3',5'-Cyclic Phenyl [^{18}O]Monophosphate (4c). Compound **2c** (54 mg, 0.13 mmol) and pyridine[12] (0.37 mmol, 30 μL) were dissolved in 2 mL of anhydrous acetonitrile. To this solution was added slowly 800 μL (6 equiv of $H_2{}^{18}O/H_2{}^{16}O$ and 1.2 equiv of I_2) of a stock solution.[13] After being stirred for 15 h the solution was filtered and evaporated in vacuo. The residue was chromatographed on a silica gel column, using a gradient of methanol (0% → 5% v/v) in acetonitrile as eluent. Pure compound **4c** was obtained as a white solid. Yield 41 mg (72%): R_f 0.24 (methanol/acetonitrile 5:95 v/v); ^{31}P NMR (CD_3OD) δ −10.29 (^{16}O) and −10.33 ppm (^{18}O) ($\Delta\delta$ = 6.6 Hz), ratio $^{18}O/^{16}O$ 90:10). The ^{1}H- and ^{13}C NMR spectra were identical to those for **3c**.

General Procedure for the Hydrolysis of 3a, 3b, and 3c.[14] The 3',5'-cyclic phosphate triester (0.026 mmol) was transferred into a 5-mm NMR sample tube and dissolved in a mixture of 300 μL of 1,4-dioxane and 161 μL of D_2O. Addition of 39 μL (3 equiv) of a 2.00 M sodium hydroxide solution initiated the hydrolysis. After vigorous shaking of the NMR tube for 5 s the reaction was monitored at 21 °C by means of ^{31}P NMR: 8 FIDs were accumulated (time domain 16K; size 16K; sweep width 3240 Hz) prior to Fourier transformation at different reaction times, and the start of the fifth scan was chosen as the reaction time. Because of the fast hydrolysis of compound **3b**, it was not possible to monitor the reaction by means of ^{31}P NMR. Therefore, we hydrolyzed **3b** in a very dilute solution (3.2×10^{-5} M, 1,4-dioxane/H_2O 3:2 v/v), and the reaction was monitored by means of UV/visible spectroscopy at 405 nm.

(**3a**): ^{31}P NMR (1,4-dioxane-d_8/D_2O 3:2 v/v) δ −1.59 (cyclic phosphate diester), −3.89 (5'-phosphate diester), −4.87 (3'-phosphate diester).

(**3b**): ^{31}P NMR (1,4-dioxane-d_8/D_2O 3:2 v/v) δ −1.65 (cyclic phosphate diester), −4.89 (5'-phosphate diester), −5.92 (3'-phosphate diester).

(**3c**): ^{31}P NMR (1,4-dioxane-d_8/D_2O 7:3 v/v) δ −1.63 (cyclic phosphate diester), −4.00 (5'-phosphate diester), −4.84 (3'-phosphate diester).

Hydrolysis of *cis*-Thymidine 3',5'-Cyclic Phenyl [^{18}O]Monophosphate (4a) and Methylation of the Products. In a 5-mm NMR tube, *cis*-thymidine 3',5'-cyclic phenyl [^{18}O]monophosphate (**4a**) (11.3 mg, 0.03 mmol) was dissolved in a mixture of 300 μL of 1,4-dioxane-d_8 and 120 μL of D_2O, resulting in a yellowish solution. Then, 80 μL (5.4 equiv, 0.16 mmol) of a 2.00 M sodium hydroxide solution was added, thereby changing the color from light yellow to pink. After 1 h, the solution adopted a purple color and **4a** was completely hydrolyzed, which was evident from ^{31}P NMR data (δ(1,4-dioxane-d_8/D_2O 3:2 v/v) −1.66 (^{16}O cyclic phosphate diester), −1.69 (^{18}O cyclic phosphate diester), −3.86 (^{16}O 5'-phosphate diester), −3.89 (^{18}O 5'-phosphate diester), −4.96 (^{16}O 3'-phosphate diester), −4.99 (^{18}O 3'-phosphate diester)).[15] To this solution 500 μL of H_2O was added, and the mixture was placed on a Dowex-H^+ column (height 2.5 cm, diameter 1 cm), in order to neutralize the excess of sodium hydroxide, and collected in 10 mL of water (solution became colorless). The solution was concentrated to approximately 1–2 mL and placed on a Dowex-K^+ column[16] (height 2 cm, diameter 1 cm), in order to exchange H^+ by K^+, and collected in 15 mL of water. To this solution, 18-crown-6 (20.5 mg, 0.08 mmol) was added and the solvents were evaporated and coevaporated (three times) with 10 mL of acetonitrile.

(12) Pyridine is used in order to neutralize the HI, which is formed during the oxidation reaction.

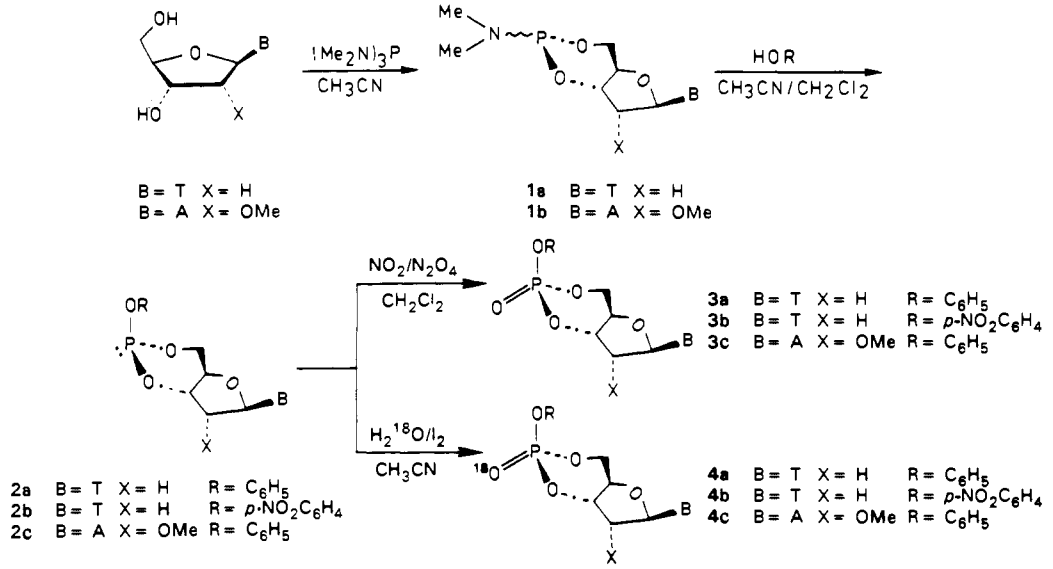
(13) The stock solution was obtained by dissolving $H_2{}^{18}O/H_2{}^{16}O$ ($^{18}O/^{16}O$ ratio of 96:4) and I_2 in a mixture of acetonitrile/diethyl ether (3:1 v/v) until the following concentration is reached: 1 M $H_2{}^{18}O/H_2{}^{16}O$ and 0.2 M I_2. Diethyl ether is used in the stock solution in order to increase the solubility of iodine in acetonitrile.

(14) Koole, L. H.; Olders, A. T. A.; Opresnik, M.; Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 55.

(15) The assignment of the 3'- and 5'-phosphate diester peaks in the ^{31}P NMR spectrum is based on a 2D ^{31}P–^{1}H correlation spectrum and on a 1D phosphorus–proton coupled spectrum of the hydrolysis products of **3a**: The 5'-phosphate diester resonates downfield from the 3'-phosphate diester.

(16) The Dowex K^+ column was prepared by percolating a Dowex H^+ column with 10 mL of a 1 M potassium chloride solution and 10 mL of a 1 M potassium hydroxide solution. Then the column was washed with water until the eluent became neutral.

Scheme I



The residue was dissolved in 500 μL of DMSO-d_6 and transferred into a 5-mm NMR tube: ^{31}P NMR (DMSO-d_6) δ −1.96 and −2.00 (^{16}O and ^{18}O cyclic phosphate diester), −3.75 and −3.79 (^{16}O and ^{18}O acyclic 5'-phosphate diester), −4.35 and −4.38 (^{16}O and ^{18}O acyclic 3'-phosphate diester) (ratio cyclic/5'/3' 58:22:20). To this solution 37 μL (20 equiv, 0.59 mmol) of methyl iodide was added. After 2–3 days the methylation was complete and the solution became brown: ^{31}P NMR (DMSO-d_6) δ −2.99, −3.01, −3.03 (*trans*-thymidine 3',5'-cyclic methyl phosphate triesters), −4.25, −4.26, −4.29 (*cis*-thymidine 3',5'-cyclic methyl phosphate triesters), −4.07, −4.08 (2×), −4.10, −4.11, −4.12 (R_P and S_P diastereoisomers of the acyclic 5'-methyl phenyl phosphate triesters), −5.13, −5.14, −5.15, −5.16, −5.17, −5.18 (R_P and S_P diastereoisomers of 3'-methyl phenyl phosphate triesters).

Hydrolysis of *cis*-Thymidine 3',5'-Cyclic *p*-Nitrophenyl [^{18}O]Monophosphate (4b) and Methylation of the Products. In a 5-mm NMR tube, *cis*-thymidine 3',5'-cyclic *p*-nitrophenyl [^{18}O]monophosphate (**4b**) (36.7 mg, 0.09 mmol) was dissolved in a mixture of 560 μL of 1,4-dioxane-d_8 and 80 μL of D_2O. To this solution was added 160 μL (3.7 equiv, 0.32 mmol) of a 2.00 M sodium hydroxide solution. After 1 h, complete hydrolysis of **4b** was observed, which was evident from ^{31}P NMR data (δ(1,4-dioxane-d_8/D_2O 7:3 v/v) −1.66 (^{16}O cyclic phosphate diester), −1.69 (^{18}O cyclic phosphate diester), −3.89 (^{16}O and ^{18}O 5'-phosphate diester), −4.66 (^{16}O and ^{18}O 3'-phosphate diester)[15] (ratio cyclic/5'/3' 91:4:5)). To this solution 1–2 mL of H_2O was added, and in order to neutralize the excess of sodium hydroxide, the mixture was placed on a Dowex-H^+ column. The solution was concentrated and placed on a Dowex-K^+ column,[16] and after addition of 18-crown-6 (130 mg, 0.49 mmol), the solvents were evaporated and coevaporated (three times) with 15 mL portions of anhydrous acetonitrile. The residue was dissolved in 1 mL of DMSO-d_6 and transferred into a 5-mm NMR tube. To this solution was added 300 μL (0.93 mmol) of methyl iodide. After 2–3 days the methylation was complete, and the solution became brown: ^{31}P NMR (DMSO-d_6) δ −3.04, −3.06, −3.09 (*trans*-thymidine 3',5'-cyclic methyl phosphate triesters), −4.43, −4.44, −4.47 (*cis*-thymidine 3',5'-cyclic methyl phosphate triesters). The acyclic phosphate triesters were not clearly identified (two broadened peaks were visible at −4.12 and −4.97 ppm).

Hydrolysis of 2'-*O*-Methyl-*cis*-adenosine 3',5'-Cyclic Phenyl [^{18}O]-Monophosphate (4c) and Methylation of the Products. In a 5-mm NMR tube, 2'-*O*-methyl-*cis*-adenosine 3',5'-cyclic phenyl [^{18}O]monophosphate (**4c**) (16 mg, 0.038 mmol) was dissolved in a mixture of 350 μL of 1,4-dioxane-d_8 and 93 μL of D_2O. To this solution was added 57 μL (3 equiv, 0.11 mmol) of a 2.00 M sodium hydroxide solution. After 1 h complete hydrolysis of **4c** was observed, which was evident from ^{31}P NMR data (δ(1,4-dioxane-d_8/D_2O 7:3 v/v) −1.62 (^{16}O cyclic phosphate diester), −1.66 (^{18}O cyclic phosphate diester), −4.06 (^{16}O 5'-phosphate diester), −4.09 (^{18}O 5'-phosphate diester), −4.84 (^{16}O 3'-phosphate diester), −4.87 (^{18}O 3'-phosphate diester)[15] (ratio cyclic/5'/3' 52:29:19)). To this solution 1 mL of H_2O was added, and in order to neutralize the excess of sodium hydroxide, the mixture was placed on a Dowex-H^+ column. The solution was concentrated and placed on a Dowex-K^+ column,[16] and after addition of 18-crown-6 (13 mg, 0.049 mmol), the solvents were evaporated and coevaporated (two times) with 10 mL portions of anhydrous acetonitrile. The residue was dissolved in 500 μL of DMSO-d_6 and transferred into a 5-mm NMR tube. To this solution was added 28 μL (0.46 mmol) of methyl iodide. After 2–3 days the methylation was complete and the solution became brown: ^{31}P NMR (DMSO-d_6) δ −2.73, −2.75, −2.77 (*trans*-2'-*O*-methyladenosine 3',5'-cyclic methyl phosphate triesters), −4.11, −4.13, −4.15 (*cis*-2'-*O*-methyladenosine 3',5'-cyclic methyl phosphate triesters), −4.17, −4.19, −4.21, −4.23, −4.25, −4.27 (R_P and S_P diastereoisomers of 5'-methyl phenyl phosphate triesters), −4.71, −4.73, −4.75, −4.79, −4.81, −4.83 (R_P and S_P diastereoisomers of 3'-methyl phenyl phosphate triesters).

Results and Discussion

Synthesis. Scheme I illustrates the method of preparation of adequate quantities of **1a,b**.[8] The trans epimers (relationship of substituent Me_2N and the base (A or T)) are formed predominantly. Compound **1a** was obtained by chromatographic purification of a mixture containing the cis/trans epimers (yield 47%, cis/trans ratio 2:98). Compound **1b** was only purified by filtration of the reaction mixture. The filtrate, which is approximately 95% pure, was used without further purification. The *cis*-3',5'-cyclic phosphite triesters **2a,b** were prepared through reaction of **1a** with phenol or *p*-nitrophenol, respectively. After washing with a sodium carbonate solution[7] to remove the phenols, the phosphites **2a,b** were isolated as pure compounds (yields 44% and 21%, respectively). Compound **2c** was formed in a reaction of **1b** with phenol. After washing with a sodium hydroxide solution to remove the phenol, **2c** was isolated as a white solid, which was approximately 90% pure (yield 47%). Compounds **3a–c** were obtained through stereospecific oxidation of the *cis*-3',5'-cyclic phosphite triesters **2a,b** with NO_2/N_2O_4[17] and **2c** with *tert*-butyl hydroperoxide.[18] These oxidations proceed with retention of the configuration at phosphorus.[17,18] After oxidation, compounds **3a,c** are obtained as white solids, whereas **3b** is isolated as a light yellow-brown solid. Compound **3a** is obtained by recrystallization from ethyl acetate, **3b** is isolated by evaporation of dichloromethane, and **3c** is obtained by means of column chromatography. Oxidation of **2a–c** by $H_2{}^{18}O/I_2$, which is also known to proceed with retention of phosphorus configuration,[19] yielded ^{18}O-labeled *cis*-3',5'-cyclic phosphate triesters **4a–c**.

Hydrolysis of 3a–c. To investigate the kinetics and product distribution of the hydrolyses of the 3',5'-cyclic phosphate triesters, we first examined the unlabeled analogs **3a–c**. The alkaline

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Table I. Kinetic Parameters for the Hydrolysis of **3a–c** at 294 K

	initial concentrations[e] (M)		rate constants[f] ($M^{-1}\cdot s^{-1}$)				product distribution[e] (%)		
compd	$10^2[P]_0$	$10^2[OH^-]_0$	10^3k_{obs}	10^3k_a	10^3k_c	r^d	cyclic	3′	5′
3a[a]	5.21	15.6	9	4	5	0.995	58	20	22
3b[b]	0.0032	5.05	160	32	128	0.998	80[c]	10[c]	10[c]
3c[a]	4.77	14.3	21	10	11	0.998	52	19	29

[a] Kinetics were performed in D_2O/1,4-dioxane-d_8 3:7 (v/v) and followed by means of ^{31}P NMR spectroscopy. [b] Kinetics were performed in D_2O/1,4-dioxane-d_8 2:3 (v/v) and monitored by means of UV/visible spectroscopy at 405 nm. [c] The product distribution is based on determining the *p*-nitrophenoxide concentration as a function of time by means of UV/visible spectroscopy. The *p*-nitrophenoxide is formed simultaneously with the 3′,5′-cyclic phosphate diester; therefore at any time, the *p*-nitrophenoxide concentration is always equal to that of the 3′,5′-cyclic phosphate diester. After a long time (approximately 1 h) the concentration of *p*-nitrophenoxide reaches a constant value. The maximum *p*-nitrophenoxide concentration which can be reached is equal to the initial concentration of the cis 3′,5′-cyclic *p*-nitrophenyl phosphate triester (assuming 100% formation of 3′,5′-cyclic phosphate diester). The sum of the concentrations of the 3′- and 5′-acyclic phosphate diesters is equal to the difference between the theoretical *p*-nitrophenoxide concentration, assuming 100% formation of the 3′,5′-cyclic phosphate diester, and the observed *p*-nitrophenoxide concentration, when reaching a constant value. The individual 3′- and 5′-acyclic phosphate diesters are assumed to be formed in equal quantity, which is derived from ^{31}P NMR spectra. ^{31}P NMR spectroscopy revealed a different product distribution (cyclic/3′/5′ 90:5:5) compared to UV/visible spectroscopy. [d] Correlation coefficient. [e] Estimated errors ±5%. [f] Estimated errors ±20%.

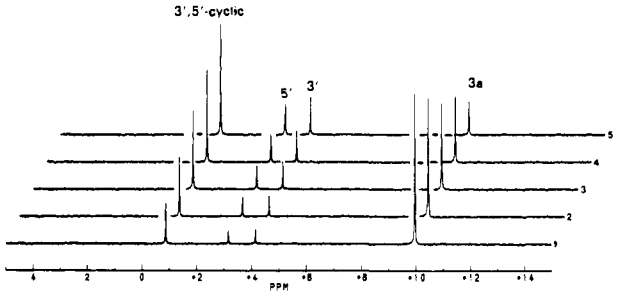


Figure 1. ^{31}P NMR spectra measured at different reaction times during alkaline hydrolysis of compound **3a** at 294 K in D_2O/1,4-dioxane-d_8 (2:3 v/v). Evidently, three different products are formed (3′,5′-cyclic phosphate diester, 5′-phenyl phosphate diester, and 3′-phenyl phosphate diester). The reaction times of the spectra 1–5 are 5, 10, 18, 27, and 60 min, respectively.

hydrolysis of compounds **3a–c** is assumed to follow second-order kinetics (first order in the cyclic phosphate triester and OH^- concentration).[20] The hydrolysis reactions were carried out by adding an exact amount (in the range 3–5 equiv) of a sodium hydroxide solution to a solution of the cyclic phosphate triesters **3a–c** in D_2O/1,4-dioxane-d_8 (2:3 or 3:7 v/v). This mixture was chosen to ensure the solubility of the cyclic phosphate triesters **3a–c** and the formed phosphate diesters. Upon hydrolysis of the phosphate triesters **3a** and **3b**, 1 equiv of OH^- is consumed instantaneously by the relatively acidic H_3 proton of the thymine base (pK_a = 9.7).[21] Compared with thymine, the adenine of **3c** contains no acidic protons. During hydrolysis three products are formed: one 3′,5′-cyclic phosphate diester and two acyclic phosphate diesters (5′- and 3′-phosphate diester) (Scheme II).[15] As an example, Figure 1 shows the ^{31}P NMR spectra at five different reaction times during hydrolysis of **3a**. Formation of the 3′,5′-cyclic phosphate diester is related to expulsion of phenol (**3a** and **3c**) or *p*-nitrophenol (**3b**). Due to the acidic character of the phenols (pK_a = 10.0 for phenol and 7.2 for *p*-nitrophenol), an additional equivalent of OH^- is consumed in order to form phenoxide or *p*-nitrophenoxide from the corresponding phenols (Scheme II). During the formation of acyclic 3′- and 5′-phosphate diesters only alkoxides were expelled, without consuming additional amounts of OH^-. Because of the nonequivalent consumption of OH^- for the formation of cyclic and acyclic phosphate diesters, we have to modify the simple second-order rate equation into a more complex equation (Appendix).

The results on the hydrolysis of **3a–c** are summarized in Table I and visualized in Figure 2. The observed straight lines from Figure 2 agree with the assumed second-order kinetics. The

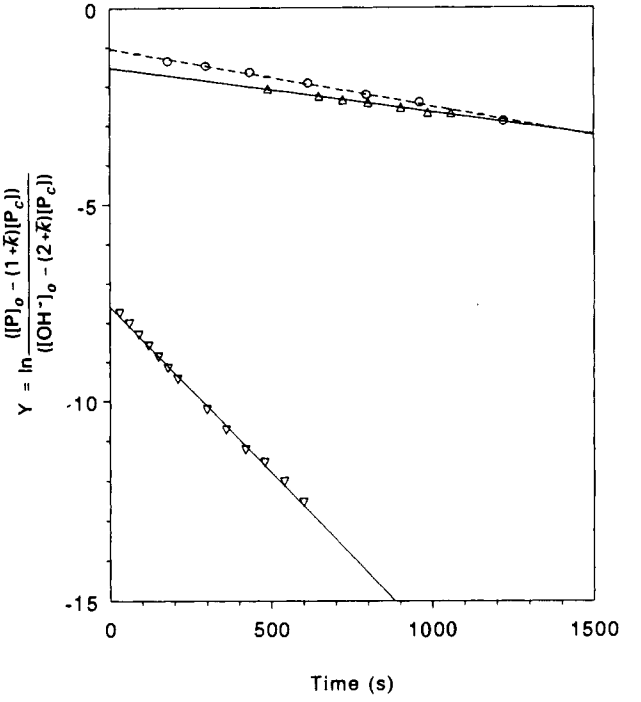
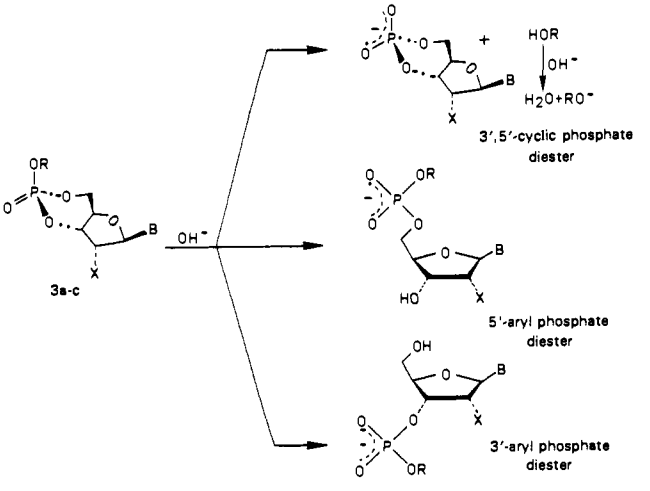


Figure 2. Visualization of the kinetics of the hydrolysis of compounds **3a** (△), **3b** (▽), and **3c** (○). The plots are obtained by using the second-order reaction equation (8) described in the Appendix. The reaction conditions are described in the Experimental Section. Evidently, the data points fit perfectly with the straight lines, indicating that second-order kinetics is very plausible (correlation coefficients: 0.995 (△), 0.998 (▽), and 0.998 (○).

Scheme II



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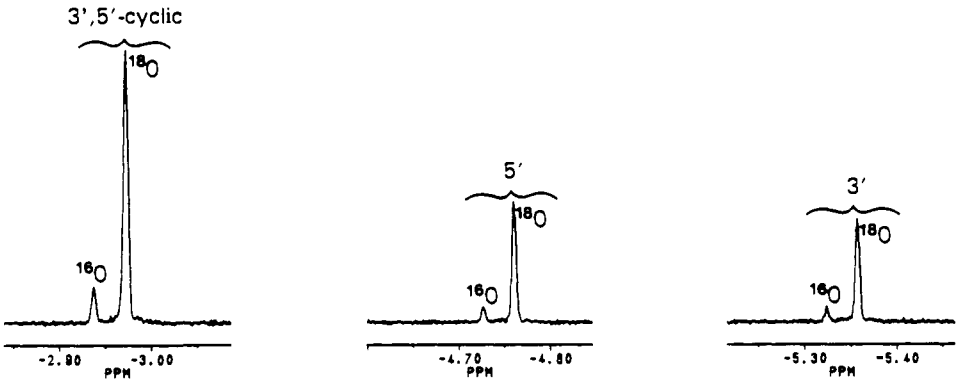


Figure 3. Expansions of the ^{31}P NMR spectrum of the hydrolysis products of compound **4a** in D_2O/1,4-dioxane-d_8 (2:3 v/v). Three different products are formed (3',5'-cyclic phosphate diester, 5'-phenyl phosphate diester, and 3'-phenyl phosphate diester). Each product consists of two isotopomers ($^{16}O/^{18}O$ 8:92) (^{18}O isotope shift, 0.035 ppm).

Table II. Product Distribution and Stereochemistry of the Hydrolysis of **4a–c**

	cyclic[a]			3'[a]			5'[a]		
compd	product distribution (%)	retention (%)	inversion (%)	product distribution (%)	retention (%)	inversion (%)	product distribution (%)	retention (%)	inversion (%)
4a	58	83	17	20	88 or 12	12 or 88	22	~50	~50
4b	90	60	40	5	*b*	*b*	5	*b*	*b*
4c	52	83	17	19	79 or 21	21 or 79	29	~50	~50

[a] Estimated errors ±5%. [b] Could not be determined because of the low resolution of the expansions of the 3'- and 5'-phosphate diesters in the ^{31}P NMR spectrum.

distribution of the reaction products and the overall second-order rate constant k_{obs} ($= k_a + k_c$) (Appendix) are slightly dependent on the base (A or T) but clearly dependent on the aryl substituent. The ligand *p*-nitrophenoxy is a better leaving group than phenoxy, resulting in faster hydrolysis of the 3',5'-cyclic phosphate triester and in formation of relatively large amounts of cyclic phosphate diester.

Hydrolysis of ^{18}O-Labeled 3',5'-Cyclic Phosphate Triesters 4a–c. Hydrolysis reactions of these compounds were studied to determine the stereochemical aspects of the reaction. Alkaline hydrolysis is carried out by adding an exact amount (in the range 3–5 equiv) of a sodium hydroxide solution to a solution of the cyclic phosphate triesters **4a**, **4b**, or **4c** in D_2O/1,4-dioxane-d_8 (**4a**, 2:3 v/v; **4b**,**4c**, 3:7 v/v). After being mixed, the initial concentration of the cyclic phosphate triesters is approximately 0.05 M. The hydrolysis reaction of **4a** and **4c** is complete within about 0.5–1 h, while complete conversion of **4b** has been obtained within 5–10 minutes. Figure 3 shows the ^{31}P NMR spectrum of the products, which have been obtained after complete hydrolysis of **4a** ($^{18}O/^{16}O$ 92:8). Obviously, three different products were derived (3',5'-cyclic phosphate diester, acyclic 3'-phosphate diester, and acyclic 5'-phosphate diester), which were assigned by comparing the hydrolysis of unlabeled **3a**.

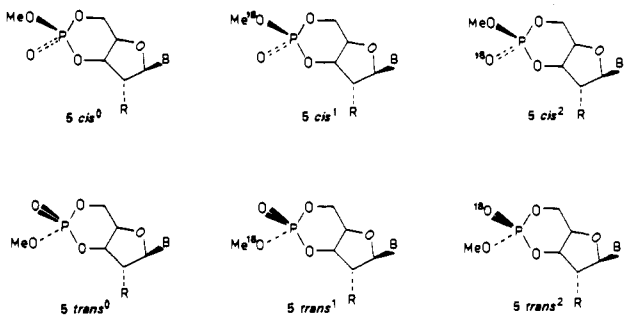
The small peaks represent the unlabeled analogs of the reaction products. The ^{18}O-induced upfield shift was expected from earlier studies.[6,22] Integration of the [^{18}O]- and [^{16}O]phosphate diester peaks revealed a ratio of approximately 92:8. This ratio is identical to the $^{16}O/^{18}O$ ratio in the 3',5'-cyclic phosphate triester. We therefore conclude that no exchange of the oxygen (^{16}O) from the solvent with the phosphate di(tri)ester oxygen (^{18}O) has occurred under the reaction conditions. Previous studies showed that hydrolysis of cdAMP proceeds with exclusive cleavage of the P–O bond[4] without breaking the C–O bond and that introduction of ^{18}O in a cyclic phosphate triester proceeds in a stereospecific and regiospecific way (no exchange between ^{18}O and ^{16}O in the phosphate triester).[19a] The hydrolysis of **4b** results predominantly in formation of the 3',5'-cyclic phosphate diester (90–93%), whereas the hydrolysis of **4c** is closely related to that of **4a**. Due to the chirality of the 3',5'-cyclic- and the acyclic 3'- and 5'-phosphate diesters, introduced by the ^{18}O-label in the 3',5'-cyclic phosphate triester, each [^{18}O]phosphate diester exists as two diastereoisomers. However, in the ^{31}P NMR spectrum these diastereoisomers are not detected separately, due to the delocalization of the negative charge on the phosphoryl oxygens. Therefore, the sodium counterion of the phosphate diesters, formed during hydrolysis, has been exchanged for potassium, using Dowex-K^+,[16] and the potassium salts have been methylated by methyl iodide in dimethyl sulfoxide-d_6 solution and in the presence of 18-crown-6, according to the procedure described by Lowe et al.[4,19a,23]

A. Stereochemical Analysis of the 3',5'-Cyclic Methyl Phosphate Triesters. Figure 4a shows the ^{31}P NMR spectrum of the methyl phosphate triesters, which have been derived after hydrolysis of **4a** and methylation of the hydrolysis products. The two patterns, each consisting of three resonances (peaks 1, 2, and 3 and 10, 11, and 12), located at −3.0 and −4.3 ppm, are assigned to be the trans and cis 3',5'-cyclic methyl phosphate triesters, respectively, since it has been found that cis (OMe pseudoaxial) phosphate triesters of substituted 3',5'-cyclic phosphate triesters resonate at ca. 1–3 ppm upfield from the trans (OMe pseudoequatorial) derivatives.[9,17c] This observation of a large ^{31}P chemical shift difference is due to the relatively large difference in geometry of the diastereoisomers at the phosphorus center (OMe cis or trans). Thus, the diastereoisomeric 3',5'-cyclic methyl phosphate triesters do not have to be physically separated. The three low field resonances (peaks 1, 2, and 3) of the 3',5'-cyclic methyl phosphate triesters are due to the ^{18}O-perturbations on the ^{31}P NMR resonances. If ^{18}O is singly bonded to phosphorus, it causes a smaller upfield isotope shift, relative to ^{16}O, than when doubly bonded to phosphorus (ca. 0.02 and 0.04 ppm, respectively).[6,22] Therefore, the ^{31}P resonance of the trans 3',5'-cyclic methyl phosphate triester **5**-trans[1] (compound **5** in a trans configuration and one P–^{18}O bond) (peak 2) will be located more downfield than that of the trans 3',5'-cyclic methyl phosphate triester **5**-trans[2] (trans configuration and two P–^{18}O bonds) (peak 3). The trans 3',5'-cyclic phosphate triester **5**-trans[0] (peak 1), containing no ^{18}O-labels, will

(22) (a) Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200. (b) Lutz, O.; Nolle, A.; Staschewski, D. *Z. Naturforsch.* **1978**, *A33*, 380. (c) Lowe, G.; Sproat, B. S. *J. Chem. Soc., Chem. Commun.* **1978**, 565. (d) Cohn, M.; Hu, A. *J. Am. Chem. Soc.* **1980**, *102*, 913.

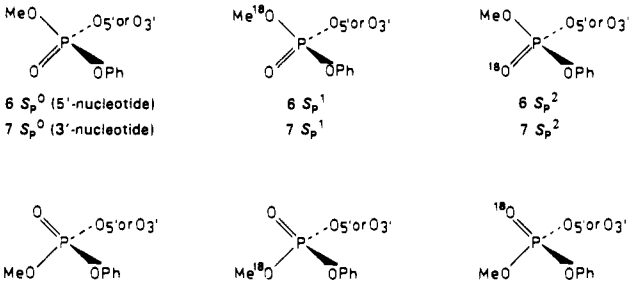
(23) Jarvest, R. L.; Lowe, G.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1981**, 3186.

be located downfield from the ^{18}O-labeled trans 3',5'-cyclic phosphate triesters (**5**-trans[1] and **5**-trans[2]). Similarly, the cis 3',5'-cyclic methyl phosphate triester **5**-cis[2] (peak 12) will resonate upfield from the cis 3',5'-cyclic methyl phosphate triester **5**-cis[1] (peak 11) in the ^{31}P NMR spectrum. The relative intensities



of the ^{18}O cis triesters (**5**-trans[1] and **5**-cis[2]) to the ^{18}O cis triesters (**5**-trans[2] and **5**-cis[1]) in the three low-field and high-field resonances (peaks 1, 2, and 3 and 10, 11, and 12, respectively) of the 3',5'-cyclic phosphate diesters indicate that during the hydrolysis of **4a** (^{18}O cis) the formation of the 3',5'-cyclic phosphate diester has occurred with 17% inversion and 83% retention of configuration at phosphorus (Table II). Hydrolysis of **4b** takes place with 40% inversion and 60% retention, related to the formation of 3',5'-cyclic phosphate diester (Figure 4b). During the hydrolysis of **4c** the formation of the 3',5'-cyclic phosphate diester occurs with 17% inversion and 83% retention of configuration at phosphorus (Figure 4c), which is similar to the hydrolysis of **4a**.

B. Stereochemical Analysis of the Acyclic 3'- and 5'-Methyl Aryl Phosphate Triesters. Compared with the ^{31}P NMR chemical shift difference between the trans and cis 3',5'-cyclic methyl phosphate triesters, the chemical shift difference between the acyclic R_P and S_P 3'- or 5'-methyl aryl phosphate triesters is relatively small (ca. 1.3 versus 0.02 ppm), because of the relatively small difference in geometry of the diastereoisomers at the phosphorus center (R_P/S_P). The chemical shift difference be-



tween the R_P and S_P diastereoisomers[24] is in the same range as the ^{31}P NMR isotope shift of the ^{18}O-label, relative to ^{16}O. Therefore, it is not possible to assign the R_P and S_P diastereoisomers of the 3'- or 5'-methyl phenyl phosphates unambiguously. For this reason, we have to separate the diastereoisomers and determine the configuration of the isolated diastereoisomers, which we are working on at present. The assignment of the acyclic 3'- and 5'-methyl aryl phosphate triesters in the ^{31}P NMR spectrum is based on the relative positions of the unlabeled 3'- and 5'-aryl phosphate diesters.[15]

In Figure 4a and 4c, the multiplets of the 5'-methyl phenyl phosphate triesters consist of six ^{31}P NMR signals, but the intensity distributions are not the same. The two small peaks (**4a**, peaks 4 and 5; **4c**, peaks 7 and 10) were assigned to be the R_P and S_P unlabeled 5'-methyl phenyl phosphate triesters (**6** S_P^0 (S_P configuration, zero P–^{18}O bonds) and **6** R_P^0), but these resonances cannot be identified unambiguously at this moment. The ^{31}P NMR multiplets of the 5'-methyl phenyl phosphate triesters, shown in Figure 4a and 4c, can be divided into two three-line patterns (**4a**, peaks 4, 6, and 8 and 5, 7, and 9; **4c**, peaks 7, 8, and 9 and 10, 11, and 12). The chemical shift difference in the ^{31}P NMR spectrum between the isotopomers **6** S_P^0 and **6** S_P^1 and between **6** R_P^0 and **6** R_P^1 is 0.016 ppm (**4a**, differences between the resonances 4 and 6 and between 5 and 7; **4c**, differences between 7 and 8 and between 10 and 11), whereas a chemical shift difference of 0.041 ppm is observed between the isotopomers **6** S_P^0 and **6** S_P^2 and between **6** R_P^0 and **6** R_P^2 (**4a**, differences between the resonances 4 and 8 and between 5 and 9; **4c**, differences between resonances 7 and 9 and between 10 and 12). These values are in excellent agreement with the values expected for the upfield isotope shift of the ^{31}P resonance (relative to ^{16}O), when ^{18}O is singly or doubly bonded to phosphorus (ca. 0.02 or 0.04 ppm, respectively). The ^{18}O-labeled 5'-methyl phenyl phosphate triesters **6** S_P^1 and **6** R_P^2 are associated with retention of configuration at phosphorus during hydrolysis of **4a** and **4c** and formation of the 5'-phenyl phosphate diesters, whereas **6** S_P^2 and **6** R_P^1 correspond with inversion of configuration. Since the intensities of the ^{18}O-labeled 5'-methyl phenyl phosphate triesters (**6** S_P^1, **6** S_P^2, **6** R_P^1, and **6** R_P^2) are almost identical, we state that during hydrolysis the formation of acyclic 5'-phenyl phosphate diesters occurs with virtually complete loss of stereochemistry.

The multiplets of the 3'-methyl phenyl phosphate triesters in Figure 4a and 4c also consist of six ^{31}P NMR signals with an unequal intensity distribution. However, four small and two large ^{31}P NMR peaks have been observed. This means that two unlabeled 3'-methyl phenyl phosphate triesters (**4a**, peaks 13 and 14; **4c**, peaks 13 and 16) and two ^{18}O-labeled 3'-methyl phenyl phosphate triesters (**4a**, peaks 15 and 18; **4c**, peaks 15 and 17) represent the small peaks and that two ^{18}O-labeled 3'-methyl phenyl phosphate triesters represent the large peaks. In Figure 4a and 4c, the multiplets of 3'-methyl phenyl phosphate triester are composed of two three-line patterns (**4a**, peaks 13, 15, and 17 and 14, 16, and 18; **4c**, peaks 13, 14, and 15 and 16, 17, and 18). The chemical shift difference in both ^{31}P NMR spectra between the isotopomers **7** S_P^0 and **7** S_P^1 and between **7** R_P^0 and **7** R_P^1 is 0.016 ppm (**4a**, differences between resonances 13 and 15 and between 14 and 16; **4c**, differences between 13 and 14 between 16 and 17). The chemical shift difference between the isotopomers **7** S_P^0 and **7** S_P^2 and between **7** R_P^0 and **7** R_P^2 is 0.041 ppm (**4a**, difference between resonances 13 and 17 and between 14 and 18; **4c**, differences between peaks 13 and 15 and between 16 and 18). These values are equal to those of the 5'-methyl phenyl phosphate triesters. The expected values for the chemical shift difference between isotopomers indicate that the assigned sequence of the isotopomers **7** S_P^0, **7** S_P^1, and **7** S_P^2 and **7** R_P^0, **7** R_P^1, and **7** R_P^2 in the ^{31}P NMR spectra in Figure 4a and 4c is correct. However, it is not possible to identify the two R_P and S_P patterns absolutely. Since the ^{18}O-labeled 3'-methyl phenyl phosphate triesters **7** S_P^2 and **7** R_P^1 are associated with retention of configuration at phosphorus and **7** S_P^1 and **7** R_P^2 are associated with inversion of configuration, no explicit statement can be made about the percentage retention or inversion of configuration. From the two three-line patterns of the 3'-methyl phenyl phosphate triester in Figure 4a two values can be extracted for inversion or retention of configuration at phosphorus: ca. 12% and 88%. Thus during hydrolysis of **4a**, formation of 3'-phenyl phosphate diester proceeds with ca. 12% (or 88%) inversion or retention. From Figure 4c, we conclude that the formation of acyclic 3'-phenyl phosphate diesters, during hydrolysis of **4c**, occurs with ca. 21% (or 79%) inversion or retention of configuration at phosphorus. The hydrolysis of **4b** results in formation of approximately 10% acyclic 3'- and 5'-*p*-nitrophenyl phosphate diesters, which were not clearly detectable in the ^{31}P NMR spectrum. Due to methylation of the acyclic aryl ^{18}O phosphate diesters the signal to noise ratio became

(24) The sequence rules of Cahn, Ingold, and Prelog state that substituents (in this case oxygen substituents) have priority over isotopes (^{18}O). Therefore, the isotopomers **6** S_P^0, **6** S_P^1, and **6** S_P^2 or **7** S_P^0, **7** S_P^1, and **7** S_P^2 adopt the same configuration (S_P), whereas **6** R_P^0, **6** R_P^1, and **6** R_P^2 or **7** R_P^0, **7** R_P^1, and **7** R_P^2 have the R_P configuration. See also: (a) Cahn, R. S.; Ingold, S. C.; Prelog, V. *Angew. Chem.* **1966**, *78*, 413. (b) Prelog, V.; Helmchen, G. *Angew. Chem.* **1982**, *94*, 614, (c) Cahn, R. S. *J. Chem. Educ.* **1964**, *41*, 116. (d) Herdering, W.; Seela, F. *J. Org. Chem.* **1985**, *50*, 5314.

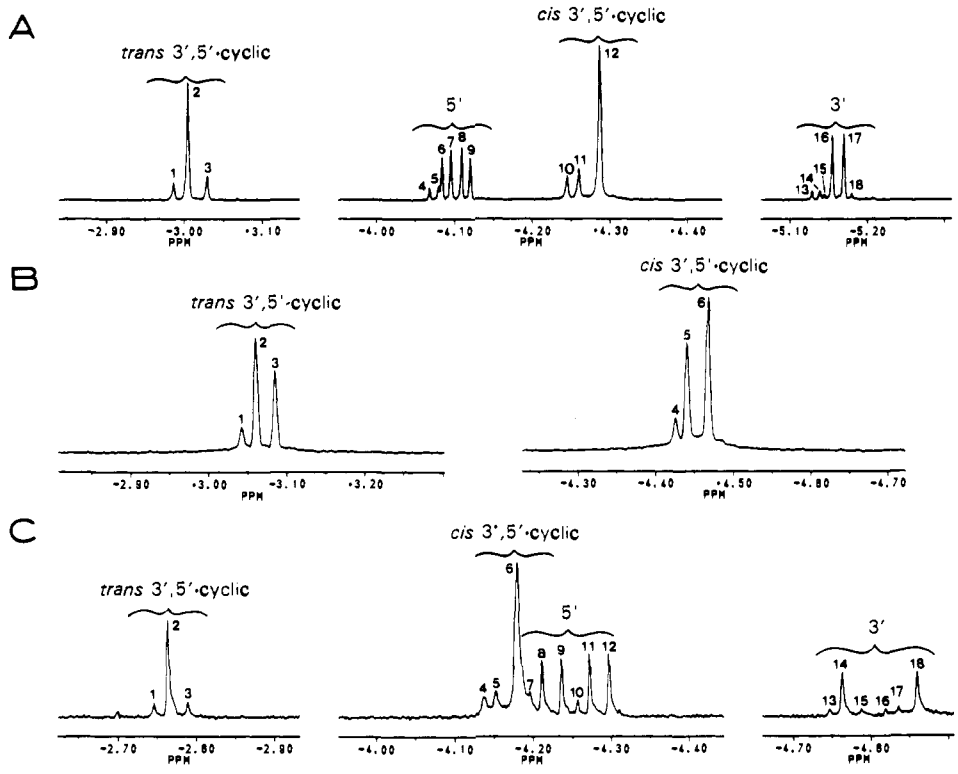


Figure 4. Expansions of the ^{31}P NMR spectrum of the methylated hydrolysis products of **4a** (A), **4b** (B), and **4c** (C) in DMSO-d_6. See text for assignment of the peaks.

worse, because of the formation of the 4 isomers (7 S_P^1, 7 S_P^2, 7 R_P^1, and 7 R_P^2). Therefore it was not possible to determine the acyclic 3'- and 5'-methyl *p*-nitrophenyl phosphate triesters.

Interpretation and Conclusions

In order to interpret our results, we have postulated a plausible reaction mechanism. Following the original ideas of Westheimer,[25a] we assume that the nucleophilic attack of an OH^- ion on the phosphorus center results in the formation of a five-coordinated phosphorus (P^V) intermediate with a trigonal bipyramidal (TBP) geometry, that the nucleophile enters the P^V-TBP at one axial site, and that cleavage also takes place axially.[25] Furthermore, it is assumed that negatively charged oxygens adopt equatorial sites in the P^V-TBP[25b,26] only, and that Berry pseudorotation[25a,27] can occur prior to bond breaking. As shown in Scheme III, the initial attack of OH^- on phosphorus can take place in three different ways (route 1, 2, and 3). Attack of the OH^- on phosphorus opposite the P=O bond is not taken into account because the P^V-TBP formed will possess a negatively charged axial oxygen atom, which is energetically unfavorable. Moreover, after protonation of the labeled axial oxygen atom this P^V-TBP could lose the oxygen label by cleavage of the axial P–^{18}O bond. This is in contradiction with our observations that no exchange takes place between the oxygen (^{16}O) from the solvent and the ^{18}O-labeled oxygen from the phosphate (di)triester.

In route 1, OH^- attacks phosphorus opposite to the P–OR bond, resulting in a P^V-TBP with an unfavorable *diequatorially* oriented dioxaphosphorinane ring.[28] Cleavage of the P–OR bond results in formation of the 3',5'-cyclic phosphate diester **8**, with inversion of configuration. In principle, shifting a proton from the axial oxygen atom to the equatorial oxygen atom simultaneously followed by Berry pseudorotation can lead to two P^V-TBPs with axially located ^{18}O-labeled hydroxyl groups (Scheme IV). However, cleavage of the P–^{18}O bond results in formation of unlabeled 3',5'-cyclic phosphate triesters. This means that the ^{18}O-label will be lost, which is in contradiction with our observations. Therefore, we can ignore the reaction routes incorporating a simultaneous Berry pseudorotation and H^+-shift, shown in Scheme IV.

In route 2, OH^- attacks phosphorus opposite the P–$O_{3'}$ bond. Initially, the aryloxy (OR) group of the P^V-TBP formed is placed equatorially and cannot be cleaved immediately. Rather, the axial P–$O_{3'}$ bond will break resulting in the formation of the acyclic 5'-phosphate diester **9**. This process proceeds with inversion of configuration at phosphorus. Berry pseudorotation of the P^V-TBP initially formed results in axial location of the OR group. In this situation, breaking of the P–OR bond results in the formation of the 3',5'-cyclic phosphate diester **11** with *retention* of configuration, whereas cleavage of the axial P–$O_{5'}$ bond leads to the acyclic 3'-phosphate diester **10**, with retention of configuration.

In route 3, OH^- attacks phosphorus opposite the P–$O_{5'}$ bond. In the P^V-TBP initially formed the axial P–$O_{5'}$ bond can be cleaved resulting in the formation of the acyclic 3'-phosphate diester **13**. This process proceeds with inversion of configuration at phosphorus. Berry pseudorotation of the P^V-TBP initially formed leads to a P^V-TBP with an axially located OR group. Cleavage of the P–OR bond leads to the 3',5'-cyclic phosphate diester **11** with retention of configuration, whereas cleavage of the axial P–$O_{3'}$ bond results in the formation of the acyclic 5'-phosphate diester **12**, with retention of configuration.

Thus, nucleophilic attack on phosphorus and direct cleavage of the P–OR bond takes place with inversion of configuration,

(25) (a) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70. (b) Emsley, J.; Hall, D. In *The Chemistry of Phosphorus*; Wiley: New York, 1976; Chapter 8. (c) Gorenstein, D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1967**, *89*, 2762. (d) Gorenstein, D. G.; Powell, R.; Findlay, J. *J. Am. Chem. Soc.* **1980**, *102*, 5077. (e) Kluger, R.; Covitz, F.; Dennis, E.; Williams, L. D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1969**, *91*, 6066. (f) van Ool, P. J. J. M.; Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 79. (g) van Ool, P. J. J. M.; Buck, H. M. *Eur. J. Biochem.* **1982**, *121*, 329. (h) van Ool, P. J. J. M.; Buck, H. M. *Recl. Trav. Chim. Pays-Bas* **1984**, *103*, 119.

(26) Holmes, R. R. In *Pentacoordinated Phosphorus*; ACS Monograph Series 175, 176; American Chemical Society: Washington, DC, 1980; Vols. I, II.

(27) Berry, P. S. *J. Chem. Phys.* **1960**, *32*, 933.

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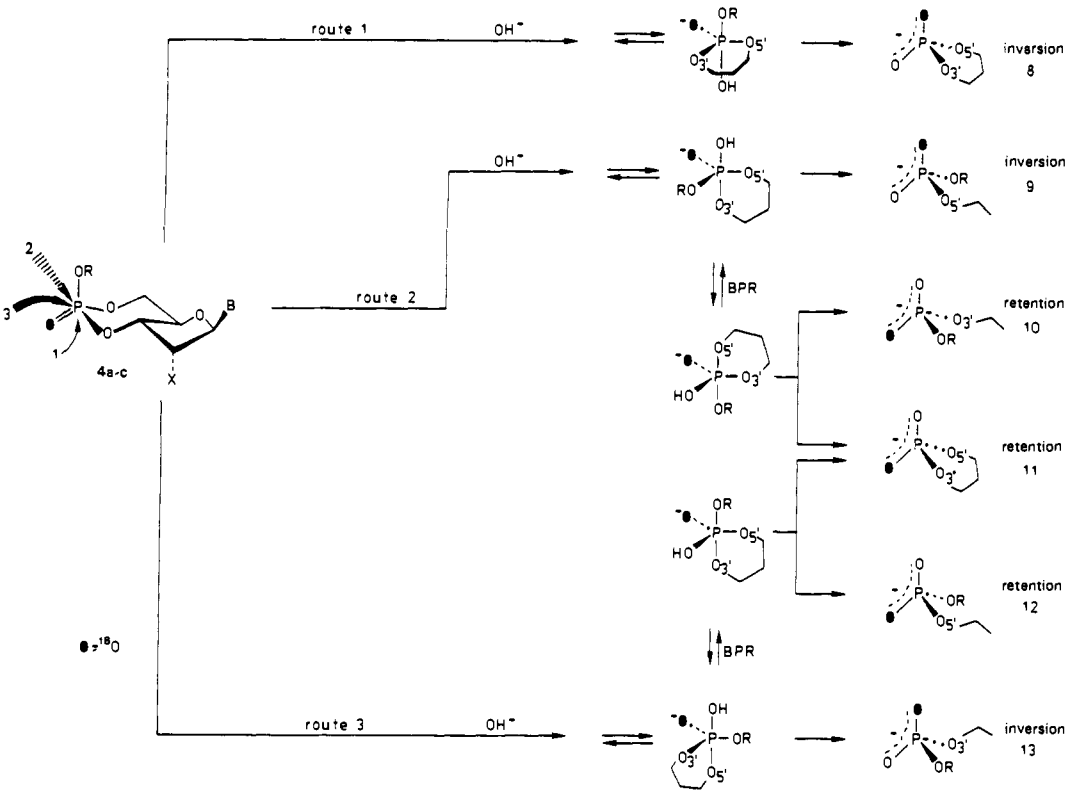
Scheme III



Scheme IV

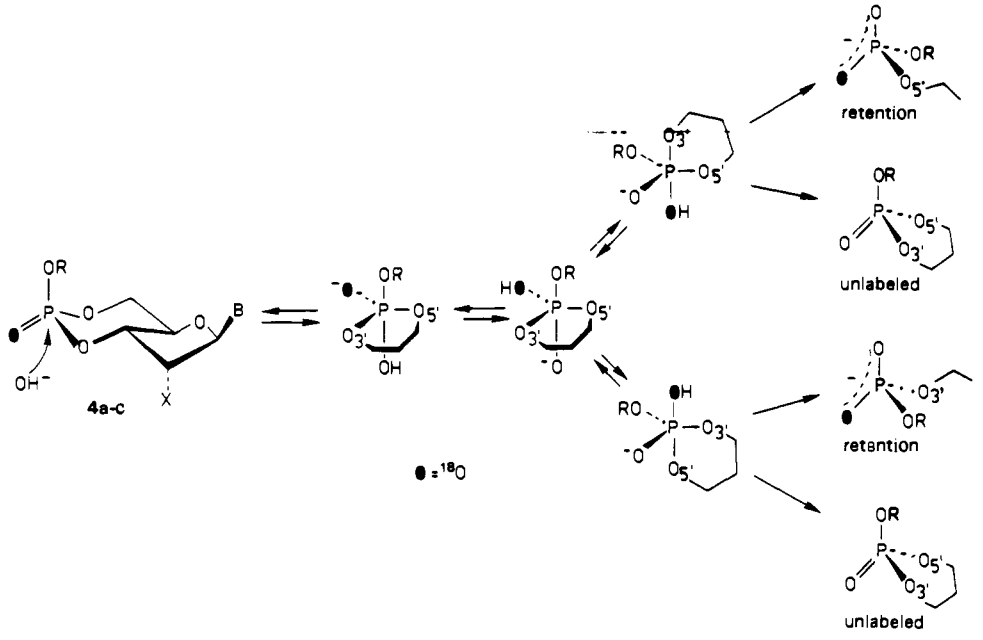


Table III. Calculated Distribution of Hydrolysis Products[a]

compd	8 (cyclic inversion) (%)	9 (5′; inversion) (%)	10 (3′; retention) (%)	11 (cyclic; retention) (%)	12 (5′; retention) (%)	13 (3′; inversion) (%)
4a	10	~11	18 or 2	48	~11	2 or 18
4b	36	*b*	*b*	54	*b*	*b*
4c	9	~14.5	15 or 4	43	~14.5	4 or 15

[a] Based on Table II. [b] Could not be determined because of the low resolution of the expansions of the 3′- and 5′-phosphate diesters in the ^{31}P NMR spectrum.

whereas breaking the axial bonds after pseudorotation leads to retention of configuration. Taking all these considerations (vide supra) into account, we believe that the reaction routes in Scheme III sufficiently describe the hydrolysis of the 3′,5′-cyclic phosphate triesters. The total product distribution of the hydrolysis of 3′,5′-cyclic phosphate triesters is shown in Table III.

From Table III it is obvious that during hydrolysis of **4a–c** the cyclic product **11** (retention of configuration at phosphorus) is

formed as the main product, whereas compound **8** (inversion of configuration) is formed in a smaller amount. We believe that the formation of compound **8** is not thermodynamically determined (based on the leaving group character), whereas the formation of compound **11** is based on the pseudorotational equilibrium which is determined by the axiophilicity of the leaving groups (OR) in the P^V-TBPs. Compared with the $O_{3'}$- or $O_{5'}$- and OH^--residues, the OR groups are better leaving groups resulting in a preferential formation of product **11**.

The formation of the 3',5'-cyclic phosphate diester during hydrolysis of **4a** and **4c** proceeds with 17% inversion of configuration at phosphorus. These results closely resemble those obtained from $H_2{}^{17}O$ hydrolysis of monocyclic phenyl phosphate triesters, reported by Gordillo et al.[7] The formation of the 3',5'-cyclic phosphate diester during hydrolysis of **4b**, containing *p*-nitrophenoxy, proceeds with 40% inversion, which is slightly less than reported by Gordillo et al.[7] for monocyclic *p*-nitrophenoxy analogs (56%) (Chart I; C and D) but much less than reported by Gorenstein et al.[6] for a bicyclic system containing an axially located dinitrophenoxy group (83%) (Chart I; A). Presumably this difference can be attributed to the fact that Gorenstein et al.[6] used 2,4-dinitrophenoxy as the leaving group, i.e., the P^V-TBP intermediate that is formed after nucleophilic attack on phosphorus has a shorter lifetime in Gorenstein's model A (Chart I)[6] as compared to our models **4a–c** and models of Gordillo et al.[7] The combined experimental data substantiate the conclusion of Gorenstein et al.[6] that the leaving group character significantly influences the inversion/retention ratio of the 3',5'-cyclic phosphodiesters (Table III).

The formation of the ^{18}O-labeled 5'-acyclic aryl phosphate diesters is not stereospecific at all (ca. 50% retention), whereas the stereospecific preference (retention or inversion) of the formation of the ^{18}O-labeled 3'-acyclic aryl phosphate diesters cannot be determined at this moment.

The observed stereospecificity in the 3'- and 5'-aryl phosphate diesters is not in agreement with the reported chemical hydrolysis of R_P cyclic [^{17}O,^{18}O]dAMP, which proceeds with complete inversion of configuration at phosphorus.[4] The difference in hydrolytic behavior between our compounds, which hydrolyze both with retention and inversion of configuration at phosphorus, and those of [^{17}O,^{18}O]cdAMP,[4] proceeding with complete inversion, can be explained in terms of formation of dianions during hydrolysis of the latter. The P^V-TBPs, initially formed in route 2 or 3 by hydrolysis of 3',5'-cyclic phosphate diesters[4] will adopt dianionic character, because of the deprotonation of the equatorial OH group (R = H). Pseudorotation places one of the O^- ligands in an axial site, which is energetically unfavorable. The only escape for the P^V-TBPs is to release energy by cleavage of the P–$O_{3'}$ or P–$O_{5'}$ bond in route 2 or 3, respectively, resulting in the observed complete inversion of configuration at phosphorus. The P^V-TBPs, initially formed in route 2 and 3 by hydrolysis of our 3',5'-cyclic phosphate triesters are monoanions with the negative charge on an equatorial site. In this case, the P^V-TBPs initially formed in route 2 and 3 can "choose" between two different routes (i) direct cleavage of the P–$O_{3'}$ or P–$O_{5'}$ leading to acyclic phosphate diesters (3'(**13**) or 5'(**9**)) with inversion of configuration at phosphorus; (ii) pseudorotation leading to P^V-TBPs with an axially located OR group and possible deprotonation of the equatorially located OH group, leading to cyclic phosphate diester **11** with retention of configuration or to acyclic phosphate diesters (3'(**10**) or 5'(**12**)) also with retention of configuration at phosphorus.

In Table III it is shown that during hydrolysis of **4a–c** significant amounts of compound **8** are observed. *Therefore we conclude that, according to Scheme III, the existence of P^V-TBPs with a diequatorially located dioxaphosphorinane ring is most likely.* This result is in agreement with the recently reported crystal structures of P^V-TBPs, containing diequatorially located six- and eight-membered rings.[29a–c] Interestingly these results indicate that the P^V-TBP containing a diequatorially located dioxaphosphorinane ring has comparable stability with respect to all isomeric P^V-TBPs in which the ring has an equatorial–axial orientation. This observation contrasts with calculational results of Holmes et al.[29c] on a model which is perhaps oversimplified. In addition it may be noted that earlier work by us already suggested a diequatorial orientation of a dioxaphosphorinane ring in a P^V-TBP.[30] *Furthermore, it is clear that during hydrolysis of 3',5'-cyclic phosphate triesters Berry pseudorotation takes place, whereas hydrolysis of 3',5'-cyclic phosphate diesters[4] proceeds without Berry pseudorotation, leading to complete inversion of configuration at phosphorus.*

Acknowledgment. The authors wish to thank Prof. Dr. E. W. Meijer and Dr. M. H. P. van Genderen for interesting and helpful discussions and Mr. H. Eding for his assistance in the layout.

Appendix

Second-order kinetics:

$$\frac{d[P_c]}{dt} = k_c[P][OH^-] \quad (1)$$

$$\frac{d[P_a]}{dt} = k_a[P][OH^-] \quad (2)$$

$[P]_0$ = initial concentration of the cyclic phosphate triester; [P] = concentration of the cyclic phosphate triester; $[P_a]$ = concentration of the acyclic phosphate diesters (3' and 5'); $[P_c]$ = concentration of the cyclic phosphate diester; $[OH^-]_0$ = initial concentration of OH^-; $[OH^-]$ = concentration of OH^-; k_c = overall reaction rate constant for the formation of cyclic phosphate diester; k_a = overall rate constant for the formation of acyclic phosphate diesters (3'- and 5'-phosphate diester); t = reaction time. During the formation of the cyclic phosphate diester from the cyclic phosphate triester 2 equiv of OH^- are consumed, while 1 equiv of OH^- is consumed during the formation of the acyclic phosphate diesters (3' and 5').

$$[P] = [P]_0 - [P_c] - [P_a] \quad (3)$$

$$[OH^-] = [OH^-]_0 - 2[P_c] - [P_a] \quad (4)$$

From eqs 1 and 2:

$$\frac{[P_a]}{[P_c]} = \frac{k_a}{k_c} = \bar{k} \quad (5)$$

Substitution of eqs 3 and 4 in eq 1, combined with eq 5 yields the following:

$$\frac{d[P_c]}{dt} = ([P]_0 - (1 + \bar{k})[P_c])([OH^-]_0 - (2 + \bar{k})[P_c])k_c \quad (6)$$

or

$$\int_0^{[P_c]} \frac{d[P_c]}{([P]_0 - (1 + \bar{k})[P_c])([OH^-]_0 - (2 + \bar{k})[P_c])} = k_c \int_0^t dt \quad (7)$$

Integration yields the following:

$$\ln \frac{([P]_0 - (1 + \bar{k})[P_c])}{([OH^-]_0 - (2 + \bar{k})[P_c])} = ((2 + \bar{k})[P]_0 - (1 + \bar{k})[OH^-]_0)k_c t + \ln \frac{[P]_0}{[OH^-]_0} \quad (8)$$

Registry No. *cis*-**1a**, 66512-18-3; *trans*-**1a**, 66512-19-4; *cis*-**1b**, 143816-26-6; *trans*-**1b**, 143791-09-7; **2a**, 87970-09-0; **2b**, 143791-10-0; **2c**, 143791-11-1; **3a**, 108146-36-7; **3b**, 143791-12-2; **3c**, 143791-13-3; **4a**, 143791-14-4; **4b**, 143791-15-5; **4c**, 143791-16-6; C_6H_5OH, 108-95-2; p-$NO_2C_6H_4OH$, 100-02-7; $(Me_2N)_3P$, 1608-26-0; thymidine, 50-89-5; 2'-*O*-methyladenosine, 2140-79-6.

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