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Nucleoside 3′,5′-Cyclic *H*-Phosphonates, New Useful Precursors for the Synthesis of Nucleoside 3′,5′-Cyclic Phosphates and Their Analogues

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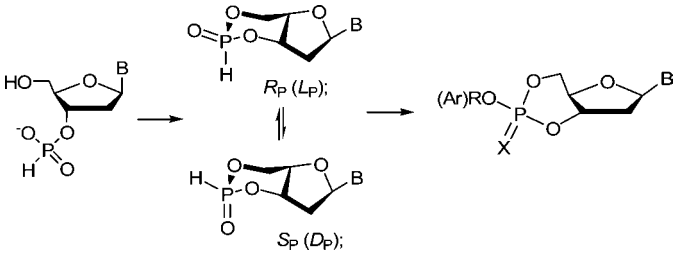
ABSTRACT



Nucleoside *H*-phosphonates activated with a condensing agent spontaneously formed nucleoside 3′,5′-cyclic *H*-phosphonates. The cyclization was stereoselective and produced one of the P-diastereomers in preponderance (*de* ca. 80%). Nucleoside 3′,5′-cyclic *H*-phosphonates were stereochemically unstable and underwent epimerization affording the thermodynamically more stable diastereomer as a major product (*de* ca. 80%). They were susceptible to hydrolysis, transesterification, and oxidation and by changing oxidation protocols nucleoside 3′,5′-cyclic phosphate analogues, e.g. phosphodiesters, phosphorothioate diesters, and phosphotriesters, were obtained.

Nucleoside 3′,5′-cyclic phosphates (e.g., cAMP, cGMP) play an important role in living organisms acting as second messengers in numerous cellular events such as activation of protein kinases, cyclic nucleotide binding proteins or cAMP receptor proteins, regulation of ion channels, etc.[1,2] Recently, some disorder in cAMP pathways and an aberrant activation of cAMP-controlled genes have been implicated in tumor progression.[3,4] This diverse biological activity of these rather simple molecules has stirred interest among chemists for the preparation of various analogues of nucleoside 3′,5′-cyclic phosphates as molecules for potential biomedical intervention. Typical examples here are nucleoside 3′,5′-cyclic phosphorothioates,[5,6] the corresponding phosphoramidates derivatives[7−9] (especially those with defined stereochemistry at the phosphorus center), and stereospecifically isotope labeled cAMP derivatives.[8,10] Many of these analogues were found to be very useful tools for elucidation of the mechanistic aspects of various enzymatic reactions.[11]

From a synthetic point of view, different chemistries were applied for the preparation of nucleoside cyclic phosphotriesters. The most common approach involves condensation of the corresponding cyclic phosphodiesters with an alcohol aided by a condensing agent, or an

(1) Cohen, S. S. *Biochem. Pharmacol.* **1975**, *24*, 1929–1932.
(2) Schultz, C.; Vajanaphanich, M.; Harootunina, A. T.; Sammak, P. J.; Barret, K. E.; Tsien, R. Y. *J. Biol. Chem.* **1993**, *268*, 6316–6322.
(3) Abramovitch, R.; Tavor, E.; Jacob-Hirsch, J.; Zeira, E.; Amariglio, N.; Pappo, O.; Rechavi, G.; Galun, E.; Hinigman, A. *Cancer Res.* **2004**, *64*, 1338–1364.
(4) Dumaz, N.; Hayward, R.; Martin, J.; Ogilvie, L.; Hedley, D.; Curtin, J. A.; Bastian, B. C.; Springer, C.; Marais, R. *Cancer Res.* **2006**, *66*, 9483–9491.
(5) Eckstein, F.; Kutzke, U. *Tetrahedron Lett.* **1986**, *27*, 1657–1660.
(6) Zielinski, W. S.; Stec, W. J. *J. Am. Chem. Soc.* **1977**, *99*, 8365–8366.
(7) Baraniak, J.; Kinas, R.; Lesiak, K.; Stec, W. J. *J. Chem. Soc., Chem. Commun.* **1979**, 940–941.
(8) Baraniak, J.; Lesiak, K.; Sochacki, M.; Stec, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 4533–4534.
(9) Baraniak, J.; Stec, W. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1645–1656.
(10) Cullis, P. M. *Tetrahedron Lett.* **1983**, *24*, 5677–5680.
(11) Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 160–166.

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alkylation reaction with a suitable alkylating agent.[12–14] Other methods make use of bifunctional phosphorylating reagents which, after phosphorylation of one hydroxyl group of a suitably protected nucleoside, form a 3′,5′ six-membered ring in a subsequent intramolecular cyclization reaction.[15–17] All these methods are multistep and afford final nucleoside 3′,5′-cyclic phosphotriesters in moderate or poor yield.[16,17] This can be a serious drawback when working with starting materials that are not readily accessible (e.g., antiviral nucleoside or nucleotide analogues) or with compounds for which diverse modifications at the phosphorus center are required.

The chemistry of phosphorus compounds with a phosphorus atom embedded in a six-membered *H*-phosphonate ring fused to a ribose moiety has been rather poorly explored in nucleotide chemistry, and nucleoside 3′,5′-cyclic *H*-phosphonates have not been prepared yet. There are few examples of the synthesis of *H*-phosphonate phosphinane derivatives of simple diols[18–22] described in literature, and these compounds have unique properties that distinguish them from tricoordinated phosphite and tetracoordinated phosphate analogues.

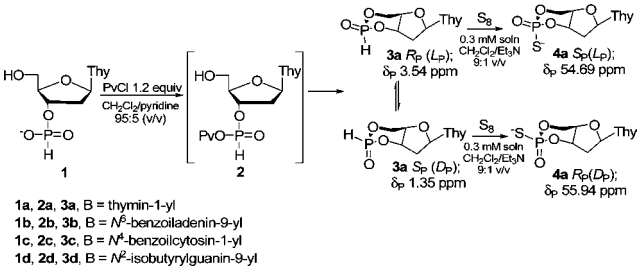
In this paper we would like to describe our studies on the synthesis and properties of nucleoside 3′,5′-cyclic *H*-phosphonates as new synthetic intermediates for the preparation of various analogues of nucleoside 3′,5′-cyclic phosphates.

Attempted synthesis of nucleoside 3′,5′-cyclic *H*-phosphonates using a bifunctional phosphonylating reagent, diphenyl *H*-phosphonate, and unprotected thymidine as a model deoxyribonucleoside gave complex reaction mixtures, in contrast to the reaction with 2′,3′-unprotected ribonucleosides which smoothly afforded the corresponding nucleosides 2′,3′-cyclic *H*-phosphonates.[23] In this situation we explored another approach that was based on a condensing agent activation of nucleoside *H*-phosphonate monoesters bearing an unprotected hydroxyl function, followed by intramolecular cyclization (Scheme 1). Since comparable results were obtained for isomeric nucleoside 3′- or 5′-*H*-phosphonate monoesters and for different condensing agents, we confined our discussion below to the reactions of nucleoside 3′-*H*-phophonates and pivaloyl chloride used as an activator.

As a model reaction for the formation of nucleoside 3′,5′-cyclic *H*-phosphonate diesters we chose activation of thymidine 3′-*H*-phosphonate monoester **1a** with pivaloyl chloride. To this end, we added pivaloyl chloride (1.2 equiv) to a 0.1 mM solution of *H*-phosphonate **1a** in dichloromethane/pyridine (95:5, v/v). The first ^{31}P NMR spectrum recorded (after ca. 3 min) revealed that the starting material **1a** had completely disappeared and two main resonances (ratio 9:1, ca. 95% of the phosphorus containing compounds), centered at δ_P = 1.4 and 3.4 ppm (2 m, $^1J_{HP}$ = 741.8 Hz, $^1J_{HP}$ = 699.7 Hz, respectively), appeared.[24] The chemical shifts and multiplicities of these signals were quite different from those expected for the putative intermediate mixed anhydride **2a** (δ_P = 0.8 ppm and 0.9 ppm, 2 dd, $^1J_{HP}$ = 745.2 and 746.2 Hz, $^3J_{HP}$ = 8.1 Hz)[25] and indicated formation of the desired thymidine cyclic *H*-phosphonate **3a**. In agreement with this, *in situ* oxidation of a diastereomeric mixture of the putative cyclic *H*-phosphonate **3a** with iodine (*vide infra*) produced thymidine 3′,5′-cyclic phosphate (cTMP, **6a** in Scheme 2) that was identical to an original sample of this compound prepared in another way.

A similar course of the reaction was also observed for other nucleoside 3′-*H*-phosphonates investigated (**1b**–**d**), and thus, the described above synthetic protocol was used for generation of nucleoside 3′,5′-cyclic *H*-phosphonates of type **3** discussed in this paper.

Scheme 1. Formation of Nucleoside 3′,5′-Cyclic *H*-Phosphonates and Their Sulfurization



We also assigned the absolute configuration at the phosphorus center in **3a** using stereochemical correlation analysis. To this end, to a diastereomeric mixture of thymidine cyclic *H*-phosphonate **3a** (ratio 9:1) preformed as described above, elemental sulfur was added (3 equiv). The reaction was fast, and after ca. 5 min the ^{31}P NMR spectrum indicated the presence of two compounds resonating at 55.94 and 54.69 ppm (ratio ca. 8:1). These were identified as the corresponding thymidine 3′,5-cyclic phosphorothioate diesters by comparison with original samples.[5] Intensities of the signals suggested that the

(12) Engels, J. W.; Schlaeger, E.-J. *J. Med. Chem.* **1977**, *20*, 907–911.
(13) Kruppa, J.; Keely, S.; Schwede, F.; Schultz, C.; Barret, K. E.; Jastorff, B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 945–948.
(14) Engels, J. W. *Bioorg. Chem.* **1979**, *8*, 9–16.
(15) Chao, Q.; Nair, V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1199–1202.
(16) Broeders, N. L. H. L.; Koole, L. H.; Buck, H. M. *J. Am. Chem. Soc.* **1990**, *112*, 7475–7482.
(17) Nelson, K. A.; Bentrude, W. G.; Stezer, W. N.; Hutchinson, J. P. *J. Am. Chem. Soc.* **1987**, *109*, 4058–4064.
(18) Oswald, A. A. *Can. J. Chem.* **1959**, *37*, 1498–1504.
(19) Cullis, P. M.; Lee, M. *J. Chem. Soc., Chem. Commun.* **1992**, 1207–1208.
(20) Kumar, N. S.; Kumaraswamy, S.; Said, M. A.; Swamy, K. C. K. *Org. Process Res. Dev.* **2003**, *7*, 925–928.
(21) Mikolajczyk, M. *Chem. Commun.* **1969**, 1221.
(22) Mikolajczyk, M.; Luczak, J. *Tetrahedron* **1972**, *28*, 5411–5422.
(23) Wenska, M.; Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **2001**, *42*, 8055–8058.

(24) Under these reaction conditions we also observed formation of variable amounts of linear condensation products (ca. 5%, signals at 6–7 ppm). However, when we decreased the concentration of **1a** to 0.01 mM, the formation of linear products was practically completely eliminated.
(25) Cieslak, J.; Sobkowski, M.; Kraszewski, A.; Stawinski, J. *Tetrahedron Lett.* **1996**, *37*, 4561–4564.

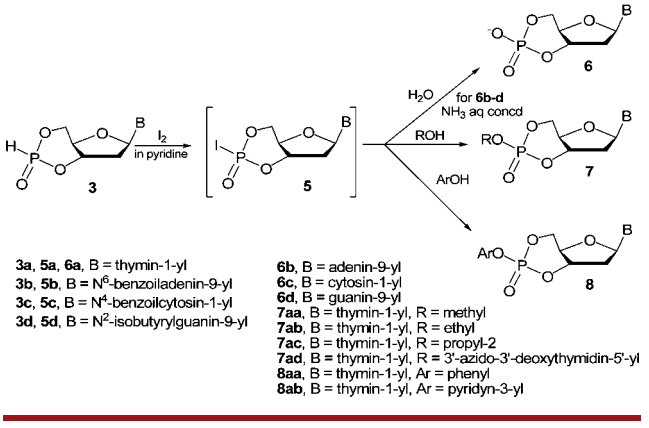
downfield signal (δ_P = 55.99 ppm, R_P-thymidin-3′,5′-diyl phosphorothioate, **4a**[5]) was formed from the major diastereomer **3a** resonating at δ_P = 1.35 ppm, and the upfield signal (δ_P = 54.69 ppm, S_P-thymidin-3′,5′-diyl phosphorothioate, **4a**[5]) arose from the minor diastereomer **3a** (δ_P = 1.35 ppm). In an analogous experiment in which the ratio of diastereomers of cyclic *H*-phosphonate **3a** was opposite (ca. 1:7, *vide infra*), a major product of the reaction was the upfield isomer S_P-thymidin-3′,5′-diyl phosphorothioate **4a** (δ_P = 54.69 ppm), and the ratio of the S_P/R_P diastereomers **4a** was inverted compared to the previous experiment (8:1 vs 6.7:1 in this experiment). The produced thymidine 3′,5′-cyclic phosphorothioates **4** were purified by silica gel column chromatography, and their structures were unambiguously determined by spectroscopic methods (^{1}H, ^{13}C, and ^{31}P NMR, HRMS).

Since the stereochemical correlation analysis above involved one stereospecific reaction (sulfurization) that proceeds with retention of configuration, it implies that the high-field resonating diastereomers of cyclic *H*-phosphonate **3a** (δ_P = 1.35 ppm) has an S_P configuration (or D_P using D_P/L_P notation[26,27]), and the low-field resonating diastereomer of **3a** (δ_P = 3.54 ppm), an R_P configuration (or L_P using D_P/L_P notation[26,27]) at the phosphorus center (Scheme 1).[28] Since a similar pattern of signals was observed also for the reactions involving other nucleosides **1b**–**d**, we assumed that the stereochemical assignments shown in Scheme 1 can be extended to other nucleoside 3′,5′-cyclic *H*-phosphonates investigated in this paper.

H-Phosphonate diesters derived from nucleosides, sugars, lipids, etc. are usually stable enough to be purified by silica gel chromatography and then stereospecifically converted into various P(V)-derivatives.[29−32] By way of contrast, attempted isolation of nucleoside 3′,5′-cyclic *H*-phosphonates of type **3** failed, due to instability of these compounds during aqueous workup. This susceptibility to hydrolysis and transesterification with alcohols (data not shown) may result from some strain or distortion of a 1,3,2-dioxaphosphinane ring imparted by the fused deoxyribose moiety. This increased reactivity of nucleoside 3′,5′-cyclic *H*-phosphonates **3** compared to acyclic *H*-phosphonate diesters is also manifested in the configurational instability of these compounds. For example, we observed that when a reaction mixture containing a mixture of R_P /S_P-diastereomers **3a** was left at room temperature for 5 h, a gradual change in the ratio of the diastereomers occurred from an initial value of ca. 1:9 to the final equilibrium ratio of ca. 9:1. This meant that the kinetic product of the reaction in Scheme 1, S_P-thymidine cyclic *H*-phosphonate **3a**, upon standing, was converted into the thermodynamic product, R_P-**3a**.[33] Mechanistic aspects of this phenomenon are under investigation.

To demonstrate the synthetic utility of the nucleoside 3′,5′-cylic *H*-phosphonates **3** and to gain additional insight into their reactivity, we subjected these compounds to various oxidation protocols. Since, during the oxidation of *H*-phosphonate diesters with iodine, reactive iodophosphates are formed, that are highly susceptible to nucleophilic substitution at the phosphorus center,[34−38] we attempted to develop this as a new method for the preparation of nucleoside 3′,5′-cyclic phosphodiesters of type **6** and also for the synthesis of less explored, until now, alkyl or aryl nucleoside 3′,5′-cyclic phosphotriesters of type **7** and **8**, respectively (Scheme 2).

Scheme 2. Synthesis of Nucleoside 3′,5′-Cyclic Phosphates and Phosphotriesters



The efficacy of such an approach was checked by generating thymidine 3′,5′-cyclic *H*-phosphonate **3a** (*vide supra*) and adding this to the reaction mixture of I_2 (1.2 equiv, dissolved in anhydrous pyridine), followed by an excess of water (after 30 s) (Scheme 2). We chose a separate addition of iodine and water, instead of a standard oxidation protocol (treatment of *H*-phosphonate diesters with an aqueous pyridine solution of iodine[36]), to minimize the risk of hydrolysis of **3a** under the reaction

(26) Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Nucleosides, Nucleotides Nucleic Acids* **2006**, *25*, 1363–1.

(27) Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Nucleosides, Nucleotides Nucleic Acids* **2006**, *25*, 1377–1389.

(28) Change from the R_P to S_P or S_P to R_P configuration in Scheme 1 for the stereoretentive sulfurization step is due to the priority order of substituents in the ICP convention.[39] Since a sense of chirality at the phosphorus center is preserved in this reaction, the D_P/L_P stereochemical descriptors remain invariant.[26,27]

(29) Stawinski, J.; Kraszewski, A. *Acc. Chem. Res.* **2002**, *35*, 952–960.

(30) Bollmark, M.; Stawinski, J. *Tetrahedron Lett.* **1996**, *37*, 5739–5742.

(31) Zain, R.; Stawinski, J. *J. Org. Chem.* **1996**, *61*, 6617–6622.

(32) Stawinski, J.; Thelin, M. *J. Org. Chem.* **1991**, *56*, 5169–5175.

(33) In 1969 Mikołajczyk[21] postulated epimerization at the phosphorus center of 4-methyl-2-oxo-1,3,2-dioxaphosphinane to explain the stereoselectivity observed during hydrolysis of the corresponding phosphorochloridite. More recently, Cullis and Lee[19] reported that during distillation at elevated temperature equilibration of the R_P and S_P diastereomers of 5-*tert*-butyl-2-oxo-1,3,2-dioxaphosphinane occurred. No mechanistic aspect of this phenomenon was discussed.

(34) von Nylen, P. *Z. Anorg. Allg. Chem.* **1938**, *235*, 161–183.

(35) Saunders, B. C.; Stark, B. P. *Tetrahedron* **1958**, *4*, 169–177.

(36) Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1269–1273.

(37) Romanowska, J.; Szymańska-Michalak, A.; Boryski, J.; Stawinski, J.; Kraszewski, A.; Loddo, R.; Sanna, G.; Collu, G.; Secci, B.; La Colla, P. *Bioorg. Med. Chem.* **2009**, *17*, 3489–3498.

(38) Romanowska, J.; Sobkowski, M.; Szymańska-Michalak, A.; Kołodziej, K.; Dąbrowska, A.; Lipniacki, A.; Piasek, A.; Pietrusiewicz, Z. M.; Figlerowicz, M.; Guranowski, A.; Boryski, J.; Stawinski, J.; Kraszewski, A. *J. Med. Chem.* **2011**, *54*, 6482–6491.

conditions. Both steps of the reaction, oxidation with iodine and hydrolysis, were rapid, and after evaporation of the solvents and aqueous workup, the desired thymidine 3′,5′-cyclic phosphate **6a** was isolated in high yield (82%) by silica gel column chromatography.

Analogous reactions for other cyclic *H*-phosphonates **3** containing standard 2′-deoxyribonucleosides (2′-deoxyadenosine **3b**, 2′-deoxycytidine **3c**, and 2′-deoxyguanosine **3d** derivatives, generated from the corresponding nucleoside 3′-*H*-phosphonates **1b**−**d**) worked equally well affording after deprotection and silica gel chromatography the corresponding nucleoside cyclic phosphates **6b**−**d** in consistently high yield (74%−82%, calculated on the starting nucleoside *H*-phosphonates **1**).

The synthetic potential of the putative cyclic iodophosphate intermediates **5** (Scheme 2) was also exploited in the preparation of alkyl and aryl nucleoside 3′,5′-cyclic phosphotriesters of type **7** or **8**, respectively. To this end, cyclic iodophosphate intermediates **5** (generated in situ from **3** as described above) were treated with a moderate excess (3.0 molar equiv) of the respective alcohol (methanol, ethanol, 2-isopropanol) dissolved in pyridine. The reactions were rapid (< 3 min) and stereoselective, affording practically quantitatively two diastereomers of triesters **7** in a ratio of ca. 3:1 (downfield/upfield resonating diastereomer in the ^{31}P NMR spectra).

A similar course of the reaction was also observed for oxidative condensations with phenols. For example, unsubstituted phenol or 3-hydroxypyridine reacted smoothly under the analogous conditions with *in situ* generated **5a** affording the corresponding cyclic triesters **8aa** and **8ab** that, after standard workup, were purified by reversed-phase column chromatography and isolated in 66% and 54% yield, respectively. The lower yields observed for the aryl phosphotriesters were, most likely, due to the incomplete stability of these compounds during chromatography.

We also applied the above oxidative coupling procedure for the reaction involving thymidine cyclic *H*-phosphonate **3a** and 3′-azido-3′-deoxythymidine (AZT) to produce the AZT thymidine 3′,5′-cyclic phosphotriester **7ad**, as a potential vehicle for delivery of an anti-HIV agent (AZT) into the infected cell. We were delighted to find that cyclic triester **7ad** indeed revealed high anti-HIV potency (EC_{50} 0.01 μM, MT-4) along with very low cytotoxicity ($CC_{50} \gg$ 350 μM; SI $\gg$ 35 000), which makes it a very promising anti-HIV pro-drug candidate. This type of compound most likely acts as an anti-HIV pro-nucleotide and potentially can be useful in AIDS therapy. Further studies are in progress in our laboratory.

To summarize, condensing agent-activated nucleoside 3′- or 5′-*H*-phosphonate monoesters undergo a stereoselective, intramolecular cyclization to produce S_P-nucleoside 3′,5′-cyclic *H*-phosphonates **3** as the major products (*de* 80%). It was found that this type of cyclic *H*-phosphonate was significantly more susceptible to hydrolysis and transesterification than its acyclic counterpart and, upon standing, underwent epimerization at the phosphorus center to afford the thermodynamically more stable R_P-diastereomers of **3** (de 80%). Absolute P-configurations of thymidine 3′,5′-cyclic *H*-phosphonate **3a** were established using stereochemical correlation analysis. To demonstrate the synthetic utility of this new type of intermediates, we converted cyclic *H*-phosphonates of type **3** under mild conditions into the corresponding cyclic phosphorothioates, phosphodiesters, and diverse cyclic phosphotriester derivatives. The procedures developed are simple, efficient, high yielding, and thus provide new entries to biologically important nucleoside 3′,5′-cyclic phosphate analogues of type **6**, **7**, **8**, and others.

We also found that a nucleoside 1,3,2-dioxaphosphinane scaffold may exert a beneficial effect on the pharmacological activity of a potential drug as exemplified by the high anti-HIV potency and very low cytotoxicity of the 3′-azido-3′-deoxythymidine (AZT) derivative **7ad**.

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Supporting Information Available. Synthetic protocols, ^{1}H, ^{13}C, ^{31}P NMR spectra, HRMS data, and HPLC (purity criteria). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(39) Prelog, V.; Helmchen, G. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 567–583.