ORGANIC LETTERS

2003
Vol. 5, No. 20
3571−3573

Aryl *H*-Phosphonates. 14. Synthesis of New Nucleotide Analogues with Phosphonate–Phosphate Internucleosidic Linkage

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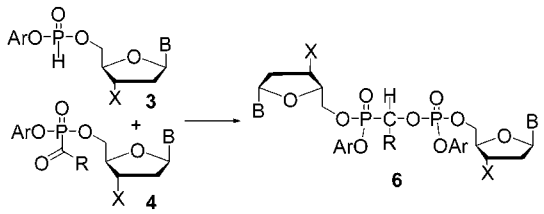
Received June 24, 2003

ABSTRACT



Aryl nucleoside *H*-phosphonates 3 and aryl nucleoside *P*-acylphosphonates 4, generated in situ from the appropriate *H*-phosphonate 1 and acylphosphonate monoesters 2, respectively, reacted rapidly in the presence of tertiary amines to produce in high yields the extended, pyrophosphate analogues, diaryl dinucleoside phosphonate–phosphate derivatives 6. These, depending on a substituent on the α-carbon of the phosphonate moiety, either underwent transformation into the dinucleoside phosphonate–phosphate 7 or afforded nucleoside *H*-phosphonates 8 and aryl nucleoside phosphate 9.

Searching for new nucleoside kinase bypass lipophilic pronucleotides,[1] we considered diaryl dinucleoside phosphonate−phosphate (**6**, Scheme 1) as unique vehicles for delivering biologically active nucleotides into the cell. A distinctive feature of this type of analogues is that their lipophilicity and susceptibility to hydrolysis are expected to be tuneable through a proper choice of substituent R on the α-carbon of the phosphonate center and the aryl ester moieties. In addition, the electronic structure of R can be instrumental in controlling possible degradation pathways of phosphonate−phosphate **6**.

Although simple tetraalkyl phosphonate−phosphates are known compounds[2] and have already found several therapeutic applications,[3] the corresponding nucleotide derivatives of type **6**, which can be viewed as extended pyrophosphate analogues, have not been investigated yet. This is most likely due to synthetic problems connected with the preparation of *gem*-diphosphonates of type **5** and harsh reaction conditions that are required for their rearrangement into the respective phosphonate−phosphates **6**.[4]

To overcome these problems we designed a new synthesis of aryl esters of dinucleoside phosphonate−phosphates **6** by

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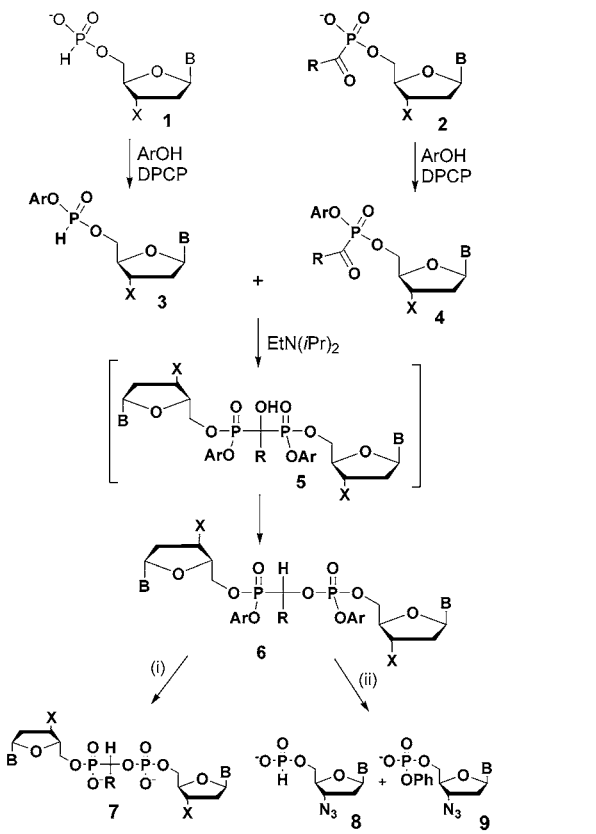
10.1021/ol035166u CCC: $25.00 © 2003 American Chemical Society
Published on Web 09/04/2003

Scheme 1. Synthesis and Degradation of Dinucleoside Phosphonate−Phosphates[a]



B = thymin-1'-yl
DPCP = diphenyl chlorophosphate
Ph = phenyl
4-ClPh = 4-chlorophenyl
Bz = benzoyl
1a, X = O-Bz
1b, X = N_3
2a, R = *t*-butyl; X = OBz
2b, R = *t*-butyl; X = N_3
2c, R = Ph; X = N_3
2d, R = 4-methoxyphenyl; X = N_3

3a, Ar = 4-ClPh; X = OBz
3b, Ar = 4-ClPh; X = N_3
3c, Ar = Ph; X = N_3
4a - 6a, Ar = 4-ClPh; R = *t*-butyl, X = OBz
4b - 6b, Ar = 4-ClPh; R = *t*-butyl, X = N_3
4c - 6c, Ar = 4-ClPh; R = Ph, X = N_3
4d - 6d, Ar = Ph; R = 4-methoxyphenyl; X = N_3
7a, R = *t*-butyl; X = OH
7b, R = *t*-butyl; X = N_3
7c, R = Ph, X = N_3

[a] Reagents and conditions: (i) conc aq NH_3, 50 °C; (ii) CH_3CN/Et_3N/H_2O 2:1:1 (v/v) or phosphate buffer, pH 7.4, 37 °C.

making use of nucleoside aryl *H*-phosphonates **3** and the corresponding *P*-acylated derivatives **4**, as shown in Scheme 1. The inspiration for this came from our previous observation that phosphonate−phosphate derivatives of type **6** were formed as side-products during aminolysis of reaction mixtures containing nucleoside aryl *H*-phosphonates and pivaloyl chloride.[5]

Starting materials for this new synthetic protocol are readily accessible. Aryl *H*-phosphonates **3** can be prepared from the corresponding nucleoside *H*-phosphonates **1** and the appropriate phenol as described previously.[6] For the preparation of the second component, acylphosphonate **4**, we elaborated an efficient method involving condensation of nucleoside *P*-acylphosphonate of type **2**[7] with 4-chlorophenol (1.2 molar equiv) in the presence of diphenyl chlorophosphate (DPCP, 1.5 molar equiv). This reaction proceeded smoothly in methylene chloride−pyridine (9:1, v/v), and after 2 h the desired 4-chlorophenyl (or phenyl) nucleoside *P*-acylphosphonate **4**[8] (two diastereoisomers, for **4a**, δ_P = −6.50 and −6.95 ppm, 2t, $^3J_{HP}$ = 6.5 Hz) was usually formed as the sole nucleotidic product (^{31}P NMR spectroscopy).

To produce phosphonate−phosphate **6a** (Scheme 1), equimolar amounts of aryl *H*-phosphonate **3a** and aryl *P*-acylphosphonate **4a** were allowed to react in methylene chloride−pyridine (9:1, v/v) in the presence of *N*,*N*-diisopropylethylamine (DIEA, 10 molar equiv). The reaction was rapid (<5 min) and afforded a new compound, which gave rise to two multiplets centered at δ_P = 16.39 and −6.22 ppm in the ^{31}P NMR spectrum. Integrals of these two groups of signals (ca 1:1) together with their chemical shifts indicated the presence of two kinds of phosphorus centers in the molecule (a phosphate triester center, δ_P = −6.22 ppm ,and an aromatic C-phosphonate center, δ_P = 16.39 ppm), and suggested a phosphonate−phosphate structure of type **6a**.[9] Compound **6a** was stable during silica gel column chromatography [gradient of propanol-2 (0−10%) in methylene chloride] and its structure was unambiguously confirmed by ^{1}H, ^{31}P, and HRMS spectroscopy (vide infra).

By monitoring the progress of this reaction by ^{31}P NMR spectroscopy we could not detect a putative intermediate involved in this transformation, namely *gem*-diphosphonate **5**. Since tetraalkyl *gem*-diphosphonates are known to be rather stable species that require harsh reaction conditions for the rearrangement,[2a] this may indicate that the presence of aromatic moieties in **5** significantly facilitates the conversion into phosphonate−phosphate product **6**.

Since a separate handling of aryl *H*-phosphonates **3** and aryl *P*-acylphosphonates **4** may cause, due to the high reactivity of these compounds, some experimental inconvenience, we attempted to develop a four-component one-pot reaction for the synthesis of phosphonate−phosphate **6**. ^{31}P NMR spectroscopy revealed that by reacting equimolar amounts of *H*-phosphonate monoester **1a** and *P*-acylphosphonate **2a** with 4-chlorophenol in the presence of a condensing agent (DPCP), the expected aryl esters **3a** and **4a** were formed cleanly and in equal amounts. Addition of DIEA to this reaction mixture triggered rapid (<5 min) formation of the desired phosphonate−phosphate **6a**, which was isolated as described above. This modification significantly simplified the synthetic protocol and, due to sup-

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(7) Compounds **2** were obtained by modification of the method of van Boom et al. (de Vroom, E.; Spierenburg, M. L.; Dreef, C. E.; Van der Marel, G. A.; Van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 65−66) that involved acylation of a nucleoside bis(trimethylsilyl)phosphite with respective acyl chloride.

(8) Compounds **4a**−**d** turned out to be rather unstable and underwent rapid hydrolysis to acylphosphonate monoester of type **2** upon the addition of water. Their structure was assigned on the basis of the spectral data (chemical shift and splitting pattern of signals in the ^{31}P NMR spectra) and the observed chemical reactivity.

(9) Due to the presence of three chiral centers in the phosphonate−phosphate backbone, compounds **6** are formed as a mixture of eight diastereomers and usually gave a complex pattern of signals in the ^{31}P NMR spectra.

pressing possible interference from adventitious water, made the method highly reproducible.

The efficacy of this approach was further assessed in the synthesis of phosphonate−phosphate **6b** (Scheme 1) bearing a nucleoside analogue with established antiviral properties, 3′-azido-3′-deoxythymidine (AZT). The replacement of thymidine by AZT did not affect the efficiency of the method and di-AZT phosphonate−phosphates **6b** was obtained in a yield comparable with that of **6a**.

To expand the array of structural variations in phosphonate−phosphate **6**, we prepared phenyl and 4-methoxyphenyl derivatives of **6** (**6c**, R = phenyl, and **6d**, R = 4-methoxyphenyl) by condensing the respective aryl *H*-phosphonate **3** (**3b** or **3c**) with different aryl *P*-acylphosphonates **4** (**4c** and **4d**, respectively). The syntheses were uneventful and produced the expected phosphonate−phosphates **6c** and **6d** as the sole nucleotidic products (^{31}P NMR). In the instance of the 4-methoxyphenyl derivative of **6** bearing the 4-chlorophenyl phosphoester group, we observed a partial decomposition during silica gel chromatography. Fortunately, the replacement of the 4-chlorophenyl by unsubstituted phenyl alleviated this problem and permitted the preparation of diAZT phosphonate−phosphate **6d** in high yield and of high purity. Compounds **6** after precipitation from methylene chloride with an excess of *n*-hexane, filtration, and drying were obtained as amorphous white powders with purity higher than 98%.

To assess the stability of a phosphonate−phosphate backbone, compounds **6** were subjected to treatment with 33% aq ammonia at 50 °C for 48 h. Phosphonate−phosphates **6** with R = *tert*-butyl or 4-chlorophenyl (**6a**, **6b**, and **6c**) under these conditions underwent only ester group hydrolysis and produced exclusively (^{31}P NMR analysis) unprotected phosphonate−phosphates **7a**, **7b**, and **7c**. On the other hand, AZT phosphonate−phosphate **6d** with R = 4-methoxyphenyl afforded a rather complex mixture of products. However, under milder conditions, e.g. in acetonitrile-water−triethylamine (2:1:1, v/v, rt), **6d** underwent rapid and clean degradation (>5 min, ^{31}P NMR) to afford an equimolar amount of AZT *H*-phosphonate **8**[10] and AZT phenyl phosphate diester **9** exclusively.[11]

The same degradation pathway of **6d** but with different kinetics ($t_{1/2}$ ca. 60 min) was also observed in 0.1 M phosphate buffer (pH 7.4, 37 °C). Since phosphonate−phosphates **6b** and **6c** were completely stable under the same conditions (overnight), it seems that the presence of a strong electron-donating R group in **6** favors the C−O bond scission of a phosphonate−phosphate backbone, most likely due to stabilization of a putative intermediate carbocation.[12] This feature can make dinucleoside phosphonate−phosphates of type **6d** potentially useful vehicles for delivery of pronucleotides into the cell, where they will undergo further chemical and enzymatic transformations into the corresponding, nucleoside triphosphates of antiviral activity.

In conclusion, we developed a new and efficient protocol for the synthesis of novel dinucleoside phosphonate−phosphate analogues. It consisted of a one-pot reaction of the nucleoside *H*-phosphonate **1** and nucleoside acylphosphonate **2** with phenols in the presence of diphenyl chlorophosphate, followed by treatment with *N*,*N*-diisopropylethylamine. The method is experimentally simple, making use of readily accessible starting materials, and permits an easy introduction of structural variations into the phosphonate−phosphates of type **6**. By a proper choice of ester groups and a substituent on the α-carbon of the phosphonate moiety, one can also control stability and the decomposition pathways of these compounds. Since di-AZT phosphonate−phosphate **6d** under mild conditions can generate AZT 5′-*H*-phosphonate **8** and AZT 5′-phenylphosphate **9**, this type of compounds can be considered as potential lipophilic prodrugs for the delivery of one or two kinds of nucleoside monophosphates into the cells. Evaluation of antiviral activity of di-AZT phosphonate−phosphates and related compounds will be reported in due course.[13]

Acknowledgment. Financial support from the State Committee for Scientific Research, Republic of Poland (project no PBZ-KBN-059/T09/19) is gratefully acknowledged.

Supporting Information Available: ^{1}H, ^{31}P NMR, and HRMS analytical data for compounds **6a**−**d** and **7a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The initial products of the degradation of **6d** are probably **9** and AZT phenyl α-hydroxy(4-methoxyphenyl)methylenephosphonate. The latter one is apparently in equilibrium with 4-anisaldehyde and AZT phenyl *H*-phosphonate diester, but due to irreversible hydrolysis of the phenyl H-phosphonate diester into AZT *H*-phosphonate **9**, the equilibrium is driven to the right and depletes the amount of α-hydroxyphosphonate in the reaction mixture. Since no intermediates were observed by ^{31}P NMR spectroscopy, probably the rate determining step in this process is the formation of the hydroxyphosphonate derivative. More detail studies on this are currently being carried out in our laboratory.

(11) The identity of compounds **8** and **9** was confirmed by comparison with authentic samples obtained in other ways. (a) Cieślak, J.; Jankowska, J.; Sobkowski, M.; Wenska, M.; Stawiński, J.; Kraszewski, A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 31−37. (b) Jankowska, J.; Sobkowski, M.; Stawiński, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355−3358.

(12) Meier, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32* (12), 1704−1706.

(13) **Typical Procedure for the Synthesis of Dinucleoside Phosphonate**−**Phosphates of Type 6.** Nucleoside *H*-phosphonate **1** (1 molar equiv), *P*-acylphosphonate **2** (1 molar equiv), and the appropriate phenol (2.5 molar equiv) were rendered anhydrous by repeated evaporation of added pyridine (3 × 20 mL/1 mmol) and then dissolved in methylene chloride/pyridine 9:1 (v/v) (10 mL/1 mmol). To this solutionwas added diphenyl chlorophosphate (2.5 molar equiv), and when the formation of aryl phosphonate esters **3** and **4** was complete (ca. 2 h), *N*,*N*-diisopropylethylamine (10 molar equiv) was added. After 5 min the reaction mixture was diluted with methylene chloride (10 times the initial volume) and washed with 5% aq $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated, and the oily residue was applied on a silica gel column preequilibrated with CH_2Cl_2. Products **6** were isolated by using a stepwise gradient of propanol-2 (0−10%) in methylene chloride. Fractions containing pure products were evaporated and the residue precipitated with an excess of petroleum ether. After filtration and drying under vacuum, compounds **6** were obtained as amorphous white powders with a purity higher than 98% (^{1}H NMR). **Synthesis of Dinucleoside Phosphonate**−**Phosphates of Type 7.** To products **6a**−**c** dissolved in pyridine (0.1 g/2 mL) was added aqueous concentrated ammonia (33%; 4 mL) and the reaction mixture was kept for 24 h at 50 °C. This time was usually sufficient to complete the deprotection of phosphonate−phosphates **6a**−**c** (^{31}P NMR). Ammonia and solvents were removed by evaporation, and the residue was dissolved in propanol-2/conc NH_3 95:5 (v/v) and applied to a silica gel column equilibrated with the same solvent. Compounds **7a**−**c** were isolated by using a linear gradient of water [0−10% (v/v)] in propanol-2/conc NH_3 95:5 (v/v). Fractions containing pure compounds were collected and evaporated with an added excess of propanol-2 to afford products **7a**−**c** (ammonium salts) as amorphous white powder.