Pergamon

Tetrahedron Letters 42 (2001) 2217–2220

TETRAHEDRON LETTERS

2-Pyridylphosphonates: a new type of modification for nucleotide analogues

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Received 28 November 2000; revised 12 January 2001; accepted 17 January 2001

Abstract—Suitably protected dithymidine H-phosphonates afforded the corresponding dinucleoside 2-pyridylphosphonates upon treatment with *N*-methoxypyridinium tosylate in acetonitrile in the presence of 1,8-diazabicylo[5.4.0]undec-7-ene (DBU). The reaction was rapid (ca. 5 min), practically quantitative and proceeded stereospecifically, most likely with retention of configuration at the phosphorus centre.

Simple dialkyl phosphonate diesters bearing a 2-pyridyl moiety (e.g. **6**, Fig. 1), apart from being widely used as corrosion inhibitors, dispersing and emulsifying agents, antistatics, and lubricant additives in various technological processes,[1] are also known as potent insecticides,[2] fungicides[3] and herbicides.[4] Some of them also possess a pronounced cytokinin activity.[5] Recently, anti-proliferating and anti-platelet activating factor (anti-PAF) activities[6] of 2-pyridylphosphonates have been reported, and quinolylphosphonic acid derivatives were found to be potent antagonists of AMPA/kainate and thus of potential use as therapeutic agents in the acute treatment of stroke and head trauma.[7]

This diverse array of biological activity of 2-pyridylphosphonates prompted us to develop a synthetic method for incorporation this functionality into nucleotides, as a potentially useful modification in designing new antisense/antigene agents.[8]

There are only a few synthetic methods for the preparation of dialkyl esters of 2-pyridylphosphonates and practically none of them can be applied to the synthesis of natural products bearing this functionality. The most common approach used is that of Redmore[9] and consists of the reaction of *N*-methoxypyridinium salts with sodium dialkyl phosphites in the corresponding dialkyl H-phosphonates as solvents. Unfortunately, the reaction is confined to simple dialkyl H-phosphonates, is highly sensitive to experimental conditions and usually affords the desired products in variable yields (low to moderate), partly due to decomposition of the reactants during the extended reaction time (overnight). Other methods, e.g. nucleophilic substitution of halides in deactivated pyridine rings with sodium dialkyl phosphite[10] or with triethyl phosphite,[11] are even less efficient and require harsh reaction conditions. The most recent approach to the preparation of 2-pyridylphosphonates using *N*-trifluoromethanesulphonylpyridinum triflate and trialkyl phosphites suffers from a rather lengthy protocol and low overall yields.[12]

As part of our programme in developing new synthetic methods for biologically important phosphate derivatives based on H-phosphonate chemistry,[13] we have recently reported an efficient conversion of H-phosphonate diesters into the corresponding 4-pyridylphospho-

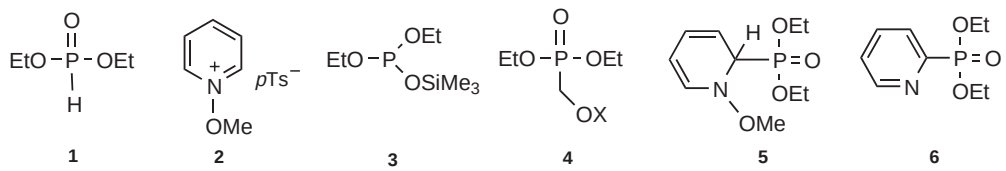

Figure 1.

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PII: S0040-4039(01)00115-0

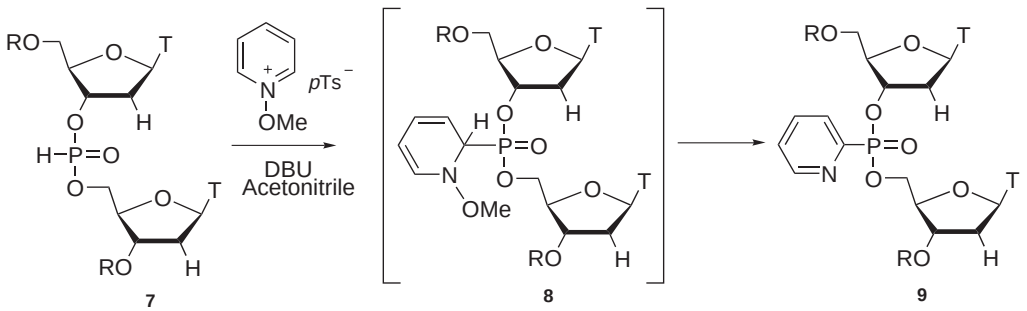
nate derivatives using a pyridine–trityl chloride–DBU reagent system.[14] Unfortunately, 2-pyridylphosphonate derivatives cannot be prepared by this method.

Since activation of the pyridine ring to nucleophilic attack by the formation of an *N*-alkoxypyridinium salt[15] seemed attractive both in terms of the availability of suitable substrates and the desired regioselectivity,[9] we decided to explore this avenue for the preparation of 2-pyridylphosphonate analogues of nucleotides.

To this end diethyl H-phosphonate **1** ($\delta_P = 7.6$ ppm) in methylene chloride was silylated with trimethylsilyl chloride (TMS-Cl, 3 equiv.) in the presence of triethylamine (TEA, 2 equiv.) to produce a nucleophilic phosphorus species, diethyl trimethylsilyl phosphite **3** ($\delta_P = 127.1$ ppm), and this was allowed to react with pyridinium salt **2** (3 equiv.). After ca. 3 h, ^{31}P NMR spectroscopy revealed in the reaction mixture the presence of several phosphorus-containing species (including 30% of the starting material), but none of them resonated in the range of chemical shifts expected for 2-pyridylphosphonate **6** (the expected δ_P ca. 10 ppm). The replacement of TMS-Cl by bis(trimethylsilyl)acetamide (BSA, 3 equiv.) as a silylating agent caused significant changes in product distribution, and after 3 h the formation of a major product (>90%) resonating at $\delta_P = 22.1$ ppm was observed. The chemical shift value suggested a dialkoxyphosphinoyl group bound to an aliphatic carbon, but an indistinct splitting pattern in the ^{1}H–^{31}P NMR coupled spectrum prevented further spectral identification. Fortunately, the compound resonating at $\delta_P = 22.1$ ppm was rather stable which permitted its isolation by silica gel chromatography. Analysis of the ^{1}H, ^{13}C and ^{31}P NMR spectra showed that the compound in question was not a possible intermediate in this reaction, 1,2-dihydropyridylphosphonate **5**, but silylated hydroxymethylphosphonate **4**[16] (X = $SiMe_3$, comparison with authentic sample). Formation of hydroxymethylphosphonate **4** derivative in this reaction can probably be traced back to known instability of *N*-alkoxypyridinium salts, which under basic conditions collapse to pyridine and the corresponding aldehydes.[17] In the instance of *N*-methoxypyridinium salt **2**, the formaldehyde produced can easily add to silylphosphite **3** present in the reaction mixture to afford silylated hydroxymethylphosphonate **4** (X = $SiMe_3$). Apparently, silyl phosphite **3** itself was basic enough to trigger the decomposition of pyridinium salt **2**, since formation of hydroxymethylphosphonate **4** (X = $SiMe_3$) was also observed (albeit in lower yield, ca. 55%) in the absence of an external base.

We found, however, that diethyl H-phosphonate **1** reacted fast with pyridinium salt **2** (2 equiv.) in acetonitrile in the presence of 1,8-diazabicylo[5.4.0]undec-7-ene (DBU) (4 equiv.) affording, within 5 min, the desired diethyl 2-pyridylphosphonate **6**[18] ($\delta_P = 10.9$ ppm, 80%) and hydroxymethylphosphonate **4**[19] (X = H, $\delta_P = 24.6$ ppm, 20%).[20] This indicated that apparently under the reaction conditions nucleophilic attack of diethyl phosphite anion (generated from **1** and DBU) on the pyridine ring of **2** was significantly faster than decomposition of **2** due the presence of DBU. In agreement with this, no formation of pyridylphosphonate **6** was observed (^{31}P NMR spectroscopy) when pyridinium salt **2** was mixed with DBU prior to the addition of H-phosphonate **1**.

The efficacy of this approach in the synthesis of a new type of nucleotide analogue with a 2-pyridylphosphonate internucleotide linkage (Scheme 1) was assessed by reacting, in acetonitrile, a suitably protected dinucleoside H-phosphonate **7** (diastereomeric mixture, $\delta_P = 8.44$ and 9.35 ppm) with *N*-methoxypyridinium salt **2** (2 equiv.) and DBU (4 equiv.). The reaction was rapid and went to completion within 5 min producing as a sole nucleotidic material a 1:1 mixture of diastereomeric 2-pyridylphosphonates derivatives **9** ($\delta_P = 10.6$ and 11.0 ppm). In contrast to 4-pyridylphosphonates,[14] no intermediacy of the corresponding dihydropyridine **8** could be detected in this reaction using ^{31}P NMR spectroscopy. After work-up (see below), 2-pyridylphosphonates **9** were isolated by silica gel column chromatography in 86% yield and their chemical identity was confirmed by MS and NMR data.



7a (R_P) ("fast") (δ_P = 8.4 ppm); R = 4,4'-dimethoxytrityl

7b (S_P) ("slow") (δ_P = 9.4 ppm); R = 4,4'-dimethoxytrityl

9a (R_P) (δ_P = 10.6 ppm); R = 4,4'-dimethoxytrityl

9b (S_P) (δ_P = 11.0 ppm); R = 4,4'-dimethoxytrityl

Scheme 1.

To assess a stereochemical course of the formation of 2-pyridylphosphonates **9** (Scheme 1), the reaction was carried out on the separate diastereomers of dithymidine H-phosphonate **7**.[21] It was found (^{31}P NMR spectroscopy) that H-phosphonate diester **7a** afforded 2-pyridylphosphonate **9a**, while the diastereomer **7b**, the isomeric 2-pyridylphosphonate **9b**, exclusively. This established the transformation as stereospecific. Assuming the reaction pathway as in Scheme 1, it seems most likely that it proceeds with overall retention of configuration.

*A typical procedure for the preparation of 2-pyridylphosphonates **9***

The separate diastereomers of H-phosphonate diester **7** (0.88 mmol) in acetonitrile (4 mL) were treated with *N*-methoxypyridinium tosylate (2 equiv.) and DBU (4 equiv.). **CAUTION**: *DBU has to be added as the last reagent to the reaction mixture to ensure the efficient formation of 2-pyridylphosphonate **9** (see also in the text).* After 5 min (^{31}P NMR) the reaction mixture was concentrated, partitioned between 10% aq. $NaHCO_3$ (20 mL) and CH_2Cl_2, (20 mL), and the aqueous layer was extracted with CH_2Cl_2, (2×20 mL). The organic layer was dried over anh. Na_2SO_4, concentrated and the residue was purified by silica gel column chromatography using toluene–ethyl acetate–methanol (49:49:2, v/v/v). Yields **9a**, 86% (white, microcrystalline solid); **9b** 85% (white, microcrystalline solid).[22]

In conclusion, we have developed an efficient protocol for the preparation of a new type of nucleotide analogues bearing 2-pyridylphosphonate internucleotide linkage. The method makes use of easily available starting materials and affords the target compounds in high yields under mild reaction conditions. The distinctive feature of 2-pyridylphosphonate modification is that it permits further functionalisation (e.g. via quaternisation of the pyridine nitrogen) and thus can be of importance in designing new antisense/antigene agents with improved biomembrane penetration and tuneable chemical, biological and pharmacokinetic properties. Apart from this, the ability of 2-pyridylphosphonates to form bidentate complexes with metal cations can be exploited in the development of new artificial nucleases or specific probes for investigation of electron transfer (ET) phenomena in nucleic acids.

Studies on elaboration other heteroaromatic phosphonate systems for the purpose of modification of nucleic acids are in progress in this Laboratory.

Acknowledgements

We are indebted to Professor P. J Garegg for his interest in this work. Financial support from the Swedish Natural Science Research Council and the Swedish Foundation for Strategic Research is gratefully acknowledged.

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16. Although undesired in the context of these investigations, this reaction may constitute a convenient way for the preparation of hydroxymethylphosphonates. Further studies are in progress.
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18. Comparison with the authentic sample. Isolated in 74% yield from the reaction mixture by silica gel chromatography.
19. Comparison with the authentic sample.
20. At this stage of the investigation no attempt was made to optimise the procedure in order to eliminate the formation of hydroxymethylphosphonate by-products. However, it seems likely that by using other *N*-alkoxypyridinium salts and varying the amount of DBU, the formation of hydroxymethylphosphonates can be eliminated or significantly suppressed. Further studies are in progress in this Laboratory.

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22. Compounds **9a** and **b** were >98% pure and their chemical identity was confirmed by ^{1}H, ^{13}C, ^{31}P, and ^{1}H–^{1}H correlated NMR spectroscopy. **9a**: HRMS $[M+H]^+$ found: 1212.4366; $C_{67}H_{67}N_5O_{15}P$ requires: 1212.4371. **9b**: HRMS $[M+H]^+$ found: 1212.4369; $C_{67}H_{67}N_5O_{15}P$ requires: 1212.4371. Some diagnostic spectral data [compound, δ_P; $\delta_{H(H\text{-}6\ py)}$; $\delta_{H1'}$; $\delta_{C(C\text{-}2\ py)}$ (J_{CP})]: **9a**: 10.63 ppm; 8.42 ppm (1H); 6.39 ppm (2H); 150.36 ppm (227 Hz). **9b**: 11.02 ppm; 8.58 ppm (1H); 6.38 ppm (2H); 150.94 ppm (228).