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Jacek Stawinski^a; Mats Thelin^a

^a Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, Stockholm, Sweden

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NUCLEOSIDE H-PHOSPHONATES. XI. A CONVENIENT METHOD FOR THE
PREPARATION OF NUCLEOSIDE H-PHOSPHONATES

Jacek Stawinski* and Mats Thelin

Department of Organic Chemistry, Arrhenius Laboratory,
University of Stockholm, 106 91 Stockholm, Sweden.

Abstract.

A simple synthetic method for the preparation of nucleoside 3'-H-phosphonates, consisting of reaction of suitably protected nucleosides with phosphonic acid in the presence of a condensing agent, has been developed.

The growing interest in hydrogenphosphonates (H-phosphonates) as useful intermediates in the synthesis of various natural products, e.g. oligonucleotides,¹⁻³ nucleopeptides,⁴ sugar phosphates,⁵ phospholipids,⁶ caused a high demand for a reliable and economical method for their preparation.

The most straightforward method for the preparation of nucleoside H-phosphonate monoesters, which relies on the condensation of suitably protected nucleosides with phosphonic acid in the presence of arene sulfonyl derivatives⁷ has been hampered by formation of bisnucleoside H-phosphonate diesters⁸ and concomitant oxidation of the desired H-phosphonate monoesters by the condensing reagent.⁹ Replacement of arene sulfonyl derivatives by pivaloyl chloride (PV-Cl), did not reduce the amount of bisnucleoside H-phosphonate formation, which still constituted ca 30% of the nucleotidic material.⁸ For this reason, most of the current methods make use of trivalent phosphitylating reagents as e.g. PCl₃/imidazole,¹⁰ PCl₃/triazole,³ salicylchlorophosphite,¹¹ which proved to be efficient in the preparation of H-phosphonate monoesters.

Other methods, based on an oxidative phosphitylation of nucleosides with phosphinic acid in the presence of mesitylenedisulfonyl chloride,⁸ and on transesterification of bis(2,2,2-trifluoroethyl)phosphonate¹² or bis(1,1,1,3,3,3-hexafluoro-2-propyl)phosphonate¹³ with nucleosides, have also been proposed.

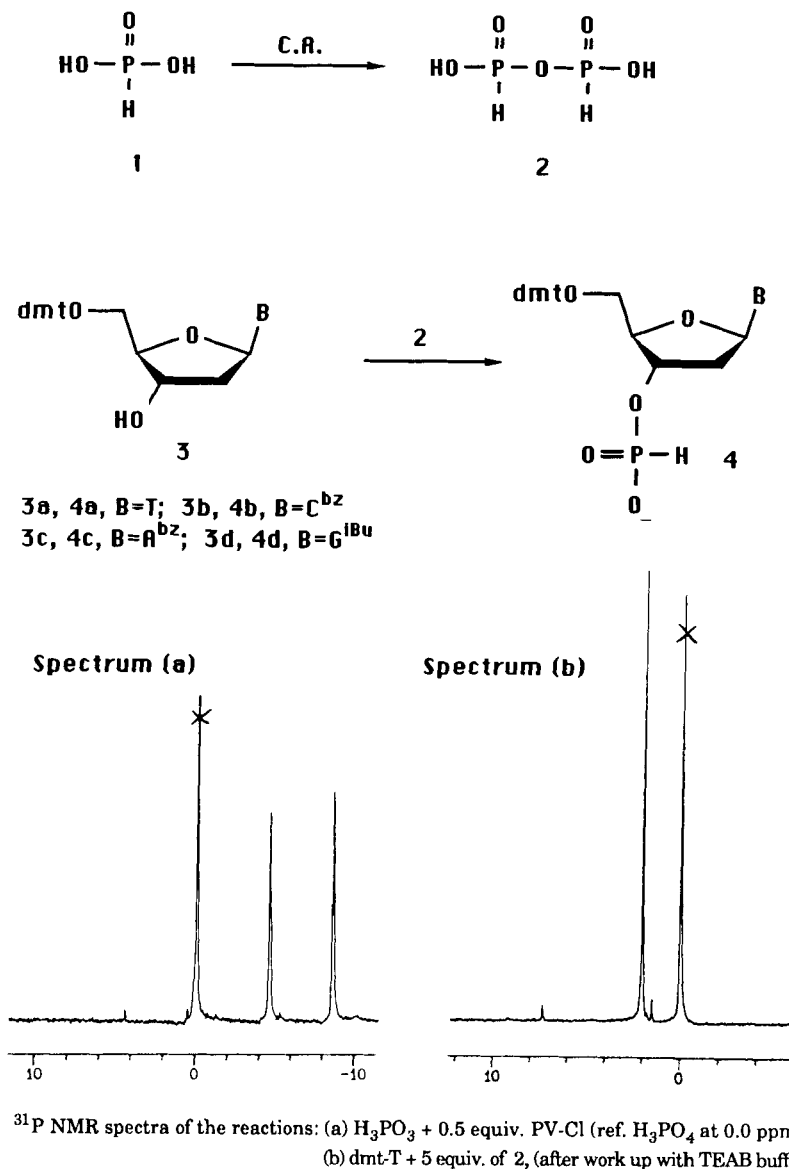
Our recent studies on the chemistry of phosphonic acid revealed, however, that depending on the ratio of phosphonic acid to a condensing agent, various distinctive reactive species can be formed in the activation process.¹⁴ Since these intermediates showed different reactivity towards nucleophiles, we found it possible to produce, under certain experimental conditions, almost exclusively the H-phosphonate monoesters in the reaction of phosphonic acid with nucleosides.

RESULTS AND DISCUSSION

When 5'-O-dimethoxytritylthymidine (dmt-T, **3a**) was allowed to react with 1 equiv. of phosphonic acid in the presence of 3 equiv. of pivaloyl chloride (PV-Cl) in pyridine, the reaction mixture consisted of dinucleoside H-phosphonate diester (ca 75%, $\delta=7.1$ ppm), an activated nucleoside 3'-H-phosphonate monoester (ca 25%, $\delta=122.3$ ppm) and unreacted (activated) phosphonic acid ($\delta=132.6$ ppm) (³¹P NMR analysis). These results are consistent with those reported by Hata *et al.*⁸ and indicate comparable coupling rates of a nucleoside with phosphonic acid and with H-phosphonate monoester.

In order to be able to steer the reaction in the direction of H-phosphonate monoester formation, detailed knowledge about the activation pathway of phosphonic acid and about reactivity of possible species formed in such a process, was necessary. Such studies have been undertaken in this laboratory¹⁴ and resulted in the selection of pyrophosphonate **2** as the most promising intermediate for H-phosphonate monoesters preparation.

The pyrophosphonate **2** can be generated practically as a single species ($\delta= - 6.63$ ppm, $^1J_{\text{PH}}=639.5$ Hz) (Scheme 1, spectrum a) from phosphonic acid in pyridine in the presence of 0.5 equiv. of a condensing reagent (acyl chlorides, chlorophosphates). Upon addition of an excess of ethanol, **2** reacts rather fast (ca 2-3 min.) affording equimolar amounts of ethyl H-phosphonate and phosphonic acid. Since the formation of **2** is almost an instantaneous process, the synthesis involving a nucleoside



Scheme 1

can be carried out by addition of a condensing reagent to the mixture of phosphonic acid and a hydroxylic component, without danger of a concomitant reaction of the latter with a coupling agent.

The reaction of equimolar amounts of the pyrophosphonate **2** and the nucleoside **3a** proved to be clean, but rather slow (ca 50-60% conversion to the product **4a** after standing over-night). To overcome this inconvenience five molar excess of **2** was used, which made the reaction

go to completion in less than 3 h. The ^{31}P NMR spectrum recorded after quenching the reaction with 1M TEAB followed by partition of the mixture between dichloromethane and 0.5M TEAB buffer, showed practically complete removal of the excess of phosphonic acid during this work up step. The mixture consisted only of the desired nucleoside H-phosphonate monoester **4a** ($\delta=2.5$ ppm) contaminated with variable amounts (0-5%) of the dinucleoside H-phosphonate ($\delta=7.1$ ppm) (TLC analysis; for the ^{31}P NMR spectrum, see Scheme 1). A similar course of reaction was observed also for the other nucleosides and after a silica gel column chromatography the 3'-H-phosphonates **4** derived from suitably protected thymidine, deoxyribocytidine, deoxyriboadenosine and deoxyribo guanosine were obtained in 86-92% yield. **4a-d** were identical in every respect with appropriate compounds produced by another method.¹⁰

The moderate reactivity of **2** makes it possible to leave the reaction mixture containing a nucleoside and 5 equiv. of pyrophosphonate overnight without danger of side reactions with heterocyclic base residues occurring (^{31}P NMR and TLC analysis). Since the condensing agent is completely consumed in the activation process of phosphonic acid and does not participate in the reaction of a nucleoside with the pyrophosphonate **2**, its chemical nature is of minor importance for the final yield of the H-phosphonate **4**. Indeed, practically the same results were obtained using pivaloyl chloride or 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane¹⁵ as condensing agents. Since the latter reagent is a stable, crystalline substance, and easy to prepare in large quantities, it was used almost exclusively in all preparative syntheses.

In conclusion, the method presented above can be considered as an alternative approach for the preparation of deoxyribonucleoside 3'-H-phosphonates. Mild reaction conditions, reasonably high yields and simple experimental procedure can make this method convenient both for small and large scale preparations. Another advantage of the method is, that it makes use of stable, cheap, and commercial available materials.

EXPERIMENTAL

Materials and Methods

^1H and ^{31}P NMR spectra were recorded on a Jeol GSX-270 FT spectrometer. ^1H NMR spectra were referenced to the internal TMS signal

and for ^{31}P NMR spectra 1% H_3PO_4 in D_2O was used as an external standard (coaxial inner tube). 2D-NMR experiments (DQF H,H-COSY; JRES) were performed in order to assign the resonances and to measure coupling constants. TLC was carried out on Merck silica gel 60 F₂₅₄ pre-coated plates using the following eluents: chloroform/methanol 8:2 (v/v)(system A); isopropanol/35% aq.ammonia/water 17:1:2 (v/v)(system B). Pyridine was refluxed with CaH_2 overnight and then distilled and stored over molecular sieves (4Å) or CaH_2 . Pivaloyl chloride and phosphonic acid were commercial grade (Aldrich). Stock solution of 2M H_3PO_3 was prepared by evaporation of added pyridine to the appropriate amount of phosphonic acid and dissolving the residue in anhydrous pyridine. 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane was prepared according to the published procedure.¹⁵

General procedure for synthesis of nucleoside H-phosphonate monoesters

Nucleoside **3** (2 mmol) was rendered anhydrous by evaporation of added pyridine and the residue was dissolved in 10 ml of 2M stock solution of phosphonic acid (20 mmol) in pyridine. To the stirred reaction mixture, 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane (11 mmol) or pivaloyl chloride (11 mmol) was added and progress of the reaction was monitored by TLC. After ca 3 h, the reaction mixture was quenched with 1M TEAB (2 ml) and then partitioned between dichloromethane (75 ml) and 0.5 M TEAB buffer (50 ml). The organic layer was evaporated, the residue dissolved in dichloromethane and purified by flash column chromatography on silica gel using a stepwise gradient of methanol in dichloromethane (containing 1% of pyridine) (0-10%, v/v). After chromatography, the product **4** was again partitioned between dichloromethane and 0.05M TEAB buffer in order to removed inorganic salts, if present. Yield 86-92%.

5'-O-dimethoxytritylthymidine 3'-H-phosphonate triethylammonium salt, **4a**

Yield: 92%. R_f=0.08 (system A), 0.44 (system B).

^{31}P NMR (in pyridine): δ =1.98 ppm, $^1J_{\text{PH}}$ =599.3 Hz, $^3J_{\text{PH}}$ =9.7 Hz.

^1H NMR (CDCl_3 , δ in ppm): 7.60 (s, H-6), 6.89 (d, $^1J_{\text{PH}}$ =617.7 Hz, H-P), 6.45 (dd, 3J =5.5 and 8.3 Hz, H-1'), 5.00 (m, $^3J_{\text{PH}}$ =8.8 Hz, H-3'), 4.27 (m, H-4'), 3.78 (s, $\text{CH}_3\text{-O}$) 3.48 (dd, 2J =10.5 Hz and 3J =2.7 Hz, H-5'), 3.38 (dd, 2J =10.5 Hz and 3J =2.7 Hz, H-5''), 2.60 (ddd, 2J =13.5 Hz, 3J =2.4 Hz, 3J =5.5 Hz, H-2'), 2.38 (ddd, 2J =13.5 Hz and 3J =5.9 Hz, 3J =8.3 Hz, H-2'), 2.92 and 1.25 (q and t, 3J =7.3 Hz, $\text{CH}_3\text{CH}_2\text{-N}$), 1.35 (s, Me).

5'-O-dimethoxytrityl-N⁴-benzoyldeoxyribocytidine 3'-H-phosphonate triethylammonium salt. 4b

Yield: 86%. Rf=0.15 (system A), 0.51 (system B).

³¹P NMR (in pyridine): δ =1.82 ppm, $^1J_{\text{PH}}$ =600.6 Hz, $^3J_{\text{PH}}$ =8.0 Hz.

¹H NMR (CDCl₃, δ in ppm): 8.25 (d, 3J =7.5 Hz, H-6), 7.10 (d, overlapp. with aromatic protons, H-5), 7.01 (d, $^1J_{\text{PH}}$ =618.8 Hz, H-P), 6.30 (dd, 3J =5.9 and 6.1 Hz, H-1'), 4.96 (m, $^3J_{\text{PH}}$ =9.0 Hz, H-3'), 4.32 (m, H-4'), 3.80 (s, CH₃-O), 3.50 (dd, 2J =10.8 Hz, 3J =2.8 Hz, H-5'), 3.43 (dd, 2J =10.8 Hz, 3J =3.7 Hz, H-5''), 2.85 (ddd, 2J =13.7 Hz, 3J =5.9 Hz, 3J =5.1 Hz, H-2'), 2.38 (ddd, 2J =13.7 Hz, 3J =6.1 Hz, 3J =5.9 Hz, H-2''), 3.04 and 1.33 (q and t, 3J =7.3 Hz, CH₃CH₂-N).

5'-O-dimethoxytrityl-N-benzoyldeoxyriboadenosine 3'-H-phosphonate triethylammonium salt. 4c

Yield: 89%. Rf= 0.12 (system A), 0.54 (system B).

³¹P NMR (in pyridine): δ =1.92 ppm, $^1J_{\text{PH}}$ =600.5 Hz, $^3J_{\text{PH}}$ =8.5 Hz.

¹H NMR (CDCl₃, δ in ppm): 8.74 and 8.18 (2 x s, H-8 and H-2), 6.94 (d, $^1J_{\text{PH}}$ =617.9 Hz, H-P), 6.58 (dd, 3J =5.9 and 7.7 Hz, H-1'), 5.08 (m, $^3J_{\text{PH}}$ =9.5 Hz, H-3'), 4.43 (m, H-4'), 3.77 (s, CH₃-O), 3.44 (dd, 2J =10.3 Hz, 3J =3.8 Hz, H-5'), 3.37 (dd, 2J =10.3 Hz, 3J =4.2 Hz, H-5''), 2.92 (ddd, 2J =13.5 Hz, 3J =7.7 Hz, 3J =5.7 Hz, H-2'), 2.80 (ddd, 2J =13.5 Hz, 3J =5.9 Hz, 3J =3.0 Hz, H-2''), 3.05 and 1.33 (q and t, 3J =7.3 Hz, CH₃CH₂-N).

5'-O-dimethoxytrityl-N²-isobutyryldeoxyriboguanosine 3'-H-phosphonate triethylammonium salt. 4d

Yield: 88%. Rf= 0.16 (system A), 0.46 (system B).

³¹P NMR (in pyridine): δ =1.49 ppm, $^1J_{\text{PH}}$ =601.8 Hz, $^3J_{\text{PH}}$ =9.4 Hz.

¹H NMR (CDCl₃, δ in ppm): 7.74 (s, H-8), 6.97 (d, $^1J_{\text{PH}}$ =620.1 Hz, H-P), 6.16 (dd, 3J =5.7 and 6.0 Hz, H-1'), 5.56 (m, $^3J_{\text{PH}}$ =8.9 Hz, H-3'), 4.20 (m, H-4'), 3.75 (s, CH₃-O), 3.37 (dd, 2J =10.4 Hz, 3J =2.9 Hz, H-5'), 3.21 (dd, 2J =10.4 Hz, 3J =4.4 Hz, H-5''), 3.11 (ddd, 2J =13.0 Hz, 3J =5.7 Hz, 3J =7.1 Hz, H-2'), 2.57 (ddd, 2J =13.0 Hz, 3J =6.0 Hz, 3J =6.5 Hz, H-2''), 2.95 and 1.25 (q and t, 3J =7.3 Hz, CH₃CH₂-N), 2.39 (hep., 3J =7.0 Hz, H-C, iBu), 1.13 and 1.05 (2 x d, 3J =7.0 Hz, Me₂-C, iBu).

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