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STUDIES ON ARYL H-PHOSPHONATES. I. AN EFFICIENT METHOD FOR THE PREPARATION OF DEOXYRIBO- AND RIBONUCLEOSIDE 3'-H-PHOSPHONATE MONOESTERS BY TRANSESTERIFICATION OF DIPHENYL H-PHOSPHONATE

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Abstract: *A convenient method for the preparation of deoxyribonucleoside and ribonucleoside 3'-H-phosphonate monoesters via transesterification of diphenyl H-phosphonate with suitable protected nucleosides in pyridine is described.*

Simplicity of the preparation of phosphate esters and their analogues *via* H-phosphonate intermediates triggered high demand for efficient and economical methods for the synthesis of H-phosphonate monoesters.

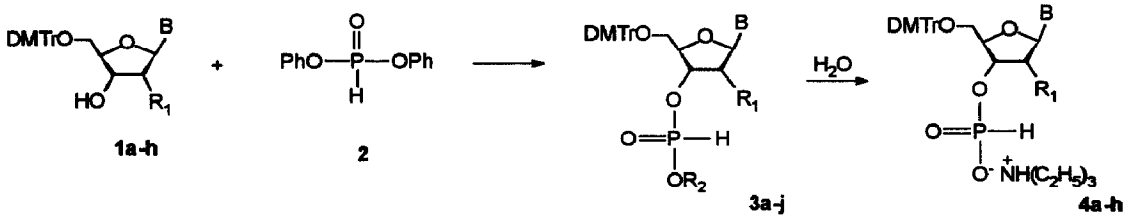
A plethora of chemical approaches have been devised for that purpose and these have been reviewed recently[1]. Unfortunately, the majority of them are not applicable to the synthesis of natural products derivatives and suffer from various shortcomings. The most recent synthetic methods designed specifically for the preparation of nucleoside H-phosphonates make use of PCl_3/azoles system[2,3], salicylchlorophosphite[4], di- or tri(2,2,2-trifluoroethyl) H-phosphonate[5,6] or pyro-H-phosphonate[7]. They differ, however, in efficiency, chemoselectivity, accessibility of the phosphonylating reagent and in cost-effectiveness.

During our studies on transesterification of nucleoside H-phosphonate diesters[8] we noticed that aryl H-phosphonate diesters are reactive enough to undergo fast and quantitative reaction with various nucleophiles at room temperature. Further, we have found that diphenyl H-phosphonate underwent rapid transesterification with suitably protected nucleosides affording nucleoside aryl and dinucleoside H-phosphonates. Since the former undergo rapid hydrolysis to alkyl H-phosphonate monoesters we attempted to exploit these findings in the development of synthetic method for the preparation of nucleoside 3' - H-phosphonates.

To this end equimolar amounts of diphenyl H-phosphonate **2** and 5'-O-dimethoxytritylthymidine **1a** were allowed to react in pyridine and progress of the reaction was followed by ^{31}P NMR spectroscopy and TLC. Within 20 min the starting nucleoside **1a** disappeared (TLC) and new resonances, assigned to nucleoside phenyl H-phosphonate **3a** (δ ~4.5 ppm) and to the corresponding symmetrical dinucleoside H-phosphonate diester **3i** (δ ~7 ppm)[9,10] appeared in the ^{31}P NMR spectrum. From the product distribution (ratio of **3a/3i** ~ 4:1) it became apparent that substitution of a phenyl group by nucleoside **1a** in the reagent **2** was faster than that in the mixed H-phosphonate diesters **3a**. It seemed likely therefore that by changing the ratio of the phosphonylating agent to a nucleoside one should be able to steer the reaction in the desired direction. Indeed, with increasing excess of the phosphonylating reagent **2** formation of the undesired symmetrical diester (**3i**) gradually decreased

until with 7 molar equivalents of **2** its amount dropped below 2%. Addition of water to the reaction mixture resulted in rapid hydrolysis of the nucleoside phenyl H-phosphonate diester **3a** (few minutes) to produce the desired product, nucleoside 3'-H-phosphonate monoester **4a**. The above reaction conditions were applied in the preparative synthesis of deoxyribonucleoside H-phosphonates to obtain after silica gel chromatography[12] **4a-d** in over 80% yield (Table 1). It is worth mentioning that excess of phosphonylating agent **2** was rapidly hydrolysed during aqueous work-up of the reaction mixtures to phenyl H-phosphonate monoester which was almost completely removed by extraction with an aqueous solution of $NaHCO_3$.

Scheme 1.



a: R_1 = H, R_2 = Ph, B = Thy
b: R_1 = H, R_2 = Ph, B = Cyt^{Bz}
c: R_1 = H, R_2 = Ph, B = Ade^{Bz}
d: R_1 = H, R_2 = Ph, B = Gua^{ibu}
e: R_1 = OTBDMSi, R_2 = Ph, B = Ura
f: R_1 = OTBDMSi, R_2 = Ph, B = Cyt^{Bz}
g: R_1 = OTBDMSi, R_2 = Ph, B = Ade^{Bz}
h: R_1 = OTIPSi, R_2 = Ph, B = Gua^{ibu}
i: R_1 = H R_2 = **1a** B = Thy
j: R_1 = OTBDMSi, R_2 = **1e** B = Ura

Abbreviations:

DMTr - 4,4'-O-dimethoxytrityl
TBDMSi - tertbutyldimethylsilyl
TIPSi - triisopropylsilyl
Ph - phenyl
Thy - thymin-1-yl
Cyt^{Bz} - N^4-benzoylcytidin-1-yl
Ade^{Bz} - N^6-benzoyladenin-9-yl
Gua^{ibu} - N^2-isobutyrylguanin-9-yl
Ura - uracil-1-yl

The ribonucleosides **1e-h** having bulky 2'-O-alkylsilyl groups were, as expected, less reactive than deoxyribonucleosides in the transesterification reaction. This provided, however, higher selectivity of the reaction (substitution of one *vs* two phenyl groups in the phosphonylating agent) but the rate of formation of the mixed H-phosphonate diesters was still reasonable high. For example, 2'-O-silylated uridine **1e** reacted with 1 equiv. of the reagent **2** affording within ~60 min a mixture of the nucleoside phenyl H-phosphonate **3e** (~95%) and the corresponding symmetrical dinucleoside H-phosphonate **3j** (~5%). Formation of the undesired symmetrical product **3j** was completely eliminated by using three molar excess of **2** and the reaction went to completion in 15 min under these conditions. Lower reactivity of the ribonucleoside phenyl H-phosphonates **3** has also been manifested in their slower hydrolysis to the H-phosphonates **4e-h** upon addition of water (ca 40 min). However, the hydrolysis was found to be substantially faster in the presence of a base and thus it was possible to convert the intermediates **3e-h** into the desired products in less than 15 min by addition of triethylamine. The yields of the purified ribonucleoside H-phosphonate **4e-h** are shown in Table 1.

Table 1. Chosen Data of Intermediates (**3**) and of Final Products (**4a-h**).

Cpds	^{31}P NMR (pyridine) δ (ppm), ^{1}J (Hz)	Cpds	Yields (%)	^{31}P NMR (pyridine) δ (ppm), ^{1}J (Hz)
3a	4.35; 726.69[b]	**4a**	85	1.84; 599.7[b]
3b	4.35, 4.47[a]; 728.5, 727.7[b]	**4b**	86	1.76; 598.8[b]
3c	4.18, 4.27[a]; 726.7, 725.8[b]	**4c**	80	1.70; 597.9[b]
3d	4.14, 4.43[a]; 726.7, 724.8[b]	**4d**	84	1.35; 600.7[b]
3e	4.51, 4.59[a]; 723.0, 739.7[b]	**4e**	82	2.12; 607.1[b]
3f	4.49, 4.47[a], 733.2, 723.9[b]	**4f**	77	1.38; 605.3[b]
3g	4.45, 4.50[a]; 725.8, 736.9[b]	**4g**	80	2.29; 609.0[b]
3h	4.50, 4.79[a]; 734.1, 721.1[b]	**4h**	90	2.19; 605.3[b]
3i	7.27; 716.5 [c]			
3j	7.41; 728.6[c]			

[a] - two diastereoisomers. [b] - doublets of doublets. [c] - doublet of triplets.

A possibility of occurrence of some side reactions during the phosphonylation was investigated by subjecting of fully protected deoxyribonucleosides (3'-O-acetylated compounds **1a-h**) to the reaction with 40 molar equivalent excess of the reagent **2** in pyridine during 8 hrs. No side products formation due to possible reaction of **2** with heterocylic bases was detected by TLC and ^{31}P NMR spectroscopy after that time.

A typical procedure for the preparation of nucleoside H-phosphonates is presented below:

General procedure for the preparation of nucleoside H-phosphonates **4a-h**

To the solution of suitably protected nucleoside **1a-h** (1 mmol) in pyridine (5 mL), 7 mmols[11] (in the case of deoxynucleoside derivatives **1a-d**) or 3 mmols (in the case of ribonucleoside derivatives **1e-h**) of diphenyl phosphite **2** was added. After 15[13] min (TLC analysis) the reaction mixture was quenched by addition of the mixture of water-triethylamine (1:1 v/v, 2 mL) and was left standing for 15 min. The solvent was evaporated and the residue was partitioned between methylene dichloride (50 mL) and 5% aq. $NaHCO_3$ (20 mL). The organic layer was extracted additionally two times with 5% aq. $NaHCO_3$ (20 mL), dried over Na_2SO_4 and finally evaporated to an oil. The products **4a-h** were purified[14] by chromatography on silica gel using a stepwise gradient of methanol (0-10%) in methylene dichloride (containing 5% of triethylamine). Yields and ^{31}P NMR are listed in Table 1.

The obtained nucleoside H-phosphonates were identical in analytical tests (TLC, ^{1}H NMR) with those prepared using the described earlier procedure[7] They have been used for synthesis of several oligomers (12 - 26 nucleotide units) which served successfully as primers for PCR and as molecular probes.

In conclusion, diphenyl H-phosphonate **2** represents an inexpensive, commercial available reagent suitable for the convenient and efficient conversion of partially protected deoxyribo- and ribonucleosides into the corresponding 3'-H-phosphonate monoesters. The reagent is stable, easy to handle and affords H-phosphonate monoesters of purity usually better than 95% even without column chromatography. Consider that phosphonylation with diphenyl phosphite occurred effectively in rather mild conditions, it could be expected that this procedure will find application(s) outside nucleotide field (e.g. peptides, carbohydrates), also.

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REFERENCES AND NOTES

1. Stawiński, J. Some Aspects of H-Phosphonate Chemistry. In *Handbook of Organophosphorus Chemistry;* R. Engel, Ed.; Marcel Dekker, Inc.: New York, 1992; pp. 377-434.
2. Garegg, P. J.; Regberg, T.; Stawiński, J.; Strömberg, R. *Chemica Scr.* **1986**, *26*, 59-62.
3. Froehler, B. C.; Ng, P. G.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 5399-5407.
4. Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661-2664.
5. Gibbs, D. E.; Larsen, C. *Synthesis-Stuttgart* **1984**, 410-413.
6. Sakatsume, O.; Ohtsuka, M.; Takaku, H., Reese, C. B. *Nucleic Acids Res.* **1989**, *17*, 3689-3696.
7. Stawinski, J.; Thelin, M. *Nucleosides & Nucleotides* **1990**, *9*, 129-135.
8. Sobkowski, M.; Sobkowska, A.; Stawiński, J.; and Kraszewski, A., *manuscript in preparation.*
9. Regberg, T.; Stawiński, J.; Strömberg, R. *Nucleosides & Nucleotides* **1988**, *7*, 23-35.
10. When 0.5 molar equiv. of **2** was used after 1.5 hr of the reaction time, symmetrical 3' - 3' dinucleoside H-phosphonate diester was obtained quantitatively.
11. To reduce the excess of diphenyl H-phosphonate in the phosphonylation of deoxynucleoside derivatives **1a** we have tested also another approach in which the solution of a nucleoside in pyridine was added dropwise (during 15 min) into pyridine containing only 3 molar equiv. of **2**. The reaction went to completion within 15 min (TLC) and formation of the symmetrical dinucleoside H-phosphonate **3i** was negligible (less than 1.5 %). Since the product **4a** was isolated in essentially the same yield as that shown in Table 1, it seems that in this way the excess of phosphonylatig reagent could be decreased without a penalty of formation of a symmetrical dinucleoside H-phosphonate diester. However, this approach is more laborious and require more attention to secure anhydrous conditions. Thus, for medium scale synthesis of deoxynucleoside H-phosphonates of type **4**, we recommend the procedure described in the text.
12. Hunt, B. I.; and Rigby, W. Chem. Ind. **1967**, 1868.
13. It is not recommended to prolong the time of phosphonylation because of diphenyl H-phosphonate, in pyridine undergoes slow transformation toward phenyl H-phosphonate monoester and triphenyl phosphite.
14. Precipitation of crude reaction products (after extraction with aqueous $NaHCO_3$) from methylene dichloride into diethyl ether - hexane (1 . 1, v/v) usually afforded nucleoside H-phosphonates of purity better than 95% (^1H, ^{31}P NMR and TLC).

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