Nucleoside Hydrogenphosphonates. Part 6.[1] Reaction of Nucleoside Hydrogenphosphonates with Arenesulphonyl Chlorides

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The reaction of nucleoside 3'-hydrogenphosphonates (**1**) with 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPS-Cl) in pyridine has been studied using ^{31}P n.m.r. spectroscopy, and a general scheme for transformations occurring during this activation process is proposed. It was found that the reaction proceeded through several intermediates affording, as final products, the pyridine adduct of nucleoside 3'-metaphosphate (**7**), P^1P^2-bis-(*S*-aryl nucleosid-3'-yl) pyrophosphate (**11a**), and nucleoside *S*-aryl 3'-phosphorochloridate (**13a**), which upon addition of water were converted into nucleoside 3'-phosphate (**2**), P^1P^2-bis-(nucleosid-3'-yl) pyrophosphate (**3**), and nucleoside *S*-aryl 3'-phosphorothioate (**4a**).

In our studies on internucleotide bond formation *via* hydrogenphosphonate intermediates, we found that nucleoside 3'-hydrogenphosphonates can be activated *inter alia* by arenesulphonyl chlorides and form dinucleoside (3'-5')-hydrogenphosphonate diesters in the presence of a suitably protected nucleoside.[2]

A characteristic of this reaction is extremely rapid hydrogenphosphonate diester formation (within a few seconds) when nucleoside hydrogenphosphonate (**1**) is activated by an arenesulphonyl chloride in the presence of the hydroxylic compound. However, when the latter is added after a few minutes to the preactivated nucleoside 3'-hydrogenphosphate, practically no hydrogenphosphonate diester formation is observed.[2] These results indicate that the reactive species generated from nucleoside hydrogenphosphonates and arenesulphonyl chlorides react rapidly with a hydroxylic compound. However, in the absence of such a nucleophile, these reactive species are converted into other intermediates that cannot produce hydrogenphosphonate diesters upon subsequent addition of a nucleoside.

Hata and his co-workers[3] have reported, in a preliminary study based on paper chromatography, that nucleoside 5'-hydrogenphosphonates react with 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPS-Cl) yielding nucleoside 5'-phosphate and the corresponding pyrophosphate, but no detailed study has been reported.

Because the formation of internucleotidic bonds *via* hydrogenphosphonate intermediates is an approach to oligonucleotide synthesis,[4–6] that may become important in the future, we wished to obtain a deeper insight into nucleoside hydrogenphosphonate chemistry and have now investigated the activation reaction of nucleoside 3'-hydrogenphosphonate (**1**) by TPS-Cl using Fourier transform ^{31}P n.m.r. spectroscopy.

Results and Discussion

Identification of Final Products in the Reaction of Nucleoside 3'-Hydrogenphosphonate (**1**) *with TPS-Cl.*—*Compound* (**1**) and TPS-Cl (1 equiv.) in pyridine were kept for 18 h and subsequently subjected to ^{31}P n.m.r. analysis. The spectrum showed a rather complicated mixture of compounds, with several resonances in the regions δ +15, −5, −10, and −20 p.p.m. (Figure, spectrum 1). However, the addition of water caused immediate simplification and only three signals were observed at δ *ca.* +15, +1, and −10 p.p.m. (spectrum 2). On the basis of chemical shifts [7,8] and splitting patterns (without ^1H heteronuclear decoupling), we could identify the final products as 5'-*O*-dimethoxytritylthymidine 3'-phosphate (**2**) (δ 1.2 p.p.m.), P^1P^2-bis-(5'-*O*-dimethoxytritylthymidin-3'-yl) pyrophosphate (**3**) (δ −10.3 p.p.m.), and 5'-*O*-dimethoxytritylthymidine *S*-2,4,6-tri-isopropylphenyl 3'-phosphorothioate (**4a**) (δ 15.4 p.p.m.). This assignment was further supported by independent synthesis (see later).

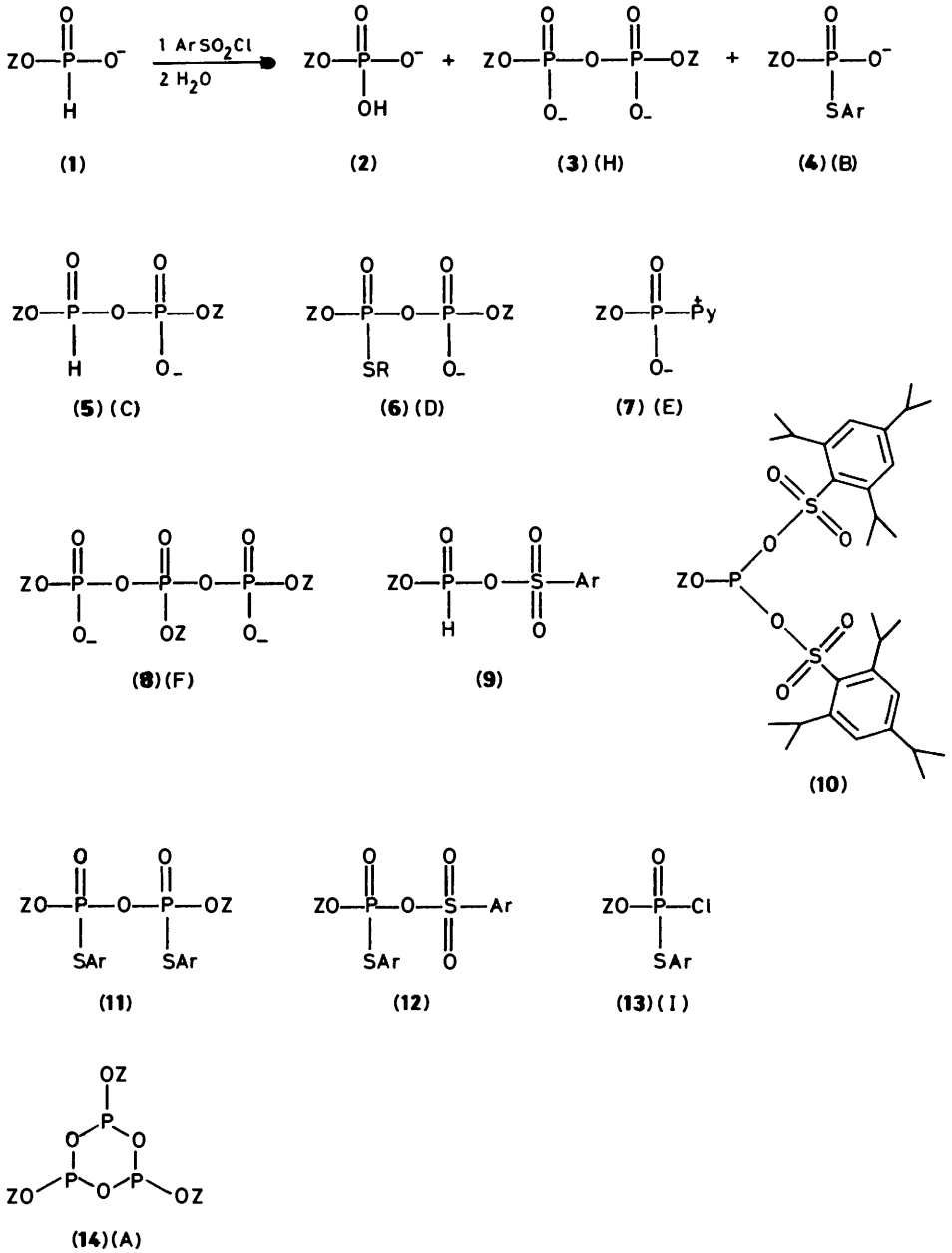
Thus, the reaction of (**1**) with TPS-Cl (1 equiv.) seems to be a redox process (Scheme 1).

These findings differ in one important respect from the results of Hata and co-workers[3] The formation of compound (**4a**) indicates that reduction of TPS-Cl does not stop at the stage of sulphinic acid, but proceeds further to the corresponding thiophenol.

The ratio of (**4a**) to the sum of compounds (**2**) and (**3**) in the final mixture was 1:2, and did not depend on the amounts of TPS-Cl used. This indicates that the subsequent oxidation steps by sulphinic and sulphenic acid derivatives are faster than oxidation by TPS-Cl itself.

The stoicheiometry for the reaction shows, that 1 equiv. of TPS-Cl should be able to oxidize 3 equiv. of (**1**), producing 2 equiv. of (**2**) and 1 equiv. of (**4a**) [compound (**3**) is formed as a side-product during the hydrolysis step and its amount varies from experiment to experiment].

Changes in the Pattern of ^{31}P *N.m.r. Signals during Reaction between* (**1**) *and TPS-Cl.*—When TPS-Cl (0.5 equiv.) was added to a pyridine solution of (**1**), the absorption signal of (**1**) shifted from δ 2.5 to 3.7 p.p.m. No oxidation products were observed during the next 25 min. Addition of another 0.5 equiv. of TPS-Cl produced substantial changes in the ^{31}P n.m.r. spectrum (spectrum 3). The singlet at δ 3.7 p.p.m. became broader and simultaneously four groups of signals appeared: eleven resonances in the region δ 120—110 p.p.m. [intermediate (A)], singlet (B) (δ 15.4 p.p.m.), two doublets (C-1) (δ −1.1 p.p.m.) and doublet (C-2) (δ −10.4 p.p.m.) (first stage of the reaction). During the next 10 min the singlet at δ 3.7 p.p.m. and the resonances from intermediate (A) gradually decreased, while signals (B), (C-1), and (C-2) increased (second stage of the reaction). When the signals from the starting material and intermediate A had disappeared completely, the third stage of the reaction started. This was characterized by gradual disappearance of the (B), (C-1), and (C-2) signals and appearance of new ones: four signals at δ 14 p.p.m. (D-1), singlet (E) at δ −4.6 p.p.m., several signals in the region δ −10 to −11 p.p.m. (D-2) and (F-1), and a triplet (F-2) at δ 21.5 p.p.m. (spectrum 4).



Scheme 1. (**1**), (**2**), (**3**), (**5**), **8**), (**10**) Z = 5′-*O*-dimethoxytritylthymidin-3′-yl (DMT-T-); (**4a**), (**6a**), (**9a**), (**11a**), (**12a**), (**13a**) Z = DMT-T-, Ar = 2,4,6-triisopropylphenyl; (**4b**), (**6b**), (**9b**), (**11b**), (**12b**), (**13b**) Z = DMT-T-, Ar = phenyl; (**7**) Z = DMT-T-, Py = pyridine; (**14a**) Z = DMT-T-; (**14b**) Z = ethyl

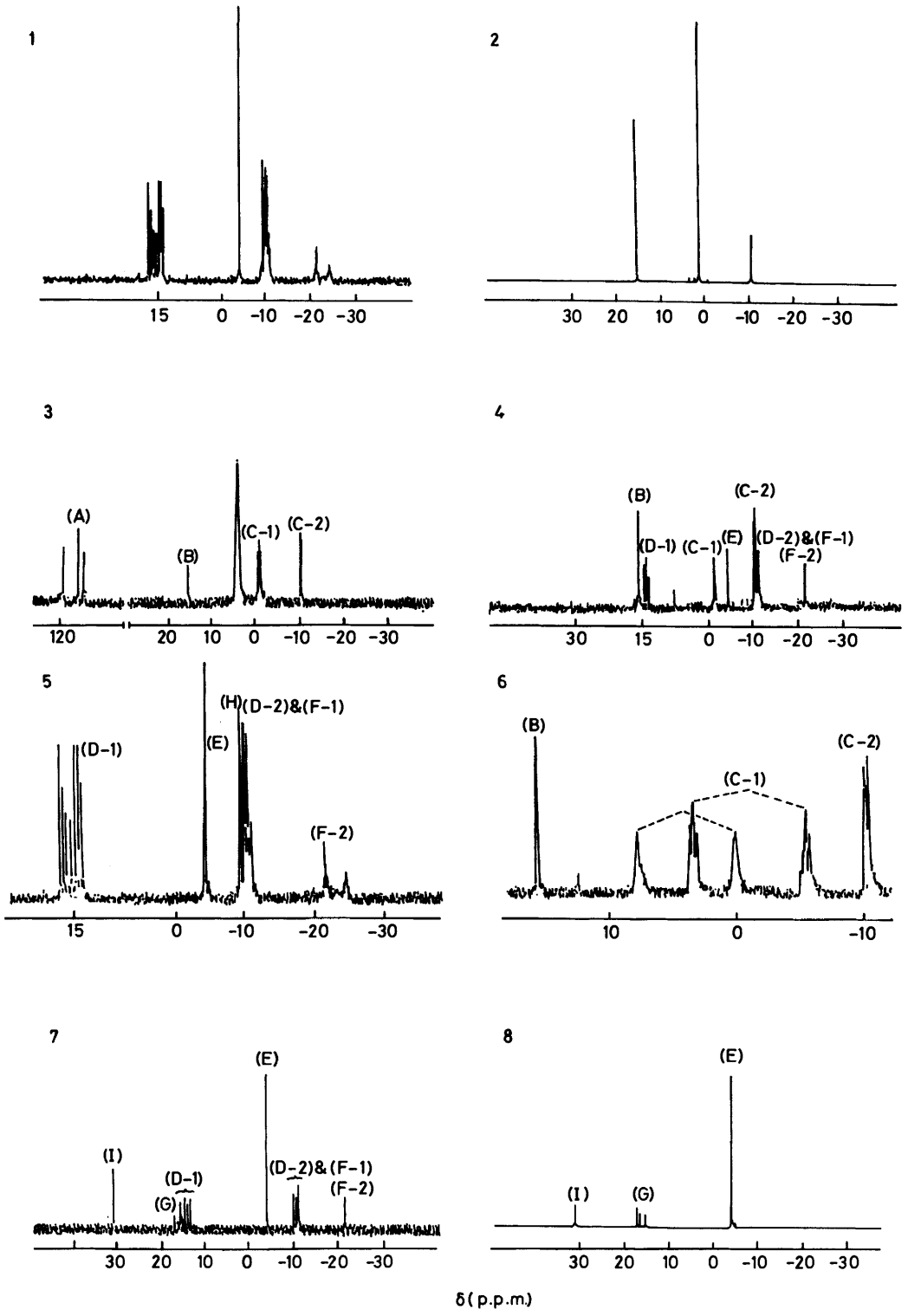
Before signals (B), (C-1), and (C-2) disappeared completely, the fourth and final stage of the reaction started. In addition to the (D-1), (E), (D-2), (F-1), and (F-2) signals, at least four absorption lines at δ +16 p.p.m. [intermediate (G)] appeared together with a singlet at δ −9.7 p.p.m. [intermediate (H)] and a rather complicated multiplet at δ −24.5 p.p.m. (spectrum 5). Because of the limited amounts of TPS-Cl in this experiment, stage 4 could not go to completion, and therefore we carried out experiments with an excess of activating agent.

The reaction of (**1**) with TPS-Cl (3 equiv.) proved to be fast. The first spectrum recorded after 2 min was similar to that recorded after 1 h during the reaction with 1 equiv. of activating agent. However, in addition to the signals from intermediates (D)—(G), two new singlets at δ *ca.* 31 p.p.m. [intermediate (I)] were observed (spectrum 7). During the following hours gradual changes occurred, and finally (after 18 h) the spectrum consisted of the two singlets at δ *ca.* 31 p.p.m. [intermediate (I)], the four signals at δ *ca.* 16 p.p.m. [intermediate (G)], and a singlet at δ −4.6 p.p.m. [intermediate (E)] (spectrum 8).

To gain more information about structural features of the observed intermediates, we recorded, in separate experiments, spectra without ^1H heteronuclear decoupling.

The broad singlet at δ 3.7 p.p.m. observed during stages 1 and 2 of the reaction then appeared as a doublet ($^1J_{PH}$ 619 Hz). A large coupling constant, characteristic of the P–H bond, was also observed for the (C-1) signal ($^1J_{PH}$ 721 Hz), but not for (B), (C-2) (spectrum 6), and intermediate (A). Addition of water at this stage produced compounds (**1**), (**2**), and (**4a**). The chemical shift of the latter was identical with signal (B).

^{31}P N.m.r. spectra ran during stages (3) and (4) showed no



^{31}P N.m.r. spectra of reaction mixtures (in pyridine): 1, (**1**) + TPS-Cl (1 equiv.), 18 h; 2, as 1, but water added; (3), (**1**) + TPS-Cl (1 equiv.), 2—3 min; 4, (**1**) + TPS-Cl (1 equiv.), 15—20 min; 5, (**1**) + TPS-Cl (1 equiv.), 1 h; 6, as 3, but without ^{1}H-heteronuclear decoupling; 7, (**1**) + TPS-Cl (3 equiv.), 2—3 min; 8, (**1**) + TPS-Cl (3 equiv.), 18 h

compounds with a P–H bond, and addition of water to the reaction mixture produced compounds (**2**), (**3**), and (**4a**).

Interpretation of the ^{31}P *N.m.r. Spectra recorded during Reaction of* (**1**) *with TPS-Cl.*—Broadening and shifting of the signal from nucleoside hydrogenphosphonate (**1**) (shift from δ 2.5 to 3.7 p.p.m.) could arise as a result of fast equilibrium between H-phosphonate (**1**) and mixed phosphonic–sulphonic anhydride (**9**), which should be the first reactive species formed in this reaction. However, since an identical effect was observed when toluene-*p*-sulphonic acid was added to a pyridine solution of hydrogenphosphonate (**1**), we

^{31}P N.m.r. data of substrates, products, and postulated intermediates in the reaction of phosphonate (**1**) with arenesulphonyl chlorides

Compound	Chemical shift [δ(p.p.m.)][a]	$^{1}J_{PH}$/ Hz[b]	$^{2}J_{PP}$/ Hz	$^{3}J_{PH}$/ Hz[b]
(**1**)	2.5 (s)	608		7.8
(**2**)	1.2 (s)			6.8
(**3**)	−10.3 (s)			3.1
(**4a**)	15.4 (s)			8.7
(**4b**)	14.7 (s)			8.5
(**5**)	P_α −1.0 (d), −1.3 (d) P_β −10.4 (d)	721	20.4	(−)
(**6a**)	P_α 14.3 (d), 13.8 (d) P_β −10.4 (d), −10.6 (d)[c]		31.5	8.0
(**6b**)	P_α 13.3 (d), 12.8 (d) P_β −10.6 (d), −10.8 (d)[c]		31.7	8.1
(**7**)	−4.6 (s)			7.6
(**8**)	P_α −21.5 (t) P_β −11.0 (d), −11.1 (d)[c]		17.3	(−)
(**11a**)	16.9 (s), 16.4 (s), 15.3 (s), 15.2 (s)			(−)
(**11b**)	15.0 (s), 14.5 (s), 13.6 (s)			(−)
(**13a**)	31.1 (s), 31.0 (s)			(−)
(**13b**)	31.0 (s), 30.9 (s)			(−)
(**14a**)	118.6 (dd)		10.6; 15.9	10.4
	112.7 (t)		11.3	11.3
	110.3 (dd)		12.2; 15.9	11.4
(**14b**)	113.9 (t), 112.2 (d)		11.1	(−)

[a] Chemical shift relative to 2% H_3PO_4 in D_2O (inner tube). The value of chemical shifts for the intermediates produces *in situ*, in some cases varied (±1 p.p.m), depending on the reaction conditions. [b] Spectra without H^1 heteronuclear decoupling; (−) means that $^{3}J_{PH}$ was not estimated. [c] Two overlapping doublets.

attributed this phenomenon to physical interactions rather than to formation of a new chemical species. The value of the coupling constant ($^{1}J_{PH}$ 619 Hz), very close to that for (**1**), supports this assumption.

The 11 signals in the region δ 120—110 p.p.m. [intermediate (A)] seem to arise from one compound since the relative intensities of all signals remained constant during the course of the reaction. The values of chemical shifts and absence of P–H bonds indicate a trivalent co-ordination state for phosphorus, and the large number of signals suggest that intermediate (A) contains more than two phosphorus atoms. The eleven resonances for intermediate (A) are arranged in three groups at δ *ca.* 119, 113, and 111 p.p.m., containing four signals of equal intensity, three of intensity ratio 1:2:1, and four signals of equal intensity, respectively. With the help of 2-D ^{31}P–^{31}P correlated (^{1}H heteronuclear decoupled) n.m.r. spectroscopy, it was found that all three groups of signals are coupled one to each other. This indicates that the phosphorus atoms in intermediate (A) are arranged in a ring. Thus, the most likely structure which is consistent with the ^{31}P n.m.r. data, is that of $P^1P^2P^3$-trinucleoside trimetaphosphite (**14a**). The rather complicated pattern of signals in the ^{31}P n.m.r. spectrum of (**14a**) is due to non-equivalent phosphorus atoms (chiral substituents in a *trans-cis-trans* relationship), this is discussed in more detail in ref. 9. Since the activation of ethyl hydrogenphosphonate with TPS-Cl followed an analogous pathway,[10] we tried to obtain further support for the trimetaphosphite structure from ^{31}P n.m.r. analysis of this reaction. An intermediate similar to (A) was indeed observed, in the same region as the chemical shifts. However, the number of signals was, as expected, reduced and only a triplet (δ 113.9 p.p.m., $^{2}J_{PP}$ 11.1 Hz) and a doublet (δ 112.2 p.p.m., $^{2}J_{PP}$ 11.1 Hz) of intensity ratio 1:2 were observed. This pattern fits $P^1P^2P^3$-triethyl trimetaphosphite (**14b**) in which the ethyl groups have a *trans-cis-trans* relationship.

The chemical shift of intermediate (B) and the splitting pattern in the spectrum without ^{1}H-heteronuclear decoupling indicates that it is the nucleoside phosphorothioate (**4a**). Signals (C-1) and (C-2) seem to represent a two-spin system (AX) and the spectrum is in agreement with the mixed phosphonic–phosphoric anhydride (**5**) [intermediate (C)].

The signals (D-1) and (D-2) also originate from a two-spin system (AX) and can be assigned to the trisubstituted pyrophosphate (**6a**) [intermediate (D)]. The chemical shift and splitting pattern of singlet (E), together with the fact that it starts to become a predominant signal in the ^{31}P n.m.r. spectra at the end of reaction, indicate that it is compound (**7**), *i.e.* the pyridine adduct of nucleoside 3′-metaphosphate [referred to as metaphosphate (**7**) further in the text].

The two groups of signals (F-1) and (F-2) are characteristic for a three-spin system (AX_2) and the ^{31}P n.m.r. data are in agreement with compound (**8**), *i.e.* $P^1P^2P^3$-tris-(5′-*O*-dimethoxytritylthymidin-3′-yl) tripolyphosphate [intermediate (F)].[7,8]

At the fourth stage of the reaction, several intermediates can be detected by ^{31}P n.m.r. spectroscopy, especially when limited amounts of TPS-Cl are used for the reaction (spectrum 5). The signal at δ −9.7 p.p.m. can be attributed to the pyrophosphonate (**3**) [intermediate (H)], and the complicated multiplet at δ −24.5 p.p.m. is probably given by a cyclic metaphosphate and/or polyphosphates.

If our tentative assignments of resonances observed in the ^{31}P n.m.r. spectra during the reaction of hydrogenphosphonate (**1**) with TPS-Cl are correct, we should not observe formation of the nucleoside 3′-phosphorothioate (**4a**) [intermediate (B)] in the presence of excess of TPS-Cl. Instead, its pyrophosphate [compound (**11a**), expected chemical shift δ *ca.* 14—20 p.p.m.] should be formed. In addition, tripolyphosphate (**8**) [intermediate (F)], higher polyphosphates (intermediate at δ −24.5 p.p.m.), and intermediate (D) should be converted into intermediate (E) [metaphosphate (**7**)] and thiopyrophosphate (**11**). However, it is possible that together with pyrophosphate (**11**), also some other reactive species derived from nucleoside phosphorothioate (**4**) [*e.g.* phosphorothioic–sulphonic anhydride (**12**)] can be formed. The chemical shift of the last compound is not known, but one can expect to observe two singlets (for the two diastereoisomers) in the region δ 14—20 p.p.m.[11] for compound (**12**).

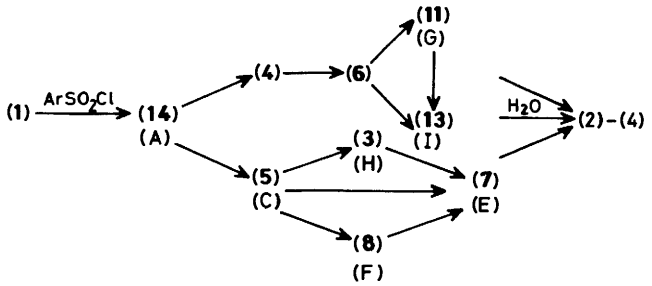
With the above in mind, we interpreted spectra 7 and 8 [reaction of (**1**) with 3 equiv. TPS-Cl] as follows. The four resonances at δ 16.9, 16.4, 15.3, and 15.2 p.p.m. [intermediate (G)] can arise from a two-spin system having two asymmetric phosphorus atoms and the values of the chemical shifts are consistent with the tetrasubstituted thiopyrophosphate (**11a**) (three phosphorus diastereoisomers *R,R*;*S,S*;*R,S*). The two singlets at δ 31.1 and 31.0 p.p.m. [intermediate (I)] are characteristic of a one-spin system of phosphorus diastereoisomers. However, the chemical shift differs substantially from what one should expect for the mixed anhydride (**12**).[11] Since the ^{31}P n.m.r. chemical shift of phosphorus compounds is determined primarily by the nature of the chemical bonds formed directly with the phosphorus atom, one may assume that these two resonances arise from a compound that contains a P–Cl bond in addition to the P–O and P–S bonds. Thus we can assign the two singlets at δ 31.1 and 31.0 p.p.m. to the chlorophosphate (**13a**).[12]

Identification of Major Intermediates in the Reaction of (**1**) *with Arenesulphonyl Chlorides.*—The identity of intermediates (A), (C), (E), (F), and (H) has been further supported by their chemical synthesis (see Experimental section). However, since it was rather difficult to prepare compounds (**4a**), (**6a**), (**11a**), and (**13a**) in a different way, for the sake of identification, we carried

out the reaction of hydrogen phosphonate (**1**) with benzenesulphonyl chloride (BS-Cl)* and compared the observed analogues of intermediates (B), (D), (G), and (I) with samples of (**4b**), (**6b**), (**11b**), and (**13b**), synthesized by independent routes.

Nucleoside *S*-phenyl 3′-phosphorothioate (**4b**) was found to be identical with the analogue of intermediate (B), produced in the reaction of hydrogenphosphonate (**1**) with BS-Cl. When compound (**4b**) was allowed to react with 1 equiv. BS-Cl, tetrasubstituted pyrophosphate (**11b**) (three signals at δ *ca.* 14 p.p.m.), analogous to intermediate (G), was formed. When more BS-Cl was added (6 equiv.), pyrophosphate (**11b**) reacted further to produce chlorophosphate (**13b**), which proved to be identical with a compound obtained in the reaction of DMT-T and *S*-phenyl phosphorodichloridate.

Proposed Reaction Pathway for the Activation of (**1**) *with* TPS-Cl.—The results allow us to suggest the reaction pathway in Scheme 2. The trinucleoside trimetaphosphate (**14a**) [intermediate (A)] is most likely formed in a multistep reaction involving hydrogenphosphonic–sulphonic anhydride (**9**) as a first intermediate, formed from nucleoside 3′-hydrogenphosphonate (**1**) and TPS-Cl. Compound (**14a**), having phosphorus atoms in the trivalent form, is much more susceptible to oxidation than tetraco-ordinated hydrogenphosphonate (**1**). Thus (**14a**) reacts with TPS-Cl, again in a multistep reaction, forming metaphosphate (**7**). Since, during the first and second stage of the reaction, hydrogenphosphonate (**1**) is present it can react with metaphosphate (**7**) to produce hydrogenphosphonic–phosphoric anhydride (**5**). Simultaneously, oxidation of



Scheme 2. Pathway for the reaction of nucleoside 3′-hydrogenphosphonate (**1**) with arenesulphonyl chlorides

trimetaphosphite (**14a**) continues affording phosphorothioate (**4a**) and more metaphosphate (**7**). However, since the latter is instantaneously trapped by hydrogenphosphonate (**1**), only phosphorothiorate (**4a**) [intermediate (B)] and a mixed anhydride (**5**) [intermediate (C)] can be detected in the ^{31}P n.m.r. spectra at this stage of the reaction.

When hydrogenphosphonate (**1**) and intermediate (A) are completely consumed, the mixed anhydride (**5**) and phosphorothioate (**4a**) undergo further activation by TPS-Cl. At this stage of reaction, the metaphosphate (**7**) [produced from compound (**5**)] reacts with phosphorothioate (**4a**) forming a trisubstituted pyrophosphate (**6a**) [intermediate (D)]. Since that reaction is apparently slower than formation of metaphosphate (**7**), the later one also starts to accumulate. At the same time several subsequent reactions occur, which lead to the formation of tetrasubstituted pyrophosphate (**11a**) [intermediate (G)] and disubstituted pyrophosphate (**3**) [intermediate (H)]. The latter can be converted into tripolyphosphate (**8**) [intermediate (F)][8] and subsequently into metaphosphate (**7**), which is the most stable species under the reaction conditions.

Interestingly, tetrasubstituted pyrophosphate (**11a**) seems to react further with chloride anion in the presence of TPS-Cl, forming diester chlorophosphate (**13a**). Formation of diester-chlorophosphates was found to be a rather general pathway in the reaction of tetrasubstituted pyrophosphates with arenesulphonyl chlorides.[12] However, it is likely that, under the reaction conditions, chlorophosphates (**13**) can also be formed directly from trisubstituted pyrophosphates (**6**) [intermediate (D)]. In agreement, only small amounts of chlorophosphate (**13a**) were observed in the reaction of tetrasubstituted pyrophosphate (**11a**) with TPS-Cl (3 equiv.).[12]

The formation of nucleoside *S*-aryl 3′-phosphorothioate (**4**) in the reaction of hydrogenphosphonate (**1**) with arenesulphonyl chlorides, unequivocally indicates that the sulphonyl function is reduced to a thio function. However, an attempted reaction of metaphosphate (**7**) with thiophenol in pyridine failed to produce any detectable amount of phosphorothioate (**4b**). This indicates that such a reaction pathway for formation of phosphorothioates (**4**) is rather unlikely. Alternatively, the reduction of arenesulphonyl chlorides produces arenesulphinyl (ArSOX) and arenesulphenyl (ArSX) derivatives (chlorides or sulphinyl–sulphonyl, sulphenyl–sulphonyl mixed anhydrides). These may oxidize hydrogenphosphonate (**1**) into phosphate (**2**) and phosphorothioate (**4**) faster than the arenesulphonyl chlorides. If sulphinyl and sulphenyl derivatives are involved in the oxidation process, their addition should influence the ratio of phosphorothiorate (**4**) to the sum of phosphate (**2**) and pyrophosphate (**3**) in the final mixture after hydrolysis. Indeed, when benzenesulphinic acid (3 equiv.) was added together with BS-Cl (3 equiv.) to the pyridine solution of hydrogenphosphonate (**1**), the ratio of (**4a**) to the sum of (**2**) and (**3**), after hydrolysis, was *ca.* 1:5. This is to compare with the 1:2 ratio in the reaction with BS-Cl alone. On the other hand, when hydrogenphosphonate (**1**) was treated with a mixture of benzenesulphenyl chloride (1 equiv.) and BS-Cl (1 equiv.), the ratio changed to 1.5:1.

Conclusions.—The ^{31}P n.m.r. experiments permit a rather complete description of the activation of nucleoside 3′-hydrogenphosphonates with arenesulphonyl chlorides (Scheme 2). Nucleoside hydrogenphosphonate (**1**) reacts with TPS-Cl producing trimetaphosphite (**14a**), which is subsequently oxidized by TPS-Cl and by the products of its reduction to metaphosphate (**7**) and phosphorothioate (**4a**). These, in turn, undergo several further transformations affording, after hydrolysis, nucleoside 3′-phosphate (**2**), a symmetrical disubstituted pyrophosphate (**3**), and nucleoside *S*-aryl 3′-phosphorothioate (**4a**).

Formation of trimetaphosphites seems to be critical for the oxidation reaction. In agreement with this, we could not detect any oxidation (within 1 h) or formation of trimetaphosphite (**14b**) in acetonitrile without pyridine (or another base).[10] Pyridine can also participate in other stages of the reaction. However, the most important role of pyridine (or other base), in the oxidation of hydrogenphosphonate monoesters by TPS-Cl, is probably to facilitate the formation of trimetaphosphites (**14**). The oxidation step, *i.e.* the reaction of trimetaphosphite (**14a**) with an arenesulphonyl chloride and with products of its reduction (benzenesulphinyl and benzenesulphenyl derivatives), probably involves a sequence of reactions with nucleophilic attack of phosphorus on electrophilic sulphur being the first. Several plausible mechanisms can be envisaged for the further transformations but more studies are needed to find out the most likely pathway for these reactions.

Bis-sulphonic anhydride (**10**), postulated by Hata and coworkers[3] as a key intermediate in this type of reaction, does not

* The reaction of nucleoside 3′-hydrogenphosphonate (**1**) with BS-Cl follows the same pathway as reaction in the presence of TPS-Cl, but is much faster.

seem to be involved in the oxidation process, but may be considered as an intermediate during the formation of trimetaphosphite (**14a**).

Fast oxidation of nucleoside hydrogenphosphonate (**1**) by TPS-Cl explains our previous findings that preactivation of a nucleoside hydrogenphosphonate, followed by addition of a nucleoside, fails to produce the corresponding hydrogenphosphonate diester.[2] The proposed mechanism also provide an explanation for the unsuccessful attempts of Hata and coworkers[3] to isolate a corresponding benzenesulphinic acid from the reaction mixture.

Experimental

Material and Methods.—5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-hydrogenphosphonate (triethylammonium salt) (**1**) was prepared as described previously[2] and purified by silica gel chromatography. Tri-isopropylbenzenesulphonyl chloride (TPS-Cl), benzenesulphonyl chloride (BS-Cl) (Aldrich), and benzenesulphinic acid (B.D.H.) were commercial grade. Benzenesulphenyl chloride[13] and *S*-phenyl phosporothiodichloridate[14] were prepared according to published procedures. Pyridine was refluxed and distilled over P_2O_5, then distilled over CaH_2, and stored over 4 Å molecular sieves. Thin layer silica gel plates (Merck) were used for t.l.c. analysis [solvent: propan-2-ol–concentrated ammonia–water 7:1:2 (v/v)]. ^{31}P N.m.r. spectra were recorded on Bruker WP 200 SY (89.01 MHz) (preliminary data), Varian XL-100 (40.48 MHz), or JEOL JNM-GX 400 FT (161.70 MHz) spectrometers. The chemical shifts are relative to an external standard of 85% H_3PO_4 or relative to 2% H_3PO_4 in D_2O (inner tube), which gives the same chemical-shift values within experimental error. Positive shift values are downfield from the reference.

Preparation of Compounds (**2**), (**3**), (**4b**), (**5**), (**6b**), (**7**), (**11b**), (**13b**), *and* (**14a**).—The following compounds were synthesized in order to compare with products or intermediates observed in ^{31}P n.m.r. spectra during the reaction of hydrogenphosphonate (**1**) with TPS-Cl or BS-Cl. 5′-*O*-Dimethoxytritylthymidine 3′-phosphate (**2**) was prepared as described in the literature[15] and was isolated using silica gel short column chromatography. The corresponding pyrophosphate (**3**) and metaphosphate (**7**) were produced from compound (**2**) in the reaction with 1 and 3 equiv. TPS-Cl, respectively. Nucleoside *S*-phenyl 3′-phosphorothioate (**4b**) was obtained in the reaction of 5′-*O*-dimethoxytritylthymidine with *S*-phenyl phosphorodichloridate 1.2 equiv. in pyridine, followed by hydrolysis and purification on a silica gel column. Compounds (**11b**) and (**13b**) were prepared from (**4b**) in the reaction with BS-Cl (1 and 6 equiv. respectively),[7] and (**13b**) was also prepared from 5′-*O*-dimethoxytritylthymidine and *S*-phenyl phosphorodichloridate. Compounds (**5**) and (**6b**) were produced in pyridine from metaphosphate (**7**) (prepared *in situ* from 2 and 2.5 equiv. of TPS-Cl), followed by addition of hydrogenphosphonate (**1**) (1.2 equiv.) and (**4b**) (1.2 equiv.), respectively.

Trinucleoside trimetaphosphite (**14a**) [intermediate (A)] was rapidly oxidized during the course of the reaction with TPS-Cl, both in pyridine and in acetonitrile with triethylamine, and thus it was difficult to record 2D ^{31}P–^{31}P correlated spectra. However, it was possible to produce (**14a**) under different reaction conditions using hydrogenphosphonate (**1**), diphenyl chlorophosphate (2 equiv.), and triethylamine (3 equiv.) in acetonitrile.[10] After standing for a couple of hours, the precipitate was removed and the clear solution was subjected to 2D ^{31}P–^{31}P correlated (^1H heteronuclear decoupled) n.m.r. analysis (JEOL JNM-GX 400 spectrometer).

General Procedure for the Reaction of (**1**) *with* TPS-Cl *or* BS-Cl.—Before reaction all reagents and n.m.r. tubes were dried overnight in a desiccator under vacuum. Compound (**1**) (0.2 mmol) was dissolved in anhydrous pyridine (3 ml) containing 30% [$^2H_5$]pyridine, or alternatively, (**1**) was dissolved in pyridine, and D_2O (inner tube) was used as source of the lock signal. Various amounts of TPS-Cl or BS-Cl (as stated in the text) were added to the solution and ^{31}P n.m.r. spectra were recorded at 25 °C.

Water (0.5 ml) was added as specified in the text and the mixtures were subsequently analysed using ^{31}P n.m.r. spectroscopy and t.l.c.

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