Reexamination of the Evidence for Solvent-Induced Intramolecular Electrophilic Catalysis by a *cis* Vicinal Hydroxyl Group in Ribonucleoside Phosphorylation Reactions

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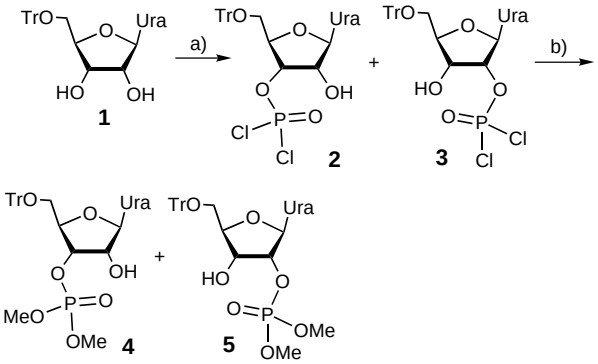
The mechanisms of enzymatic and chemical hydrolysis of RNA have been the subject of intense interest for many years. Early demonstration of a dramatically enhanced hydrolytic reactivity of RNA compared to DNA established a crucial mechanistic role for the 2′-hydroxyl group.[1] Hitherto, it has been accepted that the major role of the 2′-hydroxyl group is nucleophilic catalysis and the 2′,3′-cyclic phosphate intermediate is well established. The comparatively recent discovery of RNA catalysis (ribozymes)[2] has stimulated renewed interest in the potential role(s) of the 2′-hydroxyl group in an increasingly diverse range of reactions. Thus, the report by Roussev et al.[3] of the *electrophilic catalysis* of displacement reactions at a neighboring phosphoryl center by the *cis* vicinal hydroxyl group, if it is correct, offers a potentially novel mechanism that could be of significance to ribozymes. The importance of this claim prompted the present investigation.

Roussev and colleagues compared the reactions of 5′-protected ribonucleosides and 5′-protected-2′-deoxyribonucleosides with an excess of phosphorus oxychloride and pyridine in CH_2Cl_2 followed by treatment with an excess of an alcohol.[3] In the case of the ribonucleosides the two-stage sequence led to a mixture of the corresponding 2′- and 3′-phosphate triesters (Scheme 1) while in the case of the 2′-deoxyribonucleosides the 3′-phosphate triester was produced. Importantly the substrates with a free 2′-hydroxyl group showed much greater reactivity in the second stage, which is the alcoholysis of the intermediate chlorophosphates. This was rationalized in terms of the *cis* vicinal hydroxyl group hydrogen bonding to the P=O group of the ribonucleoside phosphorodichloridate **2** or **3**, thereby assisting the subsequent nucleophilic displacements of chlorine by electrophilic catalysis. Crucial to this rationalization was the identification of the intermediate as a mixture of the regioisomeric phosphorodichloridates **2** and **3**. This assignment was based on the ^{31}P NMR spectrum of the reaction mixture. The relevant signals in the case of 5′-O-trityl uridine were reported



Scheme 1. Reaction scheme proposed by Roussev et al.[3] for the two step synthesis of the 5′-protected ribonucleoside phosphate triesters. Reagents: a) $POCl_3$ and pyridine in CH_2Cl_2; b) MeOH.

as $\delta = 22.40$ (d, $^3J_{P,H} = 12$ Hz) and $\delta = 20.03$ (d, $^3J_{P,H} = 10$ Hz). The cyclic phosphorochloridate structure **6a/b** was assigned to a minor resonance (6–7%) at $\delta_P = 20.01$ (t, $^3J_{P,H} = 9.2$ and 8.4 Hz), and this signal apparently did not increase with time (1 h). Reaction of the 5′-O-trityl-2′-deoxyuridine under the same conditions gave the phosphorodichloridate **7** with a ^{31}P chemical shift of $\delta = 6.7$ (d, $^3J_{P,H} = 11$ Hz). Treatment of the intermediates with an excess of methanol led to the nucleoside dimethylphosphate triesters **4/5** ($\delta_P = 1.11$ and 0.92) and **8** ($\delta_P = 0.65$), respectively.

Two aspects of these observations seemed to warrant closer examination. Firstly, the resonance signals attributed to structures **2** and **3** are substantially further downfield than would be expected for an acyclic phosphorodichloridate,[4] whereas, the ^{31}P NMR chemical shift of the corresponding phosphorodichloridate **7** derived from 5′-O-trityl-2′-deoxyuridine is in line with expectation. To account for the large downfield shift for **2** and **3** relative to **7** ($\Delta\delta_P = 13-15$) the authors again invoke hydrogen bonding between the P=O group and the *cis* vicinal hydroxyl group. Noteworthy, however, is that a comparable hydrogen bond could exist in the product phosphate triesters **4** and **5** ($\delta_P = 1.11$ and 0.92) and yet the chemical shifts for these differ little from that of the corresponding 2′-deoxytriester **8** ($\delta_P = 0.65$). Secondly, if the species responsible for the minor resonance at $\delta_P = 20.01$ is indeed the cyclic phosphorochloridate **6a/b** it is not clear

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why more cyclization of **2** and **3** does not take place, since presumably **2** and **3** will be intermediates in the formation of **6a/b**. The only way around this would be to suggest that the mixture represents a thermodynamic equilibrium. At odds with this, however, are the many examples where cyclic phosphorochloridates are prepared in good yield from 1,2-diols and $POCl_3$,[5] and the demonstration of very favorable cyclization onto phosphorus when the product is a five-membered ring.[6]

In our hands, when 5′-O-trityl uridine **1** was treated with excess $POCl_3$ in dichloromethane in the presence of pyridine the ^{1}H-decoupled ^{31}P NMR spectrum was closely similar to that reported by Roussev et al.[3] Two resonance signals were observed at $\delta = 22.20$ and $\delta = 21.00$ in a ratio of approximately 60:40, apparently corresponding to the two resonances previously reported. Interestingly we did not observe the third smaller resonance that was attributed to the cyclic phosphorochloridate **6**. However, the ^{1}H-coupled ^{31}P NMR spectrum differed markedly from that previously reported.[3] The downfield signal at $\delta_P = 22.20$ appeared as a doublet of doublets with J values of 3.5 and 12.4 Hz (Figure 1). One of

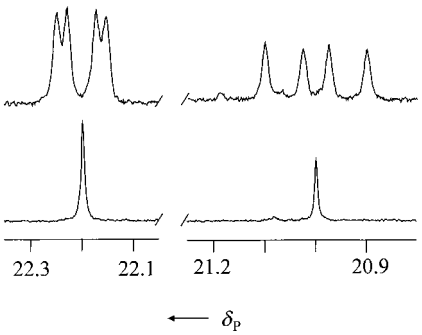
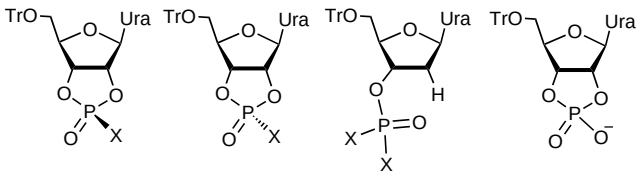
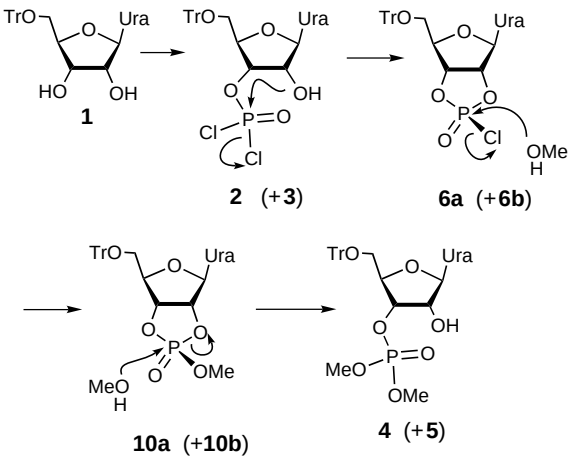


Figure 1. ^{31}P NMR spectra (162 MHz; Bruker ARX400, lower trace) of the intermediates in the reaction of 5′-O-trityl uridine with $POCl_3$, showing the proton coupling (upper trace) consistent with the cyclic phosphorochloridate compounds **6a** and **6b**.

these coupling constants is small and, with line broadening, the signal may appear as a doublet. The upfield resonance at $\delta_P = 21.0$ also appeared as a doublet of doublets, with J values of 20.3 Hz and 12.1 Hz, Figure 1, and in this case it is difficult to see how it could be interpreted as just a doublet with a J value of 10 Hz. Roussev et al. noted that this signal was close to the additional resonance assigned to the cyclic phosphorochloridate **6** ($\Delta\delta_P = 0.02$) so there would have been significant overlap of the ^{1}H-coupled ^{31}P NMR resonances which must have made the assessment of the multiplicity more difficult.[3] The straightforward explanation of our observations is that the species with resonance signals at $\delta_P = 22.20$ and 21.00 are the diastereoisomeric cyclic phosphorochloridate compounds **6a** and **6b**. Each of these would be expected to give rise to a doublet of doublets in the ^{1}H-coupled ^{31}P NMR spectrum because of coupling with both the 2′- and 3′-protons. The size of the coupling constants would, of course, depend on the conformations of the rings. Examination of the ^{1}H–^{31}P 2D NMR spectrum confirmed that both the 2′ and 3′ protons are indeed coupled to both of the signals $\delta_P = 22.20$ and 21.00. Such coupling is incompatible with structures **2** and **3**, but would accord well with the diastereoisomeric cyclic phosphorochloridates **6a** and **6b**.[7] The values of δ_P for the phosphorochloridates are consistent with structures involving a phosphoryl group in a five-membered ring so the need to invoke hydrogen-bond induced shifts of remarkable proportions is avoided.[8] Further evidence that these two intermediates are indeed the two cyclic phosphorochloridates **6a** and **6b** was obtained from hydrolysis of the mixture using an excess of water. The resulting cyclic phosphate diester **9** is dramatically more resistant to further nucleophilic attack than are the cyclic phosphate triesters **10a/b**, and gave rise to a singlet



$\delta_P(H_2O\text{-MeOH}, 9:1) = 19.89$ in the ^{1}H-decoupled ^{31}P NMR spectrum and a doublet of doublets, ($^3J_{P,H} = 6$ and 12 Hz) in the ^{1}H-coupled spectrum. The mass spectrum of the hydrolysis product confirmed the cyclic structure **9** (m/z (-ES) 547 [M^-]). Finally we confirmed that the intermediate phosphorochloridates now assigned structures **6a** and **6b** do indeed give 5′-O-trityl uridyl 2′- and 3′-dimethylphosphates **4** and **5** on treatment with an excess of methanol.[9] However, in contrast to Roussev et al. we were able to detect transient species ($\delta_P = 20.20$ and 19.80); these are almost certainly the diastereoisomers of the cyclic phosphate triesters **10a/b** which on subsequent ring opening give 5′-O-trityl uridyl 2′- and 3′-dimethylphosphate compounds **4** and **5** (Scheme 2). Furthermore, when methanol was added in portions it was possible to demonstrate by ^{31}P NMR spectroscopy the simultaneous disappearance of the cyclic phosphorochloridates **6a** and **6b** and the appearance of the cyclic phosphate triesters **10a** and **10b**.



Scheme 2. Revised reaction scheme in accord with the identification of the intermediates with resonance signals at $\delta_P = 22.20$ and 21.00 as the cyclic phosphorochloridate compounds **6a** and **6b**.

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Noteworthy is that reaction mixtures containing the cyclic phosphorochloridate compounds **6a** and **6b** showed signs of a secondary reaction characterized by a very small downfield shift of the signals $\delta_P = 22.20$ and 21.00. After about 24 h this secondary reaction appeared to be complete. It seems likely that the uracil ring is reacting with $POCl_3$/pyridine at the 3,4-positions transforming the -NHCO- moiety into -N=CX- (X = Cl or $C_5H_5N^+$). The mass spectrum of the hydrolysis product now suggested structure **9** but with the uracil 3,4-NHCO group replaced by -N=CX- (X = $C_5H_5N^+$; *m/z* (+ES) 610 [*M*+H$^+$]; *m/z* (−ES) 644, 646 [*M*+Cl$^-$]). Roussev et al. suggest that reaction with $POCl_3$/pyridine affords a convenient method for the introduction of phosphoryl groups in nucleoside chemistry; it is now clear that reaction times must be kept short if the integrity of the heterocyclic base is to be maintained.

From this reinvestigation it is clear that the reaction of 5′-O-trityl uridine with an excess of $POCl_3$ and pyridine in CH_2Cl_2 followed by an excess of methanol gives the corresponding uridyl 2′- and 3′-dimethylphosphates **4** and **5** via the stereoisomeric cyclic phosphorochloridates **6a** and **6b** (Scheme 2) rather than the regioisomeric 2′- and 3′-phosphorodichloridate compounds **2** and **3**. The rate increase seen in the formation of **4** and **5** as compared to the corresponding reaction sequence for the 5′-O-trityl 2′-deoxyuridine arises from the well documented acceleration of nucleophilic displacement reactions at phosphoryl centers held within five-membered rings.[10] Since the early work of Westheimer[10] it has been established that this rate acceleration can be up to 10^8. The initial reaction of the diastereoisomeric cyclic phosphorochloridates **6a/b** with methanol leads to the corresponding cyclic phosphate triesters **10a/b** but these are very susceptible to further nucleophilic attack by alcohol leading to the uridyl 2′- and 3′- dimethylphosphate compounds **4** and **5** (Scheme 2). None of the observations requires the recently postulated novel electrophilic catalysis of nucleophilic displacement reactions at the phosphoryl group of ribonucleosides by the vicinal *cis* hydroxyl group.[3]

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[7] Full analysis of the ^{1}H NMR spectrum (400 MHz) of the reaction mixture containing **6a** and **6b** (and some of the secondary reaction products—see text) was not possible. With the aid of the 2D ^{31}P–^{1}H HMQC (heteronuclear multiple-quantum coherence) spectrum it was possible to locate ^{31}P-coupled multiplets for *both* H$^{2'}$ and H$^{3'}$ in *both* isomers: ^{1}H NMR (400 MHz, CD_2Cl_2/pyridine, 20 °C, TMS) $\delta_H = 5.68$ and 5.41 (isomer with $\delta_P = 21.0$) and 5.59 and 5.30 (isomer with $\delta_P = 22.2$). These resonance signals are >1 ppm downfield of the corresponding signals in trityl uridine ^{1}H NMR spectrum (400 MHz, CD_2Cl_2/pyridine, 20 °C, TMS) $\delta_H = 4.46$ (dd, $J_{H,H}$ 6, 6.5 Hz, H$^{3'}$) and 4.32 (dd, $J_{H,H}$ 5, 3.5 Hz, H$^{2'}$)].

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[9] Isolated by chromatography (silica, chloroform:methanol, 95:5) as the mixture of regioisomers; **4** (60%) ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta_P = 0.01$ (d × sept, $J_{P,H} = 6$, 11 Hz); ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C, TMS) $\delta_H = 9.25$ br (s, NH), 7.70 (d, $J_{H,H} = 8$ Hz, H^6), 7.4–7.15 (m, Ph_3C), 5.91 (d, $J_{H,H} = 4$ Hz, H$^{1'}$), 5.28 (d, $J_{H,H} = 8$ Hz, H^5), 4.86–4.79 (m, H$^{3'}$), 4.41 (dd, $J_{H,H} = 4$, 5 Hz, H$^{2'}$), 4.26 (m, H$^{4'}$), 3.72 (d, $J_{P,H} = 11.5$ Hz, OMe), 3.66 (d, $J_{P,H} = 11$ Hz, OMe), and 3.52–3.37 (m, 2H$^{5'}$); **5** (40%) ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta_P = 0.59$ (d × sept, $J_{P,H} = 7$, 11.5 Hz), ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C, TMS): $\delta_H = 8.56$ (s, NH), 7.84 (d, $J_{H,H} = 8$ Hz, H^6), 7.4–7.15 (m, Ph_3C), 5.98 (d, $J_{H,H} = 2.5$ Hz, H$^{1'}$), 5.24 (d, $J_{H,H} = 8$ Hz, H^5), 4.86–4.79 (m, H$^{2'}$), 4.52 (dd, $J_{H,H} = 6.5$, 4.5 Hz, H$^{3'}$), 4.09 (m, H$^{4'}$), 3.77 (d, $J_{P,H} = 11.5$ Hz, OMe), 3.76 (d, $J_{P,H} = 12$ Hz, OMe), and 3.52–3.37 (m, 2H$^{5'}$); ES-MS: *m/z* (+ES) 617 [*M*+Na$^+$] 100% and 1211 [2*M*+Na$^+$], 75%.

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