Medium-Controlled Intramolecular Catalysis in the Direct Synthesis of 5′-O-Protected Ribonucleoside 2′- and 3′-Dialkylphosphates**

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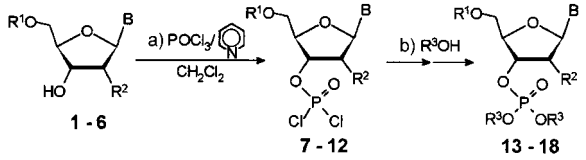
Understanding of the mechanisms of enzyme action has progressed considerably in recent years due to rapid developments in crystallography, enzymology, and chemistry. The introduction of substrate mimics[1] has become a powerful tool for the elucidation of the mechanistic aspects of RNA and enzyme catalysis. Model reactions in aprotic organic solvents are considered to accurately mimic the environment of the active site in the enzyme, which contains little or no water and where intramolecular catalysis would be favoured. In aprotic media, hydrogen-bonding interactions of the reagents with the solvent are absent or weak, which allows the catalytic or inhibititory effects on the reaction rates by hydrogen-bond donors and acceptors to be studied. Research into structure–activity relationships and solvent effects has shown that the reaction mechanisms can be dramatically altered by changing reactant substituents and/or the reaction medium.[2]

We describe here the first example of solvent-induced, intramolecular electrophilic catalysis by a *cis*-vicinal hydroxyl group. The phosphate transfer reaction in the synthesis of 5′-O-protected ribonucleoside 2′- and 3′-dialkylphosphates **13**–**15** is accelerated, relative to the corresponding 2′-deoxy- and 2′-O-protected derivatives **16**–**18** (see Scheme 1 and Table 1; isomeric mixtures of 2′-and 3′-compounds **13**–**15** were formed



1, 7, 13 B = Ura, R^1 = Tr, R^2 = OH
2, 8, 14 B = ^{m3}Ura, R^1 = Tr, R^2 = OH
3, 9, 15 B = ^{m3}Ura, R^1 = Me, R^2 = OH
4, 10, 16 B = Ura, R^1 = Tr, R^2 = H
5, 11, 17 B = Ura, R^1 = Tr, R^2 = OMe
6, 12, 18 B = Thy, R^1 = Tr, R^2 = H

Scheme 1. Two-step synthesis of the 5′-O-protected ribonucleoside derivatives. Compounds **7**–**9** and **13**–**15** are formed as an isomeric mixture of the 2′- and 3′-derivatives in ratio ca. 65:35. For simplicity the 2′-derivatives are not shown. Ura = uridine, Tr = triphenylmethyl, Thy = thymine.

but only the 3′-derivatives are shown). In the accelerated reactions, medium-strength selective microsolvation controls the participation of the adjacent hydroxyl group, 2′-OH (or 3′-OH), and prevents the usual, entropically favoured attack of the internal nucleophile. This promotes external substitution

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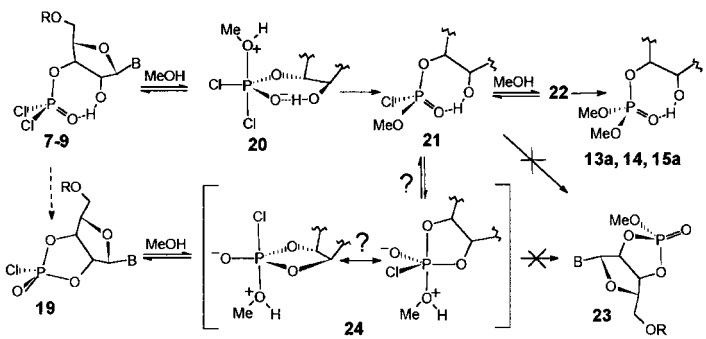
Table 1. Half-life data ($\tau_{1/2}$) showing the acceleration of the phosphate transfer reaction in the syntheses of the 5′-O-ribonucleoside derivatives

Products	R^3OH (see Scheme 1)	Yield [%]	$\tau_{1/2}$ [min][a]
13a	CH_3OH	77	5.5
13b	CH_3CH_2OH	68	12
14	CH_3OH	79	7.5
15a	CH_3OH	73	6.5
15b	$Ph(CH_2)_3OH$	69	14.5
16	CH_3OH	98	267
17	CH_3OH	96	290
18	CH_3OH	97	261

[a] $\tau_{1/2}$ is measured after the addition of R^3OH in the second step of the reaction (Scheme 1).

at the phosphorus atom and provides different phosphoryl transfer mechanisms in the aqueous and organic media. If the solvent characteristics of the enzyme active centers are better mimicked by nonaqueous solvents[3], these findings may provide relevant information as to the possible role of intramolecular catalysis in enzymatic reactions. This approach also provides a rapid, simple, and cost-effective synthetic pathway to the ribonucleoside 2′- and 3′-dialkylphosphates **13**–**15**. These compounds could be used to mimic the neutral, ionic RNA form[4, 5] in model reactions.

The triesters **13**–**18** were synthesized in two steps as shown in Scheme 1. The first step was phosphorylation of the 5′-O-protected nucleosides **1**–**6** by $POCl_3$/pyridine in dry CH_2Cl_2 (see the Experimental Section); this was complete (as judged by RPLC and ^{31}P NMR) within five minutes—the time necessary to record the first ^{31}P NMR spectrum. The second step was the alcoholysis of the formed 5′-O-protected nucleoside phosphodichloridates **7**–**12** with R^3OH. This step took only 20–40 minutes for compounds **7**–**9**, but had to be left overnight for the 2′-deoxy-derivatives **10** and **12**[4–7] and the 2′-*O*-methyl-derivative **11**.[8] Since little of the cyclic monochloridate **19**, the cyclic triester **23** (Scheme 2) or any of the possible diphosphorylated byproducts were detected on the ^{31}P NMR spectra, we tentatively assumed that the presence of the free *cis*-vicinal hydroxyl group enhanced the two consecutive alcoholyses of the ribonucleoside dichlorophosphates **7**–**9**. Our interpretation is consistent with intramolecular hydrogen-bonding participation of the adjacent hydroxy functionality, general acid catalysis induced by the aprotic medium, which promotes external alcoholysis in the ground-



Scheme 2. Possible mechanisms for the formation of the 5′-O-ribonucleoside derivatives. According to Westheimer's guidelines, the first result of nucleophilic attack on **19** should be the structures **24** which are expected to be high-energy structures.

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statestructures **7**–**9** and **21** and/or the pentacoordinated phosphorane intermediate/transition-state structures **20** and **22** (structure **22** is similar to **20** but after substitution of one of the chlorine atoms with the methoxy group).

It is known that 2′-OH nucleophilic assistance causes RNA to undergo rapid hydrolysis in neutral aqueous media and under physiological conditions.[9, 10] Here we point out the distinction between this mechanism, which involves nucleophilic participation of the vicinal hydroxyl group, and our proposed mechanism, in which the hydroxyl group activates an ester by intramolecular general acid catalysis. In polar solvents which can act as hydrogen-bond donors, such as water, the hydrogen-bond acceptor (the phosphate residue) and the intramolecular donor (the adjacent OH group) are solvated separately. Thus, such a medium prevents the intramolecular hydrogen bonding and internal nucleophilic attack at the phosphorus atom by the adjacent OH group is the only possible reaction. On the other hand, in nonpolar, aprotic organic solvents the free vicinal hydroxyl group is capable of acid catalysis[11, 12] as a hydrogen-bond donor, as explained above.

During the substitution reaction, the six-membered ring formed through hydrogen bonding may adopt the diequatorial position, and protonation of the former phosphoryl oxygen atom stabilizes the intermediate/transition state **20**. This promotes the external alcoholysis of the ribonucleoside 3′(and/or 2′)-di/monochlorophosphates **7**–**9** and **21**. The same selective microsolvation by the adjacent hydroxyl group in aprotic solvents has been shown to significantly enhance acyl transfer reactions and is known as the Henbest–Kupcham effect.[13] One example of this is the ester aminolysis of 2-hydroxyphenyl benzoate in acetonitrile relative to that of 2-methoxyphenyl benzoate.[14]

We assumed that the key intermediate structures in the reaction scheme are the derivatives **7**–**9**. To prove this we undertook ^{31}P NMR studies. We monitored the first and second steps of the reaction for 5′-*O*-trityluridine (**1**) relative to those of 5′-*O*-trityl 2′-deoxyuridine (**4**) and 5′-*O*-trityl 2′-methoxyuridine (**5**) with the alcohol being MeOH (Scheme 1). During the first step, the resonances assigned to **7** (3′: $\delta_P = 22.4$, d, $^3J_{PH} = 12$ Hz; 2′: $\delta_P = 20.03$, d, $^3J_{PH} = 10$ Hz; ratio ca. 65:35), **10** ($\delta_P = 6.7$, d, $^3J_{PH} = 11$ Hz), and **11** ($\delta_P = 8.4$, d, $^3J_{PH} = 10$ Hz) indicated a quantitative conversion five minutes after the reaction had been started. The multiplicity of these signals in the ^{31}P–^{1}H coupled spectrum and the $^3J_{PH}$ for **7**, **10** and **11** are similar and, taken in conjunction with the bigger downfield shift ($\Delta\delta_P = 15.7$ and 14) of **7** relative to **10** and **11**, this strongly supports the proposed structure **7** (Scheme 2), involving intramolecular hydrogen bonding. This interpretation is further supported by the existence of a weak hydrogen bond in the ground state of the triester product **13a**, for which a relatively small downfield shift ($\Delta\delta_H = 1.66$) was observed in ^{1}H NMR spectrum for the proton of the 2′(3′)-hydroxyl group.

The cyclization mechanism, however, is not completely avoided. The absorption assigned to the cyclic monochloridate **19** ($\delta_P = 20.01$, t, $^3J_{PH} = 9.2$ and 8.4 Hz)[15] appeared as one signal despite the chirality at the phosphorus atom. The intensity was not over 6–7% and the signal on the ^{1}H NMR spectrum overlaps with those assigned to the 2′-isomer of **7**, but on ^{31}P–^{1}H coupled spectrum they are clearly distinguishable.

The very strong evidence supporting our interpretation that the cyclization route via **19** is almost completely avoided with our conditions was found when the first step of the reaction was prolonged for one hour at room temperature (Scheme 1). There was no change in the intensities, the multiplicities or the ratio of the signals (^{31}P NMR) for the 3′- and 2′-isomers of **7**. The intensity of the cyclic monochloridate **19** remains the same (ca. 7–8%) as at the beginning of the reaction instead of increasing, which one would expect if the nucleophilic participation of the cis-vicinal hydroxyl was not avoided.

When the first-step reaction mixtures were treated with MeOH (Scheme 1) and the ^{31}P NMR spectrum monitored, the signals for **7**, **10** and **11** immediately disappeared (5 min—the time necessary to record the first spectrum). New absorptions for the products **13a**, **16**, and **17** emerged simultaneously at $\delta = 1.11$ and 0.92 (2′- and 3′-isomers in ratio ca. 35:65), 0.65, and 0.8 respectively in {^{1}H} decoupled spectra. After 15 min the intensities of the resonances assigned to **13a** indicated a quantitative yield, while those of **16** and **17** indicated 12.5% and 11% conversion only. No evidence supporting the formation of the cyclic triester **23** (Scheme 2) was found, even after 24 hours reaction time.

The kinetic parameters, presented in Table 1, were obtained by RPLC analysis of aliquots withdrawn regularly from the reaction mixture after the addition of the R^{3}OH. The time-dependent mole fractions of the products from the reaction were calculated from their respective peak areas and the half-life data ($\tau_{1/2}$) were then extracted from the kinetic curves. Yields presented for the ribo-triesters **13**–**15** are for the combined 3′- and 2′-isomers (ratio of ca. 65:35 as judged by RPLC).

By using aprotic solvents for these studies we hoped to mimic the conditions in the enzyme active sites where water molecules are either restricted or excluded. Our interpretation of the results is that the nonaqueous media change the nature of participation of the *cis*-vicinal hydroxyl group from nucleophilic to electrophilic. The in situ, easy-to-handle phosphorylation procedure described here eliminates the difficulties connected with the introduction of protecting groups and the use of complicated phosphorylating agents. Thus, the method may be of general use for the introduction of phosphoryl groups in nucleoside chemistry.

Experimental Section

Materials and Methods. Analytical RPLC was carried out on a NUCLEOSIL 100-5C_{18} column (12.5 cm × 4.6 mm) with isocratic elution (10–55% CH_3CN, depending on ROH, in 20 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.0). Detection was by UV absorbance at 254 nm. Preparative HPLC was carried out using a semi-preparative NUCLEOSIL 100-5C_{18} column (25 cm × 8 mm) with isocratic elution (20–68% CH_3CN in the same buffer). ^{1}H and ^{31}P NMR spectra were recorded on a BRUKER Avance DRX 250 spectrometer with TMS as an internal standard or 85% H_3PO_4 as an external standard at room temperature.

General procedure. Pyridine (4 mmol) was added dropwise to a cooled (5 °C) solution of $POCl_3$ (1 mmol) in dry CH_2Cl_2 (4 mL). After the initial fuming had subsided, the resulting clear solution was added to a precooled mixture (5 °C) of 5′-O-protected nucleoside (0.2 mmol) and pyridine

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(4 mmol) in dry CH_2Cl_2 (4 mL) and the reaction was stirred at room temperature for 5 – 10 min. The appropriate alcohol (4 mmol) was added and stirring continued until the reaction was completed (as judged by RPLC and ^{31}P NMR). The reaction mixture was then evaporated to dryness under reduced pressure (maximum 40 °C), dissolved in acetonitrile, and applied to a semi-preparative RP-HPLC column. Appropriate fractions (2′- and 3′-isomers can be collected separately) were evaporated immediately (reduced pressure, maximum 40 °C), dried several times by co-evaporation with dry acetonitrile or CH_2Cl_2, and kept in a desiccator. The pure samples of the triesters **13** – **18** were prepared by dry extraction from the inorganic buffer salts in acetonitrile or CH_2Cl_2. ^1H and ^{31}P NMR spectra and chromatographic properties of all isolated compounds were identical with the corresponding phosphotriesters obtained by an alternative procedure (see references [4 – 6]).

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Iron-Mediated Diastereoselective Spiroannelations with Vinylogous Urethanes – A Novel Access to Spiroannelated Carbo- and Heterocycles**

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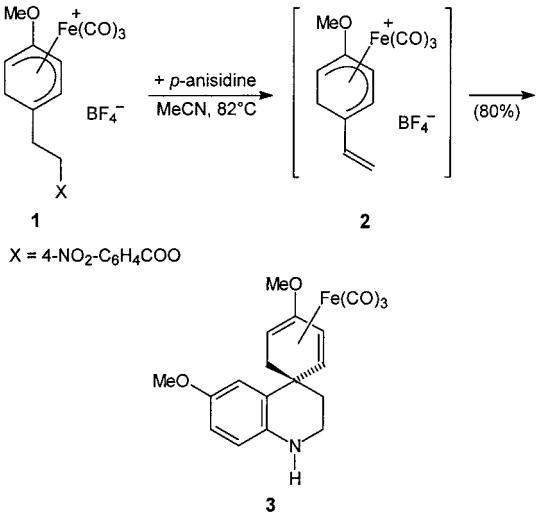
Tricarbonyl(η^4-1,3-diene)iron complexes are widely used in organic synthesis;[1] applications include stereoselective spirocyclization.[2] A few years ago, we reported the diastereoselective iron-mediated spiroannelation of arylamines to spirotetrahydroquinolines.[3, 4] The reaction of the iron complex salt **1** with *p*-anisidine provided the tricarbonyliron complex **3** containing a spiro[quinoline-4,1′-cyclohexane] framework (Scheme 1).[3] Through a deuterium labelling



Scheme 1. Iron-mediated spiroannelation of *p*-anisidine.

study, the one-pot spiroannelation was recently shown to proceed via the intermediate tricarbonyliron-complexed 1-vinyl-4-methoxycyclohexadienyl cation **2**.[5] Moreover, it was demonstrated that arylamines which are more nucleophilic in the *ortho*-amino position show a regioselectivity reversal in their reaction with **1** and afford the spiro[quinoline-2,1′-cyclohexane] framework.[4] The iron-mediated spiroannelation of arylamines was applied to the synthesis of a tetracyclic

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