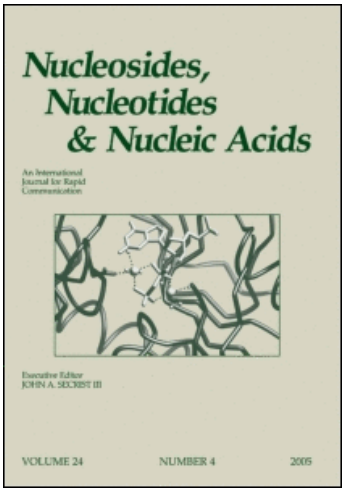

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SYNTHESIS AND STEREOCHEMICAL CHARACTERIZATION OF DIASTEREOMERIC NUCLEOSIDE-PHOSPHOROTHIOSELENOATES BY NMR METHODS

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SYNTHESIS AND STEREOCHEMICAL CHARACTERIZATION OF DIASTEREOMERIC NUCLEOSIDE-PHOSPHOROTHIOSELENOATES BY NMR METHODS

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ABSTRACT

Synthesis and stereochemical characterization of enantiomerically pure nucleoside-phosphorothioselenoates are reported. The effects of solvent and temperature on the vicinal carbon-phosphorus couplings are described and the results are interpreted in terms of conformational changes influenced by stacking interactions between the bases and the phenyl rings.

INTRODUCTION

During the past few years, due to their promising biological importance, several modified mono- di- and oligodeoxynucleotide analogues were synthesized. The modification at the phosphorus atom results in chiral phosphate derivatives, and the biological activity of some diastereomeric nucleotide analogues were found[1] to be governed by the configuration at the phosphorus atom (R_p or S_p). Different nuclear magnetic resonance spectro-

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scopic methods have developed as powerful tools to study the configurational and conformational properties of diastereomeric phosphate derivatives.

In previous papers we reported for some 5′-protected H-phosphonates, phosphoramidates[2] and phosphorothioates[3–5] that the vicinal ^{13}C-^{31}P coupling data systematically differ for the diastereomers. The difference of the $^{3}J(C4,P)$ and $^{3}J(C2,P)$ coupling values (ΔJ) of phosphoramidates and -thioates was larger for the R_p than for the S_p isomers (see the corresponding ΔJ values of representative pairs in Table 1)[2,3]. Likewise, characteristic differences in ΔJ were found for diastereomeric S-methyl-methanephosphonothiolates and Se-methyl-methanephosphonoselenolates, the absolute configurations of which were inferred from X-ray studies[6].

It was concluded from our earlier NMR results that in addition to the configurational differences other structural factors, such as the presence of protecting groups and various sidechains, influence also the conformational preferences about the C3′-O3′ bond, consequently the magnitude of the $\Delta J(=^{3}JC4',P - ^{3}JC2',P)$ values.

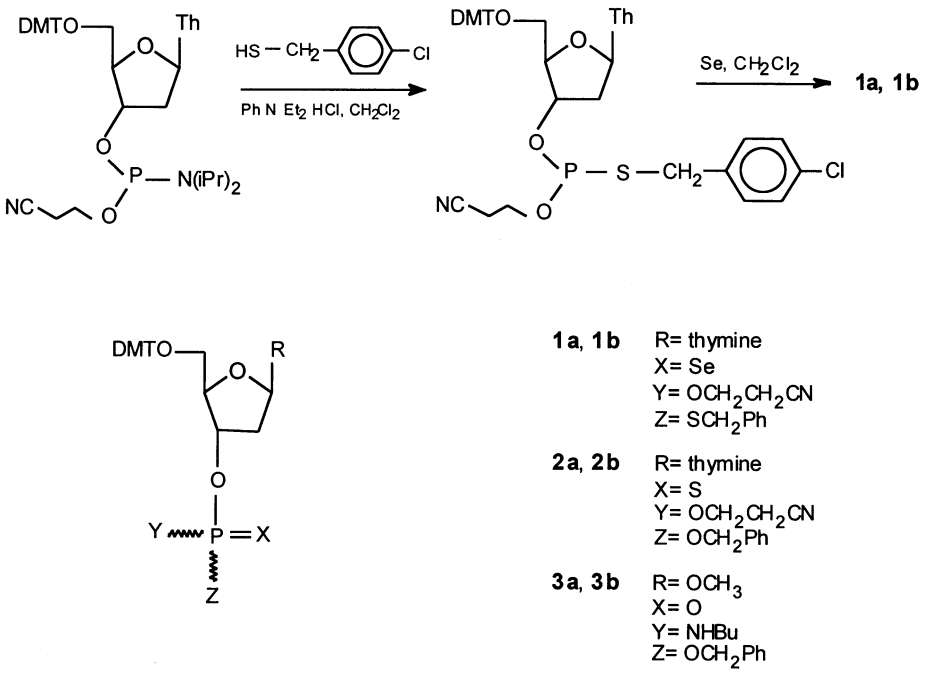
RESULTS AND DISCUSSION

With the aim to study the stereochemistry of the products obtained from the coupling reaction suggested by Caruthers and coworkers[7] using 5′-O-dimethoxytritylthymidine-3′-(S-p-chloro-benzyl)-phosphorodithioate we prepared the corresponding phosphoro-thioselenoate (**1**) by reacting the commercially available 5′-O-dimethoxy-tritylthymidine-3′[(O-β-cyanoethyl)-

Table 1. ^{13}C-^{31}P Vicinal Couplings and ΔJ Values for Diastereomers **1**–**3**[a]

	1a	**1b**	**2a**	**2b**	**3a**	**3b**
$^{3}J(C4,P)$[a]	6.1[b]	5.3[b]	5.6[b]	5.2[b]	7.5[b]	6.7[b]
	6.5[c]	5.1[c]	5.9[c]	5.5[c]	7.2[c]	6.6[c]
	6.6[d]	5.4[d]	6.3[d]	5.9[d]	7.0[d]	6.4[d]
$^{3}J(C2,P)$[a]	4.5[b]	5.4[b]	5.5[b]	5.5[b]	3.0[b]	3.6[b]
	4.0[c]	5.4[c]	5.0[c]	5.3[c]	3.3[c]	3.7[c]
	3.9[d]	5.3[d]	4.9[d]	5.1[d]	3.5[d]	3.9[d]
$\Delta J = {}^{3}J(C4,P)-{}^{3}J(C2,P)$	1.6[b]	−0.1[b]	+0.1[b]	−0.3[b]	4.5[b]	3.1[b]
	2.5[c]	−0.3[c]	+0.9[c]	+0.2[c]	3.9[c]	2.9[c]
	2.7[d]	+0.1[d]	+1.4[d]	+0.8[d]	3.5[d]	2.5[d]
$\Delta\Delta J$	1.7[b]		0.4[b]		1.4[b]	
Configuration	S_p	R_p	R_p	S_p	R_p	S_p

[a] In Hz. [b] In $CDCl_3$ solutions at room temperature. [c] In C_6D_6 solutions at 17 °C. [d] In C_6D_6 solutions at 50 °C.



Scheme 1.

N,N-diisopropyl]-phosphoramidite with p-chloro-benzyl mercaptane in the presence of diethylaniline hydrochloride in dichloromethane and subsequent oxidation with selenium powder[8]. Purification of the product and separation into diastereomers was achieved in one step by column chromatography on silica gel.

Since the X-ray analysis was not realizable for the present nucleoside-phosphorothio-selenoates, the stereochemical assignment of the isomers was based on the $^{3}J(C,P)$ coupling constants by analogy of the much studied relatives. Although the comparison of the ΔJ values allows a prediction of the configuration for diastereomeric nucleoside-phosphorothioselenoates, these parameters show differences from those of structurally related amidates and thioates. Table 1 compiles the relevant ^{13}C-^{31}P coupling constant and ΔJ values for isomers **1a** and **1b** together with those of the relatable phosphorothioate (**2a** and **2b**) and phosphoramidate (**3a** and **3b**) pairs.

Similarly to diastereomeric mono- and dinucleoside-phosphoramidates[2], mono- and di-nucleoside-phosphorothioates[3–5], S-methyl-methanephosphonothiolates and -selenolates[6], the ΔJ values showed systematic differences for the phosphorothioselenoate isomers. The trend observed in the vicinal carbon-phosphorus couplings suggests the stereochemical assignment of compounds **1a** and **1b** as shown in Table 1. It is to be noted, however, that the CIP rule settles opposite absolute configuration in the

thioselenoates (**1a**: S_p, **1b**: R_p) for the same steric arrangement as in the corresponding thioates (**2a**: R_p, **2b**: S_p) and amidates (**3a**: R_p, **3b**: S_p), which is the consequence of the presence of the different (S-benzyl vs. O-benzyl) substituent in the thioseleonates.

The variation of the ^3J(C2,P) and ^3J(C4,P) couplings with the temperature for deoxyribonucleotides in aqueous solution was extensively studied by Lankhorst and coworkers[9]. They have concluded that at room temperature the vicinal coupling constant values, much larger for ^3J(C4,P) than for ^3J(C2,P) couplings, reflect the predominancy of the ε^t conformer. At higher temperature a considerable change was noted for the vicinal ^{13}C-^{31}P couplings (the ^3J(C4,P) values decreased while those of ^3J(C2,P) increased), which was explained by a substantial increase in the amount of the ε^- conformer relative to the ε^t conformer. In our approach this means that smaller ΔJ values refer to a shift towards the ε^- conformational state.

A detailed ^1H NMR study of deoxyribonucleoside methylphosphonate dimers revealed that the conformational preferences differ in the diastereoisomers. As a consequence the configuration of the methylphosphonate group was found to exert an influence on the base stacking of the isomers[10].

According to our recent studies any modification at the phosphorus atom affects the conformational properties of the molecules as reflected by the ΔJ values. Thus e.g. in cases where the sidechain of the compounds studied was benzyl moiety, instead of a second nucleoside unit, stacking interaction between the base and the phenyl ring could also be assumed[5]. Similarly, for the present compounds the replacement of the P=O group with P=S or P=Se group and introduction of S-benzyl or O-benzyl substituents on the phosphorus atom are all expected to influence the conformational properties of the molecules. Moreover, solvent effect and change of temperature[10] are both known to influence the conformational preferences, consequently the ΔJ values. Unfortunately, the liability for decomposition (demethoxy-tritylation), especially in deuterochloroform,

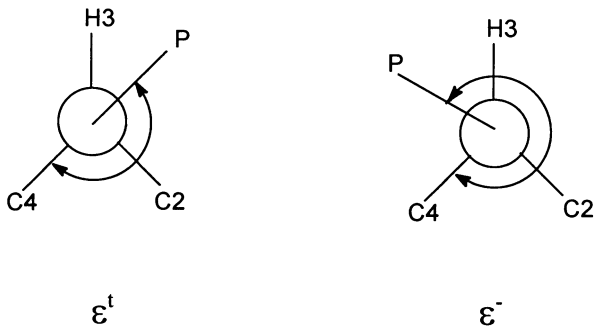


Figure 1. The ε_{trans} and ε_{gauche}(-) conformers.

prevented us from performing detailed conformational analysis of these compounds. Thus the temperature dependent ^{13}C experiments were confined to two (17 and 50 °C) degrees of temperature in deuterobenzene solutions, which allows merely a qualitative interpretation of the $^{3}J(C,P)$ couplings in terms of temperature and solvent dependent equilibrium between the ε^{t} and ε^{-} conformation.

In the phosphoramidates **3a** and **3b** the relatively large ΔJ values revealed the preponderance of the ε^{t} conformation in both diastereomers. The ΔJ values were smaller for one of the isomers in the phosphorothioates and thioselenoates (**2a** and **1a**) and even negative for their isomeric pairs (**2b** and **1b**, respectively) in deuterochloroform solutions (Table 1). The change in ΔJ values of all three pairs of isomers in deuterobenzene at room temperature indicates that solvent effects are fairly important. In the amidates (**3a** and **3b**), upon changing the solvent, the conformational equilibrium shifted away from the ε^{t} domain. At elevated temperature the ΔJ values reflect further increase of the population of the Δ^{-} conformer. On the contrary, the change of solvent and the higher temperature in deuterobenzene solutions of thioates and thioselenoates (**2a**, **2b** and **1a**, **1b**, respectively) have opposite effect i.e. the increase in the ΔJ values points to the increased weight of the ε^{t} conformer in the conformational equilibrium (Table 1). It is to be noted, however, that even under such conditions the isomers maintain their characteristic differences in the ΔJ values.

The possible explanation for the $\varepsilon^{-} \rightarrow \varepsilon^{t}$ shift of the thioates and thioselenoates at elevated temperature is connected with the presence of the O-benzyl and S-benzyl groups. The phenyl rings of compounds **1** and **2** may take part in stacking interactions with the thymin bases, moreover, the solvation in benzene obviously influence the extent of the overlap of the phenyl ring and the base. In the amidates **3a** and **3b**, having a methoxy group instead of the nucleobase, no such interaction occurs.

In dinucleotides the stacking interaction takes place between two bases which are eleven bonds apart from each other. This arrangement allows some extent of rotational freedom about the C3-O3-P-O bonds in the stacked state, while the conformational preferences definitely differ between the dinucleotide isomers. Thus relatively large ΔJ differences ($\Delta\Delta J = 4$ Hz) were found for dinucleoside-phosphoroamidate isomers[2] and dinucleoside-phosphorothioate isomers ($\Delta\Delta J = 3.3-5.1$ Hz)[4,5]. Even larger $\Delta\Delta J$ values were found for the S-methyl-methanephosphonothiolates and Se-methyl-methanephosphonoselenolate isomers (5.2 and 7.0 Hz, respectively)[6], where the lack of the benzyl unit or a second nucleoside excludes the stacking interactions.

In molecules **1** and **2** there are only eight bonds between the phenyl ring and the nucleobase. As a consequence, the conformational flexibility is more restricted for both isomers of **1** and **2** in the stacked state. The consequence of the structural constraint may be the larger weight of the ε^{-}

conformer in the conformational equilibrium relative to the dinucleotides, which results in smaller ΔJ values. It is also of note that the differences in the ΔJ values are larger between the thioselenoate diastereomers ($\Delta\Delta J = 1.7$ Hz) than in the thioate isomers ($\Delta\Delta J = 0.4$ Hz). These observations can be rationalized by considering the geometry differences existing between the S-benzyl and O-benzyl moieties. X-ray data of P-S-CH_2 units gave for the summed bond length ca. 3.8 Å, while the sum was considerably less (~2.8 Å) for the P-O-CH_2 bonds. Thus it seems reasonable to assume that in both **2a** and **2b** the conformational flexibility in the stacked form is reduced in comparison with compounds **1a** and **1b**, and is much less than in the dinucleotides.

It is well documented by the ^{1}H NMR studies of methylphosphonates that higher temperature induces destacking, which results in the detachment of the interacting rings[10]. The fact that at 50 °C in both isomers of **1** and **2** the ε^t conformational state increased in the conformational equilibrium, as observed in the temperature dependence of the ΔJ values, assumes clockwise shift of the phenyl ring with respect to the thymine base. This observation is in agreement with that found for some of the proton chemical shifts of compounds **1** and **2**. An interesting feature is the opposing trend of the H2α chemical shift for the diastereomers **1a**, **1b**, and **2a**, **2b** (Fig. 2). While most of the sugar proton resonances, though to different extents, were shifted upfield as the temperature increased from 17 °C to 77 °C, the H2α resonances of compounds **1b** and **2b** experienced downfield shifts in both deuterobenzene and deuterochloroform solutions.

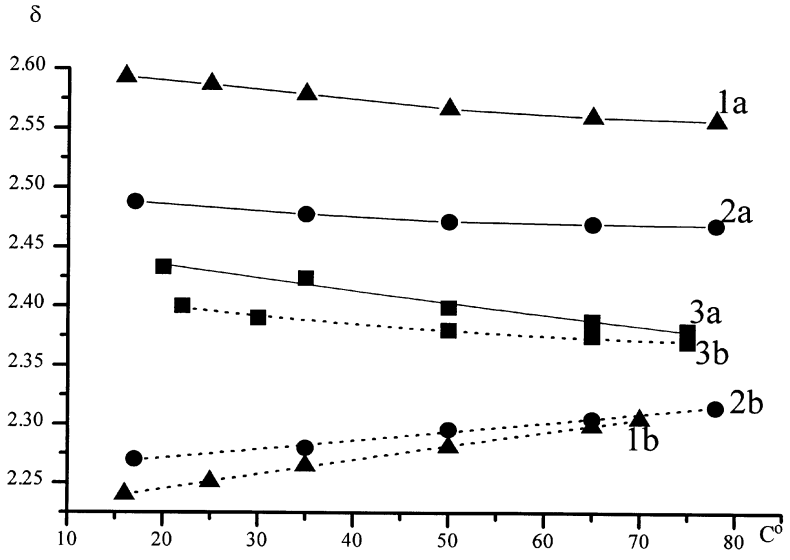


Figure 2. Temperature dependence of H2α chemical shifts in deuterobenzene solutions.

This can be interpreted by the steric arrangement of the stacked states, which differ for the diastereomers.

Molecular models of **1b** and **2b** reveal that in the stacking mode the benzyl group assumes a position where the H2α proton is in the shielding zone of the phenyl ring. As a consequence at room temperature the chemical shift value of H2α in both **1b** and **2b** is significantly upfield in comparison with the corresponding value of their isomeric pairs. Elevation of temperature and consequent destacking leads to decreased shielding of the H2α proton. At the same time the clockwise rotation about the C3-O3 bond places the H1 proton over the face of the phenyl ring. As a result, in compounds **1b** and **2b** this proton resonance shows the largest upfield shift over the temperature range studied ($\Delta\delta = 0.15$ ppm). In isomers **1a** and **2a**, according to molecular models, similar distinctive orientation of the phenyl ring can not be observed. This reasoning is corroborated by the temperature dependence of the sugar proton chemical shifts of phosphoramidate isomers **3a** and **3b**. The absence of the stacking interaction results in the uniform behaviour of the H2α proton shifts with increasing temperature for both isomers (Fig. 2), moreover, the chemical shifts of the H1 protons remain practically unchanged ($\Delta\delta = 0.02$ ppm) in the temperature range studied. In spite of the above differences between the diastereomers of compounds **1** and **2**, elevation of the temperature leads consistently to changes from ε^- to ε^t domains. It is possible that the dimethoxy-trityl moiety affects the conformational network, consequently the stacking interaction of the thymine base with the phenyl ring. The chemical shift changes upon demethoxy-tritylation support this assumption since, in addition to its obvious effect on the H5 protons, remarkable changes were noted in the H2α resonances and in the ΔJ values[5].

CONCLUSION

In summary, we have shown in this work that, similarly to phosphoramidates, phosphorothioates and Me-phosphonates, the trend observed in the ΔJ values allows prediction of the absolute configuration also for the thioselenoate diastereomers. In addition, the magnitude and the solvent and temperature dependence of the ΔJ values provide information about the stacking-destacking processes of some phosphate-modified nucleotides.

EXPERIMENTAL

General. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$ and C_6D_6 solutions using a Varian VXR 400 spectrometer. The chemical shifts were referenced to internal TMS or external H_3PO_4. The measurement of the

^{13}C spectra was repeated with a spectral width of 5000 Hz (covered by 32 K data points). Zero filling gave digital resolution of 0.16 Hz.

Materials. *5′-O-Dimethoxy-tritylthymidine-3′-[(O-β-cyanoethyl)-S-4-chlorobenzyl]-phosphorothio-selenoates* **1a** and **1b**. To the solution of 1.25 g (1.678 mmol) 5′-O-dimethoxytritylthymidine-3′-[(O-β-cyanoethyl)-N,N-diisopropyl]-phos-phoramidite in 20 ml dry dichloromethane 0.05 ml triethylamine and 0.25 ml (2 mmol) 4-chlorobenzyl mercaptane were added followed by 0.43 g (2.3 mmol) N,N-diethylaniline hydrochloride[7]. The mixture was stirred at ambient temperature for 1 h, transferred into a separatory funnel, diluted with 20 ml dichloromethane, washed with 2×10 ml water and dried over $MgSO_4$ (1 h), then filtered and treated with 0.3 g (3.8 mmol) selenium powder[8] with stirring at room temperature for 4 h. The mixture was diluted with 10 ml dichloromethane, washed with 2×10 ml water. Three drops of triethylamine was added before drying over $MgSO_4$. After filtration, the solvent was removed by rotational evaporation giving 1.82 g crude 5′-O-dimethoxy-tritylthymidine-3′-[(O-β-cyanoethyl)-S-4-chloro-benzyl]-phosphorothio-selenoate (**1**) which was purified and separeted into diastereomers by chromathography on 70 g silica gel (TLC grade) eluting with chloroform – acetone 10:1. Evaporation of the appropriate fractions afforded 0.412 g less polar (**1b**) and 0.335 g more polar (**1a**) diastereomers together with 0.130 g nonseparated mixture.

1a: ^{1}H NMR($CDCl_3$): δ 2.42(1H, $J_{gem} = 14.0$, $J_{1,2} = 8.8$, $J_{2,3} = 5.8$, $J_{2,P} = 1$ Hz, 2-H_β), 2.63(1H, $J_{gem} = 14.0$, $J_{1,2} = 5.4$, $J_{2,3} = 1.5$ Hz, 2-H_α), 2.70(2H, m, CH_2CN), 3.37+3.39(2H, $J_{gem} = 10.7$, $J_{4,5} = 2.6$ and 2.5 Hz, respectively, 5-H_2), 4.05(2H, $J_{CH_{2,P}} = 17.3$ Hz, SCH_2), 4.07+4.25(2H, m, OCH_2), 4.12(1H, $J_{3,4} = 1.5$, $J_{4,5} = 2.6+2.5$ Hz, 4-H), 5.46(1H, $J_{2,3} = 5.8+1.5$, $J_{3,4} = 1.5$, $J_{3,P} = 12.0$ Hz, 3-H), 6.40(1H, $J_{1,2} = 8.8 + 5.4$ Hz, 1-H). ^{13}C NMR ($CDCl_3$): δ 19.15($J_{C,P} = 9.2$ Hz, CH_2CN), 38.32($J_{C,P} = 3.7$ Hz, SCH_2), 39.21($J_{C,P} = 4.5$ Hz, C2), 62.52($J_{C,P} = 4.6$ Hz, OCH_2), 63.15(C5), 80.30($J_{C,P} = 6.0$ Hz, C3), 84.13($J_{C,P} = 6.1$ Hz, C4), 84.35(C1). ^{31}P NMR ($CDCl_3$): δ 97.02($J_{P,Se} = 921.1$ Hz).

1b: ^{1}H NMR($CDCl_3$): δ 2.34(1H, $J_{gem} = 14.0$, $J_{1,2} = 8.6$, $J_{2,3} = 5.7$, $J_{2,P} = 1.2$ Hz, 2-H_β), 2.40(1H, $J_{gem} = 14.0$, $J_{1,2} = 5.5$, $J_{2,3} = 1.8$ Hz, 2-H_α), 2.60(2H, t, $J_{vic} = 6.1$ Hz, CH_2CN), 3.42+3.45(2H, $J_{gem} = 10.5$, $J_{4,5} = 2.5$ Hz, 5-H_2), 4.04+4.14(2H, $J_{gem} = 10.5$, $J_{vic} = 6.2$ and 6.1, $J_{CH_{2P}} = 9.5$ and 10.0 Hz, respectively, OCH_2), 4.12(2H, $J_{CH_{2P}} = 17.4$ Hz, SCH_2), 4.24(1H, $J_{3,4} = 1.7$, $J_{4,5} = 2.5+2.5$ Hz, 4-H), 5.40(1H, $J_{2,3} = 5.7+1.8$, $J_{3,4} = 1.7$, $J_{3,P} = 12.5$ Hz, 3-H), 6.39(1H, $J_{1,2} = 8.6+5.5$ Hz,1-H). ^{13}C NMR($CDCl_3$): δ 19.11($J_{C,P} = 9.1$ Hz, CH_2CN), 38.50($J_{C,P} = 3.6$ Hz, SCH_2), 38.83($J_{C,P} = 5.4$ Hz, C2), 62.46($J_{C,P} = 4.5$ Hz, OCH_2), 63.11(C5), 80.22 ($J_{C,P} = 5.6$ Hz, C3), 84.45(Cl), 84.72($J_{C,P} = 5.3$ Hz, C4). ^{31}P NMR ($CDCl_3$): δ 97.45 ($J_{P,Se} = 921.3$ Hz).

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