SYNTHESIS OF DINUCLEOSIDE PHOSPHATES CONTAINING 5′-O-BONDED 1-(6-DEOXY-β-D-ALLOFURANOSYL)URACIL AND 1-(6-DEOXY-α-L-TALOFURANOSYL)URACIL

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1-O-Acetyl-2,3,5-tri-O-benzoyl-6-deoxy-L-talofuranose (*IV*) affords by the reaction with silylated uracil in the presence of stannic chloride, followed by the action of methanolic ammonia, 1-(6-deoxy-α-L-talofuranosyl)uracil (*Vb*). 1-(6-Deoxy-β-D-allofuranosyl)uracil (*IIb*) and/of the compound *Vb* reacts with ethyl orthoformate in the presence of hydrogen chloride under the formation of 2′,3′-O-ethoxymethylene derivatives *III* or *VI* which afford, by the reaction with pyridinium salts of 2′-O-tetrahydropyranyl-5′-O-acetyl-(N-acetyl)ribonucleoside 3′-phosphates *VIIa*—*VIId* in the presence of N,N′-dicyclohexylcarbodiimide and the removal of protecting groups, ribonucleosid-3′-phosphorylyl-(3′→5′)-1-(6-deoxy-β-D-allofuranosyl)uracils *IXa*—*IXd* and/or ribonucleosid-3′-phosphorylyl-(3′→5′)-1-(6-deoxy-α-L-talofuranosyl)uracils *Xa*—*Xd*.

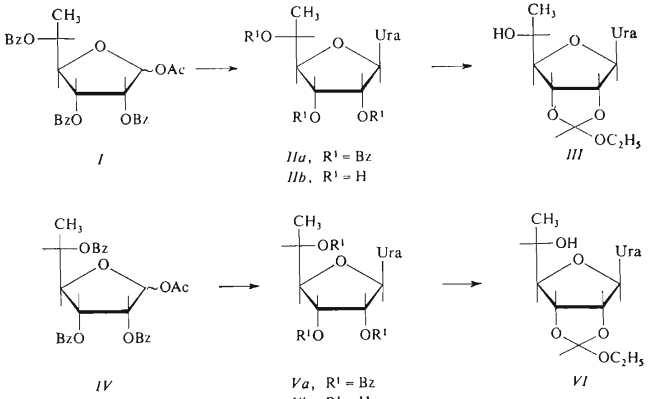
Quite a number of nucleases provided by different types of specificity are operating within and outside the cell. Important information about the structural and conformational requirements of particular enzymes may be extracted from studies using modified diribonucleoside phosphates. Modification can be achieved by chemical changes of heterocyclic base, sugar moiety, or internucleotidic bond.

We wish to report the synthesis of a new type of diribonucleoside phosphates which have been modified on sugar moiety by adding one methyl group to the 5′ carbon bonded by internucleotidic linkage. These modified diribonucleoside phosphates differ from the compounds of this class modified on sugar moiety as yet prepared[1-8] by the change of natural $OH_{primary} \rightarrow OH_{secondary}$ internucleotidic linkage to bis-$(OH_{secondary})$ phosphodiester. Introduction of the new chiral centre on the 5′ carbon would lead to two series of compounds, one of which derived from β-D-allofuranose, the second one derived from α-L-talofuranose. We prepared both series of these diribonucleoside phosphates derived from $C_{(5')}$ methyluridines (*IXa*—*IXd* and *Xa*—*Xd*).

Chemical synthesis of diribonucleoside phosphates *IX*, *X* comprises eight steps, namely, synthesis of parent nucleosides *IIb*, *Vb*, protecting of their 2′,3′-diol systems, preparation of protected ribonucleoside 3′-phosphates, synthesis of internucleotidic

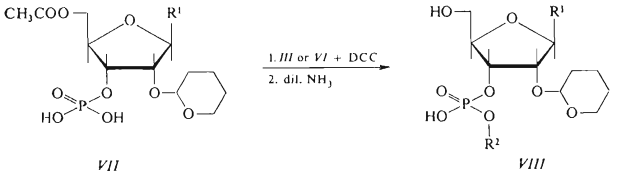
linkages by means of N,N'-dicyclohexylcarbodiimide, partial deblocking of the fully protected condensation products, isolation of alkali stabile protected diribonucleoside phosphates *VIIIa – VIIId*, action of dilute acetic acid to remove acido labile protecting groups, and, finally, the isolation of fully unprotected diribonucleoside phosphates.

As for the first task, the synthesis of analogous adenine nucleosides has been already described[9-12]. Karpeisky and Mikhailov[13] described recently the synthesis of all four nucleosides derived from 6-deoxy-D-allose using 1-O-acetyl-2,3-di-O--benzoyl-5-*p*-nitrobenzoyl-6-deoxy-D-allofuranose as key intermediate. In our work, methods according[9,10] were used. 1-O-Acetyl-2,3,5-tri-O-benzoyl-6-deoxy-β-D-allofuranose (*I*) and 1-O-acetyl-2,3,5-L-talofuranose (*IV*) were isolated in crystalline form. The compounds *I* and *IV* were treated with silylated uracil and stannic chloride[14] in dichlorethane–acetonitrile mixture at room temperature. Chromatographic separation of the products from *allo* derivative *I* afforded 58% of 1-(2,3,5-tri-O-benzoyl-6-deoxy-β-D-allofuranosyl)uracil (*IIa*), 9·5% $N_{(3)}$ isomer[13,14] and 5% of 1,3-bis--(2,3,5-tri-O-benzoyl-6-deoxy-β-D-allofuranosyl)uracil. Analogous reaction with *talo* derivative *IV* gave 51% of 1-(2,3,5-tri-O-benzoyl-6-deoxy-α-L-talofuranosyl)uracil uracil (*Vb*), 28% of 3-N-isomer and 20% of 1,3-bis-glycosilyl derivative. The ratio of $N_{(1)}$, $N_{(3)}$, and $N_{(1)}N_{(3)}$ nucleosides in the reaction of silylated uracils with peracylated sugars in the presence of stannic chloride is determined, according to Niedbala and Vorbrüggen[15], by complex combination of electronic and steric factors. In case



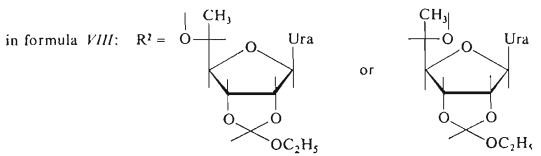
In formulae *I*–*VI*: Bz = COC_6H_5, Ac = $COCH_3$

of the reaction of one particular base with different sugar derivatives containing furanose ring, the conformation of this ring would be the main determining factor. Comparison of our results from glycosilylation of silylated uracil with diastereoisomers of $C_{(5')}$-methyl ribofuranose derivatives with these of ref.[15] showed that the conformation of 6-deoxy-D-allofuranose differed slighly from the conformation of ribofuranose derivative. On the other hand, the conformation of the diastereomeric 6-deoxy-L-talofuranose derivative seemed to be distorted much more profoundly. This distortion might affect higher yields of $N_{(3)}$ (28%) and $N_{(1)}N_{(3)}$ (20%) nucleosides.



VII *VIII*

In formulae *VII* and *VIII*: *a* R^1 = Ura, *b* R^1 = N^4-acetyl-Cyt, *c* R^1 = N^6-acetyl-Ade, *d* R^1 = Gua



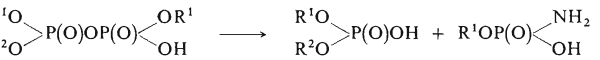
The $N_{(1)}$ nucleoside derivatives *IIa* and *Va* were then debenzoylated by the action of methanolic ammonia. At room temperature, the reaction did not go to the completion even after 70 h owing to the high stability of 5'-O-benzoyl group. Therefore the reaction had to be carried out at elevated temperature in a closed vessel. After 3 h heating to 120°C, the reaction mixtures afforded, after the removal of benzamide by chloroform extraction, 95% of 1-(6-deoxy-β-D-allofuranosyl)uracil (*IIb*) and 58% of 1-(6-deoxy-α-L-talofuranosyl)uracil (*Vb*).

Both nucleosides were then treated with ethyl orthoformate in the presence of hydrogen chloride, according to Žemlička[16], to afford 2',3'-O-ethoxymethylene derivatives *III* and *VI*. Small amount of side products with higher chromatographic mobilities were removed by ether extraction from water solution. The compounds *III* and *VI* were then isolated by extraction with ethyl acetate. Structures in both cases were established by ^{1}H-NMR spectra[17].

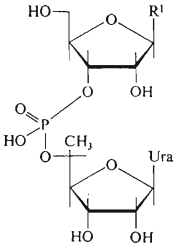
The protected ribonucleoside 3'-phosphates were synthesized according to general scheme suggested by Smrt and Šorm[18] who used the enzymatic transformation of 5'-acetylribonucleoside 2',3'-cyclic phosphates to 5'-O-acetylribonucleoside 3'-phosphates and protected the free $C_{(2')}$-hydroxyl function by reaction with 2,3-dihydropyrane in the presence of hydrogen chloride. 2'-O-Tetrahydropyranyl-5'-O-acetyluridine 3'-phosphate[18] (*VIIa*), 2'-O-tetrahydropyranyl-5'-O-acetyl-N-acetyl-cytidine 3'-phosphate[19,20] (*VIIb*), 2'-O-tetrahydropyranyl-5'-O-acetyl-N-acetyladenosine 2'-phosphate[21] (*VIIc*) and 2'-O-tetrahydropyranyl-5'-O-acetylguanosine 3'-phosphate[21,22] were used.

Synthesis of internucleotidic linkage was carried out by the action of N,N'-dicyclohexylcarbodiimide (according to Gilham and Khorana[23], on the mixture of nucleoside derivative bearing a free $C_{(5')}$-hydroxyl function (*III*, *VI*) and the pyridinium salt of protected ribonucleoside 3'-phosphate in pyridine. Preliminary experiment of N,N'-dicyclohexylcarbodiimide condensation of *III* with *VIIa* under the standard conditions (3 days, 20°C) afforded negligible yield of dinucleoside phosphate. The result was not unexpected as it was already known[12] that phosphorylation of analogous $C_{(5')}$-methyladenosine derivative by means of β-cyanoethylphosphate and N,N'-dicyclohexylcarbodiimide afforded relatively low yield of the 5'-phosphate. We therefore carried out all condensations at elevated temperature (37°C). The reaction mixtures were then diluted with water, and, after 20 h, worked up with ammonia to remove the protecting acetyl groups. Linear gradient chromatography on DEAE-cellulose (HCO_3^-) revealed the common picture of three UV-absorbing peaks, the first containing unreacted nucleoside derivative *III* or *VI*, the second one containing partially protected dinucleoside phosphate *VIIIa* – *VIIId*), and the third one containing 2'-O-tetrahydropyranylribonucleoside phosphate. The dinucleoside phosphate peaks contained the compounds *VIIIa* – *VIIId* (R_f–S_4 0·55) and varying amount of a faster moving substance (R_f–S_4 0·75). The whole mixture was worked up with 30% aqueous acetic acid at 50°C. After 40 min, the compounds *VIIIa* – *VIIId* were deblocked to dinucleoside phosphates *IXa* – *IXd* and *Xa* – *Xd*. The faster moving side products were transformed to ribonucleoside 3'-phosphates by acid treatment. The pure dinucleoside phosphates were obtained by preparative paper chromatography and isolated by lyophilisation.

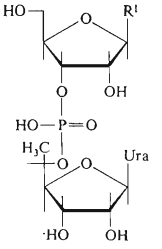
The formation of side products accompanying the dinucleoside phosphates in DEAE-cellulose chromatography might be explained by relatively high stability of asymmetric pyrophosphate formed by reaction of dinucleoside phosphate derivative with excess of phosphorylating agent, in aqueous pyridine. These pyrophosphates were transformed to dinucleoside phosphates and 2-O-tetrahydropyranyl ribonucleoside 3'-phosphoramidates by the action of ammonia. Single charged phosphoramidates wete eluted in dinucleoside phosphate peak by ion exchange chromatography, and were, in turn, transformed to ribonucleoside 3'-phosphate by acid treatment.



The modified dinucleoside phosphates were characterized by their electrophoretic mobility and by enzymatic degradation. Pancreatic ribonuclease degraded to 98% the compounds Up-(*allo*-U-6′-d) (*IXa*), Up-(*talo*-U-6′-d) (*Xa*), Cp(*allo*-U-6′-d) (*IXb*) and Cp(*talo*-U-6′-d) (*Xb*). Gp(*allo*-U-6′-d) (*IXd*) and Gp(*talo*-U-6′-d) (*Xd*) were degraded by ribonuclease T1, and Ap(*allo*-U-6′-d) (*IXc*) and Ap(*talo*-U-6′-d) (*Xc*) by ribonuclease T2. Even these qualitative degradation experiments revealed considerable differences concerning the susceptibility to enzyme action. In general, 1-(6′-deoxy-β-D-allofuranosyl)uracil containing compounds, Up(*allo*-U-6′-d) and Gp(*allo*-U-6′-d) were much better substrates for pancreatic ribonuclease, and ribonuclease T1, respectively, than 1-(6′-deoxy-α-L-talofuranosyl)uracil derivatives Up(*talo*-U-6′-d) and Gp(*talo*-U-6′-d). No significant differences were observed between Cp(*allo*-U-6′-d) and Cp(*talo*-U-6′-d) in pancreatic ribonuclease degradation and between Ap(*allo*-U-6′-d) and Ap(*talo*-U-6′-d) in ribonucelase T2 degradation. The latter enzyme easily splitted Cp(*talo*-U-6′-d) and Gp(*allo*-U-6′-d) but did not degrede Gp(*talo*-U-6′-d).



IX



X

In formulae *IX* and *X*: *a* R^1 = Ura, *b* R^1 = Cyt, *c* R^1 = Ade, *d* R^1 = Gua.

EXPERIMENTAL

TLC was performed on ready-for-use Silufol UV 254 (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_1, chloroform, S_2, chloroform–methanol (95 : 5), S_3, chloroform–methanol (8 : 2), S_3, 2-propanol–conc. ammonia–water (7 : 1 : 2), and S_5, chloroform–methanol (99 : 1). Column chromatography was performed on macroporous silica gel (produced by Service Laboratory of Institute of Organic Chemistry and Biochemistry). Unless stated otherwise, evaporations were performed at 40°C — 2 . 10^3 Pa on a rotatory eva-

porator. Melting points were determined on a heated microscope stage and are uncorrected. UV-Spectra were recorded on the apparatus Specord UV VIS (Zeiss, Jena). ^{1}H-NMR spectra were recorded on the apparatus Varian XL 100 (s singlet, d doublet, t triplet, q quadruplet, br broad; in ppm scale).

1-O-Acetyl-2,3,5-tri-O-benzoyl-6-deoxy-β-D-allofuranose (*I*)

The compound was prepared by modification of the published procedure[9]. Sulfuric acid (8·6 ml) was added to the solution of methyl 2,3,5-tri-O-benzoyl-6-deoxy-D-allofuranoside (20 g; 40·8 mmol) in a mixture of acetic acid (147 ml) and acetic anhydride (14·6 ml) cooled to 10°C under stirring. After 40 h standing, the solution was diluted with chloroform, and poured into ice cold water (500 ml). The mixture was well shaken, the chloroform layer separated and the water layer extracted with three 100 ml portions of chloroform. The chloroform extracts were washed with saturated water solution of sodium hydrogen carbonate, then with water and dried over anhydrous magnesium sulfate. The solution was evaporated, the residue evaporated with two 20 ml portions of toluene and dried at 50°C/13 Pa. Yield 19 g (90%) of compound *I* R_F (S_1) 0·23. A sample recrystallized from methanol had m.p. 149°C. ^{1}H-NMR Spectrum ($CDCl_3$, δ): H_1 6·39 d ($J_{1,2}$ = 1 Hz), H_2 5·75 dd ($J_{2,1}$ = 1 Hz, $J_{2,3}$ = 5 Hz), H_3 5·99 dd ($J_{3,4}$ = 7 Hz, $J_{3,2}$ = 5 Hz), H_4 4·60 dd ($J_{4,3}$ = 7 Hz, $J_{4,5}$ = 4·5 Hz), H_5 5·50 dq ($J_{5,4}$ = 4·5 Hz, $J_{5,6}$ = = 6·6 Hz), H_6 1·42 d ($J_{6,5}$ = 6·6 Hz). 1·80 s.

1-(2,3,5-Tri-O-benzoyl-6-deoxy-β-D-allofuranosyl)uracil (*IIa*)

A mixture of uracil (5·3 g; 48·3 mmol), hexamethyldisilazane (48 ml) and trimethylchlorsilane (2 ml) was refluxed until the solid dissolved (about 2 h). The solution was evaporated (30°C, 130 Pa), the residue dissolved in the solution of the substance *I* (18 g; 34·7 mmol) in acetonitrile (350 ml), and the solution of stannic chloride (5·7 ml; 47·3 mmol) in 1,2-dichloroethane (90ml) was added. After 16 h at 20°C, chloroform (940 ml) and saturated aqueous solution of sodium hydrogen carbonate were added, the mixture was stirred for 1 h and filtered through Celite. The chloroform layer was extracted with saturated solution of sodium hydrogen carbonate (260 ml), then with two 250 ml portions of water, dried over anhydrous magnesium sulfate and evaporated. The residue was dissolved in chloroform (20 ml) and the solution applied on top of silica gel column (3·5 × 100 cm). The column was eluted with chloroform, 15 ml fractions were taken and checked by TIC in S_2. The first substance eluted was the starting compound *IIa* (3 g). The second peak afforded 1,3-bis(2,3,5-tri-O-benzoyl-6-deoxy-β-D-allofuranosyl)uracil (0·8 g; $R_F - S_5$ 0·22). The third fraction ($R_F - S_1$ 0·70) afforded after evaporation and recrystallization from ethanol (20 ml) the compound *IIa* (12·6 g; 58%), m.p. 171°C. The last fraction from the column afforded after evaporated and recrystallization from ethanol 3-(2,3,5-tri-O-benzoyl--6-deoxy-β-D-allofuranosyl)uracil (2·2 g; $R_F - S_2$ 0·22), m.p. 207°C.

1-(6-Deoxy-β-D-allofuranosyl)uracil (*IIb*)

The mixture of the compound *IIa* (7 g) and 6M methanolic ammonia was heated in a steel autoclave to 120°C for 3 h. After cooling, the solution was exaporated, and the residue partitioned between water (100 ml) and chloroform (100 ml). The water solution was extracted with five 50 ml portions of chloroform, and evaporated. The residue was evaporated with ethanol (50 ml) and dissolved in methanol (30 ml). After 20 h at 0°C, the solid was collected, successively washed with methanol and ether and dried under diminished pressure. Yield 3 g (95%) of *IIb*, $R_F - S_3$ 0·15.

1-(2,3-O-Ethoxymethylene-6-deoxy-β-D-allofuranosyl)uracil (*III*)

A mixture of the compound *IIb* (1·03 g; 4 mmol), dimethylformamide (5 ml), ethyl orthoformate (1·6 ml), and 6M solution of hydrogen chloride in dimethylformamide (0·1 ml) was stirred for 20 h. Triethylamine (0·2 ml) was added and the mixture partitioned between water (25 ml) and ether (20 ml). The ether layer was extracted with two 10 ml portions of water, and the combined water solutions were extracted with four 45 ml portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous magnesium sulfate, evaporated and the residue dried at 40°C/12 Pa. Yield 1·24 g (98%) of *III* as a glass, $R_F - S_3$ 0·70.

1-(2,3,5-Tri-O-benzoyl-6-deoxy-α-L-talofuranosyl)uracil (*Va*)

The compound was prepared analogously to *IIa*, starting from 1-O-acetyl-2,3,5-tri-O-benzoyl-6-deoxy-α-L-talofuranose[10] in 51% yield. $R_F - S_3$ 0·82. The reaction also afforded 28% of 3-(2′,3′,5′-O-benzoyl-6′-deoxy-β-L-talofuranosyl)uracil ($R_F - S_3$ 0·65) and 20% of 1,3-bis(2′,3′,5′-tri-O-benzoyl-6′-deoxy-α-L-talofuranosyl)uracil ($R_F - S_5$ 0·61).

1-(6-Deoxy-α-L-talofuranosyl)uracil (*Vb*)

The compound was prepared analogously to *IIb* in 50% yield. M.p. 191°C, $R_F - S_3$ 0·17, $R_F - S_4$ 0·51. UV-spectrum (0·1M-HCl) λ_{max} 263 nm (ε = 10000), λ_{min} 233 nm; (0·1M-NaOH) λ_{max} 263 nm (ε = 7450), λ_{min} 248 nm. For $C_{10}H_{14}N_2O_6$ (258·2) calculated: 46·51% C, 5·47% H, 10·85% N; found: 46·74% C, 5·45% H, 11·12% N.

1-(2,3-O-Ethoxymethylene-6-deoxy-α-L-talofuranosyl)uracil (*VI*)

The compound was prepared analogously to *III*. $R_F - S_3$ 0·43.

TABLE I

Yields and Properties of Dinucleoside Phosphates

Compound	Yield, %	$R_F(S_3)$	E_{Up}
Up(*allo*-U-6′-d)	38	0·29	0·52
Up(*talo*-U-6′-d)	17	0·32	0·51
Cp(*allo*-U-6′-d)	26	0·31	0·44
Cp(*talo*-U-6′-d)	18	0·29	0·57
Ap(*allo*-U-6′-d)	10	0·41	0·51
Ap(*talo*-U-6′-d)	5	0·50	0·50
Gp(*allo*-U-6′-d)	5	0·21	0·57
Gp(*talo*-U-6′-d)	11	0·26	0·47

Synthesis of Dinucleoside Phosphates *IXa*—*IXd* and *Xa*—*Xd*

The solution of pyridinium salt of protected ribonucleoside 3'-phosphate *VIIa*—*VIId* (ref.[18-22]) (0·2 mmol) in pyridine and the solution of the protected nucleoside *III* or *VI* (0·1 mmol) in pyridine (1 ml) were mixed together and evaporated (20°C, 130 Pa. Dry Dowex-50 (pyridinium; 200 mg), pyridine (2 ml) and N,N'-dicyclohexylcarbodiimide (200 mg) were added, and the mixture incubated at 37°C for 60 h. Water (0·5 ml) was added, and, after 20 h, the mixture was diluted with conc. ammonia (8 ml), and shaken with cyclohexane (8 ml). The mixture was allowed to stand another 20 h, filtered and the solid washed with 50% aqueous pyridine (10 ml). The lower layer of the filtrate was evaporated to one half of its volume, filtered, and applied to a column (500 ml) of DEAE-cellulose (HCO_3^-). The column was washed with water (1 l) and then eluted with the use of a linear gradient (2 l of water in the mixing chamber and 2 l of 0·15M triethylammonium hydrogen carbonate in the reservoir). The peak eluate (at about 0·065M buffer concentration) was evaporated, the residue coevaporated with three portions of ethanol and dissolved in 30% aqueous acetic acid (1 ml). After heating to 50°C for 40 min, the solution was chromatographed on one sheet of Whatman 3 MM paper in S_4 for 40 h. UV-Absorbing band (R_{Up} 1·5—2) was eluted with water. Lyophilisation of the eluate afforded ammonium salt of dinucleoside phosphate. For yields, chromatographic and electrophoretic properties see Table I.

REFERENCES

1. Smrt J., Šorm F.: Biochim. Biophys. Acta *138*, 210 (1967).
2. Wechter W. J.: J. Med. Chem. *10*, 762 (1967).
3. Holý A., Šorm F.: This Journal *36*, 3282 (1971).
4. Holý A.: This Journal *38*, 100 (1973).
5. Holý A.: This Journal *39*, 310 (1974).
6. Holý A.: This Journal *40*, 187 (1975).
7. Markiewicz W. T., Wiewiórowski M.: Nucleic Acids Res. *2*, 951 (1975).
8. Ikehara M., Takatsuka Y., Uesugi S.: Chem. Pharm. Bull. *27*, 1830 (1979).
9. Reist E. J., Goodman L., Spencer R. R., Baker B. R.: J. Amer. Chem. Soc. *80*, 3962 (1958).
10. Reist E. J., Goodman L., Baker B. R.: J. Amer. Chem. Soc. *80*, 5775 (1958).
11. Howgate P., Hampton A.: Carbohyd. Res. *21*, 309 (1972).
12. Jones G. H., Moffatt J. G.: J. Org. Chem. *39*, 290 (1974).
13. Karpiesky M. Ya., Mikhailov S. N.: Bioorg. Khim. *5*, 895 (1979).
14. Niedbala U., Vorbrüggen H.: J. Org. Chem. *39*, 3654 (1974).
15. Niedbala U., Vorbrüggen H.: J. Org. Chem. *41*, 2084 (1976).
16. Žemlička J.: Chem. Ind. (London) *1964*, 581.
17. Samek Z.: Unpublished results.
18. Smrt J., Šorm F.: This Journal *27*, 73 (1972).
19. Smrt J., Šorm F.: This Journal *28*, 61 (1963).
20. Alexandrova L. A., Smrt J.: This Journal *42*, 1686 (1977).
21. Holý A.: This Journal *35*, 3686 (1970).
22. Smrt J.: This Journal *39*, 969 (1974).
23. Gilham P. T., Khorana H. G.: J. Amer. Chem. Soc. *81*, 4647 (1959).

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