DIMETHYLAMINOMETHYLENE PROTECTED PURINE H-PHOSPHONATES IN THE SYNTHESIS OF BIOLOGICALLY ACTIVE RNA (24-MER)*

Luboš ARNOLD[a], Jiří SMRT[a], Jaroslav ZAJÍČEK[a], Günther OTT[b], Michael SCHIESSWOHL[b] and Mathias SPRINZL[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia*

[b] *Laboratorium für Biochemie, Universität Bayreuth, D-8580 Bayreuth, F.R.G.*

Received December 13, 1990
Accepted February 15, 1991

Preparation of 5′-O-(4,4′-dimethoxytrityl)-2′-O-tert-butyldimethylsilyl (tBDMSi) derivatives of N^2-dimethylaminomethyleneguanosine (*IIIa*) and N^6-dimethylaminomethyleneadenosine (*IVa*) and their 3′-H-phosphonates (*IIIb*, *IVb*) is described. The compounds *IIIb* and *IVb* together with corresponding derivatives of uridine (*Vb*) and N^4-benzoylcytidine (*VIb*) were used as synthones in machine assisted synthesis of microhelix^{Ala} (5′-GGGGCUAUAGCUCUAGCU. CCACCA-3′) (*X*). The compound *X* was aminoacylated by means of alanyl-t-RNA synthetase (*E. coli*).

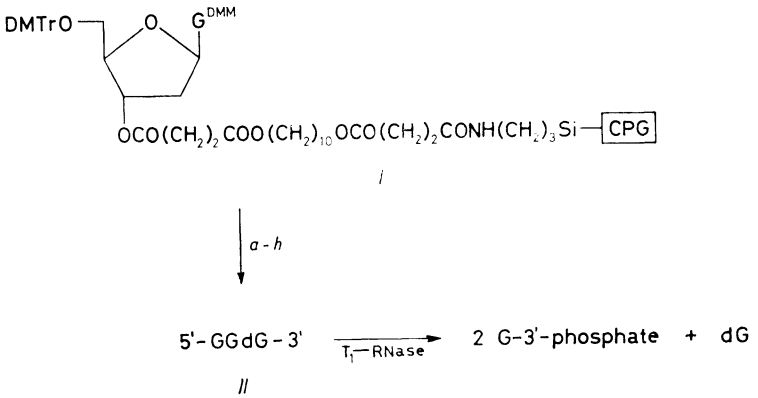
Chemical synthesis of RNA on solid support has been more difficult to evolve than DNA synthesis due to complications introduced by the necessity to find a suitable blocking group for the 2′-OH group in ribonucleosides. As a matter of fact a generally accepted method for the RNA synthesis on solid support does not exist as yet. With the classical tetrahydropyranyl[1] or methoxytetrahydropyranyl[2] 2′-O-protection short RNA chains were prepared. Photochemically sensitive 2-nitrobenzyl 2′-O--protection was described and used in several RNA syntheses[3,4]. Alkalilabile 2′-O--benzoyl derivatives were used in a synthesis of a 7-mer[5]. Recently the method which uses tert-butyldimethylsilyl protection of 2′-hydroxyl groups seems to be the most promissing one[6–10]. The original base protection, G-isobutyryl, A-benzoyl, was improved by introduction of more labile phenoxyacetyl group[11–13]. Some arguments were published about the stability of the silyl groups as 2′-O-protecting groups in the ribooligonucleotide synthesis[1,2,14,15]. Nevertheless, this approach enabled the synthesis of a t-RNA molecule[9]. The strict requirement for low temperature ammonia deprotection of synthetic oligonucleotides with 2′-silyl blocking[15] does not seem to be absolute[9,16].

* Part LXXV in the series Oligonucleotidic Compounds; Part LXXIV: Collect. Czech. Chem. Commun. *55*, 1321 (1990).

The aim of this paper is to describe the preparation of easily accessible supports and synthones for RNA synthesis. Described synthesis of a 24-ribooligomer verified the applicability of this approach.

In our laboratory the idea of the use of more labile N-protection especially for guanine was adopted and the isobutyryl group was succesfully replaced by amidine protection[17] in the machine assisted DNA-synthesis[18]. In order to investigate the applicability of N-dimethylaminomethylene protected guanosine in RNA-synthesis, we tried the silylation of 5'-O-(4,4'-dimethoxytrityl)-N^2-dimethylaminomethylene-guanosine. Surprisingly we found, that the notoriously difficult 2'-silylation of N-acyl protected guanosine proceeded in case of amidine protection with high specificity affording 70% isolated yield of the desired 2'-O-silyl compound *IIIa* which can be easily separated from the 3'-isomer. As monitored by spectroscopy at 300 nm, the dimethylaminomethylene protecting group can be removed by treatment with aqueous ammonia–ethanol mixture at room temperature during 20 h.

The compound *IIIa* was transformed to 3'-H-phosphonate (*IIIb*) by PCl_3-imidazole method[19] and the applicability of this new synthone for the automated RNA synthesis tested in a synthesis of a combined RNA–DNA triplet GGdG (*II*) (Scheme 1). The synthesis was performed starting from 0·3 μmol of blocked 2'-deoxyguanosine bound to porous glass (CPG 10)[18] (*I*) on a synthesizer Syngen-1 (constructed in this institute) using conditions according to Garegg and coworkers[20]. Coupling yield 93% in the first step and 98% in the second step was achieved. The



SCHEME 1

a 3% $CHCl_2COOH$ in 1,2-dichloroethane; *b* *IIIb* + pivaloyl chloride in pyridine–acetonitrile (1 : 1); *c* repeat *a* and *b*; *d* 0·1M I_2 in tetrahydrofuran–pyridine–water–1-methylimidazole (90 : 4 : : 5 : 1); *e* 0·1M I_2 in tetrahydrofuran–water–triethylamine (90 : 5 : 5); *f* 35% aqueous ammonia–ethanol (3 : 1); *g* 1M tetrabutylammonium fluoride in tetrahydrofuran; *h* Biogel P6. DMTr 4,4'-dimethoxytrityl; DMM $=CHN(CH_3)_2$; CPG controlled pore glass

standart oxidation, deblocking and desalting steps[20] afforded 5 O.D.$_{260}$ of *II*, R_F S4 0·03. The product was quantitatively digested by means of T1 RNase to guanosine--3'-phosphate and 2'-deoxyguanosine (ratio 2 : 1), which verified the presence of 3',5'-internucleotidic linkages only. The result showed that the amidine protected guanosine-3'-H-phosphonate derivative may be used in the automated synthesis of RNA.

The same amidine protection was adopted for adenosine. For routine preparation of both purine derivatives *IIIa* and *IVa* the "one pot" procedure was elaborated, starting from unprotected nucleoside and ending with chromatographic isolation of single 2'-O-silyl derivatives. Similar deprotection rate of N^4-benzoylcytidine to deprotection of amidine protected purines with ammonia allowed to use this derivative as a partner in the collection of blocked ribonucleosides for the preparation of 3'-H-phosphonates *IIIb*, *IVb*, *Vb* (ref.[20]), *VIb* (ref.[20]) for RNA synthesis. For ^{1}H NMR spectra see Table I.

For the automated synthesis new solid supports *IXa*–*IXd* were prepared according to the procedure published for DNA supports[18] by condensation of 5'-O-(4,4'-dimethoxytrityl) derivatives of N-phenoxyacetylguanosine and adenosine[12], N^4-benzoylcytidine and uridine (*VIIa*–*VIId*) with bis-N-hydroxysuccinimide ester of

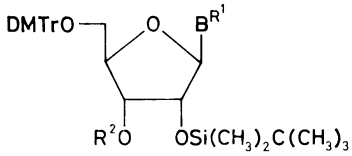
TABLE I

Selected ^{1}H NMR spectral parameters of triethylammonium salts of H-phosphonates[a]

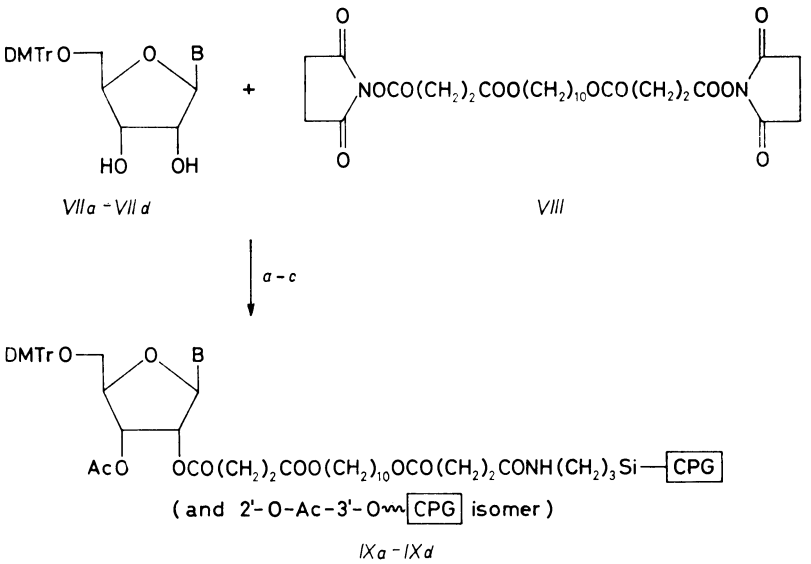
Parameter	*IIIb*	*IVb*	*Vb*	*VIb*
1'-H (d)	6·02	6·14	5·92	5·88
2'-H (m)	4·95	5·00	4·78	4·74
J(1', 2')	4·48	6·27	5·20	6·13
3'-H (m)	4·71	4·93	4·44	4·45
4'-H (m)	4·43	4·46	4·33	4·37
$C(CH_3)_3$ (s)	0·79	0·73	0·79	0·84
CH_3Si (s)	0·06	−0·05	0·06	0·15
CH_3Si (s)	−0·06	−0·23	0·05	0·08
N—CH_2—CH_3 (q)	2·98	3·07	3·06	3·00
N—CH_2—CH_3 (t)	1·25	1·38	1·35	1·28
—$N(CH_3)CH_3$ (d)	3·05	3·19	—	—
5-H	—	—	5·26	7·39
6-H	—	—	7·82	7·88
8-H	7·62	8·46	—	—

[a] Measured on Bruker AC 300 spectrometer (300 MHz) in $CDCl_3$, signals were referenced to signal of residual $CHCl_3$ (δ 7·24 ppm). Chemical shifts in ppm (δ — scale), coupling constants (*J*) in Hz.

1,10-decanediol bis-succinate (*VIII*) and aminopropylsilyltrimethylsilyl CPG 10, 500 Å, followed by capping of 2′ or 3′-OH groups (Scheme 2). With 24 h condensation time loading round 10 μmol per 1 g was achieved. Prolonged condensation time (48 h) afforded loading 20 μmol per 1 g.



IIIa, B = guanine$^-$; R^1 = =CHN(CH$_3$)$_2$; R^2 = H
IIIb, B = guanine ; R^1 = =CHN(CH$_3$)$_2$; R^2 = —PH(O)O$^-$H$^+$N(C$_2$H$_5$)$_3$
IVa, B = adenine ; R^1 = =CHN(CH$_3$)$_2$; R^2 = H
IVb, B = adenine ; R^1 = =CHN(CH$_3$)$_2$; R^2 = —PH(O)O$^-$H$^+$N(C$_2$H$_5$)$_3$
Vb, B = uracil ; R^1 = H ; R^2 = —PH(O)O$^-$H$^+$N(C$_2$H$_5$)$_3$
VIb, B = cytosine ; R^1 = —COC$_6$H$_5$; R^2 = —PH(O)O$^-$H$^+$N(C$_2$H$_5$)$_3$



SCHEME 2

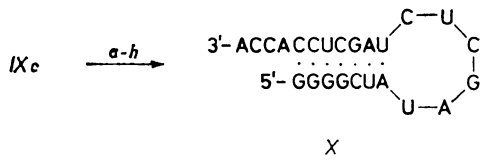
a 4-dimethylaminopyridine in pyridine; *b* 3-aminopropylsilyltrimethylsilyl-CPG 10; *c* acetic anhydride

In formulae *VII* and *IX* B = uracil
B = N^4-benzoylcytosine
B = N^6-phenoxyacetyladenine
B = N^2-phenoxyacetylguanine

The assembly of the ribooligonucleotides was performed on 381A Applied Biosystems machine starting from 1 μmol of blocked nucleoside on the support. 1-Adamantanecarbonyl chloride[21] was used as activating agent without capping procedure. On the synthesis of guanosine containing 11-mer (G_{11}) conditions were tested for optimal reaction time, reagents concentration and excess of H-phosphonates. After that coupling yields of 97% were achieved with twentyfold excess of the reagent and 20 min coupling time. The same satisfactory results were achieved in syntheses of C_{11}, A_{10} and T_{10}. After the two step oxidation procedure[19] (20 and 10 min) the product of the synthesis G_{11} was removed from the support by 20 min action of aqueous ammonia–ethanol mixture and the supernatant tested by UV-spectroscopy in one hour intervals. After 20 h at 20°C the absence of the absorbance at 300 nm indicated fully deblocking of guanine amino groups. The 20 h ammonia treatment was applied also for C_{11}. The product was then treated with 1M tetrabutylammonium fluoride in tetrahydrofurane for 12 h, desalted by means of Sephadex G-25 and a sample digested by snake venom diesterase and bacterial alkaline phosphatase. HPLC analysis showed exclusively the presence of cytidine indicating full removal of N-benzoyl and 2'-O-silyl groups (data not shown).

After finishing this preliminary study three model ribooligonucleotides: 5'-GAC.CUCG-3' (7-mer), 5'-CGGGGUCACCA-3' (11-mer), 5'-UCUGACUCCAGAUCAGA-3' (17-mer) were assembled and oxidized. The products were deblocked under the conditions found for G_{11} and C_{11}. Smear appearance of polyacrylamide gel electrophoresis indicated the presence of not fully deblocked chains. Therefore the syntheses were repeated and the ammonia treatment was prolonged to 48 h. After desilylation step sharp bands were obtained. This result showed that the ammonia deblocking of ribo-chains which may assume secondary structure was slower than deblocking of monothematic chains.

Microhelix^{Ala} (24-mer)[22] (*X*) was assembled by the same procedure starting from 1 μmol of nucleoside bound to the matrix (Scheme 3). After 48 h ammonia–ethanol deblocking and 12 h tetrabutylammonium fluoride treatment the product was desalted by gel permeation chromatography. Oligonucleotide *X* was characterized



X

SCHEME 3

a 3% $CHCl_2COOH$ in 1,2-dichloroethane; *b* *IIIb* or *IVb* or *Vb* or *VIb* + 1-adamantanecarbonyl chloride in pyridine–acetonitrile (1 : 1); *c* repeating of *a* and *b* twenty three times; *d* 0·1M I_2 in tetrahydrofuran–pyridine–water–1-methylimidazole (90 : 4 : 5 : 1); e 0·1M I_2 in tetrahydrofuran–water–triethylamine (90 : 5 : 5); *f* 35% aqueous ammonia–ethanol (3 : 1); *g* 1M tetrabutylammonium fluoride in tetrahydrofuran; *h* Biogel P6

by electrophoretic mobility and HPLC determination of nucleoside composition after digestion with snake venom phosphodiesterase and bacterial alkaline phosphatase (data not shown). Further proof of the structure was the aminoacylation of the product by specific alanine-t-RNA synthetase from *E. coli*[23]. The yield of the aminoacylation was about 16% (Fig. 1). This is in good agreement with the results of Ogilvie and coworkers[9] who described the synthesis of *E. coli* t-RNA^{fMet} with acceptance activity of 11%.

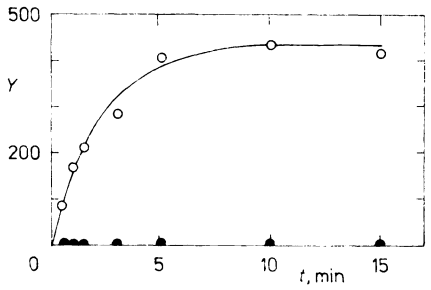
The results showed that amidine protected purine synthones can be used in machine assisted synthesis of RNA chains. H-phosphonate method applied in this paper with 97% step yields allows practical synthesis of chains containing 20–25 bases. For the synthesis of oligonucleotides of higher length, more efficient condensation method, probably phosphoramidite chemistry[9] should be applied.

EXPERIMENTAL

Nucleosides were purchased from Pharma-Waldhof, pyridine (absolute), acetonitrile (HPLC grade), dichloromethane (absolute), N,N-dimethylformamide diethylacetal, 4,4′-dimethoxytrityl-chloride, tert-butyldimethylchlorosilane, imidazole (puriss.), triethylamine (puriss.) were from Fluka and were used without further purification. Phosphorus trichloride (2M solution in dichloromethane) was purchased from Aldrich. 1-Adamantanecarbonyl chloride and 1M tetrabutylammonium fluoride in tetrahydrofuran were purchased from Fluka. Dichloroacetic acid in dichloromethane and pyridine/acetonitrile solution (1 : 1; v/v) came from Applied Biosystems. CPG-supports with spacer were prepared according to ref.[18] from 5′-O-(4,4′-dimethoxytrityl)-N-substituted ribonucleosides. 2′-O-(Tert-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)--uridine-3′-H-phosphonate and 2′-O-(tert-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-N^4-benzoylcytidine-3′-H-phosphonate were synthesized according to known procedures[15]. TLC was carried out on DC-Alufolien Kieselgel (Fluka) using the following eluents: S1, chloroform–2--propanol (9 : 1); S2, chloroform–methanol (9 : 1); S3, toluen–ethylacetate (2 : 1); S4, 2-propanol––concentrated aqueous ammonia–water (7 : 1 : 2). Column chromatography was performed on Kieselgel 60 (Fluka). UV spectra were recorded on a Shimadzu UV-160 UV/VIS spectrometer. HPLC analysis was performed on a Beckman system gold 180 pump modul using the model 167 multiwavelength UV-detector. The automated RNA synthesis was performed on a model 381A

FIG. 1

Aminoacylation (Y, pmol/A_{260} units) in the presence (○) and in the absence (●) of chemically synthesized microhelix^{Ala} with alanyl-t-RNA synthetase from *E. coli* and [^{14}C]alanine (specific activity 162 Ci/mol). At the indicated times 10 µl aliquots were removed from the reaction mixture and the total amount of TCA-precipitable radioactivity was measured



DNA-synthesizer from Applied Biosystems. Electrophoretic gels were 20% polyacrylamide containing 7M urea and were run at a field strength 200 V/cm using buffer containing 89 mM Tris-HCl, 89 mM borate and 89 mM EDTA at pH 8·2 and the oligonucleotides visualised by staining[24]. Enzymes were obtained from Boehringer and Sigma.

2′-O-(Tert-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-
-N^2-dimethylaminomethyleneguanosine (*IIIa*)

A suspension of anhydrous guanosine (2·83 g, 10 mmol) in N,N-dimethylformamide (25 ml) and N,N-dimethylformamide diethyl acetal (6 ml) was stirred overnight. To the clear solution, water (1 ml) was added and after 5 min the solution was diluted with pyridine (100 ml) and the mixture evaporated to a syrup. Pyridine (30 ml) and 4,4′-dimethoxytrityl chloride (3·74 g, 11 mmol) were added, the mixture stirred for 10 min and incubated overnight. Methanol (1 ml) was added and after 10 min the mixture was evaporated to a foam (oil pump). Pyridine (20 ml) and imidazole (26 mmol) were added, the mixture stirred 10 min and tert-butyldimethylchlorosilane (13 mmol) added and stirring continued for 1 h (R_F S1, 0·1—0·65). The mixture was partitioned between chloroform (60 ml) and water (30 ml). The water layer was extracted with chloroform (10 ml) and the combined chloroform extracts were diluted with toluene (30 ml) and evaporated. The residual solvent was removed under oil pump vacuum. The resulting foam was dissolved in chloroform (20 ml) containing triethylamine (0·5%, v/v) and applied on a silica gel column (400 ml), equilibrated in the same solvent. Elution was performed by subsequent use of 250 ml portions of chloroform–triethylamine (99·5 : 0·5), containing subsequently 1, 2, 3, 5 and 6 percent of 2-propanol. After the eluate became DMTr-positive, fractions of 50 ml were collected and checked by TLC in S1. Fractions 12—17 contained pure *IIIa* and afforded after evaporation and coevaporation with toluene 5·1 g (68%; based on guanosine). Fractions 20—22 afforded 1·8 g (24%) of 3′-O-silyl derivative (R_F S1 0·58).

2′-O-(Tert-butyldimethylsilyl)-5′-O-(4,4′-dimethoxytrityl)-N^6-
-dimethylaminomethyleneadenosine (*IVa*)

The compound was prepared from adenosine (2·67 g, 10 mmol) by the same procedure as compound *IIIa*. The terminal purification was performed in the same starting solvent using 250 ml portions of the solvent containing subsequently 0·25, 0·5, 0·75, 1·0 and 1·25 percent of 2-propanol. Appropriate fractions yielded 3·6 g (50%) of *IVa* (R_F S1 0·80) and 2·1 g (29%) of 3′-O-silyl derivative (R_F S1 0·77).

Preparation of H-Phosphonates Triethylammonium Salts *IIIb* and *IVb*

The compounds were prepared by a slightly modified method of Froehler et al.[15]. A solution of imidazole (2·04 g, 30 mmol) in dichloromethane (75 ml) was cooled under stirring on ice and 2M solution of phosphorus trichloride in dichloromethane (4·32 ml) was added by a syringe, followed by addition of triethylamine (4·2 ml, 30 mmol). The mixture was stirred 15 min and then the solution of protected ribonucleoside (2 mmol, evaporated previously with 10 ml acetonitrile), in dichloromethane (25 ml) was added dropwise during 20 min. After stirring at room temperature for 1 h TLC in S3 showed complete disappearance of the starting substance. Water (20 ml) was added, the mixture stirred for 5 min, the organic layer separated, the water layer extracted with chloroform (40 ml) and the combined extracts diluted with toluene and evaporated. The residue was dissolved in chloroform–triethylamine (99 : 1; 8 ml) and applied on a silica gel column (100 ml), equilibrated in the same solvent. Elution was performed with 100 ml portions of the starting solvent, containing successively 5, 10, 15, 20 and 25 percent of methanol.

Appropriate fractions were evaporated, the residue evaporated twice with chloroform and dried under oil pump vacuum yielding glassy foams of triethylammonium salts of H-phosphonates *IIIb* (1·46 g; 79%; R_F S3 0·55), *IVb* (1·55 g; 86%; R_F S3 0·68).

Automated RNA Synthesis

H-Phosphonates were dissolved in 50% pyridine–acetonitrile to a concentration of 0·1 mol l^{-1} each. 1-Adamantanecarbonyl chloride was dissolved in 50% pyridine–acetonitrile to reach a concentration of 0·4 mol l^{-1}. All solutions were filtered through teflon filters before use. The automated oligoribonucleotide synthesis was performed on 0·3 μmol scale according to a modified synthesizer model 381A program, developed for synthesis of DNA using H-phosphonate synthones (Applied Biosystems). The coupling time for each cycle was elevated from 1 min for DNA synthesis to 20 min for RNA synthesis. This was achieved by 6 successive pulses (6 s) of a 1 : 1 (v/v) mixture of 0·1M H-phosphonate and 0·4M 1-adamantanecarbonyl chloride solution to the CPG-support column (flow rate 1·1 ml/min). Each pulse was followed by wait steps of 200 s. At the end of the synthesis, two-step oxidation was performed[19].

Deprotection and Purification of Microhelix^{Ala} (*X*)

Cleavage of the crude oligoribonucleotide from the CPG-support and partial deprotection was performed by treatment of the CPG-support with 2 ml ethanol–35% aqueous ammonia (1 : 3; v/v) for 48 h at room temperature. After filtration, the resulting yellowish solution was evaporated to dryness. The remaining 2′-blocked oligoribonucleotide was dissolved in 2 ml 1M tetrabutylammonium fluoride in tetrahydrofurane and incubated at room temperature. After 3, 6, 9 and 12 h 10μl aliquots were removed from the reaction mixture and applied on a Nucleosil C-4 HPLC column (Macherey und Nagel, 4·6 mm × 25 cm, 300 Å, 5 μ particles) using a 70 min gradient from 100% 50 mM triethylammonium acetate pH 7·3 to 100% acetonitrile for HPLC analysis. 2′-Deprotection was complete after 12 h, as demonstrated by a single peak fraction in the HPLC profile. The solvent was evaporated, the residue dissolved in 0·1M triethylammonium hydrogen carbonate buffer (1 ml; pH 7·8) and loaded onto a Biogel-P6 column (1·5 × 40 cm). Elution with the same buffer afforded the solution of crude *X* (33 O.D.$_{260}$; 15%). The solvent was evaporated, the residue evaporated with 2 portions of ethanol (2 ml), dissolved in sterile water (1 ml) and lyophilized to white powder which was then dissolved in sterile water (165 μl) and stored at −20°C. A sample (5 μl) of the solution was diluted with deionized formamide (5 μl), the mixture heated in water bath (80°C) for 2 min and loaded into 1 cm wide lane of electrophoretic gel. After electrophoresis the gel visualised by staining showed main spot of *X* accompanied by traces of shorter chains (about 10% total intensity).

Aminoacylation of *X* (Fig. 1)

The aminoacylation reaction was carried out in a buffer containing 150 mM Tris-HCl pH 7·6, 50 mM potassium chloride, 10 mM magnesium chloride, 5 mM ATP, 5 mM 2-mercaptoethanol, 75 μM [^{14}C]alanine (specific activity 162 Ci/mol) in the presence of 0·3 A_{260} units of microhelix^{Ala} and 50 μg alanyl-tRNA synthetase from *E. coli*, at a total volume of 100 μl at 37°C. 10 μl Aliquots were removed from the reaction mixture at the indicated times and plated on Whatman 3MM filters. Trichloroacetic acid precipitable radioactivity was measured in a Beckman scintillation counter model LS 1801.

This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 213, D5 (M. Sprinzl) and Fonds der Chemischen Industrie. The authors thank Dr P. Kocis and

G. Tschech for measurement of 300 MHz ^1H NMR spectra and S. Bachmann for technical assistance.

REFERENCES

1. Kierzek R., Caruthers M. H., Longfellow C. E., Swinton D., Turner D. H., Freier S. M.: Biochemistry *25*, 7840 (1986).
2. Lehman C., Xu Y. Z., Christodoulou C., Tan Z. K., Gait M. J.: Nucleic Acids Res. *17*, 2379 (1989).
3. Tanimura H., Maeda M., Fukuzawa T., Sekine M., Hata T.: Nucleic Acids Res. *17*, 8135 (1989).
4. Sakata T., Hiroaki H., Oda Y., Tanaka T., Ikehara M., Uesugi S.: Nucleic Acids Res. *18*, 3831 (1990).
5. Rozners E., Kumpiņš V., Rekis A., Bizdena A.: Bioorg. Khim. *14*, 1580 (1988).
6. Ogilvie K. K., Sadana K. I., Thompson E. A., Quilliam M. A., Westmore J. B.: Tetrahedron Lett. *1974*, 2863.
7. Usman N., Ogilvie K. K., Jiang M. Y., Cedergren R. J.: J. Am. Chem. Soc. *109*, 7845 (1987).
8. Usman N., Pon R. T., Ogilvie K. K.: Tetrahedron Lett. *26*, 4567 (1985).
9. Ogilvie K. K., Usman N., Nicoghosian K., Cedergren G. J.: Proc. Natl. Acad. Sci. U.S.A. *85*, 5764 (1988).
10. Perreault J.-P., Pon R. T., Jiang M.-Y., Usman N., Pika J., Ogilvie K. K., Cedergren R.: Eur. J. Biochem. *186*, 87 (1989).
11. Chaix C., Duplaa A. M., Molko D., Téoule R.: Nucleic Acids Res. *17*, 7381 (1989).
12. Wu T., Ogilvie K. K., Pon R. T.: Tetrahedron Lett. *29*, 4249 (1988).
13. Wu T., Ogilvie K. K.: J. Org. Chem. *55*, 4717 (1990).
14. Reese C. B.: Nucleosides Nucleotides *4*, 117 (1985); *6*, 121 (1987).
15. Stawinski J., Strömberg R., Thelin M., Westman E.: Nucleic Acids Res. *16*, 9285 (1988).
16. Wang Y. Y., Lyttle M. H., Borer P. N.: Nucleic Acids Res. *18*, 3347 (1990); Scaringe S. A., Francklyn C., Usman N.: Nucleic Acids Res. *18*, 5433 (1990).
17. Žemlička J., Holý A.: Collect. Czech. Chem. Commun. *32*, 3159 (1967).
18. Arnold L., Točík Z., Bradková E., Hostomský Z., Pačes V., Smrt J.: Collect. Czech. Chem. Commun. *54*, 523 (1989).
19. Froehler R. C., Ng P. G., Matteucci M. D.: Nucleic Acids Res. *14*, 5399 (1986).
20. Garegg P. J., Lindh I., Regberg T., Stawinski J., Strömberg R.: Tetrahedron Lett. *27*, 4055 (1986).
21. Andrus A., Efcawitch J. W., McBride L. J., Giusti B.: Tetrahedron Lett. *29*, 861 (1988).
22. Francklyn C., Schimmel P.: Nature *337*, 478 (1989).
23. Hill K., Schimmel P.: Biochemistry *28*, 2577 (1989).
24. Dahlberg A. E., Dingman C. W., Peacock A. C.: J. Mol. Biol. *41*, 139 (1969).

Translated by the author (J.S.).