Loeb, L. (1978) *J. Supramol. Struct., Suppl. 2*, 284.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Mattern, M. R., & Cerrutti, P. A. (1975) *Nature (London) 254*, 450–456.
Medvedev, Zh. A. (1972a) *Nature (London) 237*, 453–454.
Medvedev, Zh. A. (1972b) *Exp. Gerontol. 7*, 227–238.
Merz, G. S., & Ross, J. D. (1969) *J. Cell. Physiol. 74*, 219–224.
Murray, V., & Holliday, R. (1981) *J. Mol. Biol. 146*, 55–76.
Muzyczka, N., Poland, R., & Bessman, M. (1972) *J. Biol. Chem. 247*, 7116–7122.
Painter, R. B., Clarkson, J. M., & Young, B. R. (1973) *Radiat. Res. 56*, 560–564.
Schlabach, A., Fridlender, B., Bolden, A., & Weissbach, A. (1971) *Biochem. Biophys. Res. Commun. 44*, 879–885.
Seal, G., Shearman, C. W., & Loeb, L. A. (1979) *J. Biol. Chem. 254*, 5229–5237.
Smith, J. R., & Hayflick, L. (1974) *J. Cell Biol. 62*, 48–53.
Smith, J. R., & Whitney, R. G. (1980) *Science (Washington, D.C.) 207*, 82–84.
Speyer, J., Karam, J., & Lenny, A. (1966) *Cold Spring Harbor Symp. Quant. Biol. 31*, 693–697.
Szilard, L. (1959) *Proc. Natl. Acad. Sci. U.S.A. 45*, 30–45.

Chemical Synthesis of a Messenger Ribonucleic Acid Fragment: AUGUUCUUCUUCUUCUUC†

Jack A. J. den Hartog, Gerry Wille, Rolf A. Scheublin, and Jacques H. van Boom*

ABSTRACT: The synthesis via a phosphotriester method of the octadecaribonucleotide AUG(UUC)$_5$ (**19**) is reported. The octadecanucleotide is meant to serve as a synthetic messenger in a ribosomal protein synthesizing system. A fully protected octadecamer intermediate (**18a**) was prepared by a block coupling procedure. For the introduction of the desired 3′–5′-internucleotide bonds a 3′-*O*-(2,2,2-trichloroethyl 2-chlorophenyl phosphate) function was incorporated into the monomeric building blocks which were applied in the synthesis of **18a**. Monomeric and oligomeric compounds with a thus protected 3′-*O*-phosphotriester function can be selectively deblocked to give 3′-*O*-phosphodiester derivatives suitable for condensation with 5′-hydroxyl (oligo)nucleotides. Conversion of fully protected oligomers to 5′-hydroxyl derivatives, suitable for further coupling at the 5′ end, was effected by selective removal of the levulinyl function at the 5′ end. The fully protected octadecanucleotide **18a** was deblocked with fluoride ions, followed by ammonia and acid to give the required octadecamer **19**. The condensing agent 1-(2,4,6-triisopropylphenylsulfonyl)-3-nitro-1,2,4-triazole, which was applied to effect the formation of fully protected 3′–5′-internucleotide phosphotriester functions, may give rise to side reactions with the heterocyclic bases uracil and guanine. The consequences of these side reactions for the synthesis of octadecamer **19** are reported.

The first requirement for obtaining better insight into protein synthesis of prokaryotic and eukaryotic cells is a knowledge of the precise structure and function of the ribosome. The complex system of the ribosome supplies the environment in which the correct codon–anticodon interactions between messenger RNA and the various transfer RNAs are selected, thereby assuring an ordered translation of the messenger. The complexity of the ribosome structure has restrained efforts to unravel the precise molecular mechanism of protein synthesis (Chambliss et al., 1979). Many studies on protein biosynthesis have been performed with enzymatically prepared messenger RNAs. Due to recent improvements in the chemical synthesis of RNA fragments, biochemical investigations using well-defined synthetic messengers have come within reach. We therefore decided to synthesize a messenger RNA fragment containing the initiation codon AUG followed by five successive UUC codons (see Figure 1). In translation studies with the synthetic octadecamer AUG(UUC)$_5$ (**19**), we hope to correlate errors in translation with well-defined structural alterations of the ribosome. Since UUC codes for the amino acid phenylalanine (Phe), UCU for serine (Ser), and CUU for leucine (Leu), slippage of the messenger should be detected by scoring the frequency of Ser and Leu incorporation relative to Phe.

The availability of well-defined oligonucleotide fragments is of crucial importance for studies of cellular processes. In the field of oligodeoxyribonucleotides (DNA fragments) striking examples of the application of chemically prepared oligomers have been reported. Thus, the synthesis of the structural gene for yeast alanine transfer RNA (Khorana et al., 1972), the *Escherichia coli* tyrosine transfer RNA suppressor gene (Khorana, 1978), and genes for the peptide hormones somatostatin (Itakura et al., 1977) and insulin (Crea et al., 1978) was successfully achieved by enzymatic assemblage of chemically prepared DNA fragments. The development of synthetic methods for the preparation of oligoribonucleotides (RNA fragments) has taken a much slower course. However, in the last few years considerable progress, in particular by application of phosphotriester methods, has been made in the field of oligoribonucleotide synthesis (Reese, 1978), and syntheses of several relatively large RNA fragments with heterosequences of up to 17 ribonucleoside units have been reported (Werstiuk & Neilson, 1976; Adamiak et al., 1978; van Boom et al., 1978; van Boom & Burgers, 1978; Ogilvie & Theriault, 1979; Ohtsuka et al., 1980; Jones et al., 1980). In the first part of this paper we present the synthesis of the octadecaribonucleotide AUG(UUC)$_5$ via a phosphotriester method. This octadecamer represents, up to now, the largest

† From the Department of Organic Chemistry, Gorlaeus Laboratory, State University Leiden, 2300 RA Leiden, The Netherlands. *Received March 3, 1981.*

0006-2960/82/0421-1009$01.25/0

AUGUUCUUCUUCUUCUUC (19)

FIGURE 1: Sequence of octadecamer **19**.

reported ribonucleotide sequence synthesized by pure chemical means.

Very recently, Reese & Ubasawa (1980) reported on the reaction of 1-(arenesulfonyl)-3-nitro-1,2,4-triazoles and uracil or guanine base residues. Since we applied 1-(2,4,6-triisopropylphenylsulfonyl)-3-nitro-1,2,4-triazole (TPSNT) for the condensation of partially protected (oligo)nucleotides in the synthesis of the fully protected octadecamer **18a** (see Scheme VI), the possible consequences of these side reactions for the synthesis of the desired octadecanucleotide had to be investigated. The results of this study are described in the second part of this paper.

Experimental Procedures

General Methods and Materials. UV absorption spectra were measured with a Cary C14 recording spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured at 100 MHz with a JEOL JNMPS 100 spectrometer; shifts are given in parts per million (δ) relative to tetramethylsilane (Me_4Si) as internal standard. High-performance liquid chromatography (HPLC) was carried out on a Hupe & Busch/Hewlett-Packard 1010A liquid chromatograph equipped with a gradient mixing system and UV absorption detector (254 nm). The photometer output was displayed on a Kipp & Sons flat-back recorder; peak areas were recorded by a Becker 7021 integrator. High-performance anion-exchange chromatography was performed with the weak anion-exchange resin Permaphase AAX (Du Pont) dry packed into a stainless steel column (1 m × 2.1 mm). Isocratic elution of mononucleotides was effected by buffer A (0.005 M KH_2PO_4, pH 4.1) at 20 °C with a flow rate of 1 mL/min at a pressure of 70 kPa/cm^2 (system I). Gradient elution was performed by starting with buffer A and applying 3% of buffer B (0.1 M KH_2PO_4 and 1.0 M KCl, pH 4.5) per min (system II) or 1% of buffer B per min (system III). A flow rate of 1 mL/min at a pressure of 50 kPa/cm^2 at 55 °C was standard. Retention times are measured relative to the injection peak. Short-column chromatography was performed on Merck Kieselgel 60 (230–400 mesh ASTM). Sephadex LH-60, DEAE-Sephadex A25,[1] and Sephadex G50 were purchased from Pharmacia. Schleicher & Schüll DC Fertigfolien F 1500 LS 254 was used for thin-layer chromatography (TLC) in solvent system A (chloroform–methanol, 92:8 v/v) and system B (chloroform–methanol, 88:12 v/v). Dioxane, acetonitrile, tetrahydrofuran, and pyridine were dried by refluxing with CaH_2 for 16 h and then distilled. Pyridine used in condensation reactions was redistilled from *p*-toluenesulfonyl chloride (60 g/L). Dioxane and tetrahydrofuran were stored over 5-Å molecular sieves and acetonitrile and pyridine over 4-Å molecular sieves.

Synthesis of the Protected Nucleotides **4** *and* **6**. The preparation of the suitably protected monomeric building blocks **4** and **6** via the routes described in Scheme I was performed as published before (van Boom & Burgers, 1978; den Hartog & van Boom, 1980).

General Method for the Preparation of Fully Protected Oligonucleotides. The 5′-*O*-levulinyl (oligo)nucleotide 3′-*O*-(2,2,2-trichloroethyl 2-chlorophenyl phosphate) derivative (1 mmol) and 2,4,6-triisopropylbenzenesulfonic acid (TPSOH, 0.2–0.4 mmol) were dissolved in pyridine (10 mL), and activated zinc (ca. 15 mmol) was added. The suspension was stirred magnetically. The course of the reaction was followed by monitoring the evolvement of heat with a thermocouple. After 30–40 s, the temperature of the mixture rose sharply to ca. 35 °C, and after 3 min, the mixture was filtered to remove excess zinc. TLC (system B) of the filtrate showed complete conversion of the starting compound into base-line material. The filtrate was diluted with chloroform (200 mL) and washed with 1 M triethylammonium bicarbonate (TEAB, pH 7.5, 15 mL) and 0.1 M TEAB (15 mL). In the case of mononucleotide **7** (B = U), the aqueous solution was reextracted with chloroform–pyridine (9:1 v/v, 2 × 25 mL). The organic layer was concentrated to an oil that was transferred to a smaller flask containing the 5′-hydroxy (oligo)nucleotide or nucleoside. The mixture was dried by repeated coevaporation with anhydrous pyridine (3 × 20 mL), and 1-(2,4,6-triisopropylphenylsulfonyl)-3-nitro-1,2,4-triazole (de Rooy et al., 1979a) (TPSNT, equimolar to 5′-hydroxy component) was added to the resulting solution. The reaction was monitored by TLC (system A or B). After 2 h, a second portion of TPSNT (50–100% of the original amount) was added. After the required reaction time (Table I), the mixture was diluted with chloroform (70 mL) and washed with 10% aqueous $NaHCO_3$ (40 mL) and water (25 mL). The organic layer was dried ($MgSO_4$), concentrated to a small volume, and triturated with petroleum ether (bp 40–60 °C, 150 mL). Small oligomers (**8–10**, **12**, **13**) were purified by short-column chromatography; the crude oligomers were dissolved in chloroform (2–5 mL) and applied to a column of Kieselgel (ca. 15 g/g of crude material). After elution of the column with chloroform–methanol [(98–96):(2–4) v/v], the appropriate fractions were concentrated to a small volume, and the pure oligonucleotides were precipitated with petroleum ether (bp 40–60 °C, 150 mL), filtered off, and dried in vacuo (P_2O_5). Hexamers and longer oligonucleotides (**14–18**) were purified by gel filtration (de Rooy et al., 1979b); the crude oligomers were dissolved in tetrahydrofuran–methanol (19:1 v/v, 2–4 mL) and brought onto a column (1 m × 6 cm) of Sephadex LH-60 (70 g) swollen in the same solvent mixture. Elution of the column with the same tetrahydrofuran–methanol mixture, concentration of the appropriate fractions, and precipitation from petroleum ether (bp 40–60 °C, 150 mL) afforded the pure oligomers as white solids. Data relevant to the synthesis of these fully protected oligonucleotides are summarized in Table I.

Removal of the Levulinyl Group from the Fully Protected Oligonucleotides. A solution of hydrazine hydrate (0.5 g, 10 mmol) in pyridine–acetic acid (3:2 v/v, 10 mL) was added to a solution of the 5′-*O*-levulinyl oligonucleotide derivative (1 mmol) in pyridine (10 mL). After 4 min at room temperature, pentane-2,4-dione (2 mL, 20 mmol) was added to the reaction mixture, and the reaction flask was immersed into an ice–water bath. After a further 2 min, the solution was added to chloroform (75 mL) and water (100 mL). The organic layer was separated and washed with 10% aqueous $NaHCO_3$ (75 mL) and water (50 mL). Further workup was performed as described above for the fully protected oligonucleotides. Yields and analytical data of the obtained oligonucleotides are given in Table I.

Deblocking of the Fully Protected Octadecanucleotide **18a**. *Method A (TBAF).* To a solution of **18a** (104 mg, 10 μmol) in tetrahydrofuran (11.2 mL) and pyridine (1.4 mL) was added an aqueous tetra-*n*-butylammonium fluoride solution (0.5 M TBAF, pH 75, 1.4 mL, 4 equiv per phosphotriester moiety). After 24 h at 20 °C, water (10 mL) and Dowex 50

[1] Abbreviations: DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

W cation-exchange resin (100–200 mesh, pyridinium form, 6 g) were added. The resin was filtered off, and a few drops of 1 M TEAB buffer (pH 7.5) were added to the filtrate. The solution was concentrated to an oil, and 25% aqueous ammonia (20 mL) was added. The reaction vessel was sealed and kept at 50 °C. After 50 h, the solution was concentrated to neutrality, analyzed by HPLC (system III, Figure 2A), and brought onto a column (2 m × 3 cm) of Sephadex G50 suspended in 0.05 M TEAB. Elution occurred with the same buffer at a flow rate of 14 mL/h. Fractions of 3 mL were taken. The fractions containing pure product were collected (HPLC, system III, Figure 2B) and lyophilized to give the partially deblocked octadecamer **18b** (70 mg).

Method B (Oximate). To a solution of **18a** (104 mg, 10 μmol) in dry dioxane (1 mL) and dry acetonitrile (1 mL) was added a solution of *syn*-4-nitrobenzaldoxime (282 mg, 1.7 mmol, 10 equiv per phosphotriester moiety) and N^1,N^1,N^3,N^3-tetramethylguanidine (196 mg, 1.7 mmol) in dioxane (2 mL) and acetonitrile (2 mL). After 24 h at 20 °C, an additional quantity of N^1,N^1,N^3,N^3-tetramethylguanidine (1 mmol) was added. After 72 h, water (1 mL) was added to the reaction mixture. After a further 24 h, 25% aqueous ammonia (20 mL) was added to the reaction mixture. After 150 h at 20 °C, the mixture was concentrated and analyzed by HPLC (system III).

Part of the purified partially deprotected (method A) octadecanucleotide **18b** (25 mg) was dissolved in 0.01 N HCl (5 mL), and the pH was adjusted to 2.0 by the addition of 0.1 N HCl. After 2 h at 20 °C, the solution was neutralized (pH 8) with 0.5 M ammonia and concentrated to a small volume. After HPLC analysis (system III, Figure 3A), compound **19** was purified by chromatography on Sephadex G50 as described above for **18b**. The appropriate fractions were collected (HPLC, system III, Figure 3B) and lyophilized. Pure octadecamer **19** was brought into the ammonium form and next into the sodium form by running it through a column of Dowex 50W cation-exchange resin (100–200 mesh, ammonium form and sodium form, respectively). The resulting aqueous solution was lyophilized. Extreme care must be taken to prevent hydrolysis of the deprotected octadecamer **19** by ribonucleases. Sterile water and glassware were used throughout the whole process of purification and isolation.

Enzymatic Hydrolysis of the Octadecamer **19**. A solution of **19** (1.2 mg) in 50 μL of sterile water was prepared. (a) *Spleen Phosphodiesterase (SPD).* The stock solution (5 μL) was incubated with spleen phosphodiesterase (Boehringer, 20 μg) in a solution (50 μL) containing 100 mM NH_4OAc (pH 5.7) and 0.05% Tween-80 at 37 °C for 4 h. (b) *Venom Phosphodiesterase (VPD).* The stock solution (5 μL) was incubated with snake-venom phosphodiesterase (*Crotalis terr. terr.*, Boehringer, 2 μg) in a buffer (50 μL) containing 25 mM Tris-HCl (pH 9.0) and 5 mM $MgCl_2$ at 37 °C for 2 h. (c) *Takadiastase T_1.* The stock solution (5 μL) was incubated with takadiastase T_1 (*Aspergillus oryzae*, Boehringer, 1 μg) in a buffer (50 μL) containing 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA at 37 °C for 2 h.

HPLC analysis (system II) showed that complete digestion of **19** had occurred in all cases. Quantitation (HPLC, system I) of the digestion products obtained by SPD and VPD treatment showed that the expected nucleotides were attained in the correct ratio: For SPD, calcd Ap 1.0, Gp 1.0, Up 11.0, Cp 4.0; found Ap 1.0, Gp 0.8, Up 11.0, Cp 4.2. For VPD, calcd pG 1.0, pU 11.0, pC 5.0; found pG 0.9, pU 11.1, pC 5.0. HPLC analysis (system II) of the takadiastase T_1 digest showed the formation of two products having the same retention times as the trimer AUGp (**20**) and the pentadecamer $(UUC)_5$ (**26**) (R_t 11.7 and 26.1 min, respectively).

The yield of the pure octadecamer **19** was determined spectrophotometrically. A correction for the hypochromicity was made (de Rooy et al., 1979a; den Hartog et al., 1980). The yield of **19** based on the fully protected octadecamer **18a** was 43%.

Preparation of Oligomers AUGp (**20**), *AUG* (**23**), $(UUC)_4$ (**25**), *and* $(UUC)_5$ (**26**). (*a*) *AUGp.* The fully protected trimer **9a** (214 mg, 0.1 mmol) was deblocked with fluoride ions (method A) and ammonia as described above for **18a**. After analysis by HPLC (system II), the crude product was brought onto a column (20 cm × 6 cm) of DEAE-Sephadex A25 (HCO_3^- form) suspended in 0.05 M TEAB. The column was eluted with a linear gradient of 0.05 → 0.6 M TEAB for 16 h at a flow rate of 36 mL/h. Fractions of 6 mL were taken. After HPLC analysis, the appropriate fractions were collected, concentrated to a small volume, coevaporated with water (2 × 15 mL), and lyophilized. The resulting solid was dissolved in 0.01 N HCl (20 mL) and the pH adjusted to 2.0 by the addition of 0.1 N HCl. After 2 h at 20 °C, the solution was neutralized with 0.5 M ammonia and lyophilized. After HPLC analysis (system II, Figure 4A), part of the obtained mixture (50 mg) was dissolved in 25% aqueous ammonia (5 mL). After 30 min, an aliquot of the solution was concentrated to neutrality and analyzed with HPLC (system II, Figure 4B). After 24 h, the solution was concentrated to a small volume. HPLC showed the complete disappearance of peak III (corresponding to **20a**) and the presence of two peaks, one (i.e., peak II) corresponding to the 3′- and 2′-monophosphates **20b** and the other (i.e., peak I) to the 2′,3′-phosphate **21**. Another quantity of the mixture corresponding to peaks I–III in Figure 4A (1 mg) was dissolved in a buffer (0.5 mL) containing 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA and incubated with takadiastase T_1 (*Aspergillus oryzae*, Boehringer, 10 μg) at 37 °C for 2 h. HPLC analysis (system II, Figure 4C) showed complete conversion of the mixture into the desired product **20**. This product was used in the identification of the products resulting after takadiastase T_1 digestion of **19**.

(*b*) *AUG.* The fully protected trimer AUG (**22**) was obtained analogously to the synthesis of **9a** (see Scheme II). The only alteration was the use of the nonphosphorylated guanosine derivative 2′,3′-*O*-methoxymethylene-2-*N*-benzoylguanosine (den Hartog et al., 1980) instead of **4** (B = G^{Bz}) in the first coupling step. In this way **22** was obtained in high yield [80% (first coupling step), 91% (hydrazinolysis), and 89% (final coupling step)]. Deblocking and purification, as described for the conversion of **9a** into **20** (steps 1^0–4^0), afforded pure **23**.

(*c*) $(UUC)_4$ *and* $(UUC)_5$. The fully protected pentadecamer **24** was obtained in good yield (62%) by condensation of trimer **13b** with dodecamer **17b**, using TPSNT as activating agent. Deblocking of the fully protected dodecamer **17a** and pentadecamer **24** with fluoride ions (method A), followed by ammonia and acid, as described for the preparation of the octadecamer **19**, and subsequent purification on Sephadex G50 afforded the pure dodecamer $(UUC)_4$ (**25**) and pentadecamer $(UUC)_5$ (**26**), respectively.

Synthesis and Reactions of the 3-Nitro-1,2,4-triazole Derivatives **28** *and* **31**. Compounds **28** and **31** were obtained in 80% and 30% yield, respectively, by reaction of **27** and **30** with TPSNT, according to the literature procedure (Reese & Ubasawa, 1980). Both compounds were treated with the following reagents. (a) Zinc in pyridine, in the presence of TPSOH, under the conditions described in the general method for the preparation of fully protected oligonucleotides; after

4 min, a few drops of pentane-2,4-dione were added. The products were analyzed by TLC (system B). (b) Hydrazine hydrate in pyridine–acetic acid as described for the removal of the levulinyl group from fully protected oligonucleotides. The products were analyzed by TLC (system B). The R_f values of the relevant uridine derivatives were as follows: **27**, 0.57; **28**, 0.68; **29**, 0.63. The products resulting after hydrazine treatment of **28** (4 min) and **31** (1 h) were worked up and analyzed by ^{1}H NMR spectroscopy. (c) TBAF in tetrahydrofuran–pyridine–water under the conditions described for the deblocking of **18a** (method A). The product resulting after TBAF treatment (3 h) of **28** was worked up and analyzed by ^{1}H NMR spectroscopy. (d) Oximate in dioxane–acetonitrile as described for the deblocking of **18a** (method B). The products were analyzed by TLC (system B). (e) Tetra-*n*-butylammonium acetate (TBAA) (Fieser & Fieser, 1972) in tetrahydrofuran. To a solution of **28** or **31** (0.2 mmol) in tetrahydrofuran (1 mL) was added a solution of TBAA in tetrahydrofuran (0.5 M, 4 mL). The product resulting after TBAA treatment of **28** (30 min) was worked up and analyzed by ^{1}H NMR spectroscopy. (f) Tetra-*n*-butylammonium formate (Fieser & Fieser, 1974) in tetrahydrofuran. To a solution of **28** or **31** (0.2 mmol) in tetrahydrofuran (1 mL) was added a solution of tetrabutylammonium formate in tetrahydrofuran (0.5 M, 4 mL). The products were analyzed by TLC (system B). (g) K_2CO_3 in dioxane–water. To a small quantity of **28** or **31** (0.2 mmol) was added a solution of 0.1 M K_2CO_3 in dioxane–water (4:1 v/v); the resulting solution was stirred. The products were analyzed by TLC (system B).

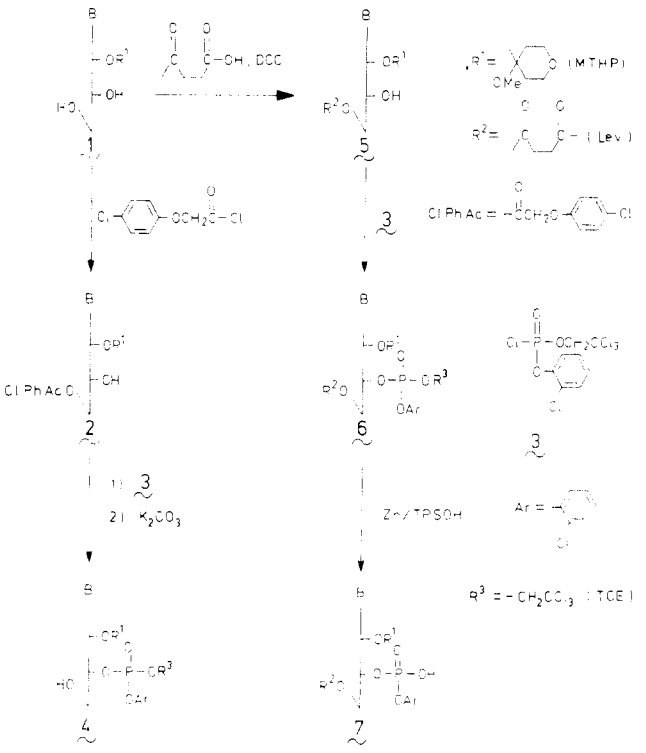
The stability of internucleotide 2-chlorophenylphosphotriester functions toward tetrabutylammonium acetate and formate and K_2CO_3 was tested by treatment with the latter reagents of a fully protected diuridine monophosphate 2-chlorophenyl triester derivative having acid-labile hydroxyl protecting groups. After 16 h, TLC (system B) showed ca. 25% conversion of the fully protected dimer into base-line material.

Results and Discussion

(I) Chemical Synthesis of Octadecaribonucleotide AUG-(UUC)$_5$. The strategy we followed to obtain our goal (i.e., the synthesis of octadecamer **19** in Figure 1) consisted of the following steps: (a) preparation of properly protected nucleotides (Scheme I); (b) introduction of 3′–5′-internucleotide phosphotriester linkages between protected nucleotides and oligonucleotide blocks to afford eventually the fully protected octadecamer **18a** (Schemes II–VI); (c) deblocking and analysis of the required compound (Scheme VI).

Protection of Ribonucleosides. In Scheme I the synthesis of suitably protected nucleotides necessary for the construction of the fully protected octadecamer **18a** is described. All building blocks (i.e., compounds **4** and **6**) necessary for the preparation of **18a**, except the 3′-terminal cytidine unit **11** (van Boom et al., 1977a) (see Scheme II), were protected at the 2′ position with the methoxytetrahydropyranyl (MTHP) group. For the selective and temporary protection of the 5′-hydroxyl group of 2′-*O*-MTHP derivatives **1** (van Boom et al., 1977b), we applied the *p*-chlorophenoxyacetyl group. Reaction of nucleosides **1** with *p*-chlorophenoxyacetyl chloride in the presence of 2,6-lutidine afforded the crystalline derivatives **2**, as previously reported (van Boom et al., 1971, 1976a, 1977a; den Hartog & van Boom, 1980). Phosphorylation of the free 3′-hydroxyl group of compounds **2** with the monofunctional reagent 2,2,2-trichloroethyl 2-chlorophenyl phosphorochloridate **3** (van Boom et al., 1976b), followed by quantitative and selective removal of the temporary 5′-acyl function by

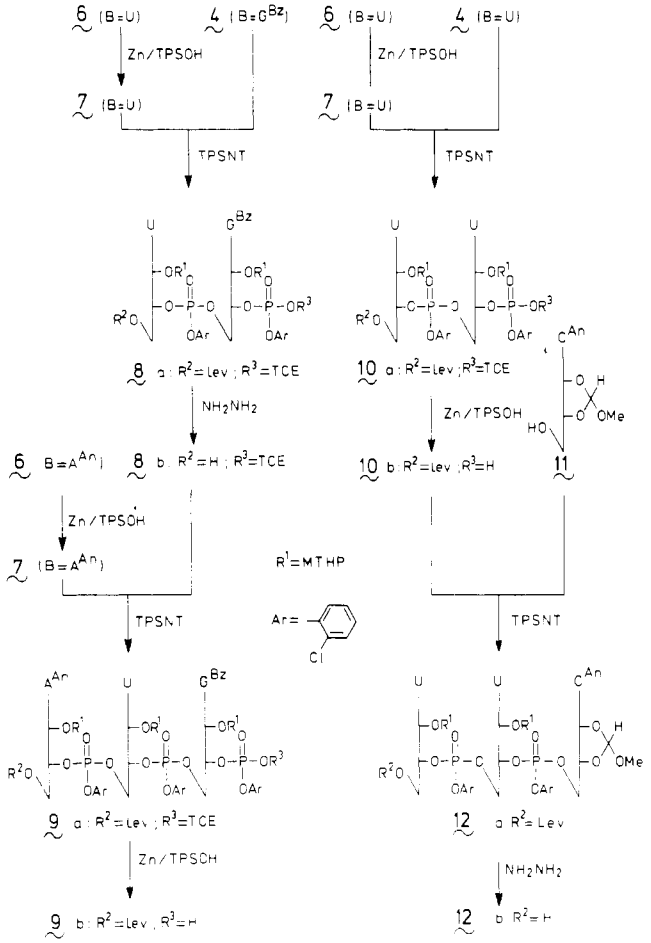
Scheme I



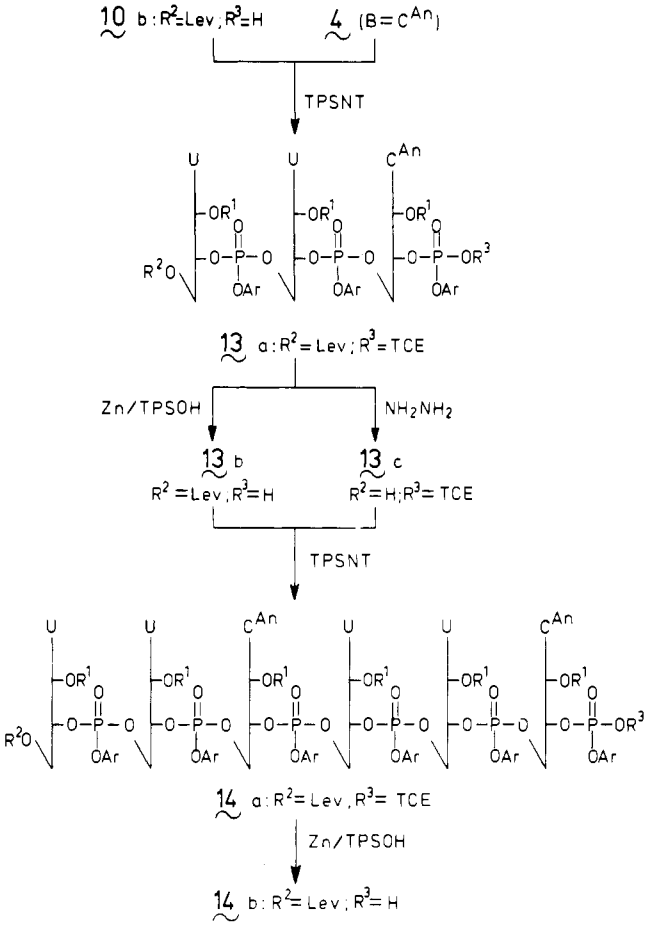
short treatment with K_2CO_3–methanol, afforded the 5′-hydroxyl derivatives **4** in good yields (den Hartog & van Boom, 1980). For the synthesis of the fully protected 5′-levulinyl derivatives **6**, we followed a previously described route (van Boom & Burgers, 1978). Thus, the required 5′-levulinyl function was introduced directly into nucleosides **1** by acylation with levulinic acid and dicyclohexylcarbodiimide (DCC) in the presence of 2,6-lutidine and 1,2-dimethylimidazole as a catalyst. Although the selectivity of this acylation procedure was not very high, compounds **5** could be isolated in reasonable yields (van Boom & Burgers, 1978). Phosphorylation of nucleosides **5** with the monofunctional reagent **3** afforded the fully protected derivatives **6**.

Synthesis of the Fully Protected Octadecanucleotide **18a**. The strategy for the synthesis of the fully protected octadecamer **18a** is depicted in Schemes II–VI. The main features of the synthetic approach are (a) the application of a phosphotriester method, which is based on the use of the monofunctional reagent 2,2,2-trichloroethyl 2-chlorophenyl phosphorochloridate (**3**) (van Boom et al., 1976b), for the formation of the desired 3′–5′-internucleotide linkages and (b) the use of the levulinyl group for protection of the 5′-hydroxy function. The internucleotide bond formation consists of the following two-step procedure. First, the selective removal of the 2,2,2-trichloroethyl group from a 3′-phosphotriester (e.g., **6**) with zinc in the presence of 2,4,6-triisopropylbenzenesulfonic acid (TPSOH) (van Boom et al., 1977c) in pyridine results in the formation of a 3′-phosphodiester intermediate (e.g., conversion of **6** into **7** in Scheme I). In the second step, condensation of the latter diester with a 5′-hydroxy derivative (e.g., **4**, see Scheme II) under the influence of the coupling agent 1-(2,4,6-triisopropylphenylsulfonyl)-3-nitro-1,2,4-triazole (TPSNT) (de Rooy et al., 1979a) affords a 3′/5′-linked oligonucleotide (e.g., dimer **8a** or **10a** in Scheme II). The incorporation of the 2,2,2-trichloroethyl 2-chlorophenyl phosphate function into all monomeric building blocks (i.e., **4** and

Scheme II



Scheme III



6 in Scheme I), except the 3′-terminal unit **11**, allows extension of monomers **6** and oligomers (e.g., **10a**) in the 3′ direction. Furthermore, the presence of the 5′-levulinyl group in the fully protected monomers **6**, which are starting compounds in the synthesis of all oligomers, allows extension of the prepared fully protected oligonucleotides (e.g., dimer **8a** in Scheme II) in the 5′ direction. The latter protecting group can be removed selectively under essentially neutral conditions, 0.5 M hydrazine in pyridine–acetic acid (4:1 v/v) (van Boom & Burgers, 1978), to afford an oligomer having a free 5′-hydroxyl group (e.g., dimer **8b**), which is suitable for subsequent condensation with a 3′-phosphodiester.

The described selective deblocking procedures for the 2,2,2-trichloroethyl group as well as the levulinyl function allow assemblage of octadecamer **18a** via a block coupling procedure. Because of the presence of repeating trimeric units of UUC in the desired fully protected octadecamer AUG(UUC)$_5$ (**18a**), construction of **18a** via suitably protected trimeric oligonucleotide blocks is the obvious strategy. Three distinct fully protected trimers were prepared according to this approach: (a) the 5′-terminal trimer AUGp (i.e., **9a** in Scheme II); (b) the 3′-terminal trinucleotide UUC with a blocked terminal 2′,3′-diol system (i.e., trimer **12a** in Scheme II); (c) the nonterminal trimer UUCp (i.e., **13a** in Scheme III). The synthesis of trimers **9a** and **12a** is visualized in Scheme II. The fully protected dimer **8a** was prepared by starting from the mononucleotides **6** (B = U) and **4** (B = G^{Bz}) via the general procedure described above. Thus, reductive removal of the 2,2,2-trichloroethyl group from **6** (B = U) gave the 3′-phosphodiester derivative **7** (B = U). Subsequent condensation of **7** with the 5′-hydroxyl derivative **4** (B = G^{Bz}), in the presence of TPSNT, afforded dimer **8a**. After hydrazinolysis of **8a**, the resulting dimer **8b** was extended to a trimer by condensation with the 3′-phosphodiester **7** (B = A^{An}). In this way, the fully protected 5′-terminal trinucleotide AUGp (**9a**) was obtained. Conversion of **9a** to the phosphodiester derivative **9b** by zinc treatment allowed further extension of this trimer in the 3′ direction. The 3′-terminal trimer **12a** was attained as follows. Coupling of monomers **6** (B = U) and **4** (B = U), via the described two-step procedure, afforded dimer **10a**. In this case the dimer was extended to a trimer by removal of the trichloroethyl group to give **10b** and subsequent condensation of **10b** with the cytidine derivative **11** (van Boom et al., 1977a), in the presence of TPSNT, resulting in the formation of the fully protected terminal trimer UUC (**12a**). Hydrazinolysis of **12a** afforded the 5′-hydroxy derivative **12b** suitable for extension on the 5′ end. In Scheme III the synthesis of the third required trimer (**13a**) is illustrated. Condensation of dimer **10b**, which was an intermediate in the synthesis of the 3′-terminal trimer **12a**, with the 3′-phosphotriester derivative **4** (B = C^{An}) afforded the fully protected nonterminal trimer UUCp (**13a**).

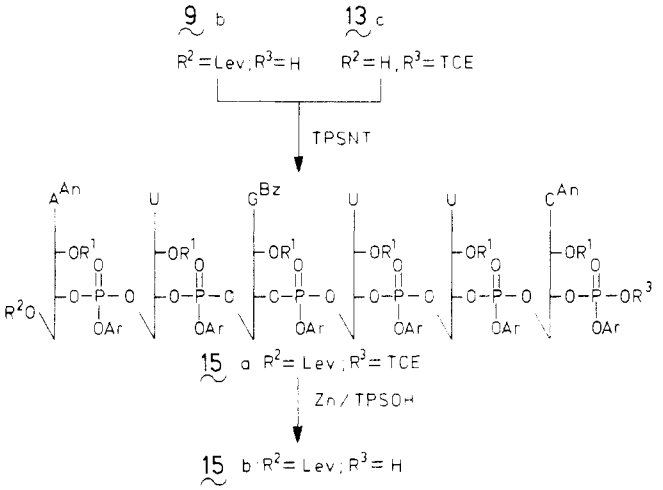
The strategy for the further assemblage of octadecamer **18a** was as follows (see Schemes III–VI). Starting from the obtained fully protected trimer blocks AUGp (**9**), UUC (**12**), and UUCp (**13**), we constructed three fully protected hexamers, i.e., the 5′-terminal hexanucleotide AUGUUCp (**15**), the nonterminal hexamer UUCUUCp (**14**), and the 3′-terminal hexanucleotide UUCUUC (**16**). Subsequent coupling of hexamers **14** and **16** gave a fully protected dodecanucleotide (**17**). Coupling of the 5′-terminal hexamer **15** and dodecamer **17** completed the synthesis of the fully protected octadecaribonucleotide AUG(UUC)$_5$ (**18a**). In the synthesis of all hexamers **14–16**, selectively deblocked derivatives of the trimer

Table I: Data on the Synthesis of Octadecanucleotide 18a

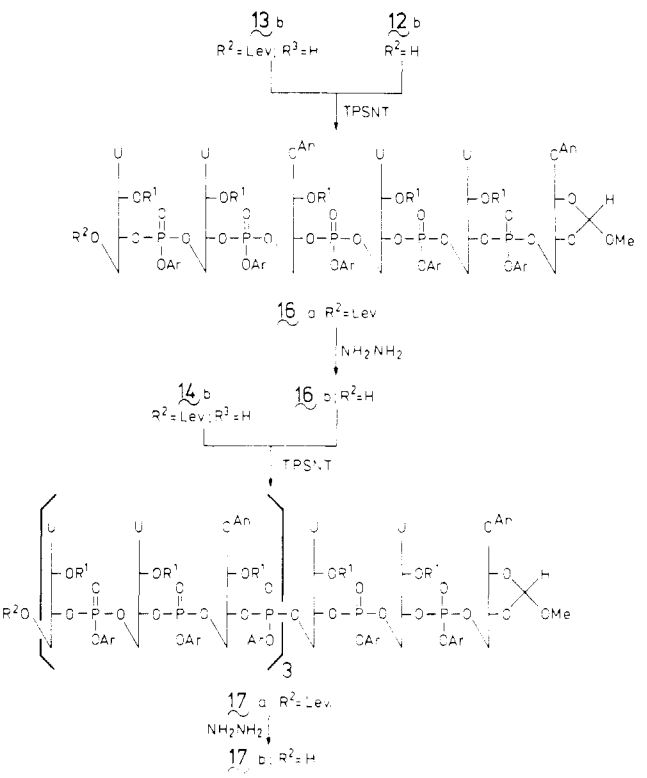
3′-phospho component		5′-hydroxy component		reaction time	product		R_f values[b]		removal of levulinyl group: product		R_f values[b]	
						yield[a]				yield		
no.	mmol	no.	mmol	(h)	no.	(%)	A	B	no.	(%)	A	B
7 (U)	2	4 (G^{Bz})	1.8	2	8a	60	0.41[c]	0.57	8b	86	0.33	0.53
							0.47	0.64				
7 (A^{An})	1.5	8b	1.35	2	9a	69	0.42	0.63				
7 (U)	4.4	4 (U)	4	2	10a	87	0.43	0.60				
10b	2.2	11	2	1.5	12a	89	0.33	0.55	12b	95	0.23	0.46
10b	3.3	4 (C^{An})	3	1	13a	74	0.40	0.64	13c	96	0.29	0.53
13b	2.4	13c	2	3	14a	75	0.37	0.59				
9b	1	13c	0.8	3	15a	60	0.35	0.64				
13b	1.2	12b	1	3	16a	51	0.28	0.54	16b	92	0.24	0.45
14b	0.3	16b	0.2	3.5	17a	50	0.25	0.57	17b	88	0.24	0.51
15b	0.08	17b	0.05	4	18a	66	0.23	0.61				

[a] Based on 5′-hydroxy component. [b] On silica gel plates in system A and B, respectively. [c] Pair of diastereoisomers.

Scheme IV



Scheme V

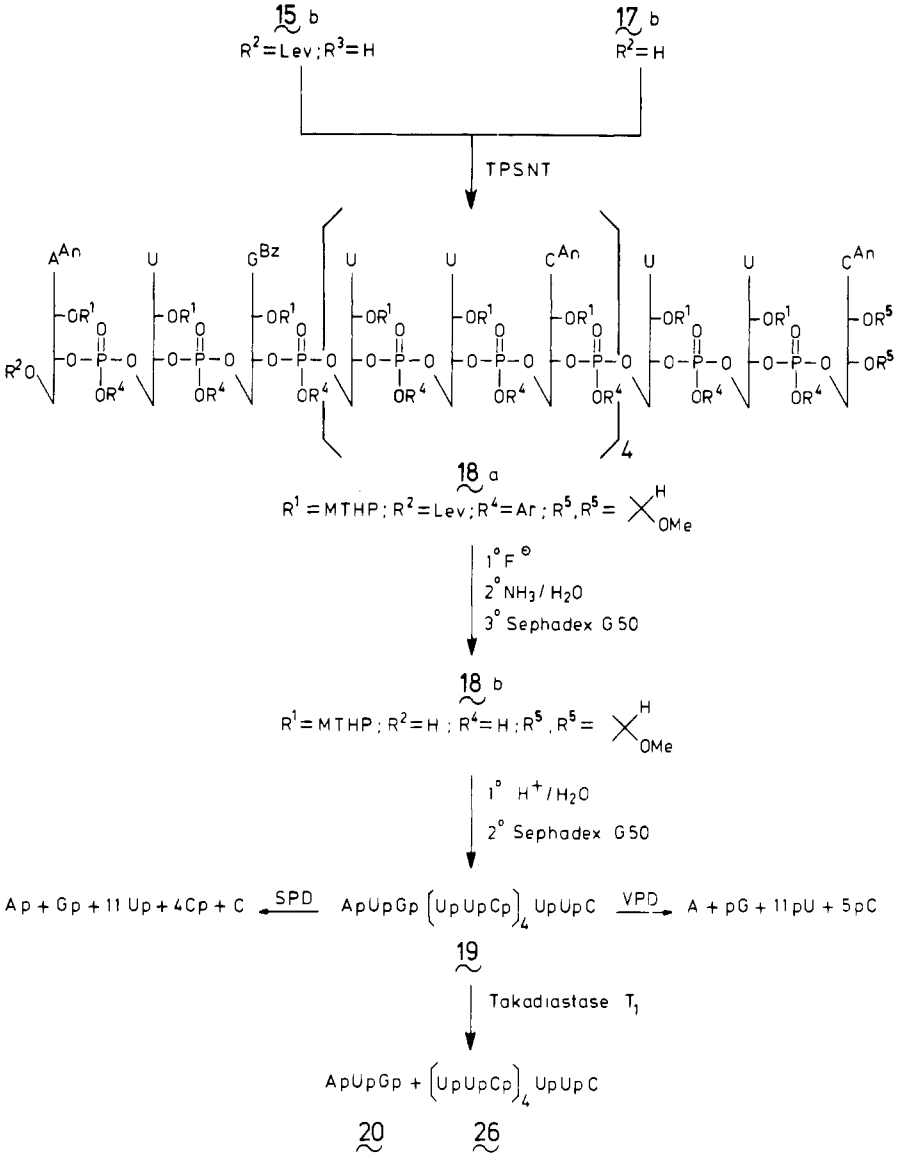


UUCp (**13a**) were applied. Zinc treatment of **13a** afforded the 3′-phosphodiester derivative **13b** (see Scheme III) while hydrazinolysis gave the 5′-hydroxy trinucleotide **13c** (Scheme III). The nonterminal hexamer **14a** was obtained by condensation of both trimers **13b** and **13c** in the presence of TPSNT. The synthesis of the fully protected 5′-terminal hexamer AUGUUCp (**15a**) is illustrated in Scheme IV. Condensation of the suitably protected 3′-phosphodiester AUGp (**9b**) with **13c** gave the 5′-terminal hexanucleotide **15a**. Removal of the trichloroethyl group from **15a** allowed further extension of the resulting hexamer **15b** in the 3′ direction. The synthesis of the third required hexamer, i.e., the fully protected 3′-terminal fragment UUCUUC (**16a**), is depicted in Scheme V. Condensation of the trimer **13b** with the 3′-terminal trinucleotide **12b**, in the presence of TPSNT, afforded hexanucleotide **16a**. The hexamers obtained after hydrazinolysis of UUCUUC (**16a**) and zinc treatment of UUCUUCp (**14a**), i.e., the 5′-hydroxyl derivative **16b** and the 3′-phosphodiester **14b**, respectively, were condensed to give the fully protected dodecamer **17a**. In the final coupling step (see Scheme VI), the hexamer **15b** was condensed with the 5′-hydroxyl derivative $(UUC)_4$ (**17b**), which was obtained by removal of the levulinyl group from the fully protected dodecamer **17a**. In this way the fully protected octadecamer $AUG(UUC)_5$ (**18a**) was obtained. Data relating to the synthesis of **18a** are collected in Table I.

Deblocking and Analysis. It was shown before (van Boom & Burgers, 1978) that relatively long oligoribonucleotide phosphotriesters containing internucleotide-phosphate 2-chlorophenyl protecting groups could be conveniently deblocked with 0.05 M tetra-*n*-butylammonium fluoride (TBAF) in tetrahydrofuran–pyridine–water (8:1:1 v/v). The stability of all other protecting groups of octadecamer **18a** toward this reagent excludes neighboring group participation of free hydroxy functions and leads to the formation of an oligonucleotide product **18b** with solely 3′–5′-internucleotide linkages. Thus, removal of the 2-chlorophenyl groups was accomplished by treating **18a** with TBAF in tetrahydrofuran–pyridine–water for 16 h at 20 °C. After complete deblocking of the phosphotriesters, the remaining base-labile levulinyl, benzoyl, and *p*-anisoyl groups were removed by treatment with 25% aqueous ammonia for 50 h at 50 °C. The resulting partially protected octadecanucleotide **18b** was analyzed by HPLC (system III, Figure 2A) and purified by chromatography on Sephadex G50 (HPLC, Figure 2B). The MTHP protecting groups on the 2′ positions of **18b** were retained to eliminate the danger of endonucleolytic cleavage of the molecule by traces of ribonucleases.

In a recent report (Reese & Yau, 1978) it was shown that deblocking of 2-chlorophenyl phosphotriesters with 0.3 M

Scheme VI



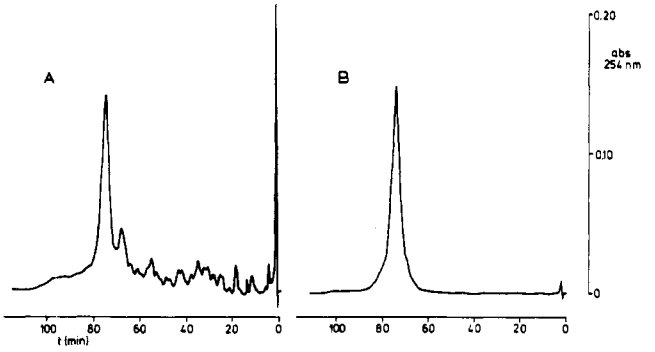


FIGURE 2: HPLC analysis (system III) of (A) the crude partially deprotected octadecamer **18b** and (B) compound **18b** after purification.

N^1,N^1,N^3,N^3-tetramethylguanidinium 4-nitrobenzaldoximate in dioxane–water (1:1 v/v) led to less internucleotide cleavage than deblocking with TBAF under the conditions described above. However, the 5′-levulinyl protecting group is not stable under the described conditions of oximate treatment (J. H. van Boom et al., unpublished observations). The removal of the 5′-protecting group before completion of the deblocking of the internucleotide linkages leads to terminal phosphoryl migration (Chattopadhyaya & Reese, 1980). On the other hand, preliminary results suggested that oximate deblocking can equally well be effected under anhydrous conditions [0.3 M oximate in acetonitrile–dioxane (1:1 v/v)]. No hydrolysis of the levulinyl group was detected under the latter deblocking conditions (J. H. van Boom et al., unpublished observations). Thus, a small portion of the octadecamer **18a** was treated with oximate in acetonitrile–dioxane at 20 °C. After 72 h, some precipitated material was observed that was redissolved by addition of water to the reaction mixture. The resulting solution of nucleotide material and oximate in acetonitrile–dioxane–water was left for another 24 h. The product obtained after subsequent ammonia treatment (150 h, 20 °C) was analyzed by HPLC (system III). From the chromatographic pattern it was concluded that slightly less internucleotide cleavage had occurred compared to that obtained with the fluoride ion deblocking procedure.

Finally, the acid-labile groups were removed. Thus, a portion of the partially deprotected (fluoride ions followed by ammonia) and purified octadecamer **18b** was dissolved in 0.01 N HCl and acidified with 0.1 N HCl until pH 2.0. After 2 h at 20 °C, the reaction mixture was neutralized with dilute ammonia and analyzed by HPLC (system III, Figure 3A). Impurities, resulting from inadequate purification of the intermediate **18b**, were removed by chromatography on Sephadex G50. In this way highly pure $AUG(UUC)_5$ (**19**) was obtained (HPLC, system III, Figure 3B) in 43% yield.

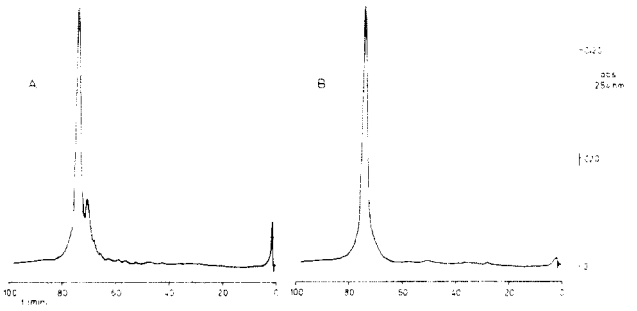


FIGURE 3: HPLC analysis (system III) of (A) the crude octadecamer **19** and (B) compound **19** after purification.

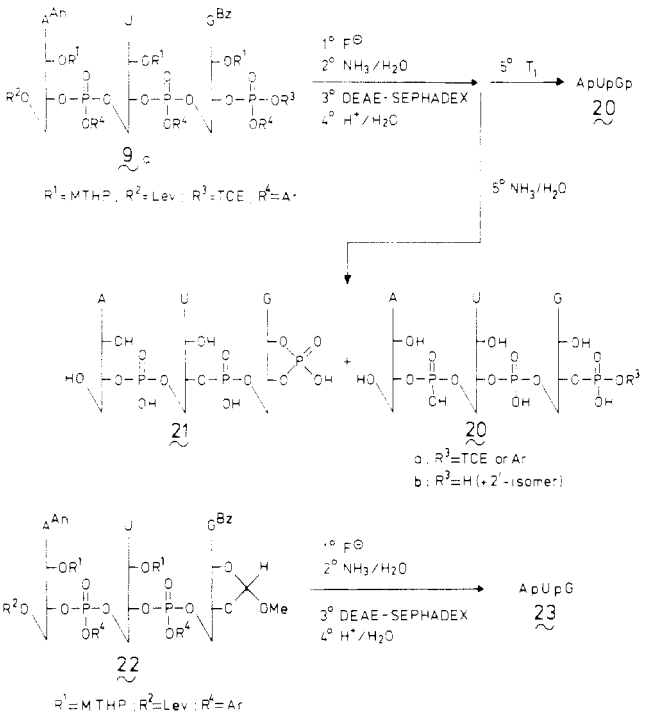
The purified and fully deprotected octadecamer **19** thus obtained was analyzed by enzymic methods. The correct nucleoside(tide) composition of **19** was corroborated by its complete digestion to nucleoside(tide) products (Scheme VI) with the exonucleases spleen (SPD) and venom (VPD) phosphodiesterase. HPLC analysis (system I) showed that the expected products were formed in the correct ratios. Hydrolysis of **19** with the specific endonuclease takadiastase T_1 gave rise to the formation of two products (HPLC, system II). Comparison with reference compounds, which were prepared from intermediates in the synthesis of **18a** (see below), showed that **19** was degraded to the expected oligomeric products: the trimer AUGp (**20**) and the pentadecamer $(UUC)_5$ (**26**).

Synthesis of Reference Compounds AUGp (**20**), *AUG* (**23**), $(UUC)_4$ (**25**), and $(UUC)_5$ (**26**). The synthesis of various oligomeric derivatives, which were meant as reference compounds in the analysis of octadecamer **19** or in biochemical studies with **19**, are depicted in Schemes VII and VIII. These compounds include the trimers AUGp and AUG as well as the $(UUC)_n$ oligomers **25** and **26**. The trimer AUGp (**20**) and pentadecamer $(UUC)_5$ (**26**) were used for the identification of the products of takadiastase T_1 digestion of **19**. The trimer AUG (**23**), dodecamer $(UUC)_4$ (**25**), and pentadecamer $(UUC)_5$ (**26**) were meant for biochemical studies.

The preparation of AUGp (**20**) is illustrated in Scheme VII. The fully protected trimer AUGp (**9a**), which was an intermediate in the synthesis of **18a**, was partially deblocked with fluoride ions followed by ammonia. The pure intermediate, which was obtained after chromatography on DEAE-Sephadex, was treated with acid. HPLC analysis (system II, Figure 4A) showed that at least three distinct products were formed. The identity of the products corresponding to peaks I–III in the chromatogram followed from analysis of the products obtained after treatment of the product mixture with aqueous ammonia or the enzyme takadiastase T_1. Short ammonia treatment (30 min) converted most of the main product, presumably the 3′-phosphodiester derivative **20a** (peak III), into the product corresponding to peak I, having the shortest retention time (HPLC, system II, Figure 4B). Therefore, the material under peak I was proposed to be the 2′,3′-phosphate **21** while the unchanged product in peak II was tentatively identified as the desired 3′-phosphate **20b** (together with the 2′). On the other hand, digestion with takadiastase T_1 of the product mixture corresponding to peaks I–III in Figure 4A afforded the product which corresponded to peak II as the sole nucleotide product (HPLC, system II, Figure 4C). From these analytical data it was concluded that quantitative conversion into the desired ApUpGp (**20**) had occurred.

For the synthesis of the nonphosphorylated trimer ApUpG (**23**), no suitably protected precursor was available from the synthesis of the octadecamer **18a**. The fully protected trimer

Scheme VII



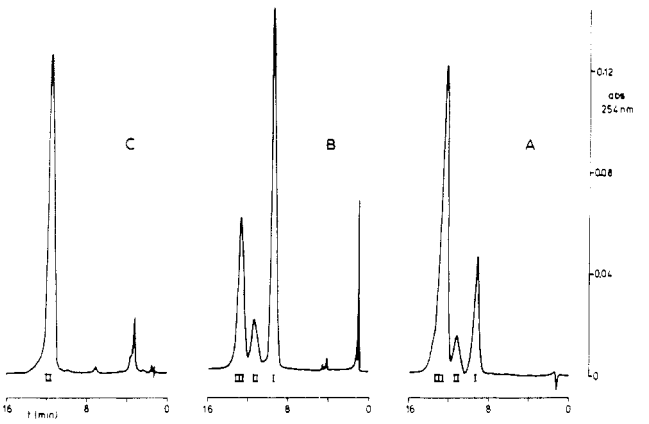
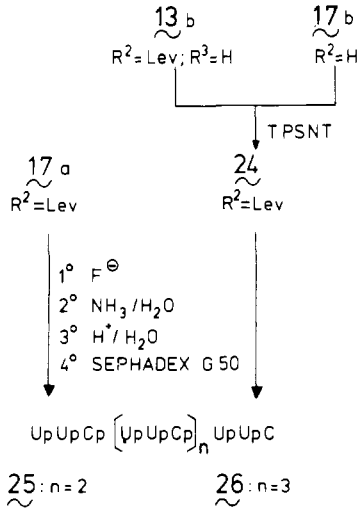


FIGURE 4: (A) HPLC analysis (system II) of the product mixture obtained after treatment of trimer **9a** with fluoride ions, followed by ammonia and acid. (B) HPLC analysis of the product mixture obtained after short ammonia treatment of the mixture corresponding to peaks I–III in Figure 4A. (C) HPLC analysis of the product obtained after digestion with takadiastase T_1 of the mixture corresponding to peaks I–III in Figure 4A.

22 (scheme VII) was prepared by a procedure analogous to the synthesis of trimer **9a** (see Scheme II), using 2′,3′-*O*-methoxymethylene-2-*N*-benzoylguanosine (den Hartog et al., 1980) instead of **4** (B = G^{Bz}) in the first coupling step. Complete deblocking (fluoride ions, ammonia, acid) of **22** gave the desired trimer ApUpG (**23**) (see Scheme VII).

Finally, the preparation of $(UUC)_4$ (**25**) and $(UUC)_5$ (**26**) is illustrated in Scheme VIII. For the preparation of $(UUC)_4$ (**25**) the fully protected dodecamer **17a** was used. The fully protected pentadecamer **24** was obtained by condensation of the suitably protected trimer UUCp (**13b**) and the 5′-hydroxy dodecamer derivative $(UUC)_4$ (**17b**). Deblocking of **17a** and **24**, followed by purification of the products, gave the desired dodecanucleotide $(UUC)_4$ (**25**) and pentadecanucleotide (**26**). Preliminary experiments performed with the synthetic RNA fragment (i.e., $AUG(UUC)_5$) indicated that normal translation occurs. A more detailed study including possible slippage of the synthetic messenger will be published soon.

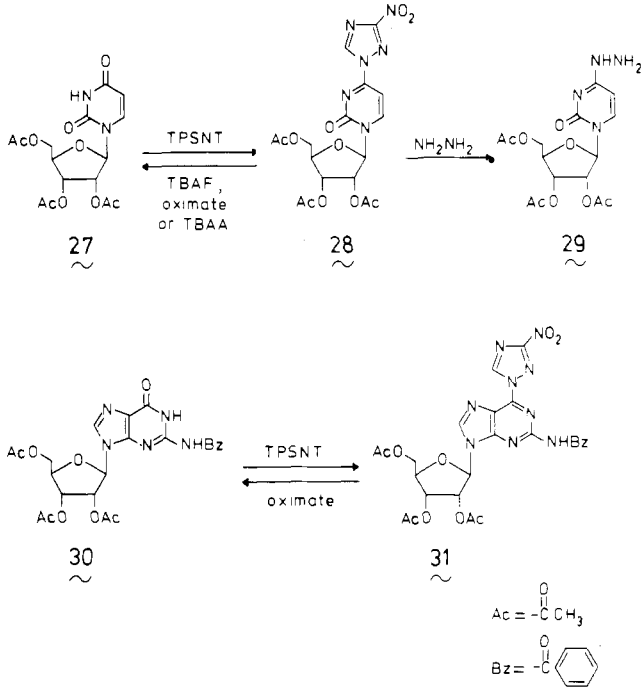
Scheme VIII



(II) Side Reactions Involving TPSNT. Recently it was reported (Reese & Ubasawa, 1980) that uridine and N^2-substituted guanosine nucleosides react with arenesulfonyl derivatives of 3-nitro-1,2,4-triazole and tetrazole. The 4-carbonyl and 6-carbonyl groups of the uracil and guanine base residues, respectively, are susceptible to reaction with, for instance, TPSNT, leading to the formation of nitrotriazole derivatives (e.g., compounds **28** and **31** in Scheme IX). Thus, when oligoribonucleotide fragments (e.g., trimers **9b** and **13c** in Scheme IV) containing uridine and/or 2-*N*-benzoylguanosine are coupled, in the presence of the condensing agent TPSNT, side reactions involving the base residues may occur. In our synthetic approach to oligoribonucleotides the obtained condensation product (i.e., a fully protected phosphotriester intermediate) is processed further in one of the following ways: (a) treatment with zinc to allow subsequent 3′ extension of the oligomer (e.g., conversion of trimer **13a** into **13b** in Scheme III); (b) hydrazinolysis to enable extension in the 5′ direction (e.g., conversion of trimer **13a** into **13c** in Scheme III); (c) complete deblocking of the fully protected oligomer (see Scheme VI), effected by treatment with TBAF, followed by ammonia and acid. To determine the consequences of possible nitrotriazole substitution of base residues during the synthesis of octadecamer **18a**, we subjected modified uridine and guanosine derivatives **28** and **31** to the reaction conditions applied in the conversion steps a–c. In Scheme IX the compounds involved in the latter experiments are depicted.

Derivatives **28** and **31** were obtained by reaction of compounds **27** and **30**, respectively, with a large excess of TPSNT in the presence of diphenyl phosphate (Reese & Ubasawa, 1980). The results of the experiments with compounds **28** and **31** were as follows. Both **28** and **31** were slowly degraded on addition of pyridine and zinc–TPSOH.[2] On hydrazine treatment the uridine compound **28** was quantitatively converted, within 4 min, into a derivative which presumably carried a hydrazine function on the uracil ring (**29**). Conversely, the nitrotriazole group of the guanosine derivative was resistant to hydrazinolysis for a much longer time (1 h). When **28** and **31** were treated with TBAF in tetrahydrofuran–pyridine–water, the first compound was completely converted into triacetyluridine (**27**) within 3 h while the latter reacted very

Scheme IX



slowly (less than 10% conversion into **30** after 16 h). Finally, the recently introduced (Reese & Yau, 1978) oximate reagent, which could be used instead of TBAF for the deblocking of internucleotide 2-chlorophenyl groups, was tested for its effect on **28** and **31**. On oximate treatment, both derivatives were rapidly (within 1 h) converted into the uridine and guanosine compounds **27** and **30**, respectively.

From these experiments the following conclusions can be drawn for the synthesis of octadecamer **18a**. When uridine is modified by nitrotriazole substitution, the product is further modified on treatment with zinc–TPSOH or hydrazine. On immediate deblocking of the nitrotriazole derivative with TBAF or oximate, the substitution is reversed. On the other hand, when guanosine is transformed into a nitrotriazole derivative, the product remains unchanged on treatment with hydrazine. Reductive treatment with zinc–TPSOH, however, leads to further modification of the nitrotriazole derivative. In case of nitrotriazole substituted guanosine derivatives, reversal of the modification does not occur when TBAF is used for deblocking of the product. Only when oximate is used for the latter purpose, the modification is reversed.[3]

Quantitation of the nitrotriazole substitution in the synthesis of octadecamer **18a** is an essential factor in the evaluation of this side reaction. For an estimation of the amount of the uracil and guanine modification ^{1}H NMR spectroscopy can be used. The presence of the characteristic nitrotriazole proton signal (δ ca. 9.5 ppm) could not be detected in the ^{1}H spectra of the fully protected hexamer $(UUC)_2$ (**16a**) and dodecamer $(UUC)_4$ (**17a**), which contain four and eight uridine residues,

[2] On addition to the reaction mixture of pentane-2,4-dione, which enhances the reduction, the remaining starting material was immediately converted into a complex mixture of fluorescent compounds with lower R_f values.

[3] The occurrence of side reactions as a consequence of possible nitrotriazole substitution on uracil and guanine ring systems is not restricted to our synthetic approach. Reactions with other nucleophilic reagents also led to further substitution reactions on nitrotriazole-substituted heterocyclic bases. For instance, when compounds **28** and **31** were treated with morpholine in acetone–water for 5 min, i.e., conditions applied in the removal of the dibromomethylbenzoyl group (Chattopadhyaya et al., 1979), complete conversion of both compounds into derivatives with lower R_f values (TLC, system B) was observed. The products were isolated and tentatively identified (^{1}H NMR) as morpholine-substituted uridine and guanosine derivatives.

respectively. Apparently, the conditions applied in the condensation reactions gave rise to a very small amount of nitrotriazole substitution in the uracil ring. Modification of guanosine was previously shown (Reese & Ubasawa, 1980) to occur even slower than modification of uridine. The following points might have contributed to suppress side reactions during condensation steps under the influence of TPSNT: (a) only a small excess of the 3′-phosphodiester component (a quantity of 1.1–1.6 equiv with respect to the 5′-hydroxy derivative) was used; (b) no large excess of TPSNT (a quantity of 1.5–2 equiv based on the 5′-hydroxy component) was applied; (c) condensation reactions were never longer than 4 h.

Although, under the condensation conditions applied by us, the reported side reactions did not occur to a significant extent, we finally investigated some ways to overcome the problems inherent to nitrotriazole substitution reactions. One approach could be the immediate removal of introduced nitrotriazole groups from the oligomers obtained after condensation reactions in the presence of TPSNT. Various systems were tested for this purpose. In experiments with the uridine derivative **28**, reversal of the nitrotriazole substitution was observed with tetrabutylammonium acetate (TBAA) or formate in tetrahydrofuran or 0.1 M K_2CO_3 in dioxane–water (Rayner et al., 1980) (within 0.5–2 h). However, none of these systems effectively removed nitrotriazole from the guanosine derivative **31**. Furthermore, the internucleotide phosphotriester functions proved to be unstable in the presence of tetrabutylammonium acetate or formate or K_2CO_3. Another approach to overcome problems caused by nitrotriazole substitution reactions would be the introduction of a suitable protection for uracil and guanine ring systems, to prevent the latter side reactions.

Acknowledgments

This project was initiated by Professor Dr. W. Möller of the Department of Physiological Chemistry, State University Leiden.

References

Adamiak, R. W., Biala, E., Grzeskowiak, K., Kierzek, W., Kraszewski, A., Markiewicz, W. T., Okupniak, J., Stawinski, J., & Wiewiorowski, M. (1978) *Nucleic Acids Res. 5*, 1889.

Chambliss, G., Craven, G. R., Davies, J., Kahan, L., & Nomura, M., Eds. (1979) *Ribosomes, Structure, Function and Genetics*, University Park Press, Baltimore, MD.

Chattopadhyaya, J. B., & Reese, C. B. (1980) *Nucleic Acids Res. 8*, 2039.

Chattopadhyaya, J. B., Reese, C. B., & Todd, A. H. (1979) *J. Chem. Soc., Chem. Commun.*, 987.

Crea, R., Kraszewski, A., Hirose, T., & Itakura, K. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5765.

den Hartog, J. A. J., & van Boom, J. H. (1980) *Recl. Trav. Chim Pays-Bas 700*, 275.

den Hartog, J. A. J., Wille, G., & van Boom, J. H. (1980) *Recl. Trav. Chim. Pays-Bas 700*, 320.

de Rooy, J. F. M., Wille-Hazeleger, G., van Deursen, P. H., Serdijn, J., & van Boom, J. H. (1979a) *Recl. Trav. Chim. Pays-Bas 98*, 537.

de Rooy, J. F. M., Arentzen, R., den Hartog, J. A. J., van der Marel, G., & van Boom, J. H. (1979b) *J. Chromatogr. 171*, 453.

Fieser, M., & Fieser, L. F. (1972) *Reagents for Organic Synthesis*, Vol. 3, p 277, Wiley-Interscience, New York.

Fieser, M., & Fieser, L. F. (1974) *Reagents for Organic Synthesis*, Vol. 4, p 478, Wiley-Interscience, New York.

Itakura, K., Hirose, T., Crea, R., Riggs, A. D., Heyneker, H. L., Bolivar, F., & Boyer, H. W. (1977) *Science (Washington, D.C.) 198*, 1056.

Jones, S. S., Rayner, B., Reese, C. B., Ubasawa, A., & Ubasawa, M. (1980) *Tetrahedron 36*, 3075.

Khorana, H. G. (1978) *Bioorg. Chem. 7*, 351.

Khorana, H. G., Agarwal, K. L., Büchi, H., Caruthers, M. H., Gupta, N. K., Kleppe, K., Kumar, A., Ohtsuka, E., RajBhandary, U. L., van de Sande, J. H., Sgaramella, V., Terao, T., Weber, H., & Yamada, T. (1972) *J. Mol. Biol. 72*, 209.

Ogilvie, K. K., & Theriault, N. Y. (1979) *Can. J. Chem. 57*, 3140.

Ohtsuka, E., Markham, A. F., Tanaka, S., Tanaka, T., Miyake, T., Nakagawa, E., Wakabayashi, T., Taniyama, Y., Fujiyama, K., Nishikawa, S., Fukumoto, R., Uemura, H., Doi, T., Tokunaya, T., & Ikehara, M. (1980) *Nucleic Acids Res., Spec. Publ. 7*, 335.

Rayner, B., Reese, C. B., & Ubasawa, A. (1980) *J. Chem. Soc., Chem. Commun.*, 972.

Reese, C. B. (1978) *Tetrahedron 34*, 3143.

Reese, C. B., & Yau, L. (1978) *Tetrahedron Lett.*, 2727.

Reese, C. B., & Ubasawa, A. (1980) *Tetrahedron Lett.*, 2265.

van Boom, J. H., & Burgers, P. M. J. (1978) *Recl. Trav. Chim. Pays-Bas 97*, 73.

van Boom, J. H., Owen, G. R., Ravindranathan, T., & Reese, C. B. (1971) *J. Chem. Soc. C*, 3230.

van Boom, J. H., Burgers, P. M. J., & Haasnoot, C. A. G. (1976a) *Nucleic Acids Res. 3*, 2731.

van Boom, J. H., Burgers, P. M. J., & van Deursen, P. H. (1976b) *Tetrahedron Lett.*, 869.

van Boom, J. H., Burgers, P. M. J., Crea, R., van der Marel, G., & Wille, G. (1977a) *Nucleic Acids Res. 4*, 747.

van Boom, J. H., Burgers, P. M. J., Haasnoot, C. A. G., & Reese, C. B. (1977b) *Recl. Trav. Chim. Pays-Bas 96*, 91.

van Boom, J. H., Burgers, P. M. J., van der Marel, G., Verdegaal, C. H. M., & Wille, G. (1977c) *Nucleic Acids Res. 4*, 1047.

van Boom, J. H., Burgers, P. M. J., Verdegaal, C. H. M., & Wille, G. (1978) *Tetrahedron 34*, 1999.

Werstiuk, E. S., & Neilson, T. (1976) *Can. J. Chem. 54*, 2689.