92. Nucleotides

Part XLIII[1])

Solid-Phase Synthesis of Oligoribonucleotides Using the 2-Dansylethoxycarbonyl (= 2-{[5-(Dimethylamino)naphthalen-1-yl]sulfonyl}ethoxycarbonyl; Dnseoc) Group for 5′-Hydroxy Protection

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(14.II.94)

A new efficient method for solid-phase synthesis of oligoribonucleotides *via* the phosphoramidite approach is described. The combination of the base-labile 2-dansylethoxycarbonyl (Dnseoc) group for 5′-OH protection with the acid-labile tetrahydro-4-methoxy-2*H*-pyran-4-yl (Thmp) group as 2′-OH blocking group is orthogonal regarding cleavage reactions and fulfills the requirements of an automated synthesis in an excellent manner if the phosphoramidite function carries the *N*,*N*-diethyl-*O*-[2-(4-nitrophenyl)ethyl] substitution.

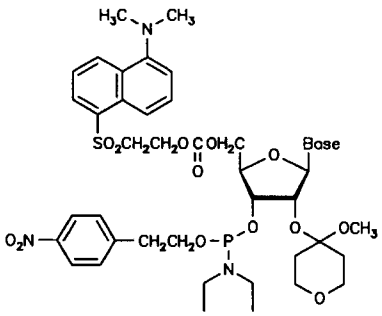
1. Introduction. – Synthetic oligoribonucleotides have achieved importance in RNA study concerning structure, catalytic activity, interactions with proteins or other reagents, and antisense approach.

The synthesis of oligoribonucleotides is difficult in comparison to that of oligodeoxyribonucleotides, since the additional 2′-OH function causes problems in compatibility of protecting groups and hence a higher synthetic expenditure to obtain the monomeric building blocks. The pronounced lability of free oligoribonucleotides against chemical isomerization and cleavage as well as enzymatic degradation creates further problems.

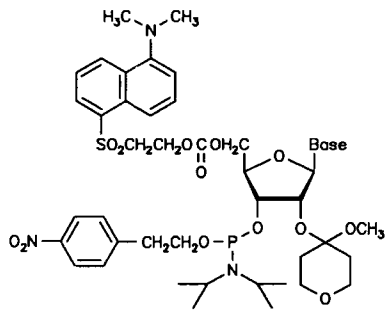
The 2′-OH protecting group must be stable during the various chemical steps of the oligoribonucleotides synthesis and should then be removed quantitatively without harming the internucleotide linkages of the oligomer. The 5′-OH protecting group, on the other hand, must be cleaved selectively and quantitatively before each chain elongation and should also be easily detectable to determine the coupling efficiency.

Today, solid-phase syntheses of oligoribonucleotides are mainly accomplished by the phosphoramidite method developed by *Caruthers* and coworkers [2–5] and *Köster* and coworkers [6]. tRNA Syntheses were reported by *Ogilvie et al.* [7], *Chamberlin* and coworkers [8], and recently *Teoule* and coworkers [9] who used the (*tert*-butyl)-dimethylsilyl group for 2′-OH protection in combination with the traditional 5′-*O*-dimethoxytrityl group. The deblocking procedure, however, were problematical [10] [11]. Shorter RNA fragments were synthesized with the photolabile 2-nitrobenzyl group

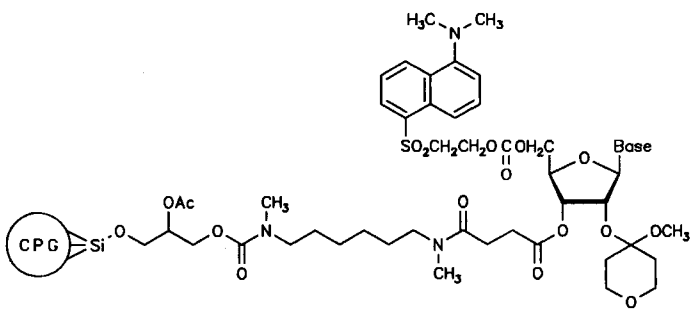
[1]) Part XLII: [1].



Base	
1	Ade^{npeoc}
2	Gua^{npe}_{npeoc}
3	Cyt^{npeoc}
4	Ura
5	Ura^{an}
6	Ura^{boc}
7	Thy
8	ψra
9	$ψra^{(npe)_2}$

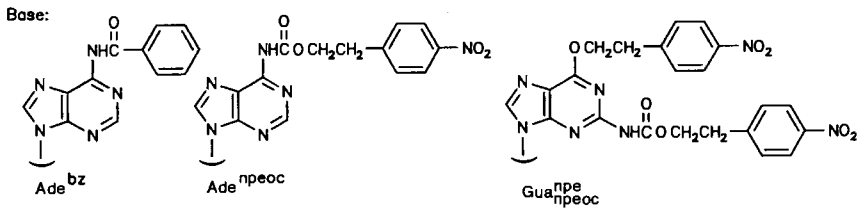


Base	
10	Ade^{bz}
11	Ade^{npeoc}

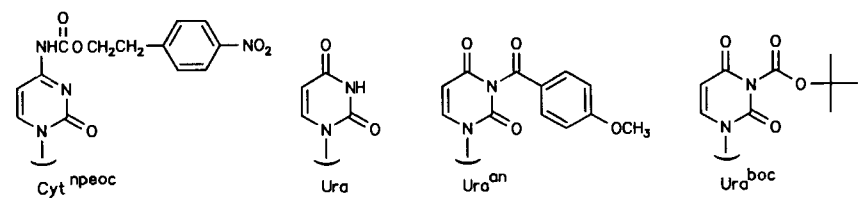


Base	
12	Ade^{npeoc}
13	Gua^{npe}_{npeoc}
14	Cyt^{npeoc}
15	Ura

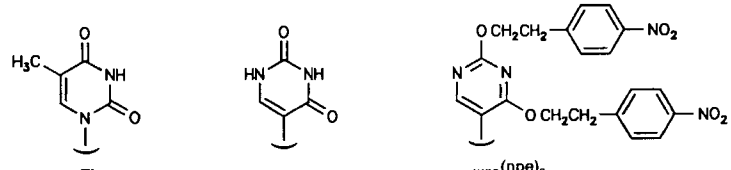
Base:



Ade^{bz} Ade^{npeoc} Gua^{npe}_{npeoc}



Cyt^{npeoc} Ura Ura^{an} Ura^{boc}



Thy ψra $ψra^{(npe)_2}$

for 2′-OH protection by *Ikehara* and coworkers [12] [13]. The use of acid-labile, acetalic 2′-OH protecting groups were put forward by *Reese* and coworkers [14–17] applying the 1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl (Ctmp) and 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp) groups in conjunction with the also acid-labile 5′-*O*-pixyl group, whereas *Gait* and coworkers [18] combined the tetrahydro-4-methoxy-2*H*-pyran-4-yl group with the base-labile 5′-*O*-[(9*H*-fluoren-9-yl)methoxycarbonyl] group.

We wish to report in this paper an efficient method for solid-phase synthesis of oligoribonucleotides *via* the phosphoramidite approach using the tetrahydro-4-methoxy-2*H*-pyran-4-yl (Thmp) group for 2′-OH protection and the recently investigated, base-labile 2-dansylethoxycarbonyl (= 2-{[5-(dimethylamino)naphthalen-1-yl]sulfonyl}-ethoxycarbonyl; Dnseoc) group for 5′-OH protection which showed good features in oligodeoxyribonucleotides synthesis [19] and in the synthesis of triadenylate in solution [20].

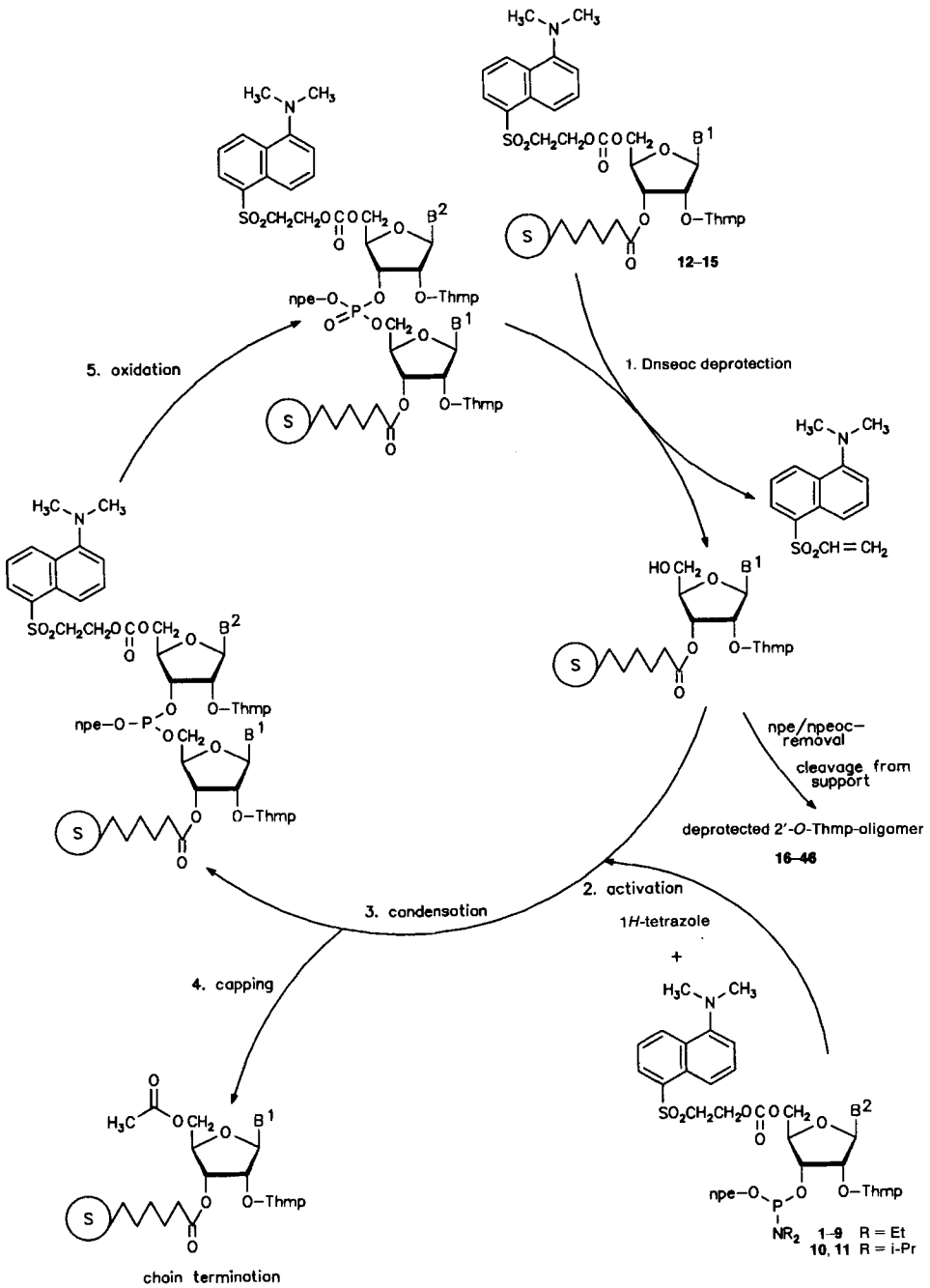
2. Syntheses. – The syntheses of the phosphoramidites **1**–**11** and the nucleoside-functionalized supports **12**–**15** were described recently [1]. The Thmp group was chosen for 2′-OH protection because it can be removed under mild acidic conditions, and it is achiral simplyfying the synthesis and characterization of the monomeric building blocks. To achieve absolute compatibility, the temporary, base-labile Dnseoc group was developed for 5′-OH protection. Its deprotection with dilute DBU (= 1,8-diazabicyclo[5.4.0]undec-7-ene) solution requires the use of the DBU-stable LCAMA-CPG (= (*l*ong-*c*hain-*a*lkyl)*m*ethyl*a*mine *c*ontrolled-*p*ore *g*lass) support and of the 2-(4-nitrophenyl)ethyl (npe) phosphate protecting group which is more stable against β-elimination than the traditional 2-cyanoethyl group. The npe/npeoc protecting group strategy (npeoc = 2-(4-nitrophenyl)ethoxycarbonyl) was used to protect the functional groups of the nucleobases because these blocking groups can be advantageously removed from the synthesized oligoribonucleotide when it is still connected with the solid phase. In principle, the acyl-protecting-group strategy can also be applied as demonstrated with N^6-benzoyladenosine revealing, however, some disadvantages during final deblocking and isolation of the oligomer.

The automated chain-elongation to various oligoribonucleotides was performed in an *Applied Biosystems 380 B* synthesizer by attachment of a small *ABI* column filled with a definite amount (mostly 0.3–0.6 μmol) of the support loaded with the desired starting nucleoside (see **12**–**15**). The oligoribonucleotide assembly consists of a programmed repetitive cycle of four chemical steps and intermediate washing steps analogous to the features of the oligodeoxyribonucleotide synthesis [19] and is documented as follows (see the *Scheme*):

1) deprotection of terminal Dnseoc groups with 0.1M DBU in MeCN for 140 s;
2) coupling with 0.1M phosphoramidite (**1**–**11**) and 0.5M 1*H*-tetrazole in MeCN for 760 s;
3) capping with Ac_2O/2,6-dimethylpyridine/1-methyl-1*H*-imidazole in THF for 50 s;
4) oxidation with 0.05M I_2 in THF/pyridine/H_2O for 53 s.

The coupling efficiency which turned out to be greater than 98% was checked by the measurement of the absorbance at 350 nm of each eluate from the Dnseoc-deprotection steps containing 5-(dimethylamino)naphthalen-1-yl vinyl sulfone. The determination of condensation yields was also possible by measurement of the fluorescence at 530 nm.

Scheme


1. Dnseoc deprotection
2. activation
1H-tetrazole
+
3. condensation
4. capping
5. oxidation
npe/npeoc–
removal
cleavage from
support
deprotected 2'-O-Thmp-oligomer
16–46
chain termination
12–15
1–9 R = Et
10, 11 R = i-Pr

After completion of the oligoribonucleotide assembly, the support was treated with 0.5M or 1M DBU in MeCN for 10 h to deblock all npe and npeoc protecting groups from the oligonucleotide. Thereafter, the 2′-*O*-Thmp-protected oligoribonucleotide was cleaved from the support by treatment with concentrated ammonia solution for 200 min. The amount of isolated 2′-*O*-Thmp-protected oligoribonucleotide was determined in a small aliquot of the ammoniacal eluate by measurement of the absorbance at 260 nm. The total oligonucleotide solution was then lyophilized in a *Speed-vac* concentrator and the quality of the isolated crude 2′-*O*-Thmp-protected oligoribonucleotide proven by reversed-phase or anion-exchange HPLC and gel electrophoresis. Short 2′-*O*-Thmp-oligoribonucleotides up to 20 bases showed in most cases a high purity, pure enough for direct use, whereas longer 2′-*O*-Thmp-oligoribonucleotides had to be purified further by prep. gel electrophoresis or anion-exchange HPLC to afford a homogeneous composition without danger of chain degradation due to the stabilizing 2′-acetal protection. The latter guarantees also an easy storage of the synthesized oligoribonucleotide in contrast to the one of the fully deprotected form. To get the free oligoribonucleotide, the 2′-*O*-Thmp-oligoribonucleotides were treated with 0.01M HCl or more advantageously with 80% AcOH/H_2O for several h.

3. Results. – *Table 1* shows the synthesized sequences of crude 2′-*O*-Thmp-oligoribonucleotides **16–46** and the isolated yields. Typical HPLC chromatograms of the four crude 2′-*O*-Thmp-protected homo-decamers of Ado (see **19**), Guo (see **22**), Cyd (see **24**), and Urd (see **26**), directly obtained from the synthesizer without purification, are given in *Fig. 1*. In addition, several 2′-*O*-Thmp-protected oligoribonucleotides were characterized by capillary gel electrophoresis showing a single peak and laser-desorption (LD) mass spectrometry[2]) revealing the correct mass of, *e.g.*, the nonaammonium salt of $[A(thmp)]_{10}$ (**19**) at 4524 (*Fig. 2*).

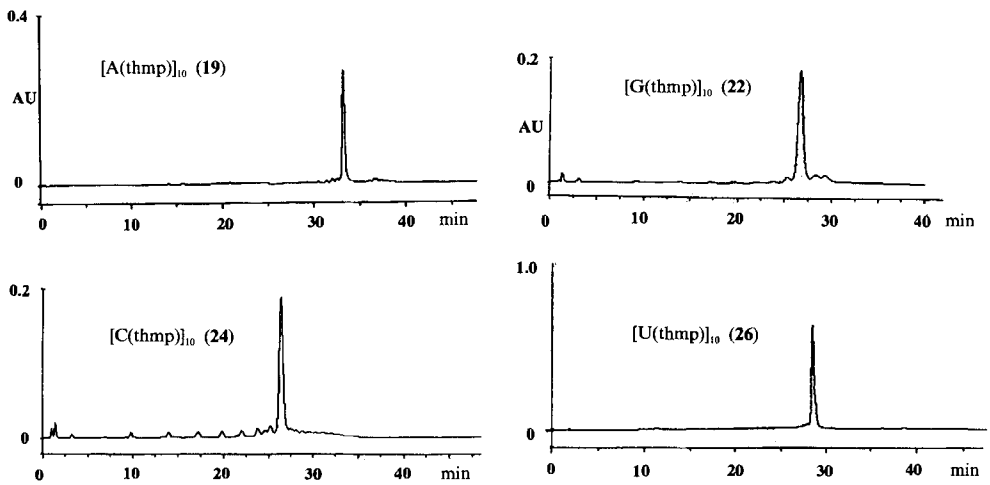


Fig. 1. *HPLC Trails of* **19** *and* **26** *on* RP-18 *and of* **22** *and* **24** *on* Nucleogen *column*. AU = arbitrary unit.

[2]) Capillary gel electrophoresis was performed by Dr. *E. Uhlmann*; Fa. *Hoechst*, Pharma Research, and LD-MS by Dr. *M. Grübler*, Fa. *Eppendorf*.

Table 1. *Synthesis of 2′-O-Thmp-Oligoribonucleotides* **16**–**46** *Using the Monomeric Building Blocks* **1**–**9** *and the Solid-Support Material* **12**–**15**. Support: LCAMA-CPG 500 Å.

Sequence (length)[a]		Amount of starting nucleoside [μmol]	Phosphoramidites	Isolated yield (in *OD*)
A_4 (4)	**16**	0.3 of **12**	**1**	3
A_5 (5)	**17**	0.6 of **12**	**1**	30[b])
A_6 (6)	**18**	0.3 of **12**	I	12
A_{10} (10)	**19**	0.6 of **12**	**1**	49[b])
G_2 (2)	**20**	0.6 of **13**	**2**	10
G_4 (4)	**21**	0.6 of **13**	**2**	22
G_{10} (10)	**22**	0.6 of **13**	**2**	50[b])
C_5 (5)	**23**	0.3 of **14**	**3**	8[b])
C_{10} (10)	**24**	0.6 of **14**	**3**	34[b])
U_5 (5)	**25**	0.3 of **15**	**4**	10
U_{10} (10)	**26**	0.6 of **15**	**4**	35
U_{10} (10)	**26**	0.6 of **15**	**5**	47[c])
T_9U (10)	**27**	0.6 of **15**	**7**	42[b])
ψ_9U (10)	**28**	0.6 of **15**	**8**	22[b])
ψ_9U (10)	**28**	0.53 of **15**	**9**	27[b])
GUGAA (5)	**29**	0.6 of **12**	**1**, **2**, **5**	31[c])
GGUUAA (6)	**30**	0.61 of **12**	**1**, **2**, **4**	28
GGUUAA (6)	**30**	0.63 of **12**	**1**, **2**, **5**	29[c])
GUGUGUG (7)	**31**	0.53 of **13**	**2**, **5**	27[c])
GUUUGAA (7)	**32**	0.64 of **12**	**1**, **2**, **4**	32
CCAAACCC (8)	**33**	0.61 of **14**	**1**, **3**	38[b])
GGUGAGUGA (9)	**34**	0.57 of **12**	**1**, **2**, **4**	41[b])
GGGGGUGGGG (10)	**35**	0.6 of **12**	**2**, **4**	45[b])
GGGUGAGUGGA (11)	**36**	0.6 of **12**	**1**, **2**, **4**	33
GGUUGGUUGGU (11)	**37**	0.6 of **15**	**2**, **4**	41
GGAAGUGUGUUGAA (14)	**38**	0.6 of **12**	**1**, **2**, **4**	37
UGGUCCUGAUUGUGA (15)	**39**	0.6 of **12**	**1**, **2**, **3**, **4**	63[b])
UAAUCCUAAUUAUAA (15)	**40**	0.63 of **12**	**1**, **3**, **4**	67[b])
AGGACCAGAAAGAGA (15)	**41**	0.6 of **12**	**1**, **2**, **3**	76[b])
GCGUGGAAGCCGUGGUUC (18)	**42**	0.6 of **14**	**1**, **2**, **3**, **4**	81[b])
ACGUUCCUCCUGCGGGAA (18)	**43**	0.6 of **12**	**1**, **2**, **3**, **4**	69
GCCGCGCUGCGCCGGAGUCCC (21)	**44**	0.6 of **14**	**1**, **2**, **3**, **4**	66[b])
GGAGAGGUCUCCGGTΨCGAUU-CCGGACUCGUCCACCA (37)	**45**[d])	0.6 of **12**	**1**, **2**, **3**, **4**, **7**, **8**	99[b])
UGUGGCUAUAUCAUAAUUGGU-UAAUGGUCCUGAUUGUGAAΨC-AGGCCUAUGUGGATΨCGAAUU-CUACUAGCCACACCA (78)	**46**[e])	0.6 of **12**[f])	**1**, **2**, **3**, **4**, **7**, **8**	217

[a]) Sequences are listed in 5′→3′ direction; for convenience, the internal Thmp and the terminal Thmp groups (all at C(2′)) as well as the hyphens for the diesterified phosphate residues between the nucleoside residues are omitted in the sequence formulae.

[b]) Further treatment of the support with conc. NH_3 soln. for 12 h at r.t.

[c]) 2′-*O*-Thmp-oligoribonucleotide contains 4-methoxybenzamide raising the absorption.

[d]) Sequence of **45**: 3′-terminal end of yeast Ala-tRNA.

[e]) Sequence of **46**: His-tRNA of phage T5.

[f]) LCAMA-CPG 1000 Å.

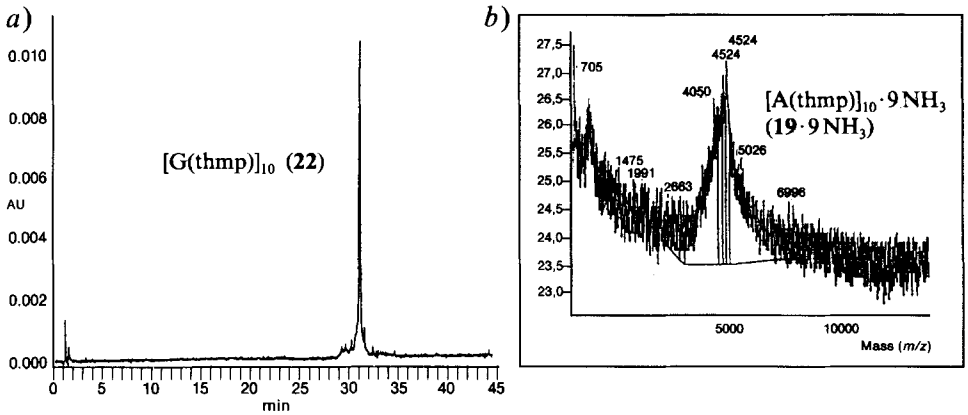


Fig. 2. a) *Capillary gel electrophoresis* (5% polyacrylamide) *of crude* $[G(thmp)]_{10}$ (**22**) *and* b) *LD-MS of crude nonaammonium salt of* $[A(thmp)]_{10}$ (**19**) *in sinapinic-acid matrix.* AU = arbitrary unit.

Best results were obtained by using a 0.1M phosphoramidite concentration in the coupling step since lower concentrations afforded clearly lower coupling yields, and higher concentrations did not lead to purer oligomers and hence were not economic. During the synthesis of the A_{10} oligomer **19**, it was realized that the newly introduced strategy worked faster and more efficiently by use of the *N,N*-diethylphosphoramidite **1** instead of the corresponding *N,N*-diisopropylphosphoramidite **11**, thus recommending the more reactive *N,N*-diethylphosphoramidites in general for this strategy. The synthesis of the A_{10} oligomer **19** using phosphoramidite **10** instead of **11** demonstrated also the advantage of the npeoc protecting group of **11** over *N*-acyl groups, due to a more efficient removal concerning completeness and time of cleavage. Furthermore, the cleaved npe and npeoc groups can be washed off as long as the oligonucleotide is still attached to the support, whereas the application of the common acyl protecting groups would afford further purification after deprotection. In the case of 2′-*O*-Thmp-protected cytidine oligomers, the *RP-18* HPLC gave, with rising chain length, broadening product peaks resulting probably from chain aggregation or secondary-structure effects. This phenomenon was also reported by *Gait* and coworkers [18]. The 2′-*O*-Thmp-protected C_{10} oligomer **24** showed an extremely broad peak on *RP-18* but a normal peak shape on an anion-exchange phase. The product peak of reversed-phase HPLC of uridine-rich sequences exhibited often a shoulder towards longer retention times of the main peak, due perhaps to some by-products resulting from back addition of 5-(dimethylamino)naphthalen-1-yl vinyl sulfone and 4-nitrostyrene into the N^3-position although an enzyme digestion did not absolutely support this assumption. The same considerations must be taken into account for oligoribonucleotide syntheses applying the unprotected ribothymidine (= ribosylthymine) and pseudouridine phosphoramidites **7** and **8**, respectively. Further investigations must be carried out applying other cleavage conditions by addition of vinyl traps such as thiocresol to establish whether base protection is necessary or not. Good results showed the O^2,O^4-di-npe-protected pseudouridine derivative **9** and the N^3-anisoyl-protected uridine derivative **5**, whereas N^3-boc protection turned out to be ineffective due to partial cleavage during chain assembly. It should, furthermore, be

noted that a removal of some npe phosphate protecting groups of the support-bound oligomer during the Dnseoc-deprotection processes cannot by totally excluded. If this side reaction occurs, it is possible that the formed phosphodiester functions might also be phosphitylated during the coupling reactions. This side reaction, however, has no influence on the quality of the synthesized oligonucleotide because subsequent treatments regenerate the desired phosphodiester functions *via* hydrolysis, *e.g.* at every oxidation process. This could be demonstrated by the synthesis of $[C(thmp)]_2$-$[A(thmp)]_3$-$[C(thmp)]_3$ (**33**) applying modified conditions (1M DBU in MeCN) to deprotect the Dnseoc groups. The HPLC of the isolated 2′-*O*-Thmp-oligoribonucleotide **33** showed no difference compared to the same oligonucleotide synthesized under normal Dnseoc-deprotection conditions using 0.1M DBU in MeCN. Particularly, in the case of long sequences, a reduction of phosphoramidite excess would be the consequence.

In general, longer sequences up to 37-mer **45** and 78-mer **46** afforded further purification of the synthesized 2′-*O*-Thmp-oligoribonucleotides by prep. polyacrylamide gel electrophoresis (PAGE) or more advantageously by anion-exchange HPLC and subsequent desalting by gel filtration without danger of degradation (*Figs. 3* and *4*). The 37-mer sequence **45** represents the 3′-terminal half of yeast alanine-tRNA, already synthesized by *Reese* and coworkers [21] [22] appyling the phosphotriester approach in solution, whereas the 78-mer **46** is the complete sequence of histidine-tRNA of phage T5.

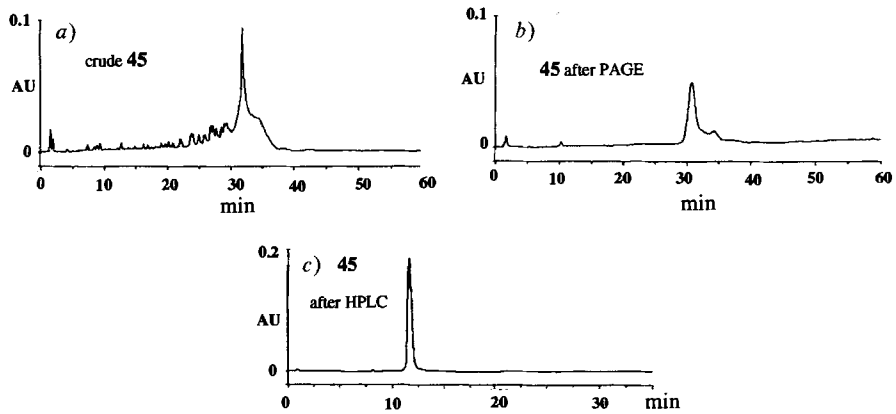


Fig. 3. *HPLC Studies of* a) *crude* **45** *on a* NucleoPak *column*, b) *PAGE-purified* **45** *on a* RP-18 *column, and* c) Gen-Pak-*purified* **45** *on a* Gen-Pak *column*. AU = arbitrary unit.

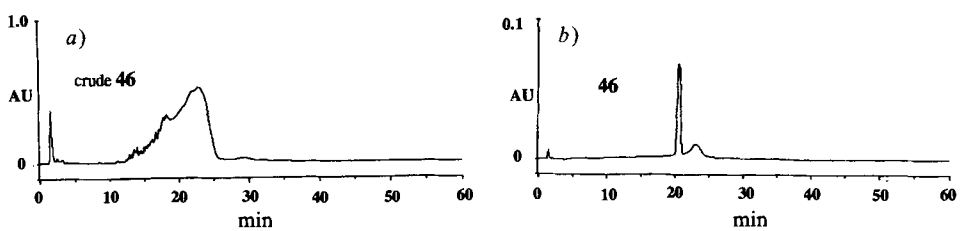


Fig. 4. *HPLC Studies of* a) *crude* **46** *and* b) *anion-exchange purified* **46** *on a* NucleoPak *column*. AU = arbitrary unit.

The 2′-*O*-Thmp-protected storage form of the oligomers could be converted into the free oligoribonucleotides by treatment with 0.01M HCl at pH 2 if needed. Treatment with 80% AcOH/H_2O gave the same results and is recommended since the HCl solution had to be neutralized after deprotection and then desalted by gel filtration, whereas the AcOH solution could be directly lyophilized. The rate of deprotection of the 2′-*O*-Thmp groups was monitored by reversed-phase HPLC. The Thmp groups of short oligomers were completely removed within 2–3 h, *e.g.* from $[A(thmp)]_{10}$ (**19**; *Fig. 5*), whereas the 37-mer tRNA half **45** did not give a pure product after 24 h of acid treatment. A purification by reversed-phase HPLC was necessary (*Fig. 6*), indicating that possibly self aggregation made complete Thmp removal more difficult.

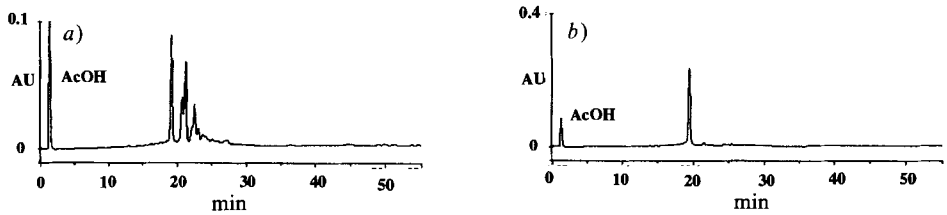


Fig. 5. *Thmp Cleavage of* **19** *in 80% AcOH/H_2O after* a) *10 min and* b) *2 h*. AU = arbitrary unit.

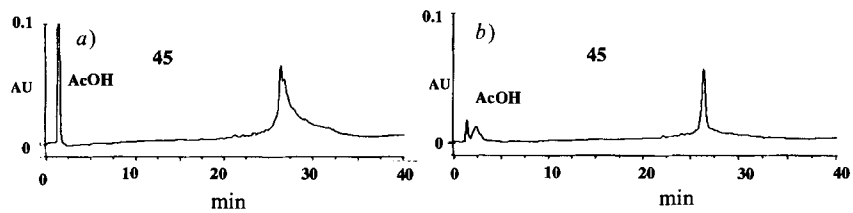


Fig. 6. *Thmp Cleavage of purified 37-mer* **45** *in 80% AcOH/H_2O after* a) *24 h and* b) *24 h followed by* RP-18 *HPLC purification*. AU = arbitrary unit.

Several free oligoribonucleotides were digested by snake-venom phosphodiesterase and alkaline phosphatase to ensure the right composition (see *Exper. Part, Table 2*).

Experimental Part

General. Synthesizer: *Applied Biosystems 380 B* und *392*. Reagents: DNA-grade MeCN (< 50 ppm H_2O); DBU *purum* was dried over molecular sieve; 1*H*-tetrazole was freshly sublimed; THF *p.a.* was freshly distilled from CaH_2; Ac_2O, 1-methyl-1*H*-imidazole, pyridine, I_2 and conc. NH_3 soln. were used in *p.a.* grade, 2,6-dimethylpyridine in *purum* grade. Lyophilization: *Savant Speed-vac* concentrator in 1.5-ml *Eppendorf* tubes under high vacuum. HPLC: *Merck/Hitachi* system, gradient pump *L-6200*, UV-detector *L-4000*, HPLV manager software. UV/VIS: *Perkin-Elmer, Lambda 15*.

1. *Assembly of Oligoribonucleotides.* A definite amount of the nucleoside-functionalized LCAMA-CPG support **12–15** (*ca.* 0.3–0.6 µmol) was weighed into a small *ABI* column, and after sealing and connecting with the flow path system of the synthesizer, a programmed repetitive series of reagent and solvent washes based on recommended procedures with the following main steps were performed: *1*) 5′-*O*-Dnseoc Deprotection: 0.1M DBU in MeCN delivered in 2 30-s and 8 10-s bursts with intermediate 1-s reverse flushes. The eluate from this step was collected and the absorbance at 350 nm measured to determine the condensation yields. *2*) Coupling: 0.1M

phosphoramidite (P) and 0.5M 1*H*-tetrazole (T) in dry MeCN delivered in a series of alternating bursts (8 s T, 4 s P + T, 3 s T, 3 s P + T, 3 s T, 60 s wait time, 3 s P + T, 3 s T) with a subsequent wait time of 700 s. *3*) Capping: soln. A: Ac_2/2,6-dimethylpyridine/THF 1:1:8; soln. B: 1-methyl-1*H*-imidazole/THF 16:84 delivered in one 20-s burst with a subsequent wait time of 30 s. *4*) Oxidation: 0.05M, I_2 in THF/H_2O/pyridine 7:2:1 delivered in one 23-s burst with a subsequent wait time of 30 s.

After completion of the oligoribonucleotide assembly, a cleavage programme was carried out: *5*) npe/npeoc Deprotection: 0.5M or 1M DBU in MeCN delivered in one 180-s burst with a consecutive wait time of 3600 s and 5 times one 120-s burst followed by another wait time of 5400 s (total wait time 10 h). *6a*) Cleavage from the support: conc. NH_3 soln. delivered in one 18-s burst with a consecutive wait time of 2400 s (5 times, total wait time 3 h 20 min). *6b*) If acyl groups (anisoyl or benzoyl) for nucleobase protection were used, the ammoniacal eluate was heated in a sealed tube at 50° for 4–48 h depending on the species (an or bz) and quantity of acyl groups. The an group is more labile than bz, thus requiring shorter treatments with NH_3 for removal than bz.

The ammoniacal oligonucleotide-containing soln. obtained after deprotection according to *6a* or *6b* was submitted to the UV absorbance measurement at 260 nm (determination of the isolated amount of oligoribonucleotide) and then lyophilized in a *Speed-vac* concentrator under high vacuum.

2. *Purification of 2′-*O*-Thmp-Oligoribonucleotides by Anion-Exchange HPLC.* Lyophilized crude 2′-*O*-Thmp-oligoribonucleotides which needed further purification were dissolved in H_2O and purified by anion-exchange HPLC: Shorter 2′-*O*-Thmp-oligoribonucleotides were purified on a *Nucleogen 60-7 DEAE* column (*Macherey-Nagel*) using the gradient 20% MeCN/0.02M AcONa/0–1M LiCl (flow rate 1 ml/min). Some longer 2′-*O*-Thmp-oligoribonucleotides were purified on a *Gen-Pak FAX* column (*Millipore-Waters*) applying the gradient 0.011M Na_2HPO_4/0.11–1.5M NaCl in MeCN/H_2O 1:4 (flow rate 0.75 ml/min). In each case, the separated product-peak fraction was desalted by gel filtration on *Sephadex G-10* (shorter) and *G-25* (longer, *Pharmacia*), quantified by UV spectroscopy, and lyophilized. Yields depended on the purity and length of the crude oligonucleotides and were in the case of a 10-mer mostly *ca.* 80%.

A portion of crude 2′-*O*-Thmp-protected 78-mer **46** (yield 23 OD_{260}) was purified on a *NucleoPak PA-100* column (*Dionex*) using the gradient 0.02M NaOH/0.02–1.5M NaCl (flow rate 1 ml/min). The isolated product-peak fraction was neutralized with 0.02M HCl, directly desalted on a *PD-10* column (*Sephadex-G-25M*, *Pharmacia*), and lyophilized. Yield 1 OD_{260}.

Besides anal. reversed-phase HPLC on a *LiChrospher 100 RP-18* column (*Merck*) with the gradient 2.5–50% MeCN in 0.1M AcO($NHEt_3$) (pH 7; flow rate 1 ml/min), the above *Nucleogen*, *Gen-Pak FAX*, and *NucleoPak PA-100* columns were also used for the anal. characterization with the same elution gradients as described above.

3. *Purification of 2′-*O*-Thmp-Oligoribonucleotides by Prep. Polyacrylamid Gel Electrophoresis (PAGE).* Crude 2′-*O*-Thmp-oligoribonucleotides which needed further purification could also be purified by prep. PAGE. The oligonucleotides were taken up in 80% formamide in electrophoresis loading buffer (*Tris* (10.8, 0.09), H_3BO_4 (5.5 g, 0.09 mol) and $Na_2EDTA \cdot 2\,H_2O$ (0.85 g, 2.3 mmol) in H_2O (1 l), adjusted to pH 8 with H_3BO_4) and applied to a 20% denaturing polyacrylamide gel (for **46** a 8% denaturing polyacrylamide gel was used). After electrophoresis, the product located by UV shadowing was cut out and eluted with 0.5 ml of a buffer consisting of 0.1M *Tris*, 0.5M NaCl, and 5 mM EDTA (pH 8) for 12–16 h at 37°. After centrifugation, the overstanding soln. was applied to a *RP-18* column and eluted with 4 ml of H_2O and 1 ml of 0.1M AcO($NHEt_3$)/MeOH 1:1 (oligonucleotide). The 2′*O*-Thmp-oligoribonucleotide was then quantified by UV spectroscopy and lyophilized. The yields were a little lower in comparison to anion-exchange HPLC purification and depended also on the purity and length of the crude oligonucleotide.

PAGE was also used for anal. characterization. The product bands were colored by the 'stains-all' method and photographed.

4. *Deprotection of 2′-*O*-Thmp Groups.* A soln. of 1 *OD* of pure enough crude (from *Section 1*) or purified (from *Sections 2* or *3*) 2′-*O*-Thmp-oligoribonucleotide in 1 ml of 80% AcOH/H_2O (pH 2) or 1 ml of 0.01M HCl (pH 2) was kept at r.t. for 2–24 depending on the length and composition of the oligonucleotide (monitoring by reversed-phase HPLC as described above). After deprotection, the AcOH soln. was directly lyophilized. In contrast to this, the HCl soln. had to be neutralized with 0.1M NH_3, desalted by a *PD-10* column (*Sephadex G-25M*, *Pharmacia*) eluting with sterile H_2O, and then lyophilized. In the case of PAGE-purified 37-mer **45**, an impure material was obtained even after 24 h treatment at pH 2 so that a purification by reversed-phase HPLC was directly performed as described above.

5. *Enzymatic Digestion.* A soln. of 1 *OD* of the fully deprotected oligoribonucleotide in 40 µl of buffer (50 mM (0.6 g/100 ml) *Tris*, 10 mM (0.2 g/100 ml) $MgCl_2$, adjusted to pH 8 with 1M HCl) and 30 µl of H_2O was treated with

Table 2. *Enzyme Digestion of Some Free Oligoribonucleotides*

Sequence[a]) from	Length	Bases	Found	Calculated
33	8	C/A	5.0:2.7	5:3
36	11	U/G/A	2.0:7.0:2.4	2:7:2
38	14	U/G/A	4.0:5.5:4.2	4:6:4
42	18	C/U/G/A	4.4:4.3:7.7:2.0	4:4:8:2
44	21	C/U/G/A	10.0:1.9:7.5:1.4	10:2:8:1
45	37	Ψ/C/U/T/G/A	0.8:12.5:5.5:0.8:10.4:5.8	1:12:6:1:11:6

[a]) Fully deprotected oligoribonucleotide.

6.0 µl of snake-venom phosphodiesterase (1 mg (0.5 ml), *Boehringer*) and 6.4 µl of alkaline phosphatase (1 u/µl, *Boehringer*) for 2–12 h at 37°. The monomeric nucleoside fragments were analyzed by reversed-phase HPLC (*LiChrospher 100 RP-18* column (*Merck*); 0–50% MeCN in 0.1M $AcO(NHEt_3)$ (pH 7); flow rate 1 ml/min). Results: *Table 2*.

The fully deprotected oligoribonucleotides from **33**, **36**, **38**, and **42** were digested as crude products. Oligonucleotide **44** was first purified in its 2′-*O*-protected form as described in *Section 2*, and after Thmp deprotection (*Section 4*) digested. Similarly, **45** was first purified in its 2′-*O*-protected form as described in *Section 3*, and after Thmp deprotection purified again as described in *Section 4*, and then digested.

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