Automated RNA-Synthesis with Photocleavable Sugar and Nucleobase Protecting Groups

Alfred Stutz, Stefan Pitsch*

Organisch-Chemisches Laboratorium der Eidgenössischen Technischen Hochschule, Universitätstrasse 16, CH-8092 Zürich, Switzerland
Fax +41(1)6321136

Received 29 January 1999

Abstract: A new synthetic method for the *N*-alkyloxycarbonylation of adenine and guanine nucleosides was developed and used for the preparation of RNA-phosphoramidites carrying photolabile sugar and nucleobase protecting groups. From these building blocks, a heptameric oligoribonucleotide was prepared by automated synthesis, followed by detachment from the solid support and photolytic deprotection under mild conditions. The presented strategy allows a simple preparation of 3'-*O*-aminoacylated RNA-sequences.

Key words: phosphoramidites, base-protecting groups, RNA-synthesis, photolabile protection, aminoacylation

A severe limitation in the chemical synthesis of oligonucleotide analogues is the requirement to remove the commonly used acyl-type nucleobase protecting groups with strong nucleophiles such as NH_3 or $MeNH_2$. For instance, some of the naturally occurring modified nucleosides, e.g. pseudouridine and dihydrouridine are not stable under the conditions required for deprotection of the standard base protecting groups benzoyl (for cytidine and adenosine) and isobutyryl (for guanosine). In this context, more labile base protecting groups such as phenoxyacetyl have been developed and extend the range of tolerated modifications significantly.[1] Another approach involves protection groups such as allyloxycarbonyl which are removed under nonnucleophilic conditions by the assistance of Pd^0-complexes.[2]

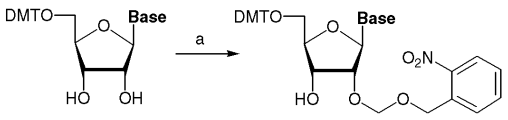
One of the mildest deprotection is offered by photolytic removal of appropriate protecting groups. We had earlier developed an efficient method for the synthesis of RNA phosphoramidites carrying the photolabile 2'-*O*-[(2-nitrobenzyl)oxy]methyl sugar protecting group, which can be removed under mild, weakly acidic conditions.[3] In the above mentioned context, we now wanted to extend the scope of RNA-synthesis by preparing phosphoramidite building blocks with photocleavable sugar and base protecting groups.

Specifically, we were interested in a general, nonenzymatic synthesis of 3'-*O*-aminoacylated t-RNA analogues which allow the biosynthetic, ribosome-mediated incorporation of unnatural amino acids into proteins.[4,5] So far, such aminoacylated t-RNA analogues were synthesized by enzymatic ligation of truncated t-RNAs with 3'-*O*-aminoacylated dimers. The latter compounds were prepared by condensation of a weekly activated, *N*-protected amino acid with a non- or partially protected ribodinucleotide.[4,5,6,7] A severe limitation is the sensitivity of the ester-linkage towards basic hydrolysis at pH-values, where the enzymatic ligation step is carried out ($t_{1/2}$ (pH 7.5) = 22 min.).[5]

Our retrosynthetic scheme for the preparation of 3'-*O*-aminoacylated t-RNA analogues differs from the known concept by excluding all enzymatic steps. We are planning to attach aminoacylated RNA-fragments to the 3'-end of truncated, chemically synthesized t-RNAs by chemical ligation.[8] The non-enzymatic ligation needs a template which is provided by the 5'-region of the truncated t-RNA; its 3'-end serves thereby as primer. This disconnection requires the synthesis of an aminoacylated riboheptanucleotide which is able to form at least three base-pairs during ligation.

Herein, we report a synthesis of novel phosphoramidite building blocks which allow the synthesis of fully or partially protected oligoribonucleotides that are deprotected under very mild conditions and can serve as precursors for the preparation of labile RNA-conjugates, such as 3'-aminoacylated oligoribonucleotides.[9]

As starting materials for the introduction of the photolabile *N*-[(2-nitrobenzyl)oxy]carbonyl base protecting groups we used 5'-*O*-dimethoxytritylated, 2'-*O*-[(2-nitrobenzyl)oxy]methylated nucleosides **5**-**7**, which we prepared from the corresponding base-acetylated compounds **1**-**3**, employing a method described in our earlier publications.[3,10] After removal of the acetyl base-protecting groups of **5**-**7** by short treatment with $MeNH_2$ in EtOH, intermediates **9**-**11** were obtained in quantitative yield (Scheme 1a).



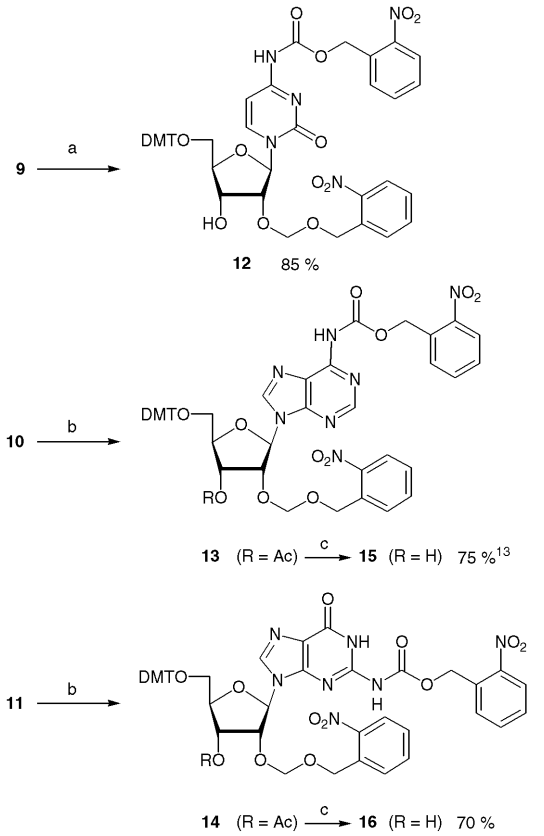
	Base
1	(N^4-Ac)**C**
2	(N^6-Ac)**A**
3	(N^2-Ac)**G**
4	**U**

	Base	Yield
5	(N^4-Ac)**C**	60 %
6	(N^6-Ac)**A**	50 %
7	(N^2-Ac)**G**	80 %
8	**U**	50 %

b (*quant.*)

	Base
9	**C**
10	**A**
11	**G**

Scheme 1a a: c(**1**-**4**) = 0.25 M, 1.1 eq. Bu_2SnCl_2, 4 eq. $EtN(iPr)_2$ in $(ClCH_2)_2$, 25 °C, 60 min; then 70 °C, addition of 1.1 - 1.6 eq. [(2-nitrobenzyl)oxy] methyl chloride[3], 30 min, extr. (aq. $NaHCO_3/CH_2Cl_2$), SiO_2-chrom. (**5**,**6**,**8**: EtOAc/hexane, **7**: CH_2Cl_2/MeOH); b: c(**5**-**7**) = 0.15 M in $MeNH_2$ (8 M in EtOH), 25 °C, 30 min, evaporation.

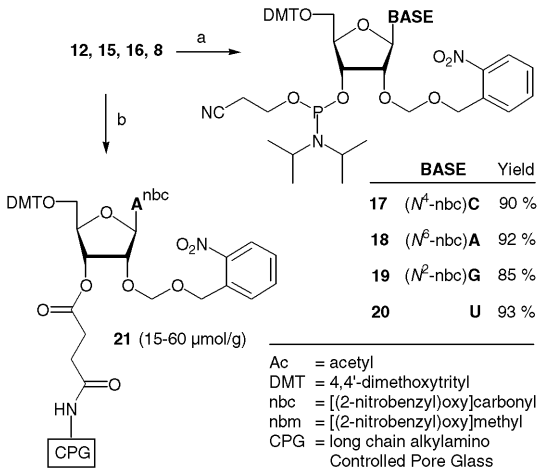


Scheme 1b a: c(9) = 0.5 M in $(ClCH_2)_2$, 2 eq. [(2-nitrobenzyl)oxy] chloroformate[11], 5 eq. aq. Na_2CO_3 (1 M), 25 °C, 60 min, extr. (aq. $NaHCO_3/CH_2Cl_2$), SiO_2-chrom. (EtOAc/hexane); b: c(**10,11**) = 0.25 M, 0.1 eq. DMAP, 1.2 eq. Ac_2O in pyridine, 4 °C, 15 min, extr. (aq. $NaHCO_3/CH_2Cl_2$), evaporation; then dissolved in CH_2Cl_2 (0.5 M), slow addition to: 1.5 eq. $COCl_2$ (1.9 M in toluene), 0.1 eq. DMAP in CH_2Cl_2/pyridine 5:1 (final c = 0.15 M), 25 °C, 10 min, then 4 (for **10**) or 6 eq. (for **11**) 2-nitrobenzyl alcohol/NEt_3 (1:1), 25 °C, overnight, extr. (aq citric acid, aq. $NaHCO_3/CH_2Cl_2$); c: c(**13,14**) = 0.03 M, 0.1 M NaOH in THF/MeOH/H_2O (5:4:1), 25 °C, 2.5 min, extr. (aq. $NaHCO_3/CH_2Cl_2$), SiO_2-chrom. (**15**: EtOAc/hexane, **16**: CH_2Cl_2/MeOH).

Carbamate-formation of the cytosine-nucleoside **9** with [(2-nitrobenzyl)oxy] chloroformate[11] in a biphasic mixture of aqueous Na_2CO_3/dichloroethane at 25 °C gave the N^4-protected derivative **12** in 85% yield. No conversion was observed by applying the same reaction conditions to the adenine- and the guanine-nucleosides **10** and **11**, respectively. Attempts to activate the reagent (in the absence of water) towards reaction with the nucleobase-moieties by addition of pyridine, DMAP, Ag^+-salts or heating the reaction mixtures failed completely (sometimes partial formation of the 3'-*O*-carbonates occurred). Instead, efficient decomposition of the reagent [(2-nitrobenzyl)oxy] chloroformate to (2-nitrobenzyl)chloride was observed.[12] In contrast, good yields of the base protected adenine and guanine nucleosides **15** and **16** were obtained by the following reaction sequence, which was carried out without purification of the intermediates.[13]

Treatment of nucleosides **10** and **11** with Ac_2O/DMAP in pyridine led to a quantitative acetylation of the 3'-*O*-position. After extractive workup these intermediates were converted into the *N*-[(2-nitrobenzyl)oxy]carbonylated derivatives **13** and **14** by subsequent treatment with $COCl_2$/DMAP and 2-nitrobenzyl alcohol/NEt_3, respectively, in CH_2Cl_2/pyridine. After extraction, selective removal of the intermediate 3'-*O*-acetyl protecting groups was achieved with NaOH in THF/MeOH/H_2O and led, after extraction and purification by silica gel chromatography, to the isolation of nucleosides **15** and **16** in 75% and 70% yield, respectively (Scheme 1b).

Following standard procedures, the protected nucleosides **8, 12, 15** and **16** were converted into the phosphoramidite building blocks **17**-**20**, which were suitable for the synthesis of the corresponding oligonucleotides with a DNA synthesizer. The solid support **21** loaded with the adenine nucleoside was synthesized using a slightly modified, known protocol by first preparing its succinic acid monoester and then immobilizing it on aminoalkyl-functionalized CPG-material (Scheme 1c).



Scheme 1c a: c(**12,15,16,8**) = 0.25 M, 1.3 eq. (2-cyanoethyl)(N,N-diisopropylamino)chlorophosphite, 2.5 eq. $EtN(iPr)_2$ in CH_2Cl_2, 25 °C, overnight, SiO_2-chrom. (**17,18,20**: EtOAc/hexane, **19**: EtOAc/hexane/EtOH); b: c(**21**) = 0.25 M, 1 eq. succinic anhydride, 1.5 eq. DMAP in CH_2Cl_2, 25 °C, 30 min, extr. (aq. citric acid/CH_2Cl_2), evaporation, then CPG (1-4 g/0.1 mmol), 3 eq. BOP, 3 eq. N-methyl imidazole in CH_3CN (c = 0.02 M), filtration.

All mentioned compounds have been characterized by NMR-spectroscopy and FAB MS-spectrometry and all data unambiguously agree with the proposed structures. Some of the relevant spectroscopic data are presented in Table 1. All FAB-MS-spectra (pos. mode, glycerol as matrix) showed beside the peak of the dimethoxytrityl-cation (m/z = 303) the product peaks as most intense signals. ^1H NMR spectra of nucleotides **12**-**19** showed the typical signals of an additional 2-nitrobenzyl-group in the aromatic region (data not shown) and the signals of an additional CH_2-group around δ = 5.6 ppm, which could be attributed

to the benzylic hydrogens. The ^1H and the ^{13}C NMR spectra showed that the signals of the nucleobase-moieties underwent no significant changes upon exchange of the base protecting groups, which illustrated the correct position of the [(2-nitrobenzyl)oxy]carbonyl groups.

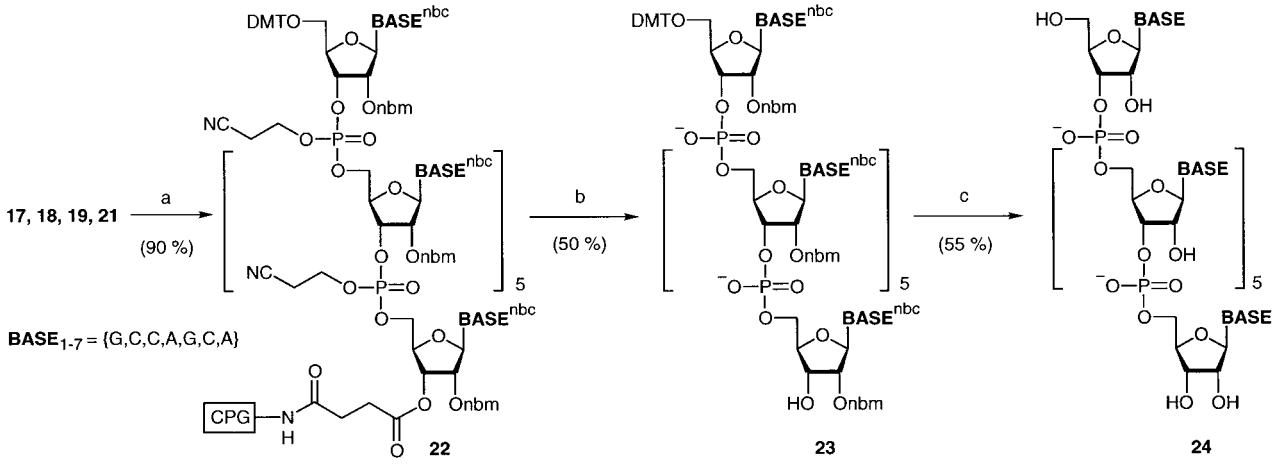
The assembly of the heptameric oligoribonucleotide **22** from the phosphoramidite building blocks **17**-**19** and the solid support **21** was carried out in the "trityl-on" mode on a "Gene Assembler" (Pharmacia) using a protocol which we had developed in a different context[10], but omitting the capping step. The individual coupling yields by detritylation assay were >99%. The removal of the phosphate-protecting groups and the cleavage from the solid support was carried out at 25° with 0.1 M NaOH in THF/MeOH/H_2O for a period of only 1.5 min followed by neutralization with aqueous Tris-HCl buffer (pH 7.4), which resulted in complete precipitation of the water-insoluble product **23** (Scheme 2).

Prior to this reaction, the stability of the *N*-[(2-nitrobenzyl)oxy]carbonylated ribonucleotides was investigated under a variety of potential detachment conditions (Table 2). In the presence of $MeNH_2$, all three nucleoside

Table 1: Selected physical data of intermediates and products

	TLC [a]	^{1}H-NMR[b] (δ[ppm])								^{13}C-NMR[c] (δ[ppm])				
	R_f	H-1'	H-2'	H-3'	H-4'	H-5'	H'-5'	CH_2(nbc)	H-5, H-6 (Pyr) H-8, H-2 (Pur)	C-2	C-4	C-5	C-6	C-8
5	0.2 (A)	6.06	4.38	4.51	4.13	3.57	3.62		8.55 }	155.1	166.4	96.9	145.2	
12	0.45 (A)	6.04	4.36	4.49	4.13	3.58	3.64	5.66	8.53 } H6[d]	155.0	169.8	97.5	144.7	
17[f]	0.65 (B)	6.08	4.51	4.42	4.31	*ca.* 3.6		5.62	8.58 }	154.8	169.7	97.2	144.9	
6	0.3 (A)	6.26	5.07	4.58	4.31	3.46	3.55		8.52, 8.12	152.7	149.4	122.5	151.4	142.1
13	0.75 (A)	6.25	5.27	5.53	4.37	3.50	3.67	5.75	8.55, 8.14	- [e]	-	-	-	-
15	0.65 (A)	6.24	5.09	4.57	4.30	3.48	3.56	5.75	8.60, 8.15	153.1	149.3	122.6	151.3	141.9
18[f]	0.85 (B)	6.18	5.21	4.65	4.35	3.65	3.85	5.77	8.51, 8.14	152.7	148.9	122.4	151.5	142.0
7	0.6 (C)	5.99	5.17	4.53	4.26	3.27	3.52		7.85	148.7	147.4	122.4	156.3	139.1
14	0.8 (C)	5.90	5.28	5.56	4.31	3.34	3.51	5.61	7.78	- [e]	-	-	-	-
16	0.65 (C)	6.04	5.00	4.55	4.28	3.37	3.48	5.61	7.81	148.7	147.5	121.3	155.8	137.9
19[f]	0.8 (C)	6.00	5.06	4.57	4.29	*ca.* 3.5		5.60	7.78	148.6	147.2	121.5	155.4	137.7
8	0.6 (A)	6.04	4.38	4.55	4.10	3.52	3.55		5.29, 7.94	150.4	163.4	102.5	140.3	
20[f]	0.5 (B)	6.08	4.48	4.55	4.25	*ca.* 3.5			5.20, 7.90	150.2	163.0	102.8	140.0	

a: Preacoated silica gel plates, stained with anisaldehyde reagent[7] ((A): EtOAc/hexane 9:1, (B): EtOAc/hexane 4:1, (C): CH_2Cl_2/MeOH 9:1); b: 300 or 500 MHz, all spectra in $CDCl_3$; c: 75 or 125 MHz, all spectra in $CDCl_3$; d: H-5 hidden; e: not measured; f: signals of major isomer.



Scheme 2. a: Pharmacia Gene Assembler Plus with **21** (1 μmol), 1.2 min detritylation with 4 % dichloroacetic acid, $(ClCH_2)_2$ / 2.5 min coupling with **17**-**19** (0.08 M × 120 μl) catalyzed by 5-benzylthio-1*H*-tetrazole (0.25 M × 360 μl), CH_3CN / 0.5 min oxidation with I_2/H_2O/pyridine/THF (3:2:20:75); b: 0.1 M NaOH in THF/MeOH/H_2O (5:4:1), 25°C, 1.5 min, then 1 M aq. Tris-HCl, RP-HPLC (Figure 1,2); c: c(**23**) = 4 o.D. / ml, Hg-lamp (250 W, Pyrex-filtered), 0.1 M sodium citrate buffer (pH 3.5)/*t*BuOH(1:1), 25°C, 2 h, RP-HPLC (Figure 2).

derivatives were degraded completely in a few minutes. In contrast, they were stable for at least 10 h in NaOH/THF/H_2O. In NH_3/EtOH and NaOH/THF/MeOH/H_2O they showed an intermediate stability. Remarkable is the relative difference in stability of the guanine derivative **16**, which is the most stable in NH_3/EtOH, but the least stable in NaOH/THF/MeOH/H_2O.[14]

In Figure 1, the HPLC-trace of the crude, still protected oligonucleotide **23** is presented. It confirms the good coupling yields observed by the detritylation assay. Apart from the dominant peak of the product, only small peaks of earlier eluting, shorter or partially deprotected compounds are visible. From the reaction mixture, sequence **23** was obtained in a yield of 50% after purification by HPLC (Figure 2).

Table 2. Half-lives ($t_{1/2}$) of N-[(2-nitrobenzyl)oxy]methylated nucleosides under potential detachment conditions[a]

	8 M NH_3 in EtOH	8 M CH_3NH_2 in EtOH	0.1 M NaOH in THF/MeOH/H_2O (5:4:1)	0.1 M NaOH in THF/H_2O (1:1)
12	24 h	< 10 min	1.5 h	>> 48 h
15	5 h	< 10 min	24 h	>> 48 h
16	>> 48 h	< 10 min	20 min	48 h

a: All experiments were carried out at 25°C; the values were estimated by TLC.

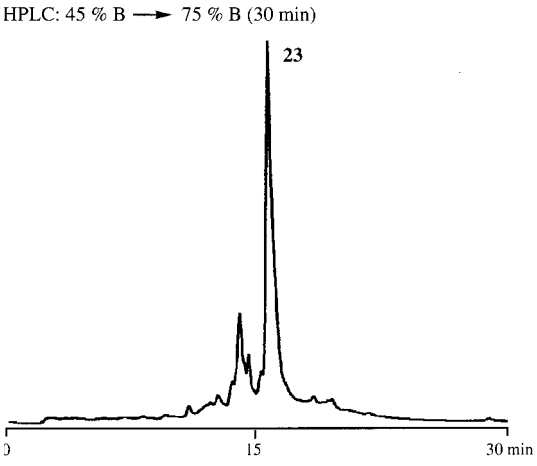


Figure 1 RP-HPLC of crude reaction mixture obtained after assembly and detachment from the solid support (Scheme 2, steps a and b). HPLC-conditions: Nucleosil 5C18 (5 x 200 mm), flow 1 ml/min, detection at 260 nm, elution at 40 °C; eluent A: 0.1 M (Et_3NH)OAc in H_2O (pH 8.0), eluent B: CH_3CN.

The purified sequence **23** was subjected to photolysis in aqueous sodium citrate buffer (pH 3.5)/*t*BuOH using Pyrex-filtered light from a mercury-lamp. Additionally, complete detritylation occurred under these acidic conditions (Scheme 2). In Figure 2 HPLC traces of the starting material **23** and the reaction mixture obtained after 2 h irradiation are shown. They reveal clean formation of a new, much earlier eluting (= much more polar) product, which was isolated by HPLC in 55% yield. The structure of this product-sequence **24** was unambiguously confirmed by the following data. Its UV-spectrum was typical for an oligonucleotide. Its chromatographic behaviour on two different HPLC-systems[15] was identical with the corresponding authentic RNA-sequence[16] as shown by coinjection experiments. Furthermore, its MALDI-TOF spectrum[17] showed the expected mass of 2202 amu.

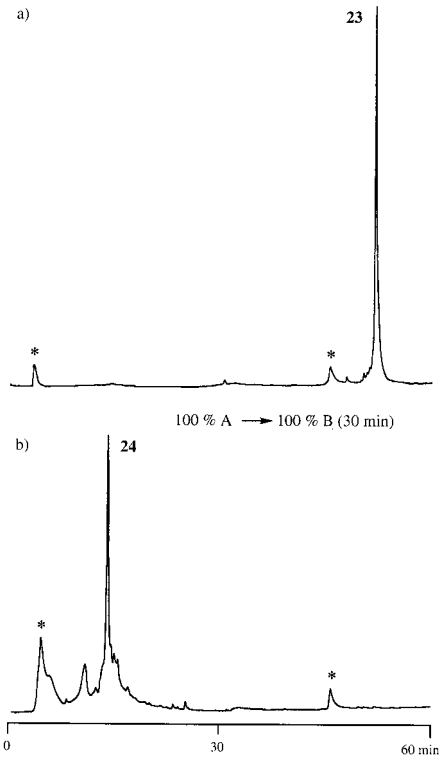


Figure 2 RP-HPLC of a): purified sequence **23**, b) reaction mixture after 2 h irradiation (Scheme 2, step c). HPLC-conditions: see Figure 1 (impurities marked with an asterisk are from the buffer).

In conclusion, a synthetic method for the introduction of photocleavable base protecting groups into RNA-phosphoramidites was developed. The presented general method can potentially be used for the introduction of any desired carbamoyl-group into ribo- and 2'-deoxyribonucleosides, because they can be formed on the nucleobase without need to preassemble the corresponding chloroformates (which are often sensitive by nature). Meanwhile, we succeeded in efficiently transforming the intermediate, protected heptamer **23** into a 3'-*O*-aminoacylated derivative, which subsequently could be deprotected under the presented conditions[18] and are now about to explore the chemical ligation with truncated t-RNAs.

Acknowledgement

We thank Patrick A. Weiss (Xeragon AG, Switzerland) for providing us with reagents, nucleoside precursors and the authentic RNA-sequence. The ETH Zürich Research Council and the Alfred Werner Foundation are gratefully acknowledged for financial support.

References and Notes

(1) Gasparutto, D.; Livache, T; Bazin, H.; Duplaa, A.-M.; Guy, A.; Khorlin, A.; Molko, D.; Roget, A.; Téoule, R. *Nucl. Acids Res* **1992**, *20*, 5159.

(2) Hayakawa, Y.; Kato, H.; Uchiyama, H. K.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2402. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691. Hayakawa, Y.; Hirose, M.; Hayakawa, M.; Noyori, R. *J. Org. Chem.* **1995**, *60*, 925.

(3) Pitsch, S. *Helv. Chim. Acta* **1997**, *80*, 2286.

(4) Noren, C. J.; Anthony-Cahill, S. J.; Griffith, M. C.; Schultz, P. G. *Science* **1989**, *244*, 182.

(5) Robertson, S. A.; Ellman, J. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 2722.

(6) Heckler, T. G.; Chang, L.; Zama Y.; Naka, T.; Chorghade, M. S.; Hecht, S. M. *Biochemistry* **1984**, *23*, 1468. Baldini, G.; Martoglio B.; Schachenmann, A.; Zugliani, C.; Brunner, J. *Biochemistry* **1988**, *27*, 7951.

(7) The synthesis of longer conjugates is not reported.

(8) The chemical synthesis of the truncated t-RNAs allows the incorporation of natural or unnatural modified nucleosides and would extend the scope of the method.

(9) The preparation of a trimeric aminoacylated RNA-sequence from a partially protected RNA-precursor has been reported. Thereby, Fmoc ([(9-fluorenyl)methoxy]carbonyl), MTHP (4-methoxytetrahydropyran-4-yl), BPOC ([2-(4-biphenylyl)-2-propyloxy]carbonyl were employed as protecting groups for the nucleobases, the sugar-moieties and the amino acid, respectively. The tedious, stepwise deprotection procedure yielded only 20-30%: Hagen, M. D.; Scalfi-Happ, C.; Happ, E.; Chladek, S. J. *J. Org. Chem.* **1988**, *53*, 5040. Hagen, M. D.; Chladek, S. J. *J. Org. Chem.* **1989**, *54*, 3189.

(10) Wu, X.; Pitsch, S. *Nucleic Acids Res.* **1998**, *26*, 4315.

(11) At 4 °C, 10.4 ml of a $COCl_2$-solution (1.9 M in toluene, 20 mmol) was treated slowly with a solution of 3.06 g (20 mmol) 2-nitrobenzyl alcohol in 20 ml THF. After 30 min at 4 °C and 2.5 h at 25 °C, it was evaporated (< 40 °C) and dried (2 h, 0.01 torr): crude [(2-nitrobenzyl)oxy] chloroformate as orange oil (90% according to ^{1}H NMR). IR ($CHCl_3$): 3009, 1776, 1530, 1347, 1142; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.75 (s, 2H), 7.25 (m, 1H), 7.55-7.74 (m, 2H), 8.19 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 69.6 (CH_2), 124.7, 129.3, 130.0, 134.5 (4 CH), 138.1, 147.5, 150.8 (3 C).

(12) This decomposition occurred very fast in polar, aprotic solvents, such as DMF, pyridine or N,N-dimethylacetamide; it also occurred during attempts to purify the reagent by distillation. The mechanism most probably involves attack of a chloride anion at the benzylic position with concomitant loss of CO_2 and chloride. The reagent is stable in biphasic reaction mixtures, where chloride ions are extracted into the aqueous phase (as realized in the preparation of **12** from **9**).

(13) Preparation of **15** from **6**: A solution of 1.175 g (1.51 mmol) **6** in 10 ml $MeNH_2$-solution (8 M in EtOH) was kept 15 min at 25 °C. After evaporation and drying (5 h, 0.01 torr), the product was dissolved in 6 ml pyridine, treated with 18 mg (0.15 mmol) DMAP and 184 mg (1.8 mmol) Ac_2O and stirred 15 min at 4 °C. After extraction (CH_2Cl_2/$NaHCO_3$), drying ($MgSO_4$), evaporation and drying (15 h, 0.01 torr), the product was dissolved in 3 ml CH_2Cl_2 and added during 20 min to a suspension obtained from 1.17 ml (2.25 mmol) $COCl_2$-solution (1.9 M in toluene) / 18 mg (0.15 mmol) DMAP / 10 ml CH_2Cl_2 / 2 ml pyridine. After 10 min at 25 °C, a solution of 915 mg (6 mmol) 2-nitrobenzyl alcohol and 607 mg (6 mmol) Et_3N in 3 ml CH_2Cl_2 was added. The mixture was stirred 15 h at 25 °C, extracted (CH_2Cl_2/10% aqueous citric acid solution, then $NaHCO_3$), dried ($MgSO_4$) and evaporated. This crude product was dissolved in 50 ml 0.1 M NaOH solution (THF/MeOH/H_2O 5:4:1), kept for 2.5 min at rt., extracted (CH_2Cl_2/$NaHCO_3$), dried ($MgSO_4$) and evaporated. The residue was subjected to chromatography (30 g SiO_2, EtOAc/hexane (+2% NEt_3) 1:1→ 9:1):1.00 g (75%) **15** as off-white, solid foam.

(14) These results are an indication of different mechanisms operating under different reaction conditions. In the presence of a strong base (such as NaOMe) formation of an isocyanate species (elimination of 2-nitrobenzyl alcohol from the [(2-nitrobenzyl)oxy]carbonyl-moiety) seems to precede the attack of a nucleophile. Weak bases, but relatively strong nucleophiles (such as NH_3), seem to react by addition to the carbonyl group, followed by elimination of 2-nitrobenzyl alcohol.

(15) The coinjections were carried out on a reversed-phase column (see Figure 1, 0 → 20% B (30 min) and on an ion-exchange column (Nucleogel SAX, 10 mM phosphate (pH 6) → 10 mM phosphate + 0.2 M KCl (30 min).

(16) Obtained from XERAGON AG (Zürich, Switzerland)

(17) Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191.

(18) Stutz, A.; Kuntze, S.; Pitsch, S. *in preparation*.

Article Identifier:
1437-2096,E;1999,0,S1,0930,0934,ftx,en;W04299ST.pdf