Novel Fluoride-Labile Nucleobase-Protecting Groups for the Synthesis of 3′(2′)-*O*-Aminoacylated RNA Sequences

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Dedicated to Prof. *Albert Eschenmoser* on the occasion of his 75th birthday

With the aim to develop a general approach to a total synthesis of aminoacylated t-RNAs and analogues, we describe the synthesis of stabilized, aminoacylated RNA fragments, which, upon ligation, could lead to aminoacylated t-RNA structures. Novel RNA phosphoramidites with fluoride-labile 2′-*O*-[(triisopropylsilyl)-oxy]methyl (=tom) sugar-protecting and *N*-{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl (=tboc) base-protecting groups were prepared (*Schemes 4* and *5*), as well as a solid support containing an immobilized *N*[6]-tboc-protected adenosine with an orthogonal (photolabile) 2′-*O*-[(*S*)-1-(2-nitrophenyl)ethoxy]methyl (=(*S*)-npeom) group (*Scheme 6*). From these building blocks, a hexameric oligoribonucleotide was prepared by automated synthesis under standard conditions (*Scheme 7*). After the detachment from the solid support, the resulting fully protected sequence **34** was aminoacylated with L-phenylalanine derivatives carrying photolabile *N*-protecting groups (→**42** and **43**; *Scheme 9*). Upon removal of the fluoride-labile sugar- and nucleobase-protecting groups, the still stabilized, partially with the photolabile group protected precursors **44** and **45**, respectively, of an aminoacylated RNA sequence were obtained (*Scheme 9* and *Fig. 3*). Photolysis of **45** under mild conditions resulted in the efficient formation of the 3′(2′)-*O*-aminoacylated RNA sequence **46** (*Fig. 4*). Additionally, we carried out model investigations concerning the stability of ester bonds of aminoacylated ribonucleotide derivatives under acidic conditions (*Table*) and established conditions for the purification and handling of 3′(2′)-*O*-aminoacylated RNA sequences and their stabilized precursors.

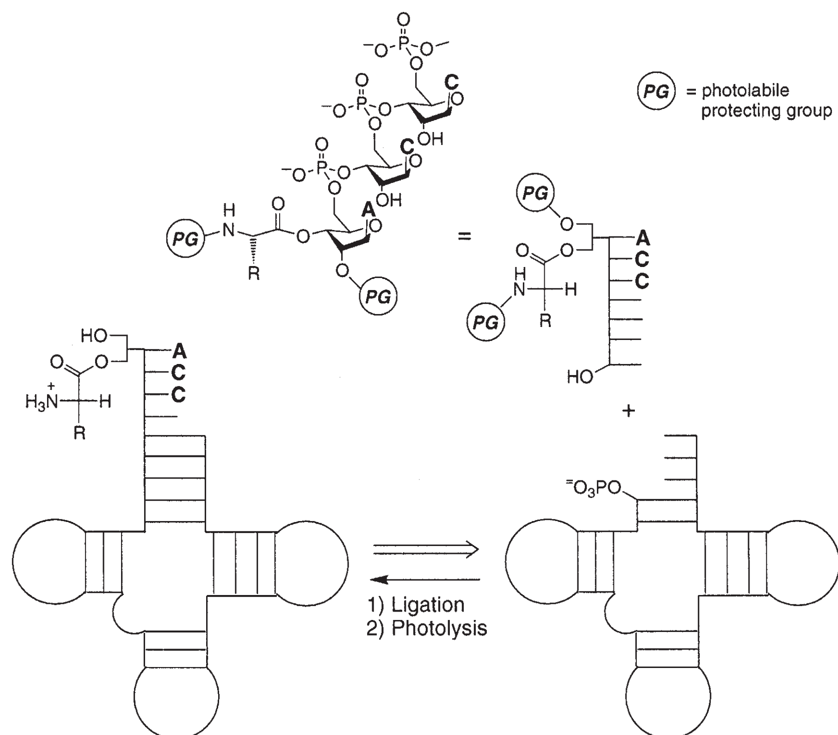
1. Introduction. – About one year ago, we introduced a concept for a total synthesis of aminoacylated t-RNAs based on the ligation of short, aminoacylated RNA fragments to truncated t-RNAs. For this purpose, we had developed novel RNA phosphoramidites with photocleavable sugar- and nucleobase-protecting groups [1]. However, we soon realized that the purification and handling of such amino acid/RNA conjugates was very difficult due to their instability towards hydrolysis. We, therefore, decided to modify the original concept by preparing stabilized precursors that could be transformed into the target structures by a final photolytic step[1]).

It is well-known that both *N*-acylation and the absence of a neighboring 2′-OH group stabilize the ester bond of aminoacylated nucleotide derivatives towards hydrolysis [4]. Our modified concept, therefore, implies the preparation of short

[1]) So far, 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 weakly activated, *N*-protected amino acid with a non-protected or partially protected ribodinucleotide [2]. The preparation of a trimeric aminoacylated RNA sequence from a partially protected RNA precursor has been reported. Thereby, [(9*H*-fluoren-9-yl)methoxy]carbonyl (=Fmoc), tetrahydro-4-methoxy-2*H*-pyran-4-yl (=Mthp), and [1-([1,1′-biphenyl]-4-yl)-1-methylethoxy]carbonyl (=Bpoc) were employed as protecting groups for the nucleobases, the sugar moieties, and the amino acid, respectively [3].

aminoacylated RNA precursors that still contain two photolabile protecting groups at these positions, which, finally, after ligation and purification, can be removed *in situ* under mild conditions (*Scheme 1*).

Scheme 1. *Retrosynthetic Analysis: Ligation of Stabilized* N- *and 2′*-O-*Protected Aminoacylated RNA Fragments to Truncated RNAs, Followed by the Liberation of the Labile Aminoacylated t-RNAs upon Photolysis*

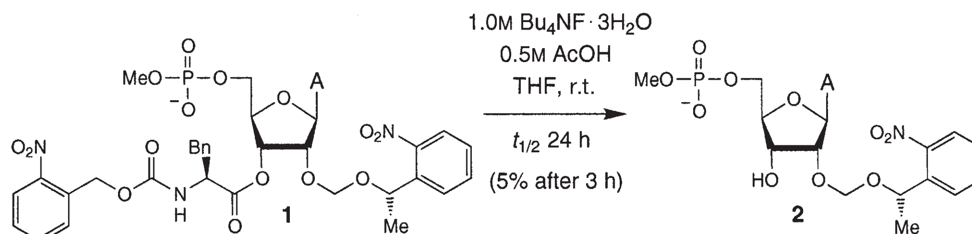


To achieve an efficient and unambiguous (regioselective) aminoacylation, the sugar and nucleobase moieties of the RNA precursors require protection by groups that, ideally, are quantitatively removable in the presence of the labile ester bond (to the amino-acid conjugate) and the remaining two photolabile protecting groups.

For this purpose, we decided to investigate fluoride-labile groups. In this work, we describe the evaluation and synthesis of novel RNA phosphoramidites carrying fluoride-labile 2′-*O*-[(triisopropylsilyl)oxy]methyl (= tom [5]) and *N*-{{[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl (= tboc) groups as sugar- and base-protecting groups, respectively, the synthesis of an immobilized adenosine nucleoside carrying an orthogonal photolabile 2′-*O*-[(*S*)-1-(2-nitrophenyl)ethoxy]methyl (= (*S*)-npeom) protecting group, and the assembly of a protected RNA fragment from these building blocks, its aminoacylation, and final deprotection. Additionally, we determined the stability of an aminoacylated model nucleotide under acidic conditions and investigated the relative influence of *N*-acylation and 2′-*O*-protection on the stability of the ester bond between an amino acid and a nucleotide.

2. Results. – 2.1. *Evaluation of Protecting Groups.* First, we evaluated the stability of the ester bond of an aminoacylated model nucleotide **1** under potential deprotection conditions required for the detachment of fluoride-labile protecting groups. In 1M $Bu_4NF \cdot 3H_2O$ in THF at room temperature, the half-life ($t_{1/2}$) of compound **1** was only *ca.* 2 h (→**2**). Upon addition of 0.5M AcOH, however, the stability of the ester bond was significantly higher ($t_{1/2}$ = 24 h), and only *ca.* 5% were cleaved to **2** within 3 h (*Scheme 2*). Under these conditions, 2′-*O*-tom groups were cleaved in less than 5 min.

Scheme 2. *Model Investigation to Determine the Stability of Protected Aminoacylated Nucleotide Derivatives under the Conditions Required for the Removal of Fluoride-Labile Protecting Groups*



Subsequently, we evaluated fluoride-labile nucleobase-protecting groups that could be cleaved under such conditions. Earlier, we [5] and others [6] had found that some nitrobenzyl-alcohol derivatives are cleaved upon treatment with F^--ions. Therefore, we first investigated such compounds as potential fluoride-labile nucleobase-protecting groups. Indeed, the [(2-nitrobenzyl)oxy]carbonyl- and [(4-nitrobenzyl)oxy]carbonyl-protected adenosine derivatives **3** and **4**, respectively, were cleaved to **8** under the conditions mentioned above (1M $Bu_4NF \cdot 3H_2O$/0.5M AcOH in THF, 20°), but were too stable to be considered for our purpose ($t_{1/2}$ of 3 h and 4 h, resp.). The isoelectronic (2-oxopropoxy)carbonyl-protected adenosine derivative **5** was more labile ($t_{1/2}$ = 1 h), but, unfortunately, still too stable. We then turned our attention towards silyl-based protecting groups. Under our conditions, the {{2-[(triisopropylsilyl)oxy]benzyl}oxy}-carbonyl- and {{4-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl-protected adenosine derivatives **6** and **7**, respectively, were both deprotected within < 2 min and were, therefore, promising candidates (*Scheme 3*)[2]).

Both adenosine derivates **6** and **7** were completely stable (for at least 2 h) under the oxidation conditions (I_2, H_2O/pyridine/THF) usually employed during the chain assembly of oligonucleotides. However, they showed completely different behaviors under the detritylation conditions (4% $CHCl_2COOH/(CH_2Cl)_2$). Whereas the *ortho*-substituted derivative **6** was completely stable for at least 30 min (= total detritylation time for 15 couplings), the *para*-substituted analogue **7** was removed quantitatively within only 2 min.

Both the stability under the conditions required for the assembly of RNA sequences and the lability towards Bu_4NF encouraged us to investigate the {{2-[(triisopropyl-silyl)oxy]benzyl}oxy}carbonyl (= tboc) moiety as fluoride-labile base-protecting group.

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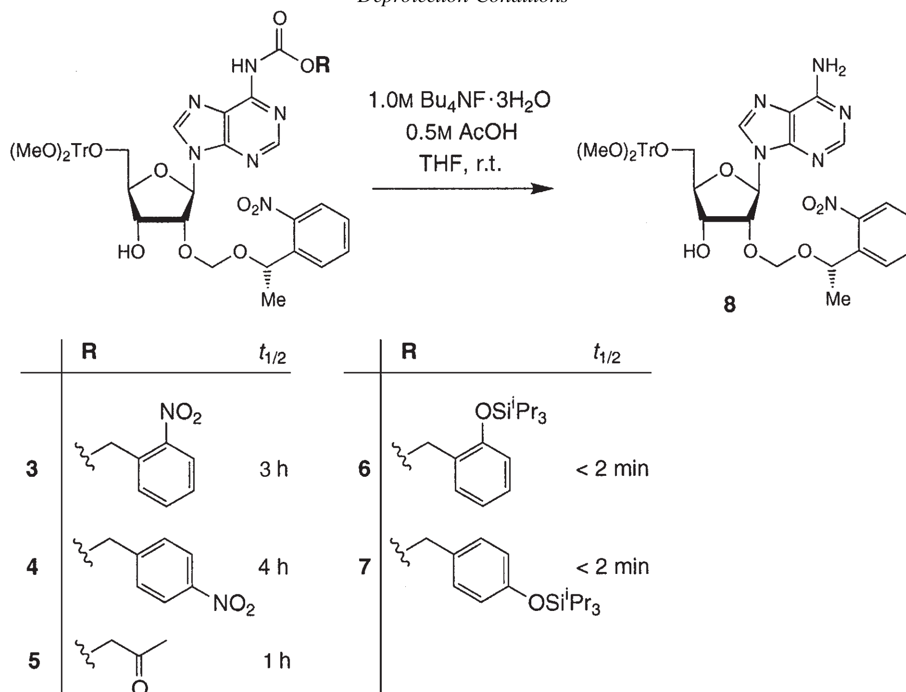
[2]) The analogous {{4-{[(*tert*-butyl)dimethylsilyl]oxy}benzyl}oxy}carbonyl protecting group was recently employed in a different context [7].

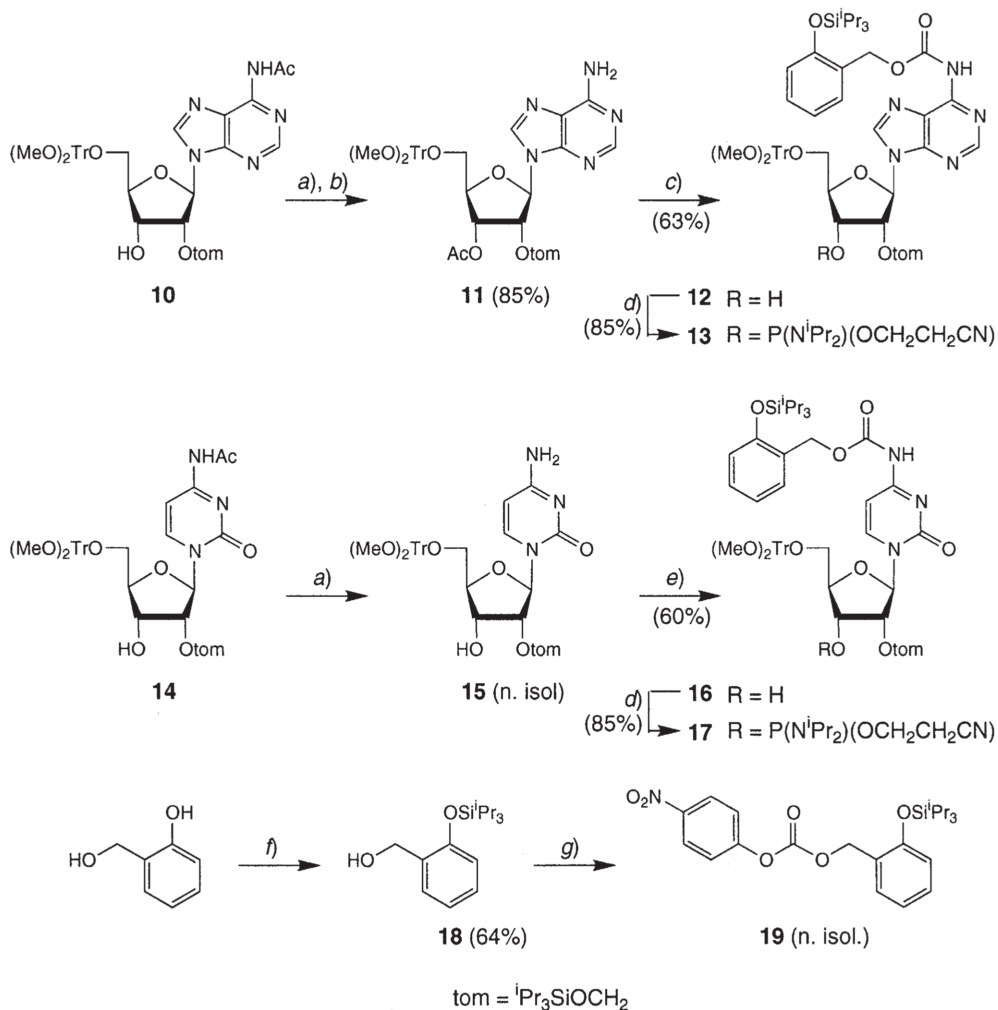
Scheme 3. *Model Investigation to Determine the Lability of Various Nucleobase-Protecting Groups under Deprotection Conditions*



2.2. *Synthesis of Building Blocks.* During the assembly of oligonucleotides, the nucleobase of uridine derivatives usually does not require protection, since it is not reacting with activated phosphoramidites. Recent investigations by *Hayakawa* and *Kataoka* have shown that unprotected guanines are also not reacting under coupling conditions [8]. We could verify these observations by performing oligoribonucleotide syntheses with the N^2-unprotected guanosine phosphoramidite **9** (see below, *Scheme 5*) and our standard 2′-*O*-tom-protected uridine, (N^4-acetylated) cytosine, and (N^6-acetylated) adenosine phosphoramidites. Crude products from such syntheses were absolutely identical to products obtained from the standard 2′-*O*-tom-protected (N^2-acetylated) guanosine phosphoramidite [5]; specifically, no indication for the formation of branched derivatives could be found. In the context of this project, we thus decided to omit nucleobase protection of the guanosine building block, and to introduce the tboc protecting group only in cytidine and adenosine derivatives.

In *Scheme 4*, the preparation of the 2′-*O*-tom- and *N*-tboc-protected adenosine and cytidine phosphoramidites **13** and **17** is shown. The reagent 2-[(triisopropylsilyl)oxy]-benzyl alcohol (**18**) was obtained by treating a DMF solution of the Na^+ salt of 2-hydroxybenzyl alcohol with $^iPr_3SiCl$ at $-70°$. The N^6-tboc-protected adenosine building block was prepared under the conditions we had developed earlier for the preparation of *N*-[(2-nitrobenzyl)oxy]carbonyl(=nboc)-protected nucleosides [1]. The regular 5′-*O*-dimethoxytritylated, N^6-acetylated, 2′-*O*-tom-protected adenosine **10** [5] was *N*-

Scheme 4



a) $MeNH_2$, EtOH, r.t. *b*) Ac_2O, DMAP, Py, r.t. *c*) 1. $COCl_2$, DMAP, Py/CH_2Cl_2; 2. **18**, Et_3N, r.t.; 3. NaOH in THF, MeOH, H_2O, 4°. *d*) $ClP(N^iPr_2)(OCH_2CH_2CN)$, $^iPr_2NEt$, CH_2Cl_2, r.t. *e*) **19**, DMAP, $^iPr_2NEt$, toluene, 80°. *f*) 1. NaH in DMF, 4°; 2. $^iPr_3SiCl$, −70°. *g*) 4-Nitrophenyl carbonochloridate, Et_3N, toluene, r.t.

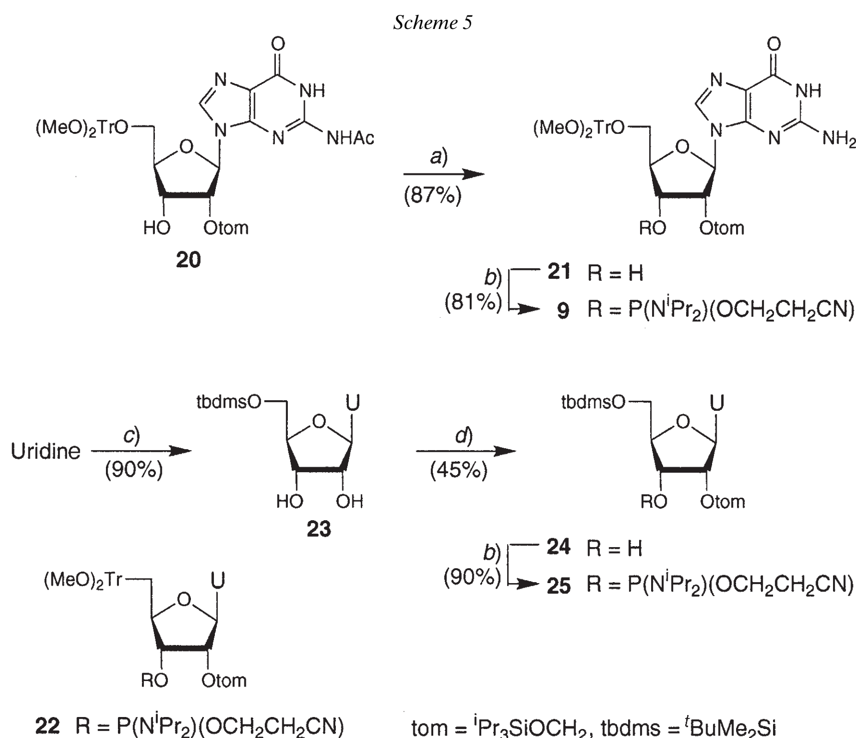
deacetylated with $MeNH_2$ in EtOH and reacetylated at the 3′-OH position with Ac_2O in pyridine. The intermediate **11** was treated first with $COCl_2$/DMAP (*N*,*N*-dimethylpyridin-4-amine) in pyridine/CH_2Cl_2 (→ formation of a reactive N^6-carbamoyl derivative [1]), and then with **18** in the presence of Et_3N. After extraction, the 3′-*O*-acetyl group was removed with NaOMe, and the resulting N^6-tboc-protected adenosine **12** was isolated by chromatography in 63% yield. The N^4-tboc-protected cytosine could not be prepared under the conditions that were successfully employed in

the synthesis of the corresponding N^4-nboc derivative [1][3]). However, *N*-deacetylation of the regular 5′-*O*-dimethoxytritylated, N^4-acetylated, 2′-*O*-tom-protected cytosine **14** [5], followed by treatment of the resulting crude nucleoside **15** with the nitrophenyl carbonate **19** at 80° in toluene gave the N^4-tboc-protected cytidine **16** in 60% yield.

The two *N*-tboc-protected nucleosides **12** and **16** were finally transformed under standard conditions into the corresponding phosphoramidites **13** and **17**, respectively.

The guanosine phosphoramidite **9** was prepared from the regular 5′-*O*-dimethoxytritylated, N^2-acetylated, 2′-*O*-tom-protected guanosine **20** [5] by removing the N^2-acetyl group with $MeNH_2$ in EtOH and subsequent phosphitylation of the intermediate **21** under standard conditions (*Scheme 5*). The phosphoramidite **9** was only poorly soluble in MeCN, and was, therefore, used as a solution in THF. For the incorporation of uridines, we could use the regular 2′-*O*-tom-protected phosphoramidite **22** [5]. For the introduction of the terminating nucleoside, we prepared the 5′-*O*-^{t}BuMe$_2$Si (=tbdms)-substituted uridine phosphoramidite **25** (*Scheme 5*). Uridine was silylated with tbdms-Cl/1*H*-imidazole in DMF. Under our established reaction conditions

Scheme 5



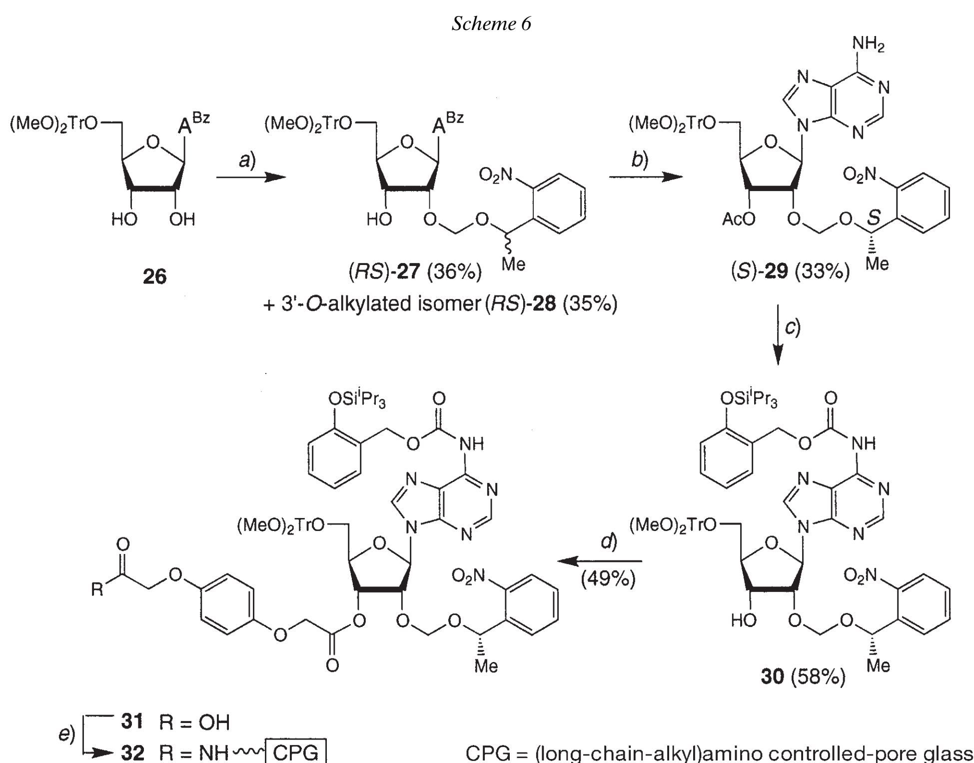
a) $MeNH_2$ in EtOH, r.t. *b*) ClP(N^{i}Pr$_2$)(OCH_2CH_2CN), ^{i}Pr$_2$NEt in CH_2Cl_2, r.t. *c*) tbdms-Cl, 1*H*-imidazole, DMF, r.t. *d*) 1. Bu_2SnCl_2, ^{i}Pr$_2$NEt, $(CH_2Cl)_2$, r.t., 2. tom-Cl [5], 80°

[3]) Reaction with 2-nitrobenzyl carbonochloridate in a biphasic mixture of aqueous Na_2CO_3 solution/dichloroethane at 25°. The analogous tboc-Cl was extremely sensitive and decomposed rapidly under such conditions.

[5][9], the resulting nucleoside **23** was activated with $Bu_2SnCl_2/^iPr_2NEt$ and subsequently alkylated with tom-Cl [5]. The 2′-*O*-tom derivative **24** was obtained in 45% yield after chromatography (together with 30% of the corresponding regioisomeric 3′-*O*-tom derivative). Phosphitylation of **24** gave the corresponding phosphoramidite **25**.

Additionally, we prepared a solid support with an immobilized adenosine, protected with a N^6-tboc group and a photolabile 2′-*O*-[(*S*)-npeom] moiety. The latter was chosen because we are using it in combination with the 2′-*O*-tom group successfully as an orthogonal 2′-*O*-protection for ribonucleosides [5]. After activation of the 5′-*O*-dimethoxytritylated, N^6-benzoylated adenosine nucleoside **26** with $Bu_2SnCl_2/^iPr_2NEt$ and alkylation with (*RS*)-npeom-Cl, the two pairs of regioisomeric 2′-*O*- and 3′-*O*-substituted derivatives (*RS*)-**27** and (*RS*)-**28** could be isolated (each as a diastereoisomer mixture) by chromatography (*Scheme 6*). The 2′-*O*-npeom derivatives (*RS*)-**27** were *N*-debenzoylated with $MeNH_2$ in EtOH and reacetylated at the 3′-OH position with Ac_2O in pyridine. At this stage, the two diastereoisomers could be easily separated by column chromatography. The product (*S*)-**29** ((*S*)-configuration of the npeom group) was transformed into the corresponding N^6-tboc-derivative by the conditions

Scheme 6



a) 1. Bu_2SnCl_2, $^iPr_2NEt$, $(CH_2Cl)_2$, r.t.; 2. (*RS*)-npeom-Cl [5], 80°. *b*) 1. $MeNH_2$, EtOH, r.t.; 2. Ac_2O, DMAP, Py, r.t.; *c*) 1. $COCl_2$, DMAP, Py/CH_2Cl_2; 2. **18**, Et_3N, r.t.; 3. NaOH, THF, MeOH, H_2O, 4°. *d*) [1,4-phenylenebis(oxy)]bis[acetic acid], BOP ((1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate), $^iPr_2NEt$, DMAP, Py, r.t. *e*) 1. LCAA-CPG, $^iPr_2NEt$, BOP, MeCN, r.t.; 2. Ac_2O/Py.

described for **11** → **12** (1. $COCl_2$, DMAP, pyridine/CH_2Cl_2; 2. **18**, Et_3N; 3. NaOMe, MeOH). The N^6-tboc-protected adenosine **30** was isolated by chromatography in 58% yield. The 3′-*O*-position of nucleoside **30** was then esterified with [1,4-phenylenebis(oxy)]bis[acetic acid] under conditions developed by *Pon* and *Yu* [10]. After chromatography, acid **31** was obtained in 49% yield. Its subsequent immobilization on LCAA-CPG ((long-chain-alkyl)amino controlled-pore glass) under peptide-coupling conditions led to the corresponding solid support **32** loaded at 20–45 μmol/g.

All compounds were characterized by their NMR and mass spectra, and all data unambiguously agreed with the proposed structures. ^{1}H-NMR Spectra of *N*-tboc-protected nucleosides showed the typical signals of an additional 2-substituted benzyl group (arom. H and benzylic CH_2 at δ *ca.* 5.3). The ^{1}H- and the ^{13}C-NMR signals of the nucleobase moieties underwent no significant changes upon exchange of the base-protecting groups (ac → tboc), which illustrated the correct position of the tboc-groups.

2.3. *Assembly of an Aminoacylated RNA Fragment.* Under our standard conditions [5], the phosphoramidites **9**, **13**, **17**, **25**, and the solid support **32** were assembled to a hexameric sequence **33** (*Scheme 7*). According to the spectrophotometrically determined amounts of the released dimethoxytrityl cation, the individual coupling yields were > 98%. The assembled sequence was subsequently detached from the solid support. Due to the lability of the [1,4-phenylenbis(oxy)]bis[acetic acid] linker, very mild conditions could be employed. The release of the sequence **34** was followed spectrophotometrically by analyzing the supernatants. With 6M NH_3 in H_2O/EtOH, the sequence was completely detached after 5 min, and with 10% Et_3N or Et_2NH in MeOH after 8 h.

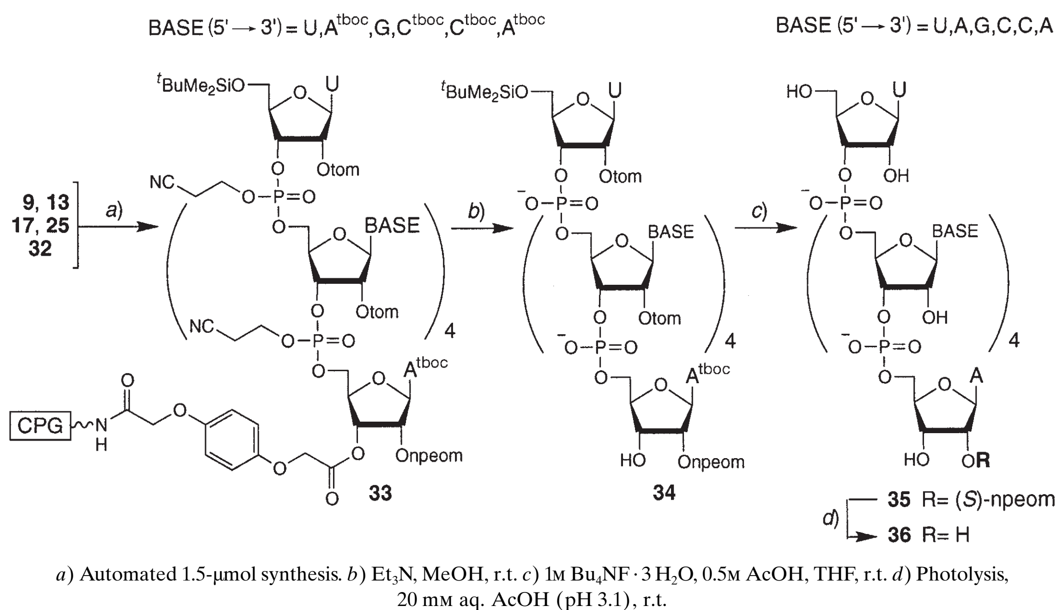
After evaporation, a small portion of the crude, still partially protected sequence **34**[4]) was treated for 2 h with an excess of 1M $Bu_4NF \cdot 3\,H_2O$/0.5M AcOH in THF. After addition of an equal volume of *Tris* · HCl buffer (pH 7.4), the mixture was desalted on *Sephadex G-10* and analyzed by reversed-phase HPLC (*Fig. 1,a*) which revealed that the sequence **35** was formed as the predominating product; the HPLC trace reflects a clean overall process, including good coupling yields, absence of side reactions, and complete removal of all fluoride-labile protecting groups. The crude product **35** was then photolyzed for 15 min with *Pyrex*-filtered light from a mercury lamp in 20 mM aq. AcOH (pH 3.1), leading in a clean, quantitative reaction to the more polar RNA sequence **36**, as shown by HPLC (*Fig. 1,b*). This product was identical (MALDI-TOF MS, HPLC[5])) to the authentic RNA sequence **36**, obtained with our standard 2′-*O*-tom-protected phosphoramidites [5].

These results demonstrated the compatibility of our novel base-protecting groups with an automated RNA synthesis under standard conditions. We subsequently investigated the preparation of aminoacylated derivatives. First, we established reaction conditions with the model nucleotide **2**. The required *N*-nboc-protected L-phenylalanine **39** was prepared from L-phenylalanine and 2-nitrobenzyl carbonochloridate [1] and the corresponding *N*-[(*RS*)-1-(2-nitrophenyl)ethoxy]carbonyl (=(*RS*)-npeoc)-protected L-phenylalanine **41** from L-phenylalanine and imidazolide **40**

[4]) Unfortunately, sequence **34** was too apolar to be analyzed by reversed-phase HPLC.

[5]) The co-injections were carried out on a reversed-phase column (0 → 20% *B* (30 min) and on an ion-exchange column (*Nucleogel SAX*, 10 mM phosphate (pH 6) → 10 mM phosphate + 0.2M NaCl (30 min)).

Scheme 7



a) Automated 1.5-µmol synthesis. *b*) Et_3N, MeOH, r.t. *c*) 1M $Bu_4NF \cdot 3\,H_2O$, 0.5M AcOH, THF, r.t. *d*) Photolysis, 20 mM aq. AcOH (pH 3.1), r.t.

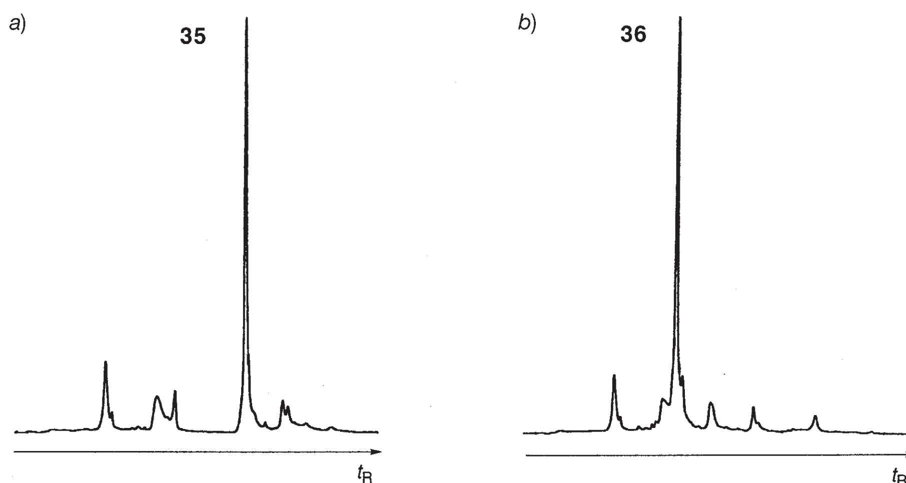
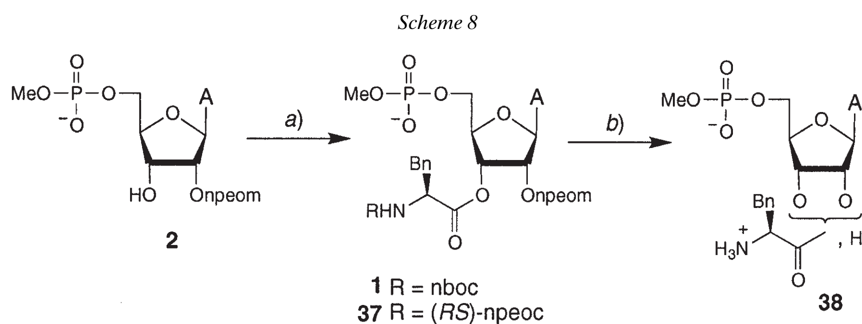
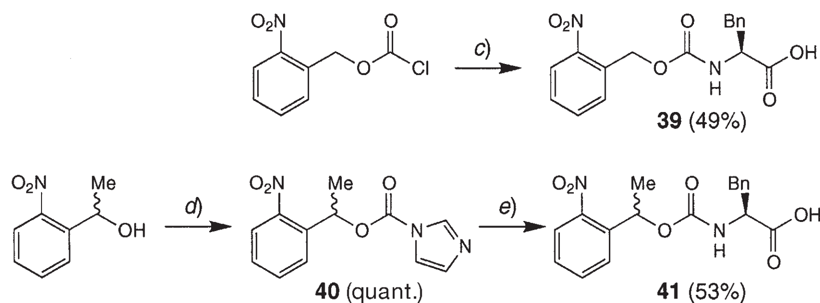


Fig. 1. *Reversed-phase HPLC traces* (measured at 260 nm) a) *of the crude 2'-*O*-[(*S*)-npeom]-protected RNA sequence* **35** *and* b) *of the crude RNA sequence* **36** *obtained from* **35** *by photolysis* (see *Scheme 7*)

(*Scheme 8*). We found that activating these two L-phenylalanine derivatives **39** and **41**, respectively, with (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate(BOP)/$^{i}Pr_2NEt$ and performing the acylation reaction at room temperature in pyridine and in the presence of 0.5 equiv. of 4-(pyrrolidin-1-yl)pyridine led to the quantitative formation of the corresponding aminoacylated products **1** and **37**

Scheme 8





a) L-(nboc)NHCH(Bn)COOH (**39**; → **1**) or L-[(*RS*)-npeoc]NHCH(Bn)COOH (**41**; → **37**), BOP, $^{i}Pr_2NEt$, 4-(pyrrolidin-1-yl)pyridine, Py, r.t. *b*) Photolysis, $^{t}BuOH$, H_2O, ammonium citrate (pH 3.6), r.t. *c*) L-Phenylalanine, NaOH, H_2O, r.t. *d*) 1,1′-Carbonylbis[1*H*-imidazole], CH_2Cl_2, r.t.; *e*) L-Phenylalanine, 1*H*-tetrazole, DMF, 80°.

within 1–2 h (*Scheme 8*, *Fig. 2,a*). We observed that nucleotide **2** (protected with a 2′-*O*-[(*S*)-npeom] group) reacted faster than the corresponding diastereoisomer (protected with a 2′-*O*-[(*R*)-npeom] group)[6]). Under these optimized conditions, racemization of the *N*-nboc-protected L-phenylalanine was observed only to a very small extent[7]). The photolysis of nucleotide **37** at pH 3.6 proceeded efficiently, resulting in a quantitative formation of the deprotected aminoacylated nucleotide **38** without concomitant hydrolysis of the ester linkage (*Fig. 2,b*)[8]).

The partially protected, crude RNA sequence **34** (see *Scheme 7*) was then aminoacylated under the same conditions, employing an excess of *N*-[(*RS*)-npeoc]- or *N*-nboc-protected L-phenylalanine **39** or **41**, respectively, and BOP/$^{i}Pr_2NEt$/4-(pyrrolidin-1-yl)pyridine in pyridine (*Scheme 9*). It was very important to dry the crude sequence **34** by repeated co-evaporation from dry pyridine or DMF. After 4 h at 20°,

[6]) Additionally, we found that the 3′-*O*-[(*R*)-npeom]-protected analogue reacted faster than nucleotide **2**.

[7]) Chromatographically, the aminoacylated analogue **53** (see below, *Scheme 10*), containing a D-phenylalanine, could be distinguished from **2**. Its position on the HPLC trace is indicated in *Fig. 2,a*.

[8]) According to ^{31}P-NMR analysis, two products in a 3:1 ratio were formed during the photolytic deprotection. This is typical for phenylalanine-substituted adenosines, which adopt 3:1 equilibrium mixtures of the 3′-*O*- and 2′-*O*-substituted derivatives, respectively [11].

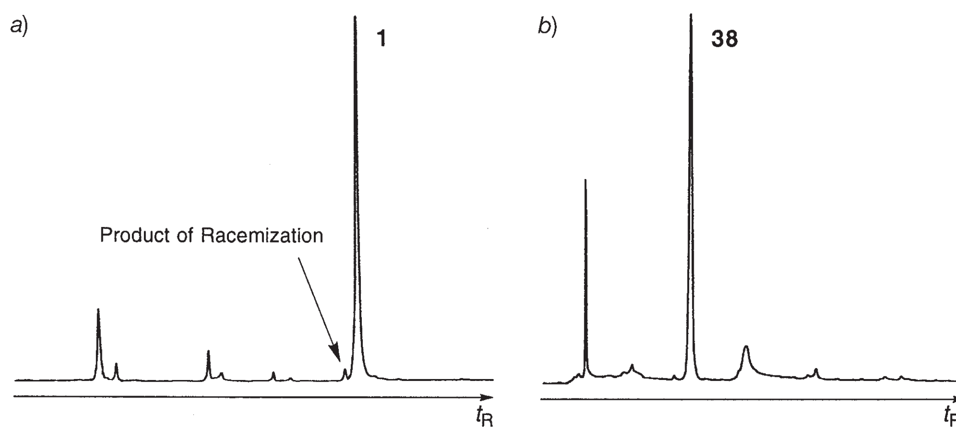
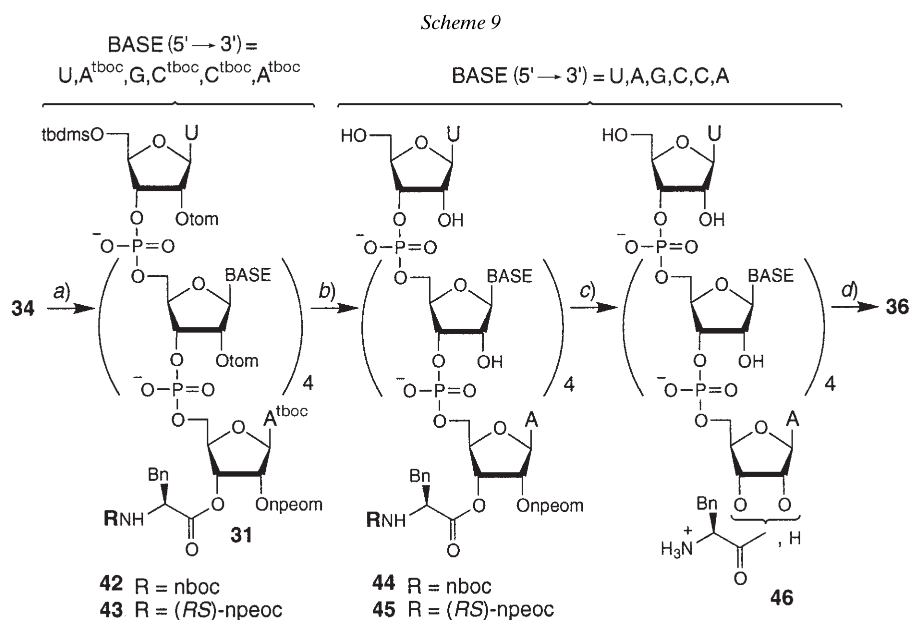


Fig. 2. *Reversed-phase HPLC traces* (measured at 260 nm) a) *of the crude* N-*(nboc)- and 2′*-O-*[(*S*)-npeom]-protected aminoacylated nucleotide* **1** *and* b) *of the crude aminoacylated nucleotide* **38** *obtained from* **1** *by photolysis* (see *Scheme 8*). The position of the corresponding protected D-phenylalanine derivative **53** (see *Scheme 10*) is indicated by the arrow.

Scheme 9



a) L-(nboc)NHCH(Bn)COOH (**39**; → **42**) or L-[(*RS*)-npeoc]NHCH(Bn)COOH (**41**; → **43**), BOP, $^{i}Pr_2NEt$, 4-(pyrrolidin-1-yl)pyridine, Py, r.t.; *b*) 1M $Bu_4NF \cdot 3 H_2O$, 0.5M AcOH, THF, r.t. *c*) Photolysis, 20 mM aq. AcOH (pH 3.1), r.t. *d*) 50 mM Phosphate buffer (pH 11.5), r.t.

the reaction mixtures (→**42** and **43**, resp.) were treated with H_2O and evaporated, and the residues were treated with 1M $Bu_4NF \cdot 3 H_2O$/0.5M AcOH in THF. An equal volume of 1M *Tris* · HCl buffer (pH 7.4) was added after 2 h, and the mixtures were desalted on

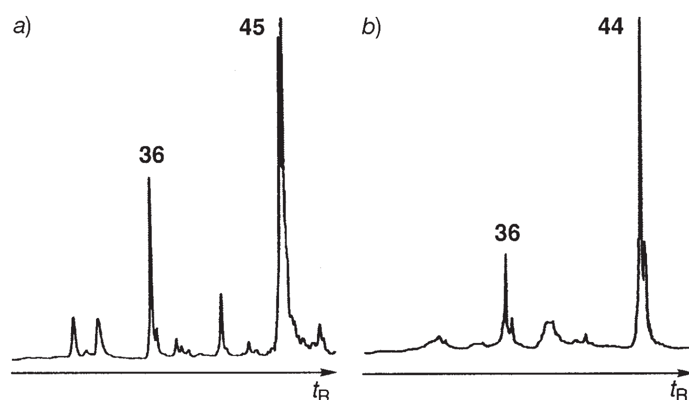


Fig. 3. *Reversed-phase HPLC traces* (measured at 260 nm) *of crude products obtained by aminoacylation of the partially protected RNA sequence* **34** a) *with* N-*[(*RS*)-npeoc]-protected* L*-phenylalanine* (→**45**) *and* b) *with* N-*nboc-protected* L*-phenylalanine* (→**44**). The remaining not-aminoacylated sequence **36** is indicated (see *Scheme 9*).

Sephadex G-10. The obtained aminoacylated crude products **44** and **45** were analyzed by reversed-phase HPLC (*Fig. 3*).

The HPLC trace of the reaction mixture obtained with the *N*-nboc-protected L-phenylalanine **39** reveals that the aminoacylation occurred to an extent of *ca.* 80%, according to the integral ratio between the peak of the product **44** and the peak of the non-acylated product **36** (see *Fig. 3,b*). A similar result was obtained with the *N*-[(*RS*)-npeoc]-protected L-phenylalanine **41**; however, in this case, two diastereoisomeric sequences **45** were formed, causing a splitting of the product peak (*Fig. 3,a*).

The aminoacylated sequence **45** was isolated by prep. reversed-phase HPLC (*Fig. 4*) and subjected to photolysis (*Scheme 9*), which yielded a more polar product **46** after 30 min (HPLC analysis). When this latter product was incubated at pH 11.5 (phosphate buffer), another more polar compound, identical to the authentic RNA sequence **36** was formed within minutes (*Fig. 4*).

When compound **45** was subjected to a short photolysis (10 min), we were able to detect by MALDI-TOF mass spectrometry not only the dominating signal of the completely deprotected aminoacylated sequence **46**, but additionally the signals of the intermediates, each having lost one of the two photolabile protecting groups (*Fig. 5*). According to this measurement, the 2′-*O*-npeom and the *N*-npeoc group of the partially protected, aminoacylated sequence **45** were cleaved at a comparable rate.

2.4. *Stability of Aminoacylated Nucleotides under Acidic Conditions.* The ester bond of aminoacylated ribonucleotide derivatives is an activated, energy-rich bond with a free energy of hydrolysis comparable to the one of ATP hydrolysis [12]. Fast and reversible migration of the amino acid occurs between the vicinal 2′-*O*- and 3′-*O*-substituents of the ribofuranose moiety, resulting in an equilibrium mixture of 2′-*O*- and 3′-*O*-aminoacylated species. Both processes, the hydrolysis under neutral and basic conditions, and the transacylation process have been studied intensively[9]). Particularly,

[9]) This subject is thoroughly reviewed [4].

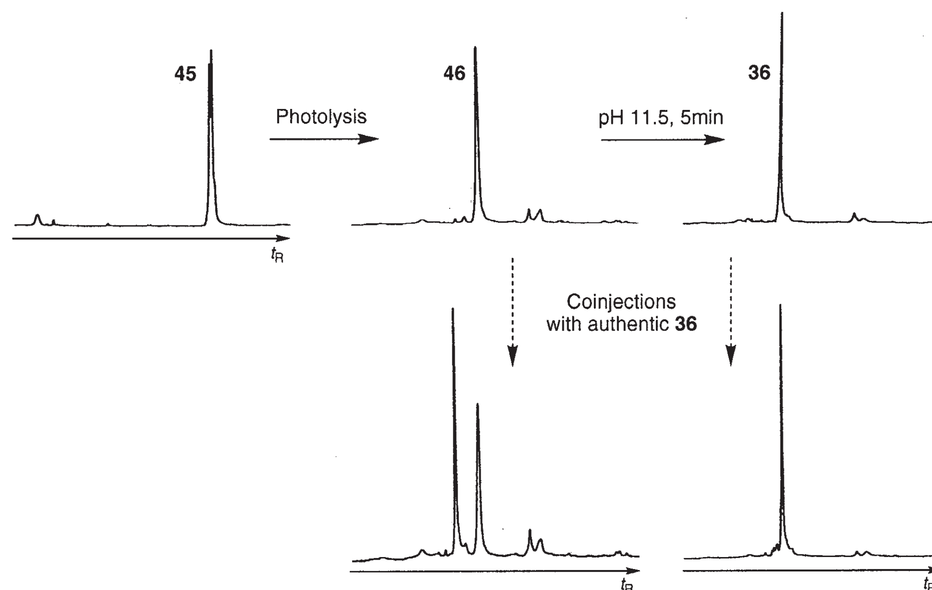


Fig. 4. *Reversed-phase HPLC traces* (measured at 260 nm) *of the purified, partially protected aminoacylated RNA sequence* **45** (top left) *of the crude product* **46** *obtained on photolysis of* **45** (top center), *and of the RNA sequence* **36** *obtained on hydrolysis of* **46** *under weakly alkaline conditions* (top right) (see *Scheme 9*)

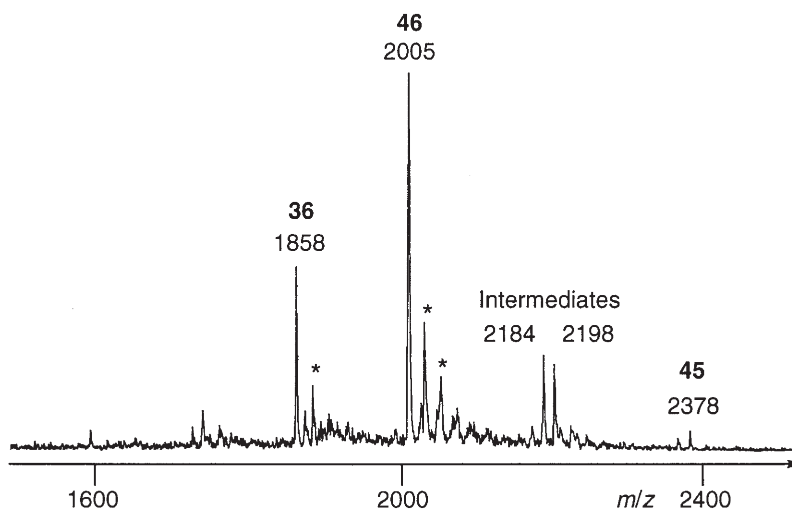


Fig. 5. *MALDI-TOF Mass spectrum of a product mixture obtained by short photolysis of the aminoacylated RNA sequence* **45**. Besides the small signal of the precursor **45** and the signal of the completely deprotected product **46**, small signals of two intermediates at *m/z* 2198 and 2184 (loss of the *N*-npeoc and the 2′-*O*-npeom group, resp.) are present. The signals marked by an asterisk are due to Na^+ adducts (+23 amu).

it was found that the t-RNA structure of aminoacylated t-RNAs has no influence on the reactivity of the ester bond, since L-valyl-t-RNA and L-valyl-AMP are hydrolyzed at a comparable rate [13]. The nucleobase of the aminoacylated nucleotide has only a very minor influence on the rate of hydrolysis, whereas the structure of the amino-acid residue affects it significantly [14][15]. Absence of a (negatively charged) 5′-phosphate group at the sugar moiety results in a higher rate of hydrolysis [13]. In their protonated forms, aminoacylated nucleotides are hydrolyzed much faster than in their unprotonated forms. From pH 6 to 7.5, where the amino groups are protonated, a linear correlation between the OH^- concentration and the rate of hydrolysis was observed; furthermore, the slope equal to 1 indicates specific base catalysis. Consequently, esters formed between nucleotides and amino-acid derivatives with a masked NH_2 group are significantly more stable [16]. Moreover, a similar stabilization of the ester bond is observed upon removal of the neighboring 2′(3′)-OH group [17].

In the context of this project, we wanted to explore conditions that would allow preparation, purification, and handling of different aminoacylated oligonucleotides without concomitant hydrolysis of the ester bond. It was known, though only in a qualitative way, that acidic conditions stabilize such compounds compared to neutral or alkaline conditions. Therefore, we performed a detailed study of the stability of aminoacylated model nucleotides under potential preparative conditions.

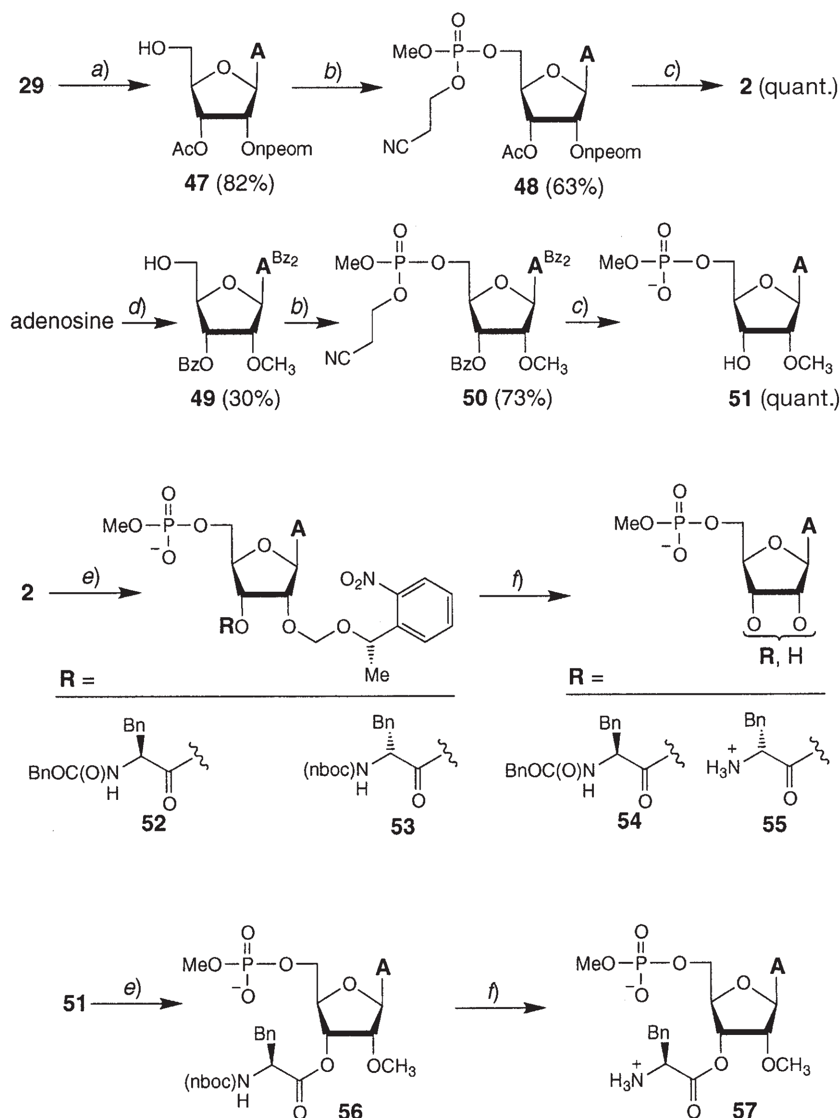
The synthesis of the model nucleotides started from **29** (see *Scheme 6*), which was detritylated under acidic conditions. The resulting alcohol **47** was transformed into the phosphotriester **48** by treatment with $(MeO)P(N^iPr_2)(OCH_2CH_2CN)$/5-(benzylthio)-1*H*-tetrazole, followed by oxidation with I_2 in H_2O/pyridine (*Scheme 10*). Removal of the 3′-*O*-acetyl and the cyanoethyl group with $MeNH_2$ in EtOH gave the nucleotide **2** which served as precursor of the aminoacylated nucleotides **1**, **37**, **52**, and **53**. Analogously, we prepared from adenosine the nucleoside **49**, which was phosphorylated (→ **50**), and subsequently transformed into the 2′-*O*-methylated nucleotide **51**. This nucleotide served as precursor for the preparation of the corresponding aminoacylated derivative **56** (*Scheme 10*).

The aminoacylated nucleotides **38**, **54**, **55**, and **57** were obtained by photolysis of the corresponding protected precursors in ^{t}BuOH/H_2O 1:1 at pH 3.6 (40 mM ammonium citrate) (*Scheme 10*). After extraction, the crude products were desalted on a plug of reversed-phase silica gel (H_2O/MeCN 4:1 (+20 mM AcOH)), diluted with an equal volume of the appropriate buffer solution, and incubated at 25°. Periodically, aliquots were removed and analyzed by reversed-phase HPLC (detection at 260 nm). From the time-dependant ratios between aminoacylated starting materials and hydrolyzed products, individual pseudo-first-order rate constants were obtained (*Table*).

The pH dependence of the hydrolysis rate of the L-phenylalanyl-nucleotide **38** is shown in *Fig. 6*. As expected, the stability increased dramatically at lower pH values, up to a half-life of 250 h at pH 2.5[10]). At a little higher value, at pH 3.6 where photolyses usually are carried out, the half-life of the ester bond was still *ca.* 150 h (0.1M formate), which translates into $< 5\%$ hydrolysis within 5 h (see *Table*). Concerning the influence of the buffer concentration on the stability, both at a pH value of 7.4 and of 3.6, the rate of hydrolysis increased with increasing buffer concentration, resulting, *e.g.*, in

[10]) Lower pH values were not considered, because of the limited stability of RNA sequences.

Scheme 10



a) $CHCl_2COOH$, MeOH, CH_2Cl_2, 4°. *b*) 1. $(MeO)P(N^iPr_2)(OCH_2CH_2CN)$, 5-(benzylthio)-1*H*-tetrazole, MeCN, r.t.; 2. I_2, H_2O, Py, THF, r.t. *c*) $MeNH_2$, EtOH, r.t. *d*) 1. NaH, MeI, DMF, 4°; 2. tbdms-Cl, 1*H*-imidazole, r.t.; 3. BzCl, Py, r.t.; 4. HCl, H_2O, THF, r.t. *e*) *N*-[(benzyloxy)carbonyl]-L-phenylalanine (→ **52**) or *ent*-**39** (→ **53**) or **39** (→ **56**), $^iPr_2NEt$, 4-(pyrrolidin-1-yl)pyridine, Py, r.t. *f*) Photolysis, ^tBuOH, H_2O, ammonium citrate (pH 3.6), r.t.

approximately a third of the half-life upon raising the formate concentration from 0.1 to 1M at pH 3.6 (see *Table*).

We were not able to detect a difference between the hydrolysis rates of the L-phenylalanine and the D-phenylalanine derivatives **38** and **55**, respectively, at pH 7.4.

Table. *Hydrolysis of Aminoacylated Nucleotides* **38**, **54**, **55**, **57**, and **1** [a])

Amino-acid conjugate	Buffer			k [10^{-5} min^{-1}] [b]	$t_{1/2}$ [h]	Hydrolysis after 5 h [%]	Time for 10% hydrolysis [h]
	pH	c					
38 L-Phe	2.5	0.1 M	citric acid, Na_2HPO_4	4.7	246	1.4	37
	3.6	0.1 M	HCOOH, NaOH	7.6	152	2.3	23
	4.7	0.1 M	AcOH, NaOH	24.7	47	7.1	7.0
	5.5	0.1 M	AcOH, NaOH	50.7	23	14	3.5
	6.5	0.1 M	NaH_2PO_4, NaOH	240	4.8	48	0.75 = 45 min
	7.4	0.1 M	*Tris* · HCl	1430	0.8	98	0.12 = 7 min
	3.1	0.02 M	AcOH	4.9	238	1.4	36
	3.6	0.05 M	HCOOH, NaOH	7.6	151	2.3	23
	3.6	0.5 M	HCOOH, NaOH	17.7	65	5.2	10
	3.6	1.0 M	HCOOH, NaOH	21.3	54	6.2	8.2
	7.4	0.5 M	*Tris* · HCl	6270	0.2	> 99	0.03
55 D-Phe	7.4	0.1 M	*Tris* · HCl	1420	0.8	98	0.12 = 7 min
54 Z-L-Phe	7.4	0.1 M	*Tris* · HCl	83.0	14	22	2.0
57 L-Phe (2′-*O*Me)	3.1	0.02 M	AcOH	2.3	505	< 1	75
	5.0	0.1 M	AcOH, NaOH	3.6	325	1.0	50
	7.4	0.1 M	*Tris* · HCl	191	6	43	0.9
1 nboc-L-Phe (2′-*O*-npeom)	7.4	0.1 M	*Tris* · HCl	20.6	56	6.0	8.5
	8.2	0.05 M	*Tris* · $B(OH)_3$)	23.6	49	6.8	7.5

[a]) All experiments were carried out at 25°. [b]) The individual k values were obtained from the ratios of starting materials and products, determined by time-dependent HPLC analyses (> 5 values). The amount of hydrolysis product was plotted against the time, and the analysis was carried out assuming pseudo-first-order kinetics. Estimated error: ±5%.

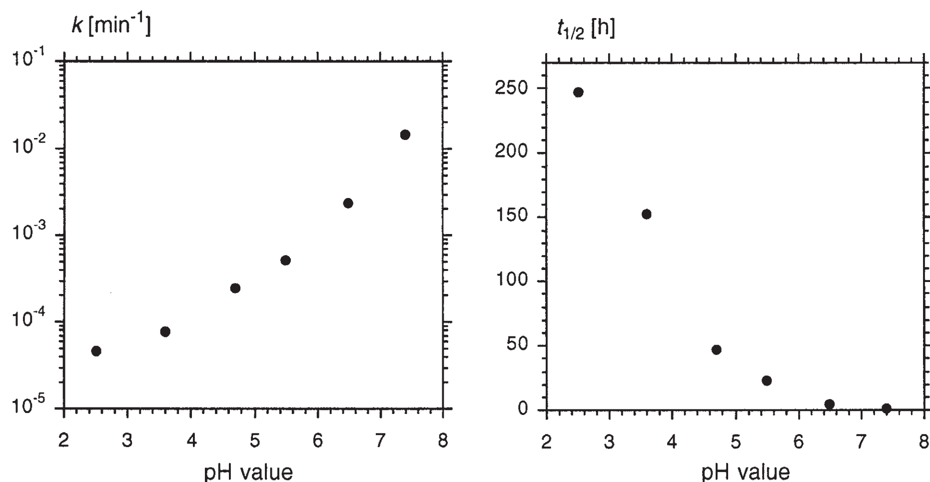


Fig. 6. *Rate constants* k *and half-lives* $t_{1/2}$ *for the hydrolysis of the aminoacylated nucleotide* **38** *at room temperature as a function of pH*. Buffer concentration: 0.1 M; estimated error: ±5% (*Table*).

Our retrosynthetic scheme includes ligation of *N*- and 2′-*O*-protected aminoacylated RNA-fragments to a truncated t-RNA (*Scheme 1*), followed by purification and deprotection. Usually, the purification of relatively long oligonucleotides is carried out by polyacrylamide-gel electrophoresis (PAGE) at pH 8.2 (50 mM *Tris* · borate buffer). Under these standard conditions, the half-life of hydrolysis of the protected model compound **1** was *ca.* 50 h, corresponding to *ca.* 5% cleavage within 3 h (the time usually required for a gel purification) (see *Table*).

As expected, the *N*- and 2′-*O*-protected compound **1** was hydrolyzed much more slowly than the completely deprotected derivative **38**. At pH 7.4, the difference amounted to a factor of 70 ($t_{1/2}$ (**1**) 56 h, $t_{1/2}$ (**38**) 48 min). To investigate the relative influence of the two separate protecting groups, we additionally prepared the aminoacylated analogues **57** and **54**, containing 2′-*O*-Me and *N*-(benzyloxy)carbonyl (= Z) groups, respectively, and determined their rates of hydrolysis at pH 7.4. It turned out that *N*-protection of the amino acid resulted in a 17, and *O*-protection of the sugar residue in a 7 times slower hydrolysis, respectively, compared to the unprotected **38**.

In general, our results concerning the stability of various aminoacylated compounds under acidic and neutral conditions are in good agreement with previously reported data obtained in the neutral and alkaline pH range. The 17-fold increase in stability of the *N*-Z-protected compound **54**, compared to the unprotected analogue **38**, at pH 7.4 and 25°, is similar to the 5-fold increase found for *N*-acetylphenylalanyl-tRNA at pH 8.8 and 37° [15][18]. Blocking the 2′-*O* group by methylation (compound **57**) resulted in a 7-fold increase in stability at pH 7.4 and 25°, whereas, with a similar aminoacylated 2′-deoxynucleotide, a 3-fold increase was found at pH 9 and 37° [17]. The known stabilization of the aminoacyl ester bond by *N*-acylation and 2′-*O*-protection seems to be even more pronounced at lower pH values.

3. Discussion. – We are interested in a general, nonenzymatic synthesis of 3′-*O*-aminoacylated t-RNA analogues for structural studies related to the process of transcription and for the ribosome-mediated incorporation of unnatural amino acids into proteins [2]. Our retrosynthetic scheme differs from the known concepts by excluding all enzymatic steps. Currently, artificial aminoacylated t-RNAs are obtained by enzymatic ligation of a truncated t-RNA (produced by T7-polymerase-mediated transcription of an appropriate gene construct) with an aminoacylated r(CA) dimer [2]. This approach results in t-RNA analogues, containing only the four canonical nucleosides. A total synthesis, in contrast, would allow the incorporation of any desired modification at any site and, therefore, enable a multitude of new investigations and applications related to the biosynthesis of proteins.

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 $MeNH_2$. A variety of modified nucleosides are not stable under the conditions required for deprotection of the standard base-protecting groups. Base-protecting groups, which are removable under milder conditions, such as phenoxyacetyl [19] or (allyloxy)carbonyl [20] have been developed and extended the range of tolerated modifications significantly. In the same context, we have recently introduced the photolabile base-protecting nboc group [1].

We now have developed novel fluoride-labile nucleobase-protecting groups that can be cleaved under very mild conditions. Their combination with photolabile *N*- and 2′-*O*-protecting groups allowed a straightforward preparation and purification of stabilized precursors of aminoacylated RNA sequences. Finally, these can be cleanly deprotected *in situ* by photolysis.

Currently, we are about to develop methods for the template-directed ligation of oligoribonucleotides, which would enable us to prepare any desired aminoacylated t-RNA by joining truncated analogues with stabilized, aminoacylated RNA fragments.

This work was supported by the ETH Zürich. We thank *Patrick A. Weiss* (*Xeragon AG*, Zürich) for providing us with reagents and an authentic RNA sequence, and *Thomas Honegger* for experimental contributions.

Experimental Part

General. Reagents and solvents (Py = pyridine) from *Fluka*, tom-Cl, nucleosides **10**, **14**, **20**, **26**, 5-(benzylthio)-1*H*-tetrazole, 'LCAA'-CPG, $^{i}Pr_3SiCl$, and the authentic RNA sequence **36** from *Xeragon AG*. Photolysis: 250-W Hg lamp, 1-cm *Pyrex* filter, r.t. Workup implies distribution of the reaction mixture between CH_2Cl_2 and sat. aq. $NaHCO_3$ soln., drying of the org. layer ($MgSO_4$), and evaporation. Column chromatography (CC): silica gel from *Fluka.* TLC: precoated silica-gel plates from *Merck* stained by dipping into a soln. of anisaldehyde (*Aldrich*; 10 ml), conc. H_2SO_4 (10 ml), and AcOH (2 ml) in EtOH (180 ml) and subsequent heating with a heat gun. Reversed-phase HPLC: *Aquapore RP 300*, 4.6 × 220 mm (*Brownlee Labs*), flow 1 ml/min; eluent *A*: 0.1M (Et_3NH)OAc in H_2O (pH 7); eluent *B*: MeCN; detection at 260 nm, elution at 40°. Ion-exchange HPLC: *Mono Q HR 5/5* (*Pharmacia*), flow 1 ml/min; eluent *A*: 10 mM sodium phosphate in H_2O (pH 11.5); eluent *B*: 10 mM sodium phosphate/1M NaCl in H_2O (pH 11.5); detection at 260 nm, elution at r.t. UV Spectra: $\lambda_{max}/\lambda_{min}$ (ε) in nm. NMR: chemical shift δ in ppm and coupling constants *J* in Hz. MS: FAB, 2-nitrobenzyl alcohol as matrix, pos. mode; ESI, neg. or pos. mode; MALDI, 3,5-dihydroxybenzoic acid as matrix, pos. mode; MALDI-TOF, 2,4,6-trihydroxyacetophenone (diammonium citrate) as matrix, pos. mode; *m/z* (rel. int. in%).

2-[(Triisopropylsilyl)oxy]benzyl Alcohol (**18**). At 4°, NaH (55%; 4.15 g, 0.095 mol) was added to a soln. of 2-hydroxybenzyl alcohol (12.4 g, 0.1 mol) in DMF (100 ml). After 15 min, the mixture was cooled to −70° (dry ice/acetone), and $^{i}Pr_3SiCl$ (14.25 g, 0.095 mmol) was added during 30 min. Workup (AcOEt/hexane 1:1, aq. $NaHCO_3$ soln.) and CC (hexane/CH_2Cl_2 9:1 → 7:3, followed by hexane/AcOEt 4:1) gave 16.2 g (64%) of **18**. Colorless viscous liquid. TLC (hexane/AcOEt 9:1): R_f 0.35. UV (MeOH): 276 (1300), 273 (1200), 270 (1300), 240 (100), 223 (4600). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.02–1.19 (*m*, 3 Me_2CH); 1.24–1.42 (*m*, 3 Me_2CH); 2.09 (*t*, *J* = 6.2, OH); 4.73 (*d*, *J* = 6.2, ArCH_2); 6.83 (*dd*, *J* = 0.8, 7.5, 1 arom. H); 6.94 (*dt*, *J* = 0.8, 7.5, 1 arom. H); 7.17 (*dt*, *J* = 1.7, 7.5, 1 arom. H); 7.31 (*dd*, *J* = 1.7, 7.5, 1 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.0 (*d*, Me_2CH); 18.1 (*q*, Me_2CH); 62.3 (*t*, ArCH_2); 118.0, 121.0, 128.6, 128.8 (4*d*, arom. C); 131.0 (*s*, arom. C); 153.9 (*s*, $^{i}Pr_3$SiO*C*). MALDI-MS: 303 (100, $[M + Na]^+$), 219 (99), 199 (82), 187 (98), 137 (60).

*3′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{[(triisopropylsilyl)oxy]methyl}adenosine* (**11**). A soln. of **10** [5] (2.0 g, 2.33 mol) in 10M $MeNH_2$/EtOH (20 ml) was kept for 30 min at r.t. After evaporation, the residue was dissolved in Py (9.3 ml) and treated with DMAP (28 mg, 0.23 mmol) and Ac_2O (286 mg, 2.8 mmol). Workup (1. 10% citric acid, 2. $NaHCO_3$ soln.) after 30 min at r.t. and CC (AcOEt/hexane 3:2 → 9:1 (+2% Et_3N)) gave **11** (1.76 g, 88%). Colorless foam. TLC (hexane/AcOEt 1:9): R_f 0.57. UV (MeOH): 258 (sh, 15900), 235 (23400), 224 (19200). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.88 (*m*, $^{i}Pr_3$Si); 2.14 (*s*, MeCO); 3.43 (*dd*, *J* = 6.5, 10.4, H−C(5′)); 3.52 (*dd*, *J* = 5.6, 10.4, H′−C(5′)); 3.78 (*s*, 2 MeO); 4.34 (*m*, H−C(4′)); 4.87 (*s*, OCH_2O); 5.19 (*dd*, *J* = 5.3, 6.9, H−C(2′)); 5.51 (*dd*, *J* = 2.5, 5.3, H−C(3′)); 5.80 (br. *s*, NH_2); 6.16 (*d*, *J* = 6.9, H−C(1′)); 6.78–6.83 (*m*, 4 arom. H); 7.20–7.45 (*m*, 9 arom. H); 7.97 (*s*, H−C(2)); 8.25 (*s*, H−C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.7 (*d*, Me_2CH); 17.6 (*q*, Me_2CH); 20.9 (*q*, *Me*CO); 55.2 (*q*, MeO); 63.4 (*t*, C(5′)); 72.1, 77.0, 82.5 (3*d*, C(2′), C(3′), C(4′)); 86.2 (*d*, C(1′)); 86.8 (*s*, Ar_2C(Ph)); 89.6 (*t*, OCH_2O); 113.2 (*d*, arom. C); 120.1 (*s*, C(5)); 127.0, 127.9, 128.2, 130.1 (4*d*, arom. C); 135.6 (*s*, arom. C); 139.2 (*d*, C(8)); 144.4 (*s*, arom. C); 150.2 (*s*, C(4)); 153.2 (*d*, C(2)); 155.4 (*s*, C(6)); 158.6 (*s*, MeO−C); 170.1 (*s*, CO). MALDI-MS: 820 (20, $[M + Na]^+$), 516 (31), 456 (16), 303 (100).

*5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{6}-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2′-*O*-{[(triisopropylsilyl)-oxy]methyl}adenosine* (**12**). A soln. of **11** (956 mg, 1.2 mmol) in CH_2Cl_2 (3.6 ml) was added during 20 min to a

suspension obtained from 1.9M $COCl_2$ in toluene (0.95 ml, 1.8 mmol), DMAP (15 mg, 0.12 mmol) in Py (2.4 ml) and CH_2Cl_2 (4.8 ml). After 15 min at r.t., **18** (1.01 g, 3.6 mmol) and Et_3N (490 mg, 4.8 mmol) were added. Workup (1. 10% citric acid, 2. $NaHCO_3$ soln.) after 24 h at r.t. gave a residue, which was dissolved in 0.2M NaOH in THF/MeOH/H_2O 5:4:1 (20 ml). Workup and CC (AcOEt/hexane 1:4 → 1:1 (+2% NEt_3)) gave **12** (805 mg, 63%). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.49. UV (MeOH): 274 (sh, 18700), 267 (21900), 252 (16800), 235 (sh, 25100). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.05 (*m*, $^iPr_3SiOCH_2$); 1.08–1.15 (*m*, 3 Me_2CH); 1.25–1.40 (*m*, 3 Me_2CH); 3.07 (*d*, *J* = 4.7, OH); 3.40, 3.52 (2*dd*, *J* = 3.3, 10.4, 2 H–C(5')); 3.78 (*s*, MeO); 4.30 (br. *q*, *J* = 3.3, H–C(4')); 4.56 (*m*, H–C(3')); 4.97 (*t*, *J* = 5.2, H–C(2')); 4.99, 5.15 (2*d*, *J* = 4.6, OCH_2O); 5.39 (*s*, $ArCH_2$); 6.20 (*d*, *J* = 5.4, H–C(1')); 6.80 (*d*, *J* = 9.1, 4 arom. H); 6.84–6.99 (*m*, 2 arom. H); 7.17–7.45 (*m*, 11 arom. H); 8.01 (br. *s*, NH); 8.13 (*s*, H–C(2)); 8.68 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8, 13.0 (2*d*, Me_2CH); 17.8, 18.0 (2*q*, Me_2CH); 55.2 (*q*, MeO); 63.3, 63.8 (2*t*, C(5'), $ArCH_2$); 70.9, 82.0, 84.3 (3*d*, C(2'), C(3'), C(4')); 86.6 (*s*, $Ar_2C(Ph)$); 87.2 (*d*, C(1')); 90.8 (*t*, OCH_2O); 113.2, 118.2, 120.8 (3*d*, arom. C); 122.5 (*s*, C(5)); 125.6 (*s*, arom. C); 126.9, 127.9, 128.2, 128.3, 129.5, 130.1, 130.2 (7*d*, arom. C); 135.6, 135.7 (2*s*, arom. C); 141.6 (*d*, C(8)); 144.5 (*s*, arom. C); 149.4 (*s*, C(4)); 150.8 (*s*, CO); 151.0 (*s*, C(6)); 152.9 (*d*, C(2)); 154.3 (*s*, arom. C); 158.5 (*s*, MeO–*C*). MALDI-MS: 1084 (5, $[M + Na]^+$), 862 (6), 736 (8), 398 (15), 303 (100), 242 (15), 101 (9).

5'-O-(4,4'-Dimethoxytrityl)-N⁶-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(triisopropylsilyl)oxy]methyl}adenosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**13**). A soln. of **12** (700 mg, 0.66 mmol), $ClP(N^iPr_2)(OCH_2CH_2CN)$ (187 mg, 0.79 mmol), and $^iPr_2NEt$ (171 mg, 1.32 mmol) in CH_2Cl_2 (2.6 ml) was kept for 14 h at r.t. CC (AcOEt/hexane 1:9 → 2:3 (+2% Et_3N)) gave **13** (704 mg, 85%; 1:1 diastereoisomer mixture). Colorless foam. TLC (hexane/AcOEt 2:3): R_f 0.40. UV (MeCN): 273 (sh, 21400), 267 (24000), 252 (19900), 236 (25200), 233 (24900). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.88 (*m*, 12 H, Me_2CH); 1.07–1.20 (*m*, 42 H, $^iPr_3Si$); 2.38 (*t*, *J* = 6.5, 0.67 H, OCH_2CH_2CN); 2.64 (*m*, 1.33 H, OCH_2CH_2CN); 3.32, 3.36 (2*t*, *J* = 4.0, 0.67 H, OCH_2CH_2CN); 3.50–3.72 (*m*, 4 H, 2 H–C(5'), OCH_2CH_2CN, Me_2CH); 3.77, 3.78 (2*s*, 6 H, MeO); 3.83–3.97 (*m*, 1.33 H, Me_2CH); 4.36 (*m*, 0.66 H, H–C(4')); 4.41 (*m*, 0.44 H, H–C(4')); 4.65 (*m*, 1 H, H–C(3')); 4.91, 4.94, 4.98 (3*d*, 2 H, OCH_2O); 5.18 (*m*, 1 H, H–C(2')); 5.38 (*s*, 2 H, $ArCH_2$); 6.16, 6.18 (2*d*, *J* = 6.4, 1 H, H–C(1')); 6.76–6.81 (*m*, 4 arom. H); 6.85 (*d*, *J* = 8.1, 1 arom. H); 6.94 (*t*, *J* = 7.5, 1 arom. H); 7.17–7.45 (*m*, 11 arom H); 8.03 (br., *s*, 1 H, NH); 8.09, 8.12 (2*s*, 1 H, H–C(2)); 8.63, 8.65 (2*s*, 1 H, H–C(8)). ^{13}C-NMR (125 MHz, $CDCl_3$): 11.8, 13.0 (2*d*, Me_2CH); 17.6, 18.0 (2*q*, Me_2CH); 20.1, 20.4 (2*t*, *J*(C,P) = 6, OCH_2CH_2CN); 24.55, 24.59, 24.65 (3*q*, Me_2CH); 43.2, 43.26, 43.34, 43.4 (4*d*, Me_2CH); 55.19, 55.22 (2*q*, MeO); 58.0, 58.9 (2*t*, *J*(C,P) = 18, OCH_2CH_2CN); 62.9, 63.3 (2*t*, C(5')); 63.8 (*t*, $ArCH_2$); 71.2 and 71.8 (2*d*, *J*(CP) = 16), 77.2 (*d*), 77.5 and 84.1 (2*d*, *J*(C,P) = 4), 84.2 (*d*), (C(2'), C(3'), C(4')); 86.5, 86.6 (2*s*, $Ar_2C(Ph)$); 87.45, 87.53 (2*d*, C(1')); 89.5, 89.6 (2*t*, OCH_2O); 112.8, 113.1 (2*d*, arom. C); 117.4, 117.6 (2*s*, CN); 118.1, 120.8 (2*d*, arom. C); 122.4, 122.5 (2*s*, arom. C); 125.6, 125.7 (2*s*, C(5)); 126.90, 126.93, 127.8, 128.2, 128.26, 128.34, 129.5, 130.04, 130.05, 130.1, 130.2 (11*d*, arom. C); 135.66, 135.70, 135.8 (3*s*, arom. C); 142.0, 142.1 (2*d*, C(8)); 144.4, 144.5 (2*s*, arom. C); 149.31, 149.33 (2*s* C(4)); 150.7 (*s*, CO); 151.0 (*s*, C(6)); 152.78, 152.81 (2*d*, C(2)); 154.3, 158.53, 158.55 (3*s*, arom. C). ^{31}P-NMR (121 MHz, $CDCl_3$): 150.6, 151.3. MALDI-MS: 1284 (18, $[M + Na]^+$), 303 (100).

5'-O-(4,4'-Dimethoxytrityl)-N⁴-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(triisopropylsilyl)oxy]methyl}cytidine (**16**). A soln. of **14** [5] (930 mg, 1.2 mmol) in 10M $MeNH_2$/EtOH (10 ml) was kept 15 min at r.t., evaporated, and co-evaporated with benzene (→ **15**). Separately, a mixture of **18** (505 mg, 1.8 mmol), Et_3N (364 mg, 3.6 mmol), and 4-nitrophenyl carbonochloridate (387 mg, 1.92 mmol) in toluene (3.6 ml) was kept for 30 min at r.t. and then filtered. The filtrate (→ **19**) was added to the soln. of crude **15** (see above) and DMAP (15 mg, 0.12 mmol) in toluene (2.4 ml) and kept for 5 h at 80°. Workup (1. 10% citric acid, 2. $NaHCO_3$ soln.) and CC (AcOEt/hexane 1:9 → 2:3 (+2% Et_3N)) gave **16** (742 mg, 60%). Colorless foam. TLC (hexane/AcOEt 6:4): R_f 0.30. UV (MeOH): 296 (sh, 4700), 281 (6000), 265 (4700), 236 (18800), 228 (17800). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.02–1.15 (*m*, $^iPr_3SiOCH_2$, 3 Me_2CH); 1.25–1.38 (*m*, 3 Me_2CH); 3.36 (*d*, *J* = 8.4, OH); 3.53 (*dd*, *J* = 2.8, 11.1, H–C(5')); 3.60 (*dd*, *J* = 1.9, 11.1, H'–C(5')); 3.81 (*s*, MeO); 4.09 (*m*, H–C(4')); 4.23 (*d*, *J* = 4.7, H–C(2')); 4.37 (*m*, H–C(3')); 5.16, 5.28 (2*d*, *J* = 4.7, OCH_2O); 5.25 (*s*, $ArCH_2$); 5.96 (*s*, H–C(1')); 6.85–6.88 (*m*, 5 arom. H); 6.91–6.96 (*m*, 1 arom. H); 6.94 (*d*, *J* = 7.5, H–C(5)); 7.19–7.44 (*m*, 11 arom. H); 8.47 (*d*, *J* = 7.5, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.9, 13.0 (2*d*, Me_2CH); 17.8, 18.0 (2*q*, Me_2CH); 55.2 (*q*, MeO); 61.1, 64.2 (2*t*, C(5'), $ArCH_2$); 67.6, 83.3, 83.4 (3*d*, C(2'), C(3'), C(4')); 87.0 (*s*, $Ar_2C(Ph)$); 90.1 (*d*, C(1')); 90.8 (*t*, OCH_2O); 94.7 (*d*, C(5)); 113.3, 118.2, 120.8 (3*d*, arom. C); 125.0 (*s*, arom. C); 127.1, 128.0, 128.3, 130.0, 130.1, 130.2, 130.4 (7*d*, arom. C); 135.4, 135.7, 144.1 (3*s*, arom. C); 144.5 (*d*, C(6)); 152.1 (*s*, CO); 154.5, 154.9 (2*s*, C(2), 1 arom. C); 158.7 (*s*, MeO–*C*); 162.3 (*s*, C(4)). MALDI-MS: 1061 (17, $[M + Na]^+$), 712 (12), 440 (40), 396 (14), 303 (100).

5'-O-(4,4'-Dimethoxytrityl)-N⁴-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(triisopropylsilyl)oxy]methyl}cytidine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**17**). A soln. of **16** (600 mg, 0.58 mmol), $ClP(N^iPr_2)(OCH_2CH_2CN)$ (166 mg, 0.7 mmol), and $^iPr_2NEt$ (150 mg, 1.16 mmol) in CH_2Cl_2 (2.3 ml) was kept

for 14 h at r.t. CC (AcOEt/hexane 1:9 → 2:3 (+2% Et_3N)) gave **17** (608 mg, 85%, 1:1 diastereoisomer mixture). Colorless foam. TLC (hexane/AcOEt 3:2): R_f 0.35, 0.42. UV (MeCN): 295 (sh, 7000), 278 (8500), 264 (6600), 236 (32400), 228 (31300). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.96–1.17 (*m*, 51 H, Me_2CH); 1.19–1.37 (*m*, 3 H, Me_2CH); 2.38 (*t*, *J* = 6.2, 1 H, OCH_2CH_2CN); 2.60, 2.61 (2*t*, *J* = 6.2, 1 H, OCH_2CH_2CN); 3.40-3.70 (*m*, 6 H, 2 H–C(5'), OCH_2CH_2CN, Me_2CH); 3.806, 3.813 (2*s*, 6 H, MeO); 4.30–4.54 (*m*, 3 H, H–C(2'), H–C(3'), H–C(4')); 5.16–5.21 (*m*, 2 H, OCH_2O); 5.24 (*s*, 2 H, $ArCH_2$); 6.14, 6.16 (2*d*, *J* = 1.9, 1 H, H–C(1')); 6.80–6.96 (*m*, 7 H, 6 arom. H, H–C(5)); 7.19–7.44 (*m*, 11 arom. H); 8.34 (*d*, *J* = 7.8, 0.5 H, H–C(6)); 8.45 (*d*, *J* = 7.5, 0.5 H, H–C(6)). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.0, 13.0 (2*d*, Me_2CH); 17.8, 18.0 (2*q*, Me_2CH); 20.1, 20.4 (2*t*, *J*(C,P) = 6, OCH_2CH_2CN); 24.47, 24.53, 24.57, 24.60, 24.66 (5*q*, Me_2CH); 43.2, 43.3 (2*d*, *J*(C,P) = 9, Me_2CH); 55.20, 55.23 (2*q*, MeO); 58.1, 58.8 (2*t*, *J*(C,P) = 17, OCH_2CH_2CN); 61.0, 61.6 (2*t*, C(5')); 64.08, 64.16 (2*t*, $ArCH_2$); 69.4, and 69.6 (2*d*, *J*(C,P) = 13); 78.4, 78.7, and 82.3 (3*d*), 82.5 (*d*, *J*(C,P) = 5, C(2'), C(3'), C(4')); 87.0, 87.1 (2*s*, Ar_2C(Ph)); 89.5 (*t*, OCH_2O); 89.48, 89.55 (2*d*, C(1')); 94.66, 94.73 (2*d*, C(5)); 113.2, 113.3 (2*d*, arom. C); 117.4 117.7 (2*s*, CN); 118.7, 120.8 (2*d*, arom. C); 125.1 (*s*, arom. C); 127.17, 127.19, 128.0, 128.3, 128.5, 129.9, 130.1, 130.18, 130.22, 130.25, 130.27, 130.4, 130.1 (13*d*, arom. C); 135.3, 135.4, 135.6, 144.0, 144.1 (5*s*, arom. C); 144.76, 144.84 (2*d*, C(6)); 152.1 (*s*, CO); 154.5 (*s*, arom. C); 154.86, 154.90 (2*s*, C(2)); 158.6 (*s*, arom. C); 162.1, 162.3 (2*s*, C(4)). ^{31}P-NMR (121 MHz, $CDCl_3$): 150.7, 150.3. MALDI-MS: 1260 (25, $[M + Na]^+$), 790 (5), 772 (14), 489 (10), 440 (5), 303 (100).

*5'-*O*-(4,4'-Dimethoxytrityl)-2'-*O*-{[(triisopropylsilyl)oxy]methyl}guanosine* (**21**). A soln. of **20** [5] (977 mg, 1.2 mmol) in 10M $MeNH_2$/EtOH (10 ml) was kept for 1 h at r.t. and was then evaporated. CC (CH_2Cl_2 → CH_2Cl_2/MeOH 47:3) gave **21** (806 mg, 87%). Off-white foam. TLC ($MeCl_2$/MeOH 9:1): R_f 0.42. UV (MeOH): 270 (sh, 11800), 236 (25800), 223 (20900). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.93–1.16 (*m*, $^iPr_3$Si); 3.16 (*d*, *J* = 2.1, OH); 3.34 (*dd*, *J* = 4.4, 10.4, H–C(5')); 3.48 (*dd*, *J* = 4.0, 10.4, H'–C(5')); 3.75, 3.76 (2*s*, 2 MeO); 4.25 (*m*, H–C(4')); 4.54 (*m*, H–C(3')); 4.84 (*t*, *J* = 5.5, H–C(2')); 4.96, 5.15 (2*d*, *J* = 5.0, OCH_2O); 5.89 (br. *s*, NH_2); 5.95 (*d*, *J* = 5.5, H–C(1')); 6.8 (*d*, *J* = 8.7, 4 arom. H); 7.16–7.45 (*m*, 9 arom. H); 7.65 (*s*, H–C(8)); 11.97 (br. *s*, NH–C(1)). ^{13}C-NMR (75 MHz, $CDCl_3$): 11.8 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 55.2 (*q*, MeO); 63.4 (*t*, C(5')); 70.9, 81.8, 83.9 (3*d*, C(2'), C(3'), C(4')); 86.3 (*s*, Ar_2C(Ph)); 86.5 (*d*, C(1')); 90.8 (*t*, OCH_2O); 113.2 (*d*, arom. C); 117.7 (*s*, C(5)); 126.9, 127.9, 128.2, 129.8, 130.1 (5*d*, arom. C); 135.69, 135.72 (2*s*, arom. C); 136.4 (*d*, C(8)); 144.6 (*s*, arom. C); 151.8, 153.5 (2*s*, C(2), C(4)); 158.5 (*s*, MeO–C); 159.1 (*s*, C(6)). MALDI-MS: 810 (2, $[M + K]^+$), 794 (14, $[M + Na]^+$), 303 (100), 273 (6), 151 (5).

*5'-*O*-(4,4'-Dimethoxytrityl)-2'-*O*-{[(triisopropylsilyl)oxy]methyl}guanosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**9**). A soln. of **21** (750 mg, 0.97 mmol), $ClP(N^iPr_2)(OCH_2CH_2CN)$ (166 mg, 0.7 mmol) and $^iPr_2NEt$ (251 mg, 1.94 mmol) in CH_2Cl_2 (3.9 ml) was kept for 14 h at r.t. CC (CH_2Cl_2 → $MeCl_2$/MeOH 9:1 (+2% Et_3N)) gave **9** (761 mg, 81%; 1:1 diastereoisomer mixture). Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.44. UV (MeCN): 269 (sh, 12800), 237 (26400), 222 (19200). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.88–1.22 (*m*, 33 H, $^iPr_3$Si, Me_2CH); 2.36 (*t*, *J* = 6.5, 1 H, OCH_2CH_2CN); 2.652 (*t*, *J* = 5.6, 0.5 H, OCH_2CH_2CN); 2.672 (*t*, *J* = 7.5, 0.5 H, OCH_2CH_2CN); 3.28–3.35 (*m*, 1 H, Me_2CH); 3.50–3.75 (*m*, 4 H, 2 H–C(5'), OCH_2CH_2CN, Me_2CH); 3.749, 3.751, 3.755 (3*s*, 2 MeO); 3.87–3.99 (*m*, 1 H, OCH_2CH_2CN); 4.31 (*m*, 0.5 H, H–C(4')); 4.38 (*m*, 0.5 H, H–C(4')); 4.59–4.69 (*m*, 1 H, H–C(3')); 4.92 (*d*, *J* = 6.0, 0.5 H, OCH_2O); 4.96 (*s*, 1 H, OCH_2O); 4.97 (*d*, *J* = 6.0, 0.5 H, OCH_2O); 5.04–5.11 (*m*, 1 H, H–C(2')); 5.68 (br. *s*, NH_2); 5.92 (*d*, *J* = 6.2, 0.5 H, H–C(1')); 5.95 (*d*, *J* = 5.5, 0.5 H, H–C(1')); 6.79, 6.80, 6.81 (3*d*, *J* = 9.0, 4 arom. H); 7.13–7.47 (*m*, 9 arom. H); 7.64, 7.65 (2*s*, 1 H, H–C(8)); 12.08 (br. *s*, H–N(1)). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.9 (*d*, Me_2CH); 18.22, 18.25 (2*q*, Me_2CH); 20.5, 20.7 (2*t*, *J*(C,P) = 7, OCH_2CH_2CN); 24.9, 24.93, 24.99, 25.03, 25.2 (5*q*, Me_2CH); 44.1, 44.3 (2*d*, *J*(C,P) = 13, Me_2CH); 55.4 (*q*, MeO); 59.4 (*t*, *J*(C,P) = 19, OCH_2CH_2CN); 60.4 (*t*, *J*(C,P) = 16, OCH_2CH_2CN); 64.5, 64.6 (2*t*, C(5')); 72.5 (*d*, *J*(C,P) = 8), 73.2 (*d*, *J*(C,P) = 14), 77.8 (*d*, *J*(C,P) = 5), 78.4, 84.6, 84.9 (3*d*, C(2'), C(3'), C(4')); 87.1, 87.2 (2*s*, Ar_2C(Ph)); 87.28, 87.34 (2*d*, C(1')); 90.1, 90.4 (2*t*, OCH_2O); 113.8 (*d*, arom. C); 118.2 (*s*, C(5)); 118.4, 118.7 (2*s*, CN); 127.4, 128.5, 129.17, 129.23, 130.9, 130.98, 131.05 (7*d*, arom C); 136.73, 136.75 (2*s*, arom. C); 136.9 (*d*, C(8)); 146.0, 146.1 (2*s*, C(4)), 152.7 (*s*, C(2)); 154.8 (*s*, C(6)); 159.7 (*s*, MeO–C). ^{31}P-NMR (121 MHz, $CDCl_3$): 150.9, 151.5. MALDI-MS: 994 (13, $[M + Na]^+$), 304 (22), 303 (100).

*5'-*O*-[(*tert*-Butyl)dimethylsilyl]uridine* (**23**). A soln. of uridine (1.0 g, 4.1 mmol) and 1*H*-imidazole (558 mg, 8.2 mmol) in DMF (16 ml) was treated at r.t. with tbdms-Cl (548 mg, 4.3 mmol). Workup after 1 h and CC (CH_2Cl_2 → CH_2Cl_2/MeOH 24:1) gave **23** (1.2 g, 80%). Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.55. UV (MeOH): 262 (9000), 231 (1800). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.11 (*s*, Me_2Si); 0.92 (*s*, ^{t}BuSi); 3.63 (br. *s*, OH); 3.84 (*dd*, *J* = 1.6, 11.5, H–C(5')); 4.03 (*dd*, *J* = 1.6, 11.5, H'–C(5')); 4.41 (*m*, H–C(4')); 4.22–4.28 (*m*, H–C(2'), H–C(3')); 5.54 (br. *s*, OH); 5.65 (*d*, *J* = 8.7, H–C(5)); 5.91 (*d*, *J* = 2.5, H–C(1')); 8.09 (*d*, *J* = 8.7, H–C(5)); 10.54 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.5 (*q*, Me_2Si); 18.4 (*s*, Me_3CSi); 25.9 (*q*, Me_3C); 61.7 (*t*, C(5'));

69.1, 75.6, 84.8 (3*d*, C(2′), 3C(′), C(4′)); 90.3 (*d*, C(1′)); 102.1 (*d*, C(5)); 140.6 (*d*, C(6)); 151.3 (*s*, C(2)); 164.1 (*s*, C(4)). MALDI-MS: 381 (100, $[M + Na]^+$), 274 (7), 251 (5), 229 (8), 213 (14), 191 (7), 177 (7), 137 (9), 127 (9).

5′-O-[(tert-Butyl)dimethylsilyl]-2′-O-{[(triisopropylsilyl)oxy]methyl}uridine (**24**). A soln. of **23** (800 mg, 2.23 mmol) and $^iPr_2NEt$ (1.53 ml, 8.9 mmol) in $(CH_2Cl)_2$ (9 ml) was treated with Bu_2SnCl_2 (745 mg, 2.45 mmol) at r.t. for 1 h and then treated with tom-Cl ([5], 596 mg, 2.68 mmol) at 80° for 15 min. Workup and CC (hexane/AcOEt 4 : 1 → 3 : 2) gave **24** (510 mg, 45%). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.46. UV (MeOH): 261 (9000), 230 (1900). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.12 (*s*, Me_2Si); 0.93 (*s*, ^tBuSi); 1.01 – 1.14 (*m*, $^iPr_3Si$); 3.12 (*d*, *J* = 4.7, OH); 3.83, 3.99 (2*dd*, *J* = 1.9, 11.8, 2 H−C(5′)); 4.10 (*dd*, *J* = 1.9, 4.7, H−C(4′)); 4.13 (*t*, *J* = 4.7, H−C(2′)); 4.24 (*q*, *J* = 4.7, H−C(3′)); 4.94, 5.18 (2*d*, *J* = 5.0, OCH_2O); 5.68 (*d*, *J* = 8.1, H−C(5)); 6.08 (*d*, *J* = 4.1, H−C(1′)); 8.00 (*d*, *J* = 8.1, H−C(6)); 8.81 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): −5.56, −5.51 (2*q*, Me_2Si); 11.8 (*d*, Me_2CH); 17.8 (*q*, Me_2CH); 18.4 (*s*, Me_3C); 25.9 (*q*, Me_3C); 62.5 (*t*, C(5′)); 69.4, 83.1, 85.0 (3*d*, C(2′), C(3′), C(4′)); 87.4 (*d*, C(1′)); 90.6 (*t*, OCH_2O); 102.3 (*d*, C(5)); 140.1 (*d*, C(6)); 150.2 (*s*, C(2)); 163.1 (*s*, C(4)). MALDI-MS: 567 (100, $[M + Na]^+$), 323 (6), 283 (6), 269 (10), 267 (24), 225 (5), 189 (9).

5′-O-[(tert-Butyl)dimethylsilyl]-2′-O-{[(triisopropylsilyl)oxy]methyl}uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**25**). A soln. of **24** (260 mg, 0.48 mmol), $ClP(N^iPr_2)(OCH_2CH_2CN)$ (137 mg, 0.58 mmol), and $^iPr_2NEt$ (124 mg, 0.96 mmol) in CH_2Cl_2 (1.9 ml) was kept for 14 h at r.t. CC (hexane/AcOEt 9 : 1 → 7 : 3 (+2% Et_3N)) gave **25** (303 mg, 85%, 3 : 2 diastereoisomer mixture). Colorless foam. TLC (hexane/AcOEt 7:3): R_f 0.38. UV (MeCN): 259 (9500), 229 (2300). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.110, 0.116, 0.119, 0.13 (4*s*, 6 H, Me_2Si); 0.93, 0.94 (2*s*, 9 H, ^tBuSi); 0.96 – 1.11 (*m*, 21 H, $^iPr_3Si$); 1.14 – 1.22 (*m*, 12 H, Me_2CH); 2.61, 2.65, 2.66 (3*t*, *J* = 6.2, 2 H, OCH_2CH_2CN); 3.57 – 3.95 (*m*, 6 H, 2 H−C(5′), OCH_2CH_2CN, Me_2CH); 4.18 (*m*, 0.4 H, H−C(4′)); 4.31 – 4.42 (*m*, 2.6 H, H−C(2′), C(3′), C(4′)); 4.88, 4.93, 4.97, 4.99 (4*d*, *J* = 5.3, 2 H, OCH_2O); 5.67 (*d*, *J* = 8.1, 1 H, H−C(5)); 6.16, 6.18 (2*d*, *J* = 5.6, 1 H, H−C(1′)); 7.84, 7.90 (2*d*, *J* = 8.1, 1 H, H−C(6)); 8.14 (br. *s*, 1 H, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): −5.6, −5.5, −5.5 (3*q*, Me_2Si); 11.9, 11.9 (2*d*, Me_2CH); 17.73, 17.76, 17.78 (3*q*, Me_2CH); 18.4, 18.5 (2*s*, Me_3C); 20.4, 20.5 (2*t*, *J*(C,P) = 8, OCH_2CH_2CN); 24.53, 24.55, 24.57, 24.59, 24.63, 24.66 (6*q*, Me_2CH); 25.9, 26.0 (2*q*, Me_3C); 43.2, 43.4 (2*d*, *J*(C,P) = 12, Me_2CH); 57.6, 59.0 (2*t*, *J*(C,P) = 16, OCH_2CH_2CN); 63.0, 63.2 (2*t*, C(5′)); 70.2, 71.8 (2*d*, *J*(C,P) = 16), 77.2, 77.9 (*d*, *J*(C,P) = 3), 84.9, 85.4 (2*d*, *J*(C,P) = 4) (C(2′), C(3′), C(4′)); 86.6, 86.6 (2*d*, C(1′)); 88.5, 88.9 (2*t*, OCH_2O); 102.4, 102.5 (2*d*, C(5)); 117.6, 117.7 (2*s*, CN); 140.2, 140.4 (2*d*, C(6)); 150.1, 150.2 (2*s*, C(2)); 162.8, 162.9 (2*s*, C(4)). ^{31}P-NMR (121 MHz, $CDCl_3$): 150.4, 150.8. MALDI-MS: 767 (100, $[M + Na]^+$), 722 (11), 684 (7), 613 (14), 599 (15), 261 (7).

N^6*-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-{[(RS)-1-(2-nitrophenyl)ethoxy]methyl}adenosine* ((*RS*)-**27**), N^6*-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-3′-O-{[(R)-1-(2-nitrophenyl)ethoxy]methyl}adenosine* ((*S*)-**28**), *and* N^6*-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-3′-O-{[(R)-1-(2-nitrophenyl)ethoxy]methyl}adenosine* ((*R*)-**28**). A soln. of **26** (10.1 g, 15 mmol) and $^iPr_2NEt$ (10.5 ml, 60 mmol) in $(CH_2Cl)_2$ (60 ml) was treated with Bu_2SnCl_2 (5.01 g, 16.5 mmol) at r.t. for 1 h and then treated with (*RS*)-npeom-Cl [5] (3.2 g, 18 mmol) at 80° for 15 min. Workup and CC (hexane/AcOEt 2 : 3 → AcOEt (+2% Et_3N)) gave (*RS*)-**27** (4.54 g, 36%), (*R*)-**28** (2.15 g, 17%), and (*S*)-**28** (2.27 g, 18%). Pale yellow foams.

*Data of (RS)-***27**: TLC (hexane/AcOEt 9 : 1): R_f 0.6. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.35, 1.49 (2*d*, *J* = 6.5, 3 H, *Me*CHAr); 2.52, 2.71 (2*d*, *J* = 5.0, 1 H, OH); 3.35 – 3.45 (2*dd*, *J* = 4.3, 11.0, 1 H, H−C(5′)); 3.47 – 3.55 (2*dd*, *J* = 3.7, 11.0, 1 H, H′−C(5′)); 3.78 (*s*, 6 H, MeO); 4.20 – 4.26 (*m*, 1 H, H−C(4′)); 4.36, 4.58 (2 br. *q*, *J* = 5, 1 H, H−C(3′)); 4.69, 4.72, 4.83, 4.85 (4*d*, *J* = 6.8, 2 H, OCH_2O); 5.01 (*t*, *J* = 4.7, 0.5 H, H−C(2′)); 5.03 (*t*, *J* = 5.0, 0.5 H, H−C(2′)); 5.22, 5.36 (2 br. *q*, *J* = 6.5, 1 H, MeC*H*Ar); 6.12 (*d*, *J* = 4.7, 0.5 H, H−C(1′)); 6.23 (*d*, *J* = 5.0, 0.5 H, H−C(1′)); 6.81 (*d*, *J* = 6.8, 4 arom. H); 7.19 – 7.64 (*m*, 15 arom. H); 7.87 (*dd*, = 1.5, 8.4, 1 arom. H); 8.03 (br. *d*, *J* = 7, 2 arom. H); 8.12, 8.22 (2*s*, 1 H, H−C(2)); 8.67, 8.75 (2*s*, 1 H, H−C(8)); 8.96, 9.00 (2 br. *s*, 1 H, NH). MALDI-MS: 975 (20, $[M + Na]^+$), 696 (9), 303 (100).

*Data of (S)-***28**: TLC (hexane/AcOEt 9 : 1): R_f 0.47. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.56 (*d*, *J* = 6.2, *Me*CHAr); 3.15, 3.40 (2*dd*, *J* = 3.7, 10.5, H′−C(5)); 3.77 (*s*, MeO); 3.95 (*d*, *J* = 5.6, OH); 4.10 – 4.16 (*m*, H−C(4′)); 4.45 (*dd*, *J* = 3.5, 5.0, H−C(3′)); 4.75, 4.79 (2*d*, *J* = 6.8, OCH_2O); 4.96 (br. *q*, *J* = 5.5, H−C(2′)); 5.36 (*q*, *J* = 6.2, MeC*H*Ar); 5.99 (*d*, *J* = 5.3, H−C(1′); 6.77 (2*d*, *J* = 6.8, 4 arom. H); 7.20 – 7.68 (*m*, 15 arom. H); 7.88 (*dd*, *J* = 1.2, 8.1, 1 arom. H); 8.03 (br. *d*, *J* = 7.2, 2 arom. H); 8.22 (*s*, H−C(2)); 8.77 (*s*, H−C(8)); 8.99 (br. *s*, NH).

*Data of (R)-***28**: TLC (hexane/AcOEt 9 : 1): R_f 0.36. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.46 (*d*, *J* = 6.5, *Me*CHAr); 3.33, 3.50 (2*dd*, *J* = 4.0, 10.6, 2 H−C(5′)); 3.78 (*s*, MeO); 3.82 (*m*, OH); 4.35 – 4.40 (*m*, H−C(4′)); 4.46 – 4.50 (*m*, H−C(3′)); 4.64, 4.83 (2*d*, *J* = 6.8, OCH_2O); 4.73 – 4.81 (*m*, H−C(2′)); 5.34 (*q*, *J* = 6.5, MeC*H*Ar); 5.98 (*d*, *J* = 5.3, H−C(1′)); 6.78 (*d*, *J* = 6.2, 2 arom. H); 6.79 (*d*, *J* = 6.8, 2 arom. H); 7.19 – 7.73 (*m*, 15 arom. H); 7.89 (*dd*, *J* = 1.2, 8.1, 1 arom. H); 8.02 (br. *d*, *J* = 7, 2 arom. H); 8.20 (*s*, H−C(2)); 8.75 (*s*, H−C(8)); 9.00 (br. *s*, NH).

3′-O-Acetyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-{[(S)-1-(2-nitrophenyl)ethoxy]methyl}adenosine ((*S*)-**29**) *and 3′-O-Acetyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-{[(R)-1-(2-nitrophenyl)ethoxy]methyl}adenosine* ((*R*)-**29**). A soln. of (*RS*)-**27** (4.40 g, 5.2 mmol) in 10M $MeNH_2$/EtOH (44 ml) was kept for 15 min at r.t. After evaporation, the residue was dissolved in Py (20 ml) and treated with DMAP (63 mg, 0.52 mmol) and Ac_2O (0.6 ml, 6.2 mmol). Workup (1. 10% citric acid; 2. $NaHCO_3$ soln.) after 30 min at r.t., and CC (AcOEt/hexane 3:2 → AcOEt (+2% Et_3N)) gave (*S*)-**29** (1.34 g, 33%) and (*R*)-**29** (1.51 g, 37%). Off-white foams.

Data of (S)-**29**: TLC (hexane/AcOEt 1:9): R_f 0.46. UV (MeOH): 255 (20100), 235 (26600), 225 (24400). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.08 (*d*, *J* = 6.2, *Me*CHAr); 2.1 (*s*, *Me*CO); 3.40–3.59 (2*dd*, *J* = 4.0, 10.5, 2 H–C(5′)); 3.81 (*s*, 2 MeO); 4.30–4.40 (*m*, H–C(4′)); 4.35, 4.69 (2*d*, *J* = 7.5, OCH_2O); 4.91 (*q*, *J* = 6.2 C*H*Ar); 5.28 (*dd*, *J* = 5.0, 7.0, H–C(2′)); 5.41 (*dd*, *J* = 2.0, 5.0, H–C(3′)); 5.62 (br. *s*, NH_2); 6.17 (*d*, *J* = 7.0, H–C(1′)); 6.85 (*d*, *J* = 6.8, 4 arom. H); 7.24–7.57 (*m*, 12 arom. H); 7.87 (br. *d*, *J* = 7.9, 1 arom. H); 8.06 (*s*, H–C(2)); 8.37 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.8 (*q*, *Me*CO); 23.0 (*q*, *Me*CHAr); 55.2 (*q*, MeO); 63.4 (*t*, C(5′)); 69.9, 71.7, 76.9 (3*d*, C(2′), C(3′), Me*C*HAr); 82.4 (*d*, C(4′)); 86.0 (*d*, C(1′)); 86.9 (*s*, Ar_2*C*(Ph)); 92.7 (*t*, OCH_2O); 113.3 (*d*, arom. C); 120.0 (*s*, C(5)); 124.3, 127.0, 127.7, 128.0, 128.2, 129.5, 130.1, 133.5 (8*d*, arom. C); 135.4, 135.5, 138.7 (3*s*, arom. C); 139.2 (*d*, C(8)); 144.4 (*s*, arom. C); 148.2 (*s*, C(4)); 150.2 (*s*, arom. C); 153.5 (*d*, C(2)); 155.6 (*s*, C(6)); 158.6 (*s*, MeO–*C*); 170.2 (*s*, Me*C*O). MALDI-MS: 819 (15, $[M + K]^+$), 813 (5, $[M + Na]^+$), 791 (3, $[M + H]^+$), 624 (11), 570 (22), 303 (100), 164 (38).

Data of (R)-**29**: TLC (hexane/AcOEt 1:9): R_f 0.34. UV (MeOH): 265 (18100), 235 (26000), 225 (23200). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.40 (*d*, *J* = 6.2, *Me*CHAr); 2.21 (*s*, *Me*CO); 3.43 (*dd*, *J* = 3.7, 10.6, H–C(5′)); 3.51 (*dd*, *J* = 3.7, 10.6, H′–C(5′)); 3.78, 3.79 (2*s*, MeO); 4.29–4.33 (*m*, H–C(4′)); 4.54, 4.71 (2*d*, *J* = 7.1, OCH_2O); 5.18 (*t*, *J* = 5.8, H–C(2′)); 5.29 (*q*, *J* = 6.2, C*H*Ar); 5.52 (*dd*, *J* = 3.1, 5.3, H–C(3′)); 5.76 (br. *s*, NH_2); 6.06 (*d*, *J* = 6.2, H–C(1′)); 6.81 (*d*, *J* = 6.8, 4 arom. H); 7.19–7.58 (*m*, 12 arom. H); 7.83 (*s*, H–C(2)); 7.86 (*dd*, *J* = 1.2, 8.1, 1 arom. H); 8.18 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.9 (*q*, *Me*CO); 23.0 (*q*, *Me*CHAr); 55.2 (*q*, MeO); 63.0 (*t*, C(5′)); 69.4, 72.1, 77.4 (3*d*, C(2′), C(3′), Me*C*HAr); 82.2 (*d*, C(4′)); 86.4 (*d*, C(1′)); 86.8 (*s*, Ar_2*C*(Ph)); 92.7 (*t*, OCH_2O); 113.2 (*d*, arom. C); 119.9 (*s*, C(5)); 124.3, 127.0, 127.5, 127.9, 128.1, 128.2, 130.1, 133.6 (8*d*, arom. C); 135.4, 135.5, 138.8 (*s*, arom. C); 138.9 (*d*, C(8)); 144.4 (*s*, arom. C); 147.6 (*s*, C(4)); 149.8 (*s*, arom. C); 153.1 (*d*, C(2)); 155.5 (*s*, C(6)); 158.6 (*s*, MeO–*C*); 170.4 (*s*, Me*C*O). MALDI-MS: 819 (4, $[M + K]^+$), 813 (10, $[M + Na]^+$), 791 (2, $[M + H]^+$), 624 (8), 509 (26), 303 (100), 164 (28).

5′-O-(4,4′-Dimethoxytrityl)-2′-O-{[(S)-1-(2-nitrophenyl)ethoxy]methyl}-N^6-{{[2-[(triisoproysilyl)oxy]benzyl}oxy}carbonyl}adenosine (**30**). A soln. of (*S*)-**29** (389 mg, 0.49 mmol) in CH_2Cl_2 (1 ml) was added during 20 min to a suspension obtained from 1.9M $COCl_2$ in toluene (0.39 ml, 0.74 mmol), DMAP (12 mg, 0.1 mmol) in Py (1.4 ml) and CH_2Cl_2 (1 ml). After 15 min at r.t., **18** (412 mg, 1.47 mmol) and Et_3N (198 mg, 1.96 mmol) were added. Workup (1. 10% citric acid; 2. $NaHCO_3$ soln.) after 18 h at r.t. gave a residue, which was dissolved in 0.2M NaOH in THF/MeOH/H_2O 5:4:1 (10 ml). Workup after 5 min at 4°, and CC (AcOEt/hexane 3:7 → 3:2 (+2% Et_3N)) gave **30** (300 mg, 58%). Colorless foam. TLC (hexane/AcOEt 1:1): R_f 0.17. UV (MeOH): 273 (sh, 18100), 270 (sh, 19300), 277 (20500), 252 (16800), 234 (sh, 22800). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.12 (*d*, *J* = 7.1, 18 H, Me_2CH); 1.33 (*d*, *J* = 6.2, *Me*CHAr); 1.22–1.38 (*m*, 3 H, Me_2CH); 2.52 (br. *d*, *J* = 5.0, OH); 3.37 (*dd*, *J* = 4.1, 10.6, H–C(5′)); 3.51 (*dd*, *J* = 6.8, 10.6, H′–C(5′); 3.78 (*s*, MeO); 4.21 (*m*, H–C(4′)); 4.34 (*m*, H–C(3′)); 4.67, 4.81 (2*d*, *J* = 6.5, OCH_2O); 4.98 (*t*, *J* = 5.0, H–C(2′)); 5.21 (*q*, *J* = 6.2, Me*CH*Ar); 5.38 (*s*, ArCH_2); 6.18 (*d*, *J* = 5.0, H–C(1′)); 6.80 (*d*, *J* = 9.0, 4 arom. H); 6.78–6.98 (*m*, 2 arom. H); 7.17–7.44 (*m*, 12 arom. H); 7.51–7.62 (*m*, 2 arom. H); 7.86 (*dd*, *J* = 1.3, 8.1, 1 arom. H); 8.12 (br. *s*, NH); 8.13 (*s*, C(2)); 8.69 (*s*, C(8)); ^{13}C-NMR (75 MHz, $CDCl_3$): 13.0 (*d*, Me_2CH); 18.0 (*q*, Me_2CH); 23.2 (*q*, *Me*CHAr); 55.2 (*q*, MeO); 63.1, 63.8 (2*t*, ArCH_2, C(5′)); 70.4, 70.9, 79.9, 84.1 (4*d*, C(2′), C(3′), C(4′), Me*C*HAr); 86.7 (*s*, Ar_2*C*(Ph)); 87.2 (*d*, C(1′)); 94.0 (*t*, OCH_2O); 113.2, 118.2, 120.8 (3*d*, arom. C); 122.5 (*s*, C(5)); 124.3 (*d*, arom. C); 125.6 (*s*, arom. C); 127.0, 127.8, 127.9, 128.2, 128.4, 129.6, 130.1, 130.2, 133.6 (9*d*, arom. C); 135.5, 135.6, 138.5 (3*s*, arom. C); 141.6 (*d*, C(8)); 144.5, 148.0 (2*s*, arom. C); 149.5 (*s*, C(4)); 150.8, 150.9 (2*s*, C(6), CO); 153.0 (*d*, C(2)); 154.3 (*s*, arom. C); 158.6 (*s*, MeO–C). MALDI-MS: 1077 (5, $[M + Na]^+$), 855 (5), 729 (4), 535 (4), 303 (100), 242 (11).

5′-O-(4,4′-Dimethoxytrityl)-2′-O-{[(S)-1-(2-nitrophenyl)ethoxy]methyl}-N^6-{{[2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}adenosine 3′-{Triethylammonium 2,2′-(1,4-Phenylenebis(oxy))bis[acetate] (**31** · Et_3N). A soln. of **30** (160 mg, 0.152 mmol), [1,4-phenylenebis(oxy)bis[acetic acid] (68 mg, 0.3 mmol), DMAP (4 mg, 0.03 mmol), and ^{i}Pr_2NEt (98 mg, 0.76 mmol) in Py (1.5 ml) was treated with BOP (133 mg, 0.3 mmol) and kept 30 min at r.t. Workup (1. 10% citric acid, 2. $NaHCO_3$ soln.) and CC (CH_2Cl_2 → CH_2Cl_2/MeOH 19:1 (+2% Et_3N)) gave **31** (103 mg, 49%). Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.49. UV (MeOH): 275 (sh, 24200), 267 (28900), 252 (22900), 236 (sh, 33300). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.94 (*d*, *J* = 6.3, *Me*CHAr); 1.11 (*d*, *J* = 7.2, 3 Me_2CH); 1.25 (*t*, *J* = 7.4, 9 H, Et_3N); 1.23–1.38 (*m*, 3 Me_2C*H*); 3.05 (*q*, *J* = 7.4, 6 H, Et_3N); 3.43 (*dd*, *J* = 4.1, 10.5, H–C(5′)); 3.57 (*dd*, *J* = 4.0, 10.5, H′–C(5′)); 3.77 (*s*, MeO); 4.25 (*d*, *J* = 7.5, 1 H, OCH_2O); 4.35 (*m*,

H–C(4′)); 4.40 (*s*, OCH_2COO); 4.56–4.62 (*m*, 3 H, OCH_2O, OCH_2COO); 4.8 (*q*, *J* = 6.3, MeC*H*Ar); 5.38 (*s*, $ArCH_2$); 5.93 (*dd*, *J* = 5.3, 7.3, H–C(2′)); 5.50 (*dd*, *J* = 1.9, 5.3, H–C(3′)); 6.08 (*d*, *J* = 7.3, H–C(1′)); 6.76–6.96 (*m*, 10 arom. H); 7.16–7.55 (*m*, 12 arom H); 7.82 (*dd*, *J* = 1.2, 8.2, 1 arom H); 8.14 (*s*, H–C(2)); 8.18 (br. *s*, NH); 8.70 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 8.5 (*q*, Et_3N); 13.0 (*d*, Me_2CH); 18.0 (*q*, *Me*$_2$CH); 22.9 (*q*, *Me*CHAr); 45.0 (*t*, Et_3N); 55.2 (*q*, MeO); 63.2, 63.8, 65.8, 67.8 (4*t*, C(5′), Ar*C*H$_2$, 2 ArO*C*H$_2$); 69.8, 72.5, 76.5, 77.2, 82.3 (4*d*, C(2′), C(3′), C(4′), Me*C*HAr); 86.3 (*d*, C(1′)); 87.0 (*s*, Ar$_2$*C*(Ph)); 92.8 (*t*, OCH_2O); 113.3, 115.5, 115.6, 118.1, 120.8 (5*d*, arom. C); 122.4 (*s*, C(5)); 124.3 (*d*, arom. C); 125.6 (*s*, arom. C); 127.1, 127.6, 128.0, 128.1, 128.4, 129.5, 130.1, 130.2 (8*d*, arom. C); 133.6, 135.3 (2*s*, arom. C); 135.4 (*d*, arom. C); 138.1 (*s*, arom. C); 141.7 (*d*, C(8)); 144.3, 148.3 (2*s*, arom. C); 149.6 (*s*, C(4)); 150.7, 151.2 (2*s*, NCOO, arom. C); 151.7 (*s*, C(6)); 153.2 (*s*, arom. C); 153.9 (*d*, C(2)); 154.3 (*s*, arom. C); 158.7 (*s*, MeO–*C*); 168.5, 174.6 (2*s*, CH$_2$*C*OO). FAB-MS: 1263 (44, $[M+H]^+$), 1219 (16), 338 (11), 303 (100).

Solid Support **32**. A suspension of LCAA-CPG (1 g), **31** (68 mg, 0.05 mmol), ^{i}Pr$_2$NEt (0.1 ml, 0.58 mmol), and BOP (22.1 mg, 0.05 mmol), in MeCN (4 ml) was shaken 20 h at r.t. After filtration, the solid was washed with MeCN and CH_2Cl_2, suspended in Py (3 ml) and Ac_2O (1 ml), and shaken for 2 h at r.t. After filtration, the solid was washed with DMF and CH_2Cl_2. Loading: 43 µmol/g.

N-*{[(2-Nitrobenzyl)oxy]carbonyl}*-L-*phenylalanine* (**39**). A soln. of L-phenylalanine (250 mg, 1.5 mmol) in 2M aq. NaOH (0.75 ml) was treated consecutively (during 15 min) with small portions of nboc-Cl [1] (90%; 440 mg, 1.8 mmol) and 2M aq. NaOH (0.75 ml). After 2.5 h at r.t., H_2O (15 ml) and Et_2O (15 ml) were added; the aq. phase was acidified with HCl, extracted with CH_2Cl_2, and evaporated. Crystallization (benzene) gave **39** (253 mg, 49%). Colorless crystals. TLC (CH_2Cl_2/MeOH 9 : 1): R_f 0.2. UV (MeOH): 300 (1500), 258 (5500), 231 (2200). ^{1}H-NMR (300 MHz, $CDCl_3$): 3.14 (*dd*, *J* = 6.0, 14.0, 1 H, H–C(β)); 3.25 (*dd*, *J* = 5.4, 14.0, 1 H, H–C(β)); 4.72 (br. *q*, *J* = 6, H–C(α)); 5.29 (br. *d*, *J* = 8, NH); 5.52 (*s*, ArC*H*$_2$O); 7.18–7.12 (*m*, 2 arom. H); 7.27–7.36 (*m*, 3 arom. H); 7.44–7.53 (*m*, 2 arom. H); 7.58–7.66 (*m*, 1 arom. H); 8.10 (*d*, *J* = 8, 1 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 37.7 (*t*, C(β)); 54.6 (*d*, C(α)); 63.7 (*t*, Ar*C*H$_2$O); 125.0, 127.3, 128.5, 128.6, 128.7, 129.4 (6*d*, arom. C); 132.9 (*s*, arom. C); 133.8 (*d*, arom. C); 135.4, 147.2 (2*s*, arom. C); 155.3 (*s*, O*C*ON); 176.2 (*s*, *C*OO). ESI-MS (neg.): 687.4 (53, $[2M-H]^-$), 343.2 (100, $[M-H]^-$). ESI-MS (pos.): 727.4 (20, $[2M+K]^+$), 711.4 (70, $[2M+Na]^+$), 383.1 (18, $[M+Na]^+$), 367.1 (100, $[M+Na]^+$).

*1-{[(*RS*)-1-(2-Nitrophenyl)ethoxy]carbonyl}-1*H*-imidazole* (**40**). A soln. of *rac*-1-(2-nitrophenyl)ethanol (2.0 g, 12 mmol) and 1,1′-carbonylbis[1*H*-imidazole] (2.14 g, 13.2 mmol) in CH_2Cl_2 (12 ml) was kept for 30 min at r.t. Workup gave crude **40** (quant.) as an off-white solid, which was used without further purification. TLC (hexane/AcOEt 3:7): R_f 0.30. UV (MeCN): 334 (sh, 500), 261 (sh, 4700), 237 (sh, 6300). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.84 (*d*, *J* = 6.5, *Me*CHAr); 6.58 (*q*, *J* = 6.5, MeC*H*Ar); 7.07 (*dd*, *J* = 0.9, 1.6, 1 arom. H); 7.42 (*t*, *J* = 1.5, 1 arom. H); 7.48–7.52 (*m*, 1 arom. H); 7.65–7.69 (*m*, 2 arom. H); 7.99–8.03 (*m*, 1 arom. H); 8.15 (*t*, *J* = 0.9, 1 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 21.9 (*q*, *Me*CHAr); 72.7 (*d*, Me*C*HAr); 117.1, 124.8, 126.9, 129.3, 130.9, 134.0 (6*d*, arom. C); 136.1 (*s*, arom. C); 137.0 (*d*, arom. C); 147.7 (*s*, arom. C); 182.2 (*s*, CO). MALDI-MS: 284 (20, $[M+Na]^+$), 221 (53), 218 (100), 150 (83).

N-*{[(*RS*)-1-(2-Nitrophenyl)ethoxy]carbonyl}*-L-*phenylalanine* (**41**). A soln. of L-phenylalanine (1.0 g, 6.05 mmol), **40** (1.74 g, 6.66 mmol), and 1*H*-tetrazole (212 mg, 3.03 mmol) in DMF (6 ml) was kept for 14 h at 80°. DMF was evaporated and the residue extracted (Et_2O, 0.5M phosphate buffer, pH 12.5). The aq. phase was acidified with HCl (→ pH 2) and extracted with CH_2Cl_2: **41** (1.15 g, 53%; 1 : 1 diastereoisomer mixture). Yellow solid, which was used without further purification. TLC (CH_2Cl_2/MeOH 9 : 1): R_f 0.37. UV (MeOH): 335 (sh, 400), 306 (sh, 900), 257 (4200), 235 (2900). ^{1}H-NMR (300 MHz, (D_6) DMSO): 1.46, 1.59 (2*d*, *J* = 6.5, 3 H, *Me*CHAr); 2.79 (*dd*, *J* = 7.5, 9.3, 0.5 H, H–C(β)); 2.91–3.12 (*m*, 1 H, H′–C(β)); 3.93–4.07 (*m*, 1 H, H–C(α)); 4.3 (*m*, 1 H, NH); 5.90, 5.91 (2*q*, *J* = 6.5, 1 H, MeC*H*Ar); 7.10–7.29 (*m*, 5 arom. H); 7.42–7.96 (*m*, 4 arom. H). ^{13}C-NMR (75 MHz, (D_6)DMSO): 21.7 (*q*, *Me*CHAr); 36.3 (*t*, C(β)); 55.5, 55.6 (2*d*, C(α)); 67.0 (*d*, Me*C*HAr); 124.0, 126.2, 127.9, 128.0, 129.0, 129.2, 133.9 (7*d*, arom. C); 137.8, 137.9, 147.0, 147.1 (4*s*, arom. C); 154.7, 154.9 (2*s*, OCON); 173 (COO). MALDI-MS: 381 (29, $[M+Na]^+$), 335 (56), 313 (100), 295 (29), 150 (32), 12 (38).

*3′-*O*-Acetyl-2′-*O*-{[(*S*)-1-(2-nitrophenyl)ethoxy]methyl}adenosine* (**47**). At 4°, a soln. of (*S*)-**29** (600 mg, 0.76 mmol) in CH_2Cl_2 (14 ml) was treated with $CHCl_2COOH$ (0.56 ml). After 10 min, MeOH (2 ml) was added, and the mixture was kept for 20 min. Workup and CC (CH_2Cl_2 → CH_2Cl_2/MeOH 19 : 1) gave **47** (306 mg, 82%). Yellow foam. TLC (CH_2Cl_2/MeOH 9 : 1): R_f 0.48. UV (MeOH): 257 (19200), 229 (6700). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.77 (*d*, *J* = 6.2, *Me*CHAr); 2.11 (*s*, *Me*CO); 3.77 (br. *d*, *J* = 13, H–C(5′)); 3.98 (br. *d*, *J* = 13, H′–C(5′)); 4.15 (*d*, *J* = 7.4, 1 H, OCH_2O); 4.36 (br. *s*, H–C(4′)); 4.56 (*d*, *J* = 7.4, 1 H, OCH_2O); 4.78 (*q*, *J* = 6.2, *MeCH*Ar); 5.22 (*dd*, *J* = 5.0, 7.8, H–C(2′)); 5.52 (*d*, *J* = 5, H–C(3′)); 5.87 (*d*, *J* = 8.1, H–C(1′)); 6.2 (br. *s*, NH_2); *ca.* 6.6 (br. *s*, OH); 7.34–7.40 (*m*, 1 arom. H); 7.46 (br. *d*, *J* = 8.1, 1 arom. H); 7.56 (br. *t*, *J* = 8.1, 1 arom. H); 7.85 (br. *d*, *J* = 8.1, 1 arom. H); 7.97 (*s*, H–C(2)); 8.38 (*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.8 (*q*, MeCO); 22.7 (*q*,

MeCHAr); 63.1 (*t*, C(5′)); 68.8, 72.5, 75.8 (3*d*, C(2′), C(3′), Me*C*HAr); 86.1, 89.3 (2*d*, C(4′), C(1′)); 91.9 (*t*, OCH_2O); 121.2 (*s*, C(5)); 124.2, 127.7, 128.3, 133.8 (4*d*, arom. C); 138.5 (*s*, arom. C); 140.9 (*d*, C(8)); 148.3, 148.4 (2*s*, C(4), arom. C); 152.6 (*d*, C(2)); 156.3 (*s*, C(6)); 170.1 (*s*, Me*C*O). MALDI-MS: 511 (74, $[M+Na]^+$), 498 (100, $[M+H]^+$), 332 (59), 310 (52), 136 (83).

*3′-*O*-Acetyl-2′-*O*-{[(*S*)-1-(2-nitrophenyl)ethoxy]methyl}adenosine 5′-(2-Cyanoethyl Methyl Phosphate]* (**48**). A soln. of **47** (200 mg, 0.41 mmol) and 5-(benzylthio)-1*H*-tetrazole (175 mg, 0.9 mmol) in MeCN (2 ml) was treated with 0.25M $(MeO)P(N^iPr_2)(OCH_2CH_2CN)$ in MeCN (2 ml, 0.5 mmol) and kept for 20 min at r.t. After evaporation, the residue was treated with a soln. of I_2 (0.5 g) in H_2O (0.4 ml), Py (4 ml), and THF (15 ml). Workup (1. $Na_2S_2O_3$ soln; 2. $NaHCO_3$ soln.) and CC ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 2 : 3) gave **48** (164 mg, 63%, 1 : 1 diastereoisomer mixture). Colorless foam. TLC ($MeCl_2$/MeOH 9 : 1): R_f 0.52. UV (MeOH): 258 (18000), 229 (6300). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.06 (*d*, *J* = 6.2, *Me*CHAr); 2.1 (*s*, MeCO); 2.75 (*m*, OCH_2CH_2CN); 3.80, 3.84 (2*d*, *J* = 3.1, POMe); 4.24, 4.27 (2*q*, *J* = 6.1, OCH_2CH_2CN); 4.33 (*d*, *J* = 7.4, 1 H, OCH_2O); 4.36 – 4.48 (*m*, H−C(4′), 2 H−C(5′)); 4.62 (*d*, *J* = 7.4, 1 H, OCH_2O); 4.93 (*q*, *J* = 6.2, Me*CH*Ar); 5.14 (*m*, H−C(2′)); 5.37 – 5.42 (*m*, H−C(3′)); 6.11, 6.12 (2*d*, *J* = 6.5, H−C(1′)); 6.2 (br. *s*, NH_2); 7.37 (*ddd*, *J* = 1.5, 6.8, 8.1, 1 arom. H); 7.49 – 7.60 (*m*, 2 arom. H); 7.84 (*dd*, *J* = 1.2, 8.1, 1 arom. H); 8.127, 8.131 (2*s*, H−C(2)); 8.413, 8.416 (2*s*, H−C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 19.6 (*t*, *J*(C,P) = 7.6, OCH_2CH_2CN); 20.7 (*q*, *Me*CO); 22.9 (*q*, *Me*CHAr); 55.0 (*q*, *J*(CP) = 4.1, POMe); 62.2 (*t*, *J*(C,P) = 4.8, OCH_2CH_2CN); 66.70, 66.76 (2*t*, *J*(C,P) = 6, C(5′)); 69.99, 70.02, 70.71, 70.75, 76.42, 76.46 (6*d*, Me*C*HAr, C(2′), C(3′)); 81.0 (*d*, *J*(C,P) = 7.3, C(4′)); 86.97, 87.02 (2*d*, C(1′)); 92.85 (*t*, OCH_2O); 116.39 (*s*, CN); 120.14 (*s*, C(5)); 124.28, 127.71, 128.31, 133.63 (4*d*, arom. C); 138.47 (*s*, arom. C); 139.54, 139.57 (2*d*, C(8)); 148.18 (*s*, C(4)); 149.82 (*s*, arom. C)); 153.06, 153.10 (2*d*, C(2)); 155.55 (*s*, C(6)); 170.06 (Me*C*O). ^{31}P-NMR (121 MHz, $CDCl_3$): 0.026, 0.066. MALDI-MS: 636 (11, $[M+H]^+$), 471 (20), 150 (100).

*2′-*O*-{[(1*S*)-1-(2-Nitrophenyl)ethoxy]methyl}adenosine 5′-(Triethylammonium Methyl Phosphate)* (**2** · Et_3NH^+). A soln. of **48** (120 mg, 0.19 mmol) in 10M $MeNH_2$/EtOH (1.2 ml) was kept for 20 min at r.t., and then evaporated. The residue was dissolved in H_2O/EtOH 4 : 1 and extracted with CH_2Cl_2. The aq. phase was treated with Et_3N, evaporated, and co-evaporated with Py: **2** (quant.). TLC (AcOEt/EtOH/H_2O 5 : 4 : 1): R_f 0.31. ^{1}H-NMR (300 MHz, D_2O): 0.97 (*d*, *J* = 6.2, *Me*CHAr); 1.24 (*t*, *J* = 7.2, 9 H, Et_3N); 3.16 (*q*, *J* = 7.2, 6 H, Et_3N); 3.54 (*d*, *J* = 10.9, POMe); 4.07 (br. *s*, 2 H−C(5′)); 4.36 (br. *s*, H−C(4′)); 4.49 (*m*, H−C(2′)); 4.55 (br. *d*, *J* = 7.1, 1 H, OCH_2O); 4.67 – 4.75 (*m*, 3 H, H−C(3′), Me*CH*Ar, OCH_2O); 6.1 (*d*, *J* = 7.1, H−C(1′)); 7.33 – 7.45 (*m*, 2 arom. H); 7.57 (br. *t*, *J* = 8, 1 arom. H); 7.74 (br. *d*, *J* = 8, 1 arom. H); 8.21 (*s*, H−C(2)); 8.46 (*s*, H−C(8)). ^{13}C-NMR (100 MHz, D_2O): 10.9 (*q*, Et_3N); 24.2 (*q*, *Me*CHAr); 49.3 (*t*, Et_3N); 55.5 (*q*, *J*(C,P) = 5.8, POMe); 67.8 (*t*, C(5′)); 72.2, 73.0, 82.5 (3*d*, C(2′), C(3′), Me*C*HAr); 87.7 (*d*, *J*(C,P) = 8.7, C(4′)); 87.9 (*d*, C(1′)); 95.3 (*t*, OCH_2O); 121.2 (*s*, C(5)); 126.8, 130.0, 131.3, 136.6 (4 *d*, arom. C); 139.4 (*s*, arom. C); 142.3 (*d*, C(8)); 150.3, 151.6 (2*s*, C(4), 1 arom. C); 155.9 (*d*, C(2)); 158.3 (*s*, C(6)). ^{31}P-NMR (121 MHz, D_2O): 2.269. ESI-MS (neg.): 593.3 (100, $[M-H]^-$).

N^6,N^6,*3′-*O*-Tribenzoyl-2′-*O*-methyladenosine* (**49**). At 4°, a soln. of adenosine (4 g, 15 mmol) in DMF (60 ml) was treated with NaH (758 mg, 18 mmol). After 45 min at 4°, MeI (3.4 g, 24 mmol) was added during 3 h [21]. At r.t., 1*H*-imidazole (3.0 g, 45 mmol) and tbdms-Cl (2.26 g, 15 mmol) were added. Workup (AcOEt/hexane 1 : 1, $NaHCO_3$ soln.) after 20 min and filtration over silica gel ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 93 : 7) gave a mixture of products (2.5 g; TLC (CH_2Cl_2/MeOH 95 : 5): R_f *ca.* 0.5). Part of this mixture (1.8 g) was dissolved in CH_2Cl_2/Py 3 : 1 (20 ml), treated with BzCl (2.64 g, 18.4 mmol), and stirred for 14 h at r.t. Workup gave a residue (TLC (hexane/AcOEt 1 : 1): R_f *ca.* 0.7), which was dissolved in THF (90 ml), treated with 10M aq. HCl (10 ml) and stirred for 5 min at r.t. Workup and CC (hexane/AcOEt 4 : 1 → 2 : 3) gave **49** (1.9 g, 30%). Colorless solid. TLC (hexane/AcOEt 1 : 1): R_f 0.32. UV (MeOH): 270 (18500), 248 (23800), 230 (28400). ^{1}H-NMR (300 MHz, $CDCl_3$): 3.35 (*s*, MeO); 3.99 (*d*, *J* = 13, H−C(5′)); 3.91 (*dd*, *J* = 10, 13, H−C(5′)); 4.51 (*s*, H−C(4′)); 4.85 (*dd*, *J* = 5, 8.1, H−C(2′)); 5.88 (*d*, *J* = 5, H−C(3′)); 5.93 (br. *d*, *J* = 10, OH); 6.05 (*d*, *J* = 8.1, H−C(1′)); 7.34 – 7.39 (*m*, 5 arom. H); 7.47 – 7.50 (*m*, 3 arom. H); 7.63 (*m*, 1 arom. H); 7.86 – 7.88 (*m*, 4 arom. H); 8.12 – 8.15 (*m*, 2 arom. H); 8.20 (*s*, H−C(2)); 8.68 (*s*, H−C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 59.1 (*q*, MeO); 62.9 (*t*, C(5′)); 72.5, 81.3, 86.7 (3*d*, C(2′), C(3′), C(4′)); 89.8 (*d*, C(1′)); 128.3, 128.6, 128.8 (3*d*, arom. H); 129.0, 129.3 (2*s*, C(5), arom. C); 129.5, 129.9, 133.2, 133.7 (4*d*, arom. C); 133.9 (*s*, arom. C); 145.1 (*d*, C(8)); 151.6 (*d*, C(2)); 151.8 (*s*, C(4)); 152.9 (*s*, C(6)); 165.7 (*s*, O*C*OPh); 172.2 (N*C*OPh). MALDI-MS: 616 (100, $[M+Na]^+$), 594 (5, $[M+H]^+$), 512 (14), 472 (38), 366 (86), 344 (40), 251 (71), 222 (53).

N^6,N^6,*3′-*O*-Tribenzoyl-2′-*O*-methyladenosine 5′-(2-Cyanoethyl Methyl Phosphate)* (**50**). A soln. of **49** (300 mg, 0.5 mmol) and 5-(benzylthio)-1*H*-tetrazole (211 mg, 1.1 mmol) in MeCN (1.5 ml) was treated with a soln. of 0.25M $(MeO)P(N^iPr_2)(OCH_2CH_2CN)$ in MeCN, (2.4 ml, 0.6 mmol) and kept for 20 min at r.t. After evaporation, the residue was treated with a soln. of I_2 (0.5 g) in H_2O (0.4 ml), Py (4 ml), and THF (15 ml). Workup (1. $Na_2S_2O_3$ soln.; 2. $NaHCO_3$ soln.) and CC ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 2 : 3) gave **48** (264 mg, 72%, 1 : 1

diastereoisomer mixture). Colorless foam. TLC (CH_2Cl_2/MeOH 93:7): R_f 0.4. UV (MeOH): 270 (20000), 248 (25900), 231 (30700). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.70 (*m*, OCH_2CH_2CN); 3.44 (*s*, MeO); 3.77, 3.81 (2*d*, *J* = 3.1, POMe); 4.21 (*m*, OCH_2CH_2CN); 4.46 (*m*, 2 H–C(5')); 4.58 (*m*, H–C(4')); 4.81, 4.82 (2*t*, *J* = 5.6, H–C(2')); 5.75 (*m*, H–C(3')); 6.26, 6.27 (2*d*, *J* = 5.9, H–C(1')); 7.37 (*m*, 4 arom. H); 7.50 (*m*, 4 arom. H); 7.64 (*m*, 1 arom. H); 7.86 (*m*, 4 arom. H); 8.12 (*m*, 2 arom. H); 8.36, 8.37 (2*s*, H–C(2)); 8.699, 8.702 (2*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 19.7 (*t*, *J*(C,P) = 7.3, OCH_2CH_2CN); 55.1 (*q*, *J*(C,P) = 6.1, POMe); 59.4 (*q*, MeO); 62.37, 62.46 (2*t*, *J*(C,P) = 4.8, OCH_2CH_2CN); 66.65, 66.75 (2*t*, *J*(C,P) = 5.9, C(5')); 70.94, 70.98 (2*d*, C(2')); 81.41, 81.46 (2*d*, C(3')); 81.51, 81.61 (2*d*, *J*(C,P) = 7.3, C(4')); 87.33, 87.46 (2*d*, C(1')); 116.58, 116.61 (2*s*, CN); 128.2 (*s*, C(5)); 128.89, 129.02 (2*d*, arom. C); 129.2 (*s*, arom. C); 129.7, 130.2, 133.3, 134.1 (4*d*, arom. C); 134.3 (*s*, arom. C); 143.71, 143.76 (2*d*, C(8)); 152.4 (*s*, C(4)); 152.7 (br. *d*, C(2)); 153.2 (*s*, C(6)); 165.99 (*s*, OCOPh); 172.62 (*s*, 2C, NCOPh). ^{31}P-NMR (121 MHz, $CDCl_3$): 0.133, 0.161. MALDI-MS: 763 (100, $[M + Na]^+$), 659 (22), 588 (10), 275 (12).

2'-O-Methyladenosine 5'-[Triethylammonium Methyl Phosphate] (**51** · Et_3NH^+). A soln. of **50** (200 mg, 0.27 mmol) in 10M $MeNH_2$/EtOH (2 ml) was kept for 20 min at r.t. and then evaporated. The residue was dissolved in H_2O/EtOH 4:1 and the soln. extracted with CH_2Cl_2. The aq. phase was treated with Et_3N, evaporated, and co-evaporated with Py: **51** (quant.). TLC (BuOH/AcOH/H_2O 5:2:3): R_f 0.48. ^{1}H-NMR (300 MHz, D_2O): 1.23 (*t*, Et_3N); 3.16 (*q*, Et_3N); 3.44 (*s*, MeO); 3.48 (*d*, *J* = 10.9, POMe); 4.06 (*dd*, *J* = 3.1, 4.7, 2 H–C(5')); 4.34 (*q*, *J* = 3.1, H–C(4')); 4.47 (*t*, *J* = 5.3, H–C(2')); 4.63 (*dd*, *J* = 3.7, 5, H–C(3')); 6.14 (*d*, *J* = 5.9, H–C(1')); 8.18 (*s*, H–C(2)); 8.41 (*s*, H–C(8)). ^{31}P-NMR (121 MHz, D_2O): 2.18.

5'-O-[(tert-Butyl)dimethylsilyl]-2'-O-{[(triisopropylsilyl)oxy]methyl}uridylyl-(3'→5')-N⁶-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(triisopropylsilyl)oxy]methyl}adenylyl-(3'→5')-2'-O-{[(triisopropylsilyl)oxy]methyl}guanylyl-(3'→5')-N⁴-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(triisopropysilyl)oxy]methyl}cytidylyl-(3'→5')-N⁴-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(triisopropylsilyl)oxy]methyl}cytidyl-(3'→5')-N⁴-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}-2'-O-{[(S)-1-(2-nitrophenyl)ethoxy]methyl}-N⁶-{{{2-[(triisopropylsilyl)oxy]benzyl}oxy}carbonyl}adenosine (**34**). An automated 1.5-μmol synthesis ('trityl-on' mode) was carried out on a *Gene Assembler* (*Pharmacia*) by the following protocol: 1) 1.5 min detritylation with 4% $CHCl_2COOH$ in $(CH_2Cl)_2$; 2) 2.5 min coupling with the appropriate phosphoramidite (0.12 ml of a 0.1M soln. in MeCN; **9**: 0.1M in THF) and 5-(benzylthio)-1*H*-tetrazole (0.36 ml of a 0.35M soln. in MeCN); 3) 1 min capping with a 1:1 mixture of Ac_2O/2,6-dimethylpyridine/THF 1:1:8 and 16% 1-methyl-1*H*-imidazole in THF; 4) 0.5 min oxidation with I_2/H_2O/Py/THF 2:2:20:75. According to the trityl assay, the average coupling yields were > 98%. The immobilized sequence (10 mg) was shaken with MeOH/Et_3N 9:1 for 8 h at r.t. The solid materials were removed by filtration and washed with MeOH. The filtrate was freeze-dried, dissolved in Py/DMF 1:1 (0.1 ml), and freeze-dried again: crude **34** as an off-white solid.

Uridylyl-(3'→5')-adenylyl-(3'→5')-guanylyl-(3'→5')-cytidylyl-(3'→5')-cytidylyl-(3'→5')-2'-O-{[(S)-1-(2-nitrophenyl)ethoxy]methyl}adenosine 3'-{N-{[(2-Nitrobenzyl)oxy]carbonyl}-L-phenylalaninate} (**44**) *and Uridylyl-(3'→5')-adenylyl-(3'→5')-guanylyl-(3'→5')-cytidylyl-(3'→5')-cytidylyl-(3'→5')-2'-O-{[(S)-1-(2-nitrophenyl)ethoxy]methyl}adenosine 3'-{N-{[(RS)-1-(2-Nitrophenyl)ethoxy]carbonyl}-L-phenylalaninate}* (**45**). Crude **34** (obtained from 10 mg of solid support) was treated with 50 μl of a soln. prepared from $^iPr_3NEt$ (0.24M), 4-(pyrrolidin-1-yl)pyridine (0.1M), BOP (0.2M), and **39** or **41** (0.2M) in Py. After 4 h at r.t., H_2O (50 μl) was added and the mixture freeze-dried. The residue (→**42** or **43**, resp.) was dissolved in 0.5 ml of 1M $Bu_4NF \cdot 3 H_2O$/0.5M AcOH in THF. After 2 h at r.t., 0.5 ml of *Tris* · HCl buffer (pH 7.4) was added, and the mixture was desalted on a *Sephadex G-10* column (H_2O). The product-containing fractions were analyzed by reversed-phase HPLC (*Fig. 3*). The sequence **45** was additionally purified by reversed-phase chromatography. After desalting the pooled product-containing fractions, 6 OD_{260nm} (20%) of pure **45** was obtained (*Fig. 4*). MALDI-TOF MS: 2378 ($[M + H]^+$).

Aminoacylated Nucleotides **1**, **37**, **52**, **53**, *and* **56**: *General Procedure* (*G.P.*). The nucleotide **2** or **51** (25 μmol for each experiment), was treated with 400 μl of a soln. prepared from $^iPr_3NEt$ (0.24M), 4-(pyrrolidin-1-yl)pyridine (0.1M), BOP (0.2M), and **39**, **41**, *ent*-**39**, or *N*-[(benzyloxy)carbonyl]-L-phenylalanine (0.2M), in Py. After 2 h at r.t., the mixture was diluted with AcOEt and separated by CC (AcOEt → AcOEt/EtOH 11:9 (+1% Et_3N), then → AcOEt/EtOH/H_2O 11:9:1): 45–50% yields.

2'-O-{[(S)-1-(2-Nitrophenyl)ethoxy]methyl}adenosine 5'-(Methyl Phosphate) 3'-{N-{[(2-Nitrobenzyl)oxy]carbonyl}-L-phenylalaninate} (**1**). According to the *G.P.* from **2** and **39**: 55% yield. TLC (BuOH/AcOH/H_2O 5:2:3): R_f 0.62. Reversed-phase HPLC (*A* → 50% *B*): t_R 28.1 min. ^{31}P-NMR (121 MHz, D_2O/^{t}BuOH 7:3, ammonium citrate (pH 3.6)): 1.77. ESI-MS (neg.): 865.5 (100, $[M - H]^-$), 343.2 (44), 269 (16), 134 (32).

2'-O-{[(S)-1-(2-Nitrophenyl)ethoxy]methyl}adenosine (5'-Methyl Phosphate) 3'-{N-{[(RS)-1-(2-Nitrophenyl)ethoxy]carbonyl}-L-phenylalaninate} (**37**). According to the *G.P.* from **2** and **41**: 45% yield. TLC (BuOH/AcOH/H_2O 5:2:3): R_f 0.60. Reversed-phase HPLC (*A* → 50% *B*): t_R 28.5 min.

*2′-*O*-{[(*S*)-1-(2-Nitrophenyl)ethoxy]methyl}adenosine 3′-{*N*-[(Benzyloxy)carbonyl]-*L*-phenylalaninate} 5′-(Methyl Phosphate)* (**52**). According to the *G.P.*, from **2** and *N*-[(benzyloxy)carbonyl]-L-phenylalanine: 52% yield. TLC (BuOH/AcOH/H_2O 5:2:3): R_f 0.65. Reversed-phase HPLC ($A \rightarrow 50\%$ B): t_R 28.2 min.

*2′-*O*-{[(*S*)-1-(2-Nitrophenyl)ethoxy]methyl}adenosine 5′-(Methyl Phosphate) 3′-*O*-{[*N*-{[(2-Nitrobenzyl)-oxy]carbonyl}-*D*-phenylalaninate}* (**53**). According to the *G.P.*, from **2** and *ent*-**39**: 48% yield. TLC (BuOH/AcOH/H_2O 5:2:3): R_f 0.61. Reversed-phase HPLC ($A \rightarrow 50\%$ B): t_R 27.6 min.

*2′-*O*-Methyladenosine 5′-(Methyl Phosphate) 3′-{*N*-{[(2-Nitrobenzyl)oxy]carbonyl}-*L*-phenylalaninate}* (**56**). According to the *G.P.,* from **51** and **39**: 55% yield. TLC (BuOH/AcOH/H_2O 5:2:3): R_f 0.48. Reversed-phase HPLC ($A \rightarrow 50\%$ B): t_R 24.0 min.

*Aminoacylated Nucleotides Adenosine 5′-(Methyl Phosphate) 2′(3′)-(*L*-Phenylalaninate)* (**38**), *Adenosine 2′(3′)-{*N*-[(Benzyloxy)carbonyl]-*L*-phenylalaninate} 5′-(Methyl Phosphate)* (**54**), *Adenosine 5′-(Methyl Phosphate) 2′(3′)-(*D*-Phenylalaninate)* (**55**), *and 2′-*O*-Methyladenosine 5′-(Methyl Phosphate) 2′(3′)-(*L*-Phenylalaninate)* (**57**): *General Procedure.* The aminoacylated nucleotides **38**, **54**, **55**, and **57** were obtained by photolysis (30 min to 1.5 h, depending on the substrate and the concentration) of the corresponding protected precursors **1** or **37**, **52**, **53**, and **56** ($c = 150 - 250$ μM), resp., in ^{t}BuOH/H_2O 1:1 at pH 3.4 (40 mM ammonium citrate). After extraction with CH_2Cl_2, the crude products were desalted on 1 g of *C18-RP* silica gel (H_2O/MeCN 4:1 (+20 mM AcOH, pH 3.1)). The product-containing fractions (UV; yields typically >70%) were pooled and analyzed by reversed-phase HPLC; the purity of such samples usually exceeded 95%. Aliquots of these stock solns. were used directly for hydrolysis studies.

Additionally, a semi-prep. photolysis experiment was carried out with **1**: a 2 mM soln. of **1** in D_2O/^{t}BuOH 7:3 (0.7 ml) (40 mM ammonium citrate (pH 3.4)) was photolyzed in a NMR tube. Periodically, ^{31}P-NMR spectra (121 MHz) and HPLCs were recorded. The deprotection was nearly finished after 3.5 h (HPLC) and gave **38** as the only product. However, the ^{31}P-NMR spectrum showed two signals at δ 1.77 and 1.88 (ratio 3:1), which were attributed to the regioisomeric 3′-*O*- and 2′-*O*-aminoacylated nucleotides **38**.

Hydrolysis Studies. Aliquots of the stock solns. containing each *ca.* 0.1 mM aminoacylated nucleotide **38**, **54**, **55**, or **57** (preparation, see above) were diluted with an equal volume of the appropriate buffer soln. and incubated at 25°. In the course of every individual experiment, 5–8 samples were removed at periodic intervals, acidified with AcOH (→ pH *ca.* 3), and analyzed by reversed-phase HPLC (detection at 260 nm). The integral ratios between the signals of aminoacylated starting materials and hydrolyzed products were translated into individual pseudo-first-order rate constants (*Table*).

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Received June 9, 2000