

# RNA mimetics: oligoribonucleotide N3'→P5' phosphoramidates

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## ABSTRACT

The synthesis and properties of novel RNA mimetics, oligoribonucleotide N3'→P5' phosphoramidates, are described. These oligonucleotides contain 3'-aminoribonucleosides connected via N3'→P5' phosphoramidate linkages, replacing the native RNA O3'→P5' phosphodiester counterparts. The key monomers 2'-*t*-butyldimethylsilyl-3'-(monomethoxytrityl)-amino-5'-phosphoramidites were synthesized and used to prepare the oligonucleotide phosphoramidates using a solid phase methodology based on the phosphoramidite transfer reaction. Oligoribophosphoramidates are very resistant to enzymatic hydrolysis by snake venom phosphodiesterase. These compounds form stable duplexes with complementary natural phosphodiester DNA and RNA strands, as well as with 2'-deoxy N3'→P5' phosphoramidates. The increase in melting temperature,  $\Delta T_m$ , was 5–14°C relative to the 2'-deoxy phosphoramidates for decanucleotides. Also, the thermal stability of the ribophosphoramidate homoduplex was noticeably higher ( $\Delta T_m$  +9.5°C) than that for the isosequential 2'-deoxy phosphoramidate complex. Furthermore, the oligopyrimidine ribo N3'→P5' phosphoramidate formed an extremely stable triplex with an oligopurine/oligopyrimidine DNA duplex with  $\Delta T_m$  +14.3°C relative to the 2'-deoxy N3'→P5' phosphoramidate counterpart. The properties of the oligoribonucleotide N3'→P5' phosphoramidates indicate that these compounds can be used as hydrolytically stable structural and functional RNA mimetics.

## INTRODUCTION

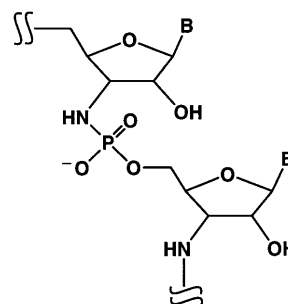
Studies involving various RNA molecules have recently attracted significant attention because, in part, of the unique properties of some RNA oligomers. Thus, these compounds can act catalytically as RNA and DNA cleaving agents, as well as ligating enzymes (1–5). Aptamers made from ribonucleosides and their derivatives produced by *in vitro* selection exhibit high affinity binding and sequence-specific recognition of various ligands (6–9). Natural RNA regulatory elements, recognized by RNA-binding proteins, play an important role in regulation of HIV viral proliferation (10,11). Additionally, RNA molecules have been strongly

implicated in the origin of life on Earth (12,13). However, native RNA molecules are chemically unstable and readily degraded by cellular nucleases. Significant effort has been made to prepare hydrolytically stable RNA analogs, which could be of interest for various therapeutic and diagnostic applications (14). Among the prepared compounds are phosphodiester and phosphorothioate RNA oligonucleotide analogs containing 2'-fluoro, 2'-amino and 2'-alkoxy substituents, which are stabilized against hydrolysis by nucleases and functionally similar to native RNA molecules (15,16).

Recently, the synthesis of 2'-deoxyoligonucleotide N3'→P5' phosphoramidate analogs of DNA was described (17). These compounds are resistant to nucleases, form stable duplexes with complementary single-stranded (ss)RNA and DNA, as well as stable triplexes with double-stranded (ds)DNA targets (18,19). Moreover, 2'-deoxy N3'→P5' phosphoramidate duplexes adopt an A-type of helix and these compounds can spatially and functionally mimic isosequential RNA structural elements (20,21).

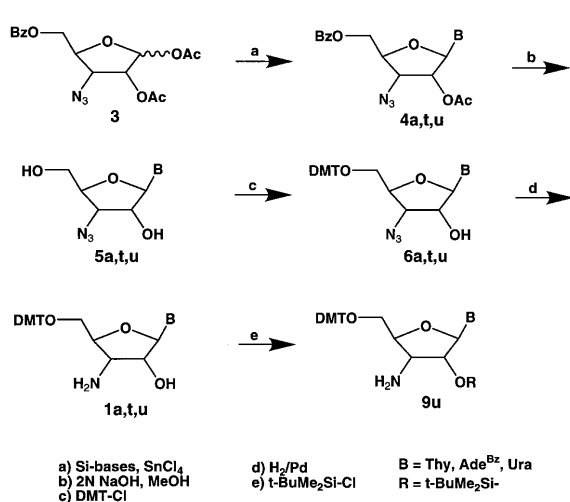
Random size short CG-containing oligoribonucleotide N3'→P5' phosphoramidates were obtained via self-polymerization of preformed dimer blocks in aqueous buffers (22). Ribo N3'→P5' phosphoramidate dimers and trimers were also synthesized in aqueous solutions (23).

Here we report the efficient synthesis of RNA mimetic oligoribonucleotide N3'→P5' phosphoramidates (Fig. 1) and describe some of their hydrolytic characteristics and hybridization properties.



**Figure 1.** Structure of the oligoribonucleotide N3'→P5' phosphoramidate internucleoside groups.

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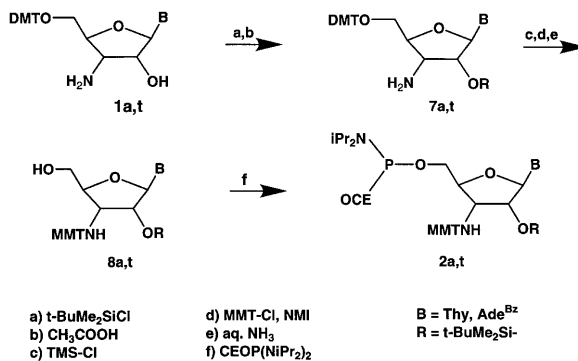
Scheme 1.

## RESULTS AND DISCUSSION

### Preparation of the monomers

The synthesis of the oligoribonucleotide N3'→P5'-phosphoramidates was accomplished by using two different types of monomers, **1** and **2** (Schemes 1 and 2), depending on the oligonucleotide assembly protocol. Thus, key 5'-O-DMT-3'-aminoribonucleosides **1a**, **1t** and **1u** were prepared according to Scheme 1. These compounds were incorporated into the oligonucleotide phosphoramidates via the oxidative phosphorylation method (17,24). First, the 3'-azido compounds **4a**, **4t** and **4u** were synthesized by condensing the trimethylsilylated bases with 3-azido-1,2-di-O-acetyl-5-O-benzoyl-3-deoxy-D-ribofuranose **3** in the presence of tin(IV) chloride (25). Removal of the sugar protecting groups with either ammonia/ethanol or sodium hydroxide afforded compounds **5a**, **5t** and **5u**, which were converted into the corresponding 3'-amino-5'-O-dimethoxytrityl nucleosides **1a**, **1t** and **1u** via tritylation with DMT chloride, followed by catalytic hydrogenation. Additionally, 2'-O-protected **9u** was obtained through silylation of **1u** with t-butyltrimethylsilyl chloride (Scheme 1).

Alternatively, 5'-(2-cyanoethyl-*N,N'*-diisopropyl)phosphoramidite building blocks **2a** and **2t** were prepared (Scheme 2). Thus, compounds **1a** and **1t** were treated with t-butyltrimethylsilyl chloride to protect the 2'-hydroxyl group and subsequently detritylated with 80% aqueous acetic acid to afford the 3'-amino-2'-silylated derivatives **7a** and **7t**. In order to selectively protect the 3'-amino group, the 5'-hydroxyl group was transiently protected with a trimethylsilyl residue (26), followed by addition of monomethoxytrityl chloride and *N*-methylimidazole. The reaction mixture was stirred at 39°C for 48 h. Anhydrous conditions and temperature control are crucial in order to avoid possible 5'-O-monomethoxytritylation. Removal of the 5'-O-trimethylsilyl group with ammonia and purification on silica gel resulted in the precursors **8a** and **8t**, which were subsequently phosphitylated to obtain the desired building blocks **2a** and **2t**. The structure of the synthesized nucleosides was confirmed by <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P NMR spectroscopy (Materials and Methods).



Scheme 2.

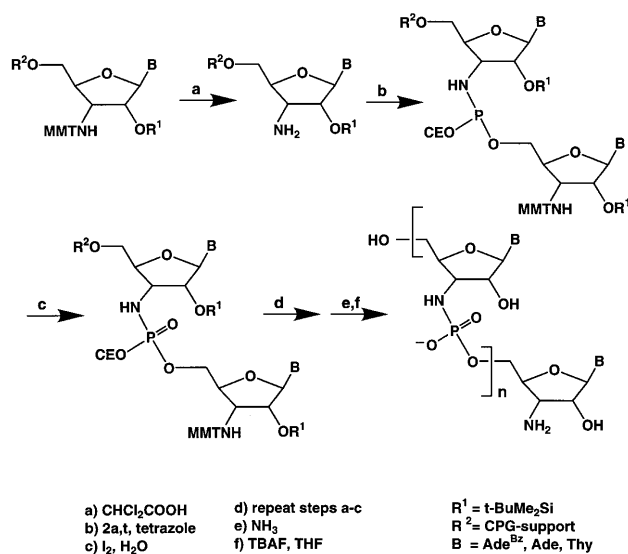
### Synthesis of oligoribonucleotide N3'→P5' phosphoramidates

Two different approaches for the synthesis of the title oligonucleotides were employed. We first tried the oxidative phosphorylation method, based on carbon tetrachloride-driven oxidative phosphorylation of the 3'-aminonucleosides in solution by the 5'-H-phosphonate diester groups of the growing oligonucleotide on a solid phase support, as originally described for the preparation of the 2'-deoxynucleotide N3'→P5' phosphoramidates (17,24). Thus, the model dimers r(Unp)dT and r(Anp)dT were prepared using 2'-hydroxyl unprotected compounds **1a** and **1u**. The products were analyzed and purified by RP HPLC. The <sup>31</sup>P NMR and mass spectra of the dimers confirmed the presence of an internucleotide phosphoramidate linkage with a characteristic resonance at ~7.0 p.p.m. and molecular composition of the synthesized compounds (Materials and Methods). Coupling times for 3'-aminoribonucleosides were extended up to 1 h and coupling efficiency was estimated to be in the range 85–91% as judged by RP HPLC analysis. It is important to note that the formation of dinucleosides with 2'-5' phosphodiester links, as a result of coupling of unprotected 2'-hydroxyl rather than 3'-amino groups, was not detected by <sup>31</sup>P NMR analysis of the reaction mixtures.

The same synthetic protocol was used to introduce one or two 3'-aminoribonucleosides into longer oligodeoxynucleotide N3'→P5' phosphoramidates. The coupling step of ribonucleoside **1u** was followed by capping with acetic or phenoxyacetic anhydride to avoid phosphitylation of the unprotected 2'-hydroxyl group during the next chain elongation cycle. Thus, compound **16** (Table 1) was prepared and isolated by IE HPLC.

One of the main drawbacks of using the 2'-OH unprotected nucleosides **1a** and **1u** for synthesis of ribophosphoramidates is the accompanying cleavage of the internucleoside phosphoramidate linkage during oligomer deprotection with ammonia. Approximately 20% of the internucleoside ribophosphoramidate groups in compound **16** (Table 1) were cleaved upon treatment with concentrated aqueous ammonia for 1 h at 55°C, as judged by IE HPLC analysis of the reaction mixture. To overcome this problem we synthesized the dimer r(Unp)dT using the 2'-O-TBDMS-protected nucleoside **9u**. After deprotecting ammonolysis, the 2'-O-TBDMS group was selectively and cleanly removed with 1 M tetrabutylammonium fluoride in THF, resulting in a fully deprotected dimer. Unfortunately, the reduced observed coupling yields for the 2'-O-TBDMS-3'-aminonucleosides (~60% as was judged by RP and IE HPLC analysis), which is presumably due to steric hindrance by the bulky TBDMS group, limit the utility of this approach.

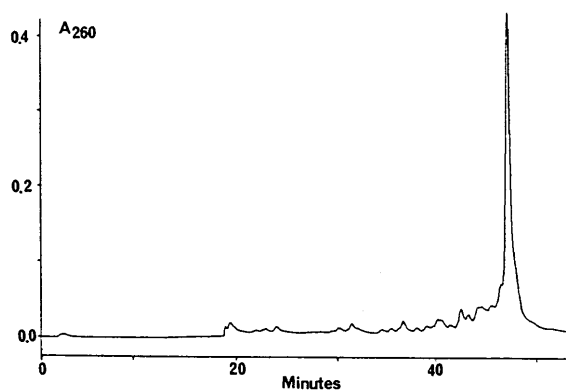
An alternative chain assembly method, based on a phosphoramidite transfer reaction, has recently been introduced for the synthesis of oligo-2'-fluoro-2'-deoxynucleoside N3'→P5' phosphoramidates and oligo-2'-deoxynucleoside N3'→P5' phosphoramidates, using 3'-(protected)amino 5'-O-(cyanoethyl-N,N'-diisopropylamino)phosphoramidite nucleosides (27–29). Thus, this approach was extended to synthesis of the ribophosphoramidates. The key aminonucleoside building blocks **2a** and **2t** were prepared according to Scheme 2. The oligoribonucleotide N3'→P5' phosphoramidates were synthesized using these monomers and the assembly protocol outlined in Scheme 3. Thus, the diisopropylamino group of incoming 5'-O-phosphoramidite **2** was exchanged for the 3'-amino group of the solid support-bound 3'-amino-2'-O-TBDMS-ribonucleoside. Coupling time was 10 min using 1H-tetrazole as activator. The newly formed internucleoside phosphoramidite diester group was then oxidized with aqueous iodine to the phosphoramidate one. Following detritylation of the 3'-amino group, the oligonucleotide chain elongation cycle can be repeated until the desired oligomer is assembled. The prepared fully protected oligoribonucleotide phosphoramidate was cleaved from the solid support and base protecting and phosphate protecting groups were removed with ammonia. Finally, 2'-O-TBDMS groups were removed with tetrabutylammonium fluoride and the completely deprotected oligoribonucleotide N3'→P5' phosphoramidates were analyzed and purified by IE HPLC (Fig. 2 and Materials and Methods). The structure of the prepared oligonucleotide phosphoramidates was confirmed by <sup>31</sup>P NMR analysis, where the phosphoramidate group characteristic resonance peaks at 6.5–7.0 p.p.m. were observed.



Scheme 3.

### Hydrolytic stability of oligoribonucleotide N3'→P5' phosphoramidates

The stability of the oligoribophosphoramidates toward hydrolysis by a mixture of snake venom phosphodiesterase and alkaline phosphatase was evaluated. Thus, 0.2 OD<sub>260 nm</sub> oligonucleotide **12** (Table 1) were treated with 0.02 U snake venom phosphodiesterase and 0.8 U alkaline phosphatase in 0.2 ml 10 mM Tris-HCl buffer, pH 7.8, at room temperature. The reaction mixture was analyzed



**Figure 2.** Ion exchange HPLC profile of the crude reaction mixture from synthesis of oligonucleotide **12** (Table 1). A Pharmacia MonoQ 5/5 column and a 1%/min gradient of 1.5 M NaCl, 0.01 M NaOH in 0.01 M NaOH were used for the analysis.

by IE HPLC at several time points. The analysis demonstrated that the half-life of the full-length ribodecanucleotide **12** was 26 h. A similar resistance to enzymatic hydrolysis *in vitro* and *in vivo* was observed for the 2'-deoxy N3'→P5' phosphoramidate counterparts, where the presence of a terminal 3'-amino group also significantly increased hydrolytic stability (17,31). For comparison, the iso-sequential all phosphodiester 2'-deoxydecathymidilate was completely hydrolyzed to the thymidine nucleoside within 20 min under identical reaction conditions. It would be interesting in the future to study stability of oligoribophosphoramidates towards various RNases. In addition, the stability of the ribophosphoramidates under basic and acidic conditions was studied. The ribophosphoramidate dimers r(Anp)dT and r(Unp)dT were relatively stable in concentrated aqueous ammonia. Incubation of r(Unp)dT and r(Anp)dT for 15 h at 55°C in concentrated aqueous ammonia resulted in ~10 and 5% hydrolysis of the starting compounds, respectively, as judged by RP HPLC. Unlike the dimers, oligomer **12** (Table 1) was completely hydrolyzed to multiple products after incubation under similar conditions for 6 h at 55°C. Additionally, treatment of r(Anp)dT dimer with 40% aqueous acetic acid for 20 h at room temperature followed by 2 h at 55°C resulted in 85% cleavage of the 3'-N-P bond and formation of 3'-aminoriboadenosine and 5'-thymidylic acid with 15% of starting material remaining, as determined by RP HPLC analysis. Similarly, 10mer **12** (Table 1) was completely hydrolyzed by 40% acetic acid within 3 h at 55°C. Hydrolysis resulted in formation of 3'-aminoribothymidine and 3'-amino-5'-ribothymidylic acid at an ~2:3 ratio, along with two minor unidentified products. This indicates that the acid-catalyzed hydrolysis of the internucleoside ribophosphoramidate group in oligomers may proceed via cleavage of both 3'-N-P and P-O-5' bonds, since a significant amount of 3'-aminoribothymidine nucleoside was formed. This acid-catalyzed hydrolysis reaction may involve participation of neighboring phosphates, unlike the dimers, where only the 3'-N-P linkage was cleaved.

### Thermal stability of the phosphoramidate duplexes and triplexes

The ability of oligoribonucleoside N3'→P5' phosphoramidates to form complexes with complementary DNA and RNA strands

was evaluated using thermal dissociation experiments. Melting temperatures ( $T_m$ ) were determined for duplexes and triplexes formed by the ribophosphoramidates under close to physiological salt and buffer conditions. The results are summarized in Table 1. Substitution of the 3'-amino-2'-deoxyfuranose rings by 3'-amino-2'-ribo counterparts in uniformly modified thymidine oligomers resulted in significant stabilization of the duplexes formed with both DNA and RNA complements. The increases in melting temperatures of the duplexes ( $\Delta T_m$ ) were up to 14.4 and 4.1 °C for the complexes with DNA and RNA strands, respectively (compare experiments 3 and 4 and 6 and 7, Table 1). These correspond to  $\Delta T_m$  of 1.8 and 0.5 °C per single nucleoside substitution. The melting temperature of the ribophosphoramidate duplexes was 20.1–30.3 °C higher than that for the isosequential 2'-deoxyphosphodiester 10mer **10** (compare experiments 1 and 2 and 6 and 7, Table 1). Additionally, oligoribophosphoramidate **12** forms a very stable triple-stranded complex with the dA<sub>10</sub>-dT<sub>10</sub> duplex region of the hairpin target (experiment 8, Table 1). Melting temperatures for the triplex were 44 and 61.5 °C in 150 mM NaCl buffer, pH 7, without or with additional 10 mM MgCl<sub>2</sub>, respectively. These  $T_m$  values were 12.0–14.3 °C higher than those for the isosequential 2'-deoxyphosphoramidate counterpart (compare experiments 5 and 8, Table 1). Parent phosphodiester decamer **10** did not form a triplex under identical experimental conditions. Triplex thermal dissociation curves were recorded at

both 260 and 282 nm, where change in absorbency is characteristic for dissociation of T·AT triplets.

Unlike the oligopyrimidines, oligopurine ribophosphoramidate **14** formed more stable duplexes with DNA and less stable duplexes with RNA complements than the isosequential 2'-deoxyphosphoramidate, with  $\Delta T_m$  values of 4.9 and –13.1 °C, respectively (compare experiments 9 and 10 and 11 and 12, Table 1). Detailed NMR and X-ray structural analysis will be needed to explain the difference in thermal stability of duplexes formed by purine- and pyrimidine-containing oligoribophosphoramidates.

Interestingly, incorporation of one or two 3'-aminoribonucleosides into 2'-deoxyphosphoramidate oligomers resulted in a 3.5–10 °C reduction in duplex thermal stability (compare experiments 15 and 16 and 17 and 18, Table 1). This was unexpected in view of similar sugar puckering of 3'-aminoribo and 3'-amino-2'-deoxynucleosides. Thus, <sup>1</sup>H NMR spectra of the synthesized 3'-aminoribonucleosides and 3'-aminoriboadenosine in the r(Anp)dT dimer indicate a C3'-endo or N-type of sugar puckering for the ribofuranose ring: the  $J^3(\text{H1}'\text{--H2}')$  coupling constants for the 3'-aminoribonucleosides and for 3'-aminoriboadenosine within the dimer were <1 Hz and similar to those for 3'-amino-2'-deoxynucleosides (30). Furthermore, the opposite effect was observed for 2'-fluoro N3'→P5' phosphoramidates, where substitution of one or two 2'-deoxynucleosides by 2'-fluoro counterparts resulted in duplex stabilization by ~2 °C per substitution (27).

**Table 1.** Oligonucleotides and  $T_m$  values of their complexes

Experiment	Oligonucleotide	Target	$T_m$ (°C) <sup>a</sup>
1	d-TTTTTTTTTT, <b>10</b>	poly(dA)	28.3
2	As experiment 1	poly(A)	26.2
3	d-T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T, <b>11</b>	poly(dA)	34.0
4	As experiment 3	poly(A)	50.7
5	As experiment 3	d-A <sub>10</sub> C <sub>4</sub> T <sub>10</sub>	32.0, 47.2 <sup>b</sup>
6	dT-r-T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>np</sub> T <sub>n</sub> , <b>12</b>	poly(dA)	48.4
7	As experiment 6	poly(A)	56.5
8	As experiment 6	d-A <sub>10</sub> C <sub>4</sub> T <sub>10</sub>	44.0, 61.5 <sup>b</sup>
9	d-A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A, <b>13</b>	poly(dT)	20.0
10	As experiment 9	poly(U)	50.6
11	As experiment 9	<b>11</b> <sup>c</sup>	43.4
12	dA-r-A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>np</sub> A <sub>n</sub> , <b>14</b>	poly(dT)	24.9
13	As experiment 12	poly(U)	37.7
14	As experiment 12	<b>12</b> <sup>c</sup>	52.9
15	d-U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> U <sub>np</sub> T, <b>15</b>	poly(dA)	18.5, 38.2 <sup>b</sup>
16	As experiment 15	poly(A)	38.1, 47.2 <sup>b</sup>
17	d-(U <sub>np</sub> ) <sub>4</sub> -r-(U <sub>np</sub> ) <sub>2</sub> -D-(U <sub>np</sub> ) <sub>3</sub> T, <b>16</b>	poly(dA)	15.0, 28.9 <sup>b</sup>
18	As experiment 17	poly(A)	30.0, 37.4 <sup>b</sup>

$T_m$  is temperature at the midpoint of the melting curve; n and np represent terminal 3'-amino groups and 3'-NHP(O)(O-)O-5' internucleoside linkages, respectively. The concentrations of the oligomers were 2 μM each strand. Buffer A, 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.04. Buffer B, buffer A containing 10 mM magnesium chloride.

<sup>a</sup> $T_m$  in buffer A.

<sup>b</sup> $T_m$  in buffer B.

<sup>c</sup>The targets were decanucleotides **11** and **12** from experiments 3 and 6, respectively.

We think that the increase in the thermal stability of duplexes and triplexes of ribophosphoramidates relative to the 2'-deoxyphosphoramidate counterparts, as well as the parent phosphodiester, is due to the further increase in the population of N-type sugar conformations for the 3'-amino-2'-hydroxyl nucleosides, which is determined by a cooperative and additive effect of 3'-amino and 2'-hydroxyl groups on furanose puckering. Additionally, improved hydration of the phosphoramidate duplexes, due to the presence of the 3'-amino group as an additional donor and acceptor of hydrogen bonds, as well as 2'-hydroxyl, may contribute to the increase in thermal stability (21).

In summary, an efficient method for the synthesis of enzyme-resistant RNA analogs, oligoribonucleotide N3'→P5' phosphoramidates, has been developed. These compounds could be potentially used as hydrolytically stable structural and functional mimetics of native RNA for preparation of ribozymes, aptamers and RNA decoys, as well as other molecules.

## MATERIALS AND METHODS

### General methods

Oligonucleotide N3'→P5' phosphoramidates, containing one or two 3'-aminoribonucleosides, were synthesized using the oxidative phosphorylation method on an ABI 394 synthesizer as previously described (18). The coupling step was followed by capping with 10% phenoxyacetic anhydride in the presence of 12.5% *N*-methylimidazole in THF if 2'-(unprotected)hydroxyl-3'-aminonucleosides were used. Uniformly modified oligoribonucleotide N3'→P5' phosphoramidates were prepared manually in a syringe using the amidite transfer reaction (27,28) and the following protocol: (i) detritylation, 3% dichloroacetic acid in dichloromethane, 1.5 min; (ii) coupling, 0.1 M phosphoramidite and 0.5 M tetrazole in acetonitrile, 10 min; (iii) oxidation, 0.1 M iodine in tetrahydrofuran/pyridine/water, 10/10/1 (v/v/v), 2 min. Chemical steps within the cycle were followed by acetonitrile or dichloromethane washing depending on the reaction solvent. Cleavage from the solid support and deprotection were accomplished with concentrated aqueous ammonia in ethanol, 3/1 (v/v), 1.5 h, 55°C for homothymidine or uracil-containing oligomers and 7.5 h, 55°C for purine-containing compounds. The oligonucleotides were concentrated to dryness *in vacuo* and the 2'-*t*-butyldimethylsilyl groups were removed by treatment with 0.5 ml 1 M tetrabutylammonium fluoride in THF for 16 h. The solvent was removed *in vacuo* and the oligonucleotides were desalted by gel filtration on a Pharmacia NAP-5 column. Oligonucleotides were analyzed and purified by IE HPLC and were desalted by gel filtration immediately after purification and stored frozen at -18°C. Thermal denaturation experiments were performed as described previously (24) in the buffers listed in Table 1.

Thin layer chromatography (TLC) was carried out on DC-Fertigplatten SIL G-25 UV254 silica gel plates (Macherey-Nagel) in dichloromethane/methanol systems. The nucleosides used for oligonucleotide chain assembly were practically pure according to TLC analysis.

The model dimers r(Unp)dT and r(Anp)dT were synthesized on an automated DNA synthesizer on the 1 μmol scale using 2'-unprotected or 2'-*O*-TBDMS-protected 3'-aminonucleosides. Coupling times were 1 and 2 h for 2'-unprotected and 2'-*O*-TBDMS-protected nucleosides, respectively. After deblocking with NH<sub>3</sub>/ethanol (3:1 v/v), the dimers were analyzed and purified by RP HPLC. In the case of nucleoside **9u**, the

2'-*O*-TBDMS group was removed by treatment with 0.5 ml 1 M tetrabutylammonium fluoride in THF at room temperature for 4 h. The observed coupling yields were 85–91 and 55–60% for 2'-unprotected and 2'-protected 3'-aminonucleosides, respectively. <sup>31</sup>P NMR: (D<sub>2</sub>O) δ 7.75 p.p.m. for r(Unp)dT and 7.59 p.p.m. for r(Anp)dT. Mass spectrum for r(AnpT): (M-H)<sup>-</sup>, *m/z* calculated and observed, 569. Acid hydrolysis of 0.6 OD of the dimer r(Unp)dT was carried out in 0.5 ml 80% AcOH at room temperature, 100 μl stock solution were evaporated and analyzed by RP HPLC. After 24 h, the dimer [retention time (RT) 13.5 min] was hydrolyzed to 3'-aminouridine (RT 3.7 min), 5'-thymidylic acid (RT 7.6 min) and dT (RT 9.3 min). For enzymatic digestion, 0.2 A<sub>260</sub> U oligonucleotide were treated with 0.02 U snake venom phosphodiesterase and 0.8 U alkaline phosphatase (Sigma, St Louis, MO) in 0.2 ml 10 mM Tris-HCl, pH 7.8, at room temperature. Aliquots from the reaction were taken at multiple points and analyzed by IE HPLC.

### 3-Azido-1,2-bis-*O*-acetyl-5-*O*-benzoyl-3-deoxy- $\beta$ -D-ribofuranose (3)

Compound **3** was synthesized starting from 1,2-*O*-isopropylidene- $\alpha$ -D-xylofuranose analogously to the described procedure for 3-azido-1,2-di-*O*-acetyl-5-*O*-(4-methylbenzoyl)-3-deoxy- $\beta$ -ribofuranose (32).

### 3'-Azido-2'-*O*-acetyl-5'-*O*-benzoyl-3'-deoxy- $\beta$ -D-ribofuranosylthymine (4t)

To a solution of 2,4-bis(trimethylsilyl)thymine prepared from 2.0 g thymine and **3** (1.40 g, 3.85 mmol) in 80 ml dry acetonitrile was added 2 ml 1 M solution of stannic chloride in dichloroethane and the stirred reaction mixture was refluxed for 4 h. The solvent was evaporated and 100 ml CH<sub>2</sub>Cl<sub>2</sub> and 100 ml saturated NaHCO<sub>3</sub> were added to the reaction mixture. The organic phase was separated and the aqueous phase was back-extracted with 100 ml CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (95:5 v/v) to afford **4t** as a white foam (1.39 g, 84.1%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 1.68 (s, 3H), 2.19 (s, 3H), 4.28–4.74 (3 m, 4H), 5.56 (dd, *J* = 5.7 Hz and *J* = 4.4), 5.87 (d, *J* = 4.4 Hz, 1H), 7.28–8.10 (m, 6H), 8.75 (s, 1H).

### 3'-Azido-3'-deoxy- $\beta$ -D-ribofuranosylthymine (5t)

Compound **4t** (1.35 g, 3.14 mmol) was dissolved in 20 ml ethanol and 40 ml 25% aqueous NH<sub>3</sub> was added. The solution was stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (95:5 v/v) to yield **5t** as a white foam (650 mg, 73.1%). <sup>1</sup>H NMR: (D<sub>2</sub>O) δ 1.76 (s, 3H), 3.70 (m, 1H), 3.80 (m, 1H), 4.01 (m, 2H), 4.46 (dd, *J* = 4.4 Hz and *J* = 5.1 Hz, 1H), 5.75 (d, *J* = 4.4 Hz, 1H), 7.57 (s, 1H). <sup>13</sup>C NMR: (D<sub>2</sub>O) δ 11.88, 60.89, 74.43, 82.48, 89.53, 111.68, 137.55, 151.94, 166.66.

### 3'-Azido-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-3'-deoxy- $\beta$ -D-ribofuranosylthymine (6t)

To compound **5t** (620 mg, 2.19 mmol), co-evaporated twice from anhydrous pyridine and redissolved in 30 ml pyridine, was added 4,4'-dimethoxytriphenylmethyl chloride (1.44 g, 4.24 mmol) and 4-dimethylaminopyridine (150 mg, 1.22 mmol). The reaction

mixture was stirred for 24 h at room temperature, 100 ml 5% aqueous NaHCO<sub>3</sub> were added and the reaction mixture extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 ml). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated *in vacuo*. The residue was co-evaporated twice with toluene and then purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (95:5 v/v) to yield **6t** as a white foam (920 mg, 71.7%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 1.43 (s, 3H), 3.38 (m, 1H), 3.66 (m, 1H), 3.79 (s, 6H), 4.14 (m, 1H), 4.32 (m, 1H), 4.65 (m, 1H), 5.97 (d, *J* = 3.3 Hz, 1H), 6.87–7.44 (m, 13H), 7.75 (s, 1H), 10.49 (s, 1H).

### 3'-Amino-5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-deoxy-β-D-ribofuranosylthymine (**1t**)

Compound **6t** (1.35 mg, 2.31 mmol) was dissolved in 50 ml EtOH, Pd/C (100 mg, 10% Pd) was added and the reaction mixture was hydrogenated at room temperature for 72 h. The catalyst was removed by filtration and the filtrate evaporated. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (9:1 v/v) to yield **1t** as a white foam (1.05 g, 81.5%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 1.42 (s, 3H), 3.41 (m, 1H), 3.65 (m, 2H), 3.79 (s, 6H), 4.04 (m, 1H), 4.26 (d, *J* = 4.9 Hz, 1H), 5.82 (s, 1H), 6.84–7.47 (m, 13H), 7.79 (s, 1H).

### 3'-Amino-2'-O-(t-butyltrimethylsilyl)-3'-deoxy-β-D-ribofuranosylthymine (**7t**)

Compound **1t** (1.05 g, 1.88 mmol) was co-evaporated twice with pyridine and redissolved in 20 ml pyridine. To this stirring mixture t-butyltrimethylsilyl chloride (345 mg, 2.29 mmol) was added at room temperature and, 24 h later, 25 ml saturated aqueous NaHCO<sub>3</sub> was added and the solution was extracted with 3 × 100 ml CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The residue was co-evaporated twice with toluene and dissolved in 100 ml 80% aqueous acetic acid. The solution was stirred at room temperature for 45 min and then 150 ml methanol were added and the solvents were evaporated. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (95:5 v/v) to yield **7t** as a white foam (505 mg, 72.3%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 0.14 (s, 3H), 0.20 (s, 3H), 0.90 (s, 9H), 1.90 (s, 3H), 3.13 (m, 1H), 3.50 (m, 1H), 3.91–4.22 (3 m, 3H), 5.67 (s, 1H), 7.76 (s, 1H).

### 2'-O-(t-Butyltrimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-3'-deoxy-β-D-ribofuranosylthymine (**8t**)

Compound **7t** (250 mg, 0.67 mmol) was co-evaporated twice with pyridine and redissolved in 20 ml pyridine. Trimethylsilyl chloride (0.75 ml, 8.6 mmol) was added and the solution was stirred at room temperature for 2 h. 4-Methoxytriphenylmethyl chloride (492 mg, 1.59 mmol) and *N*-methylimidazole (0.13 ml, 1.6 mmol) were added and the solution was stirred at 38°C for 48 h. Saturated aqueous NaHCO<sub>3</sub> (25 ml) was then added and the solution was extracted with 3 × 100 ml CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo*. The residue was dissolved in 50 ml methanol and 5 ml 25% aqueous NH<sub>3</sub> was added. The solution was stirred at room temperature for 15 min and then evaporated. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (95:5 v/v) to yield a light yellow foam. The foam was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and precipitated from hexane to yield **8t** as a white solid (270 mg, 62.6%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 0.04

(s, 3H), 0.14 (s, 3H), 0.90 (s, 9H), 1.87 (s, 3H), 2.91–3.07 (m, 3H), 3.79 (s, 3H), 3.95–4.05 (3 m 3H), 5.58 (s, 1H), 6.80–7.53 (m, 14H), 7.77 (s, 1H), 8.85 (s, 1H). LSIMS (MH<sup>+</sup>) *m/z* found 644.3; calculated for C<sub>36</sub>H<sub>45</sub>N<sub>3</sub>O<sub>6</sub>Si 643.3.

### 2'-O-(t-Butyltrimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-3'-deoxy-β-D-ribofuranosylthymine 5'-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (**2t**)

Compound **8t** (240 mg, 0.37 mmol) was dissolved in 5 ml anhydrous CH<sub>2</sub>Cl<sub>2</sub>. Diisopropylammonium tetrazolide (62.4 mg, 0.38 mmol) and 2-(cyanoethyl)-*N,N,N,N'*-tetraisopropylphosphorodiamidite (0.38 ml, 1.12 mmol) were added and the solution was stirred at room temperature for 2 h. Saturated aqueous NaHCO<sub>3</sub> (5 ml), H<sub>2</sub>O (10 ml) and CH<sub>2</sub>Cl<sub>2</sub> (30 ml) were added and, following extraction, the organic phase was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo*. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:Et<sub>3</sub>N (99:1 v/v) to yield **2t** as a white foam (208 mg, 66.6%). <sup>31</sup>P NMR: (CDCl<sub>3</sub>) δ 148.45, 149.81.

### 3'-Azido-2'-O-acetyl-5'-O-benzoyl-N<sup>6</sup>-benzoyl-3'-deoxyadenosine (**4a**)

To a solution of N<sup>6</sup>-benzoyl-N<sup>6</sup>,9-bis(trimethylsilyl)adenine prepared from 3.0 g N<sup>6</sup>-benzoyladenine and **3** (1.95 g, 5.37 mmol) in 150 ml dry 1,2-dichloroethane was added 4 ml 1 M stannic chloride solution in dichloroethane. The stirred reaction mixture was refluxed for 8 h, then cooled to room temperature, diluted with 100 ml CH<sub>2</sub>Cl<sub>2</sub> and 100 ml saturated aqueous NaHCO<sub>3</sub> were added. The organic phase was separated and the aqueous phase was back-extracted with an additional 100 ml CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (95:5 v/v) to yield **4a** as a white foam (2.05 g, 70.5%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 2.19 (s, 3H), 4.39–5.00 (4 m, 4H), 6.13 (m, 2H), 7.28–8.10 (m, 10H), 8.61 (s, 1H), 9.19 (s, 1H).

### 3'-Azido-N<sup>6</sup>-benzoyl-3'-deoxyadenosine (**5a**)

Compound **4a** (870 mg, 1.59 mmol) was dissolved in 80 ml methanol:water (1:1 v/v). The solution was cooled to 0°C and 10 ml 2 M NaOH were added. The solution was stirred at 0°C for 30 min, the reaction mixture was neutralized with 2 M HCl and the solvents were evaporated. The residue was extracted with 3 × 150 ml CH<sub>2</sub>Cl<sub>2</sub>:MeOH (9:1 v/v), the solvent evaporated and the residue purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (9:1 v/v) to yield **5a** as a white solid (480 mg, 76.2%). <sup>1</sup>H NMR: (DMSO) δ 3.65 (m, 1H), 3.75 (m, 1H), 4.05 (m, 1H), 4.35 (m, 1H), 5.05 (dd, *J* = 3.4 Hz and *J* = 5.4 Hz, 1H), 6.08 (d, *J* = 3.4 Hz, 1H), 7.54–8.07 (2 m, 5H), 8.72 (s, 1H), 8.77 (s, 1H), 11.21 (s, 1H).

### 3'-Azido-N<sup>6</sup>-benzoyl-5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-deoxyadenosine (**6a**)

Compound **5a** (450 mg, 1.14 mmol) was co-evaporated twice with pyridine and then dissolved in 30 ml pyridine. 4,4'-Dimethoxytriphenylmethyl chloride (0.75 g, 2.20 mmol) and 4-dimethylaminopyridine (75 mg, 0.61 mmol) were added. The reaction mixture was stirred for 24 h at room temperature, 100 ml 5% aqueous NaHCO<sub>3</sub> were added and the reaction mixture was

extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 100$  ml). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$  and evaporated *in vacuo*. The residue was co-evaporated twice with toluene and then purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (94:6 v/v) to yield **6a** as a white foam (620 mg, 77.8%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  3.35 (m, 1H), 3.52 (m, 1H), 3.79 (s, 6H), 4.25 (m, 1H), 4.39 (m, 1H), 5.22 (dd,  $J = 5.2$  Hz and  $J = 5.5$  Hz, 1H), 6.08 (d,  $J = 5.3$  Hz, 1H), 6.79–7.98 (m, 18H), 8.20 (s, 1H), 8.60 (s, 1H), 9.31 (s, 1H).

### 3'-Amino-*N*<sup>6</sup>-benzoyl-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-3'-deoxyadenosine (**1a**)

Compound **6a** (1.80 g, 2.58 mmol) was dissolved in 60 ml ethanol, Pd/C (100 mg, 10% Pd) was added and the reaction mixture was hydrogenated at room temperature for 24 h. The catalyst was removed by filtration, the filtrate was evaporated and the residue was purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (9:1, v/v) to yield **2a** as a white foam (1.61 g, 92.7%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  3.42 (m, 1H), 3.52 (m, 1H), 3.79 (s, 6H), 3.80 (m, 1H), 4.08 (m, 1H), 4.60 (dd,  $J = 1.7$  Hz and  $J = 5.3$  Hz, 1H), 6.08 (d,  $J = 1.6$  Hz, 1H), 6.80–8.00 (m, 18H), 8.29 (s, 1H), 8.62 (s, 1H). LSIMS ( $\text{MH}^+$ )  $m/z$  found 673.3; calculated for  $\text{C}_{38}\text{H}_{36}\text{N}_6\text{O}_6$  672.3.

### 3'-Amino-*N*<sup>6</sup>-benzoyl-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-2'-*O*-(*t*-butyldimethylsilyl)-3'-deoxyadenosine (**9a**)

To compound **2a** (1.61 g, 2.39 mmol), co-evaporated twice from anhydrous pyridine and redissolved in 40 ml pyridine, were added *t*-butyldimethylsilyl chloride (390 mg, 2.59 mmol) and *N*-methylimidazole (0.25 ml, 3.03 mmol). The solution was stirred at room temperature for 24 h, followed by addition of 25 ml saturated aqueous  $\text{NaHCO}_3$ . The reaction mixture was extracted with  $3 \times 100$  ml  $\text{CH}_2\text{Cl}_2$ . The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated. The residue was co-evaporated twice with toluene and purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (95:5 v/v) to yield **9a** as a white foam (1.71 g, 90.5%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  0.15 (s, 3H), 0.21 (s, 3H), 0.90 (s, 9H), 3.48 (m, 1H), 3.60 (m, 1H), 3.66 (m, 1H), 3.79 (s, 6H), 4.08 (m, 1H), 4.65 (d,  $J = 4.2$  Hz, 1H), 6.10 (s, 1H), 6.80–8.00 (m, 18H), 8.36 (s, 1H), 8.79 (s, 1H), 9.29 (s, 1H).

### 3'-Amino-*N*<sup>6</sup>-benzoyl-2'-*O*-(*t*-butyldimethylsilyl)-3'-deoxyadenosine (**7a**)

Compound **9a** (1.68 g, 2.13 mmol) was dissolved in 100 ml 80% aqueous acetic acid and stirred at room temperature for 60 min. Methanol (100 ml) was added and the solvents were evaporated *in vacuo*. The residue was co-evaporated twice with methanol and then purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (95:5 v/v) to yield **7a** as a foam (790 mg, 76.7%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  0.07 (s, 6H), 0.90 (s, 3H), 3.48 (m, 1H), 3.60 (m, 1H), 3.80 (m, 2H), 4.51 (m, 1H), 6.09 (d,  $J = 2.4$  Hz, 1H), 7.54–8.07 (m, 5H), 8.76 (s, 1H), 8.81 (s, 1H).

### *N*<sup>6</sup>-Benzoyl-2'-*O*-(*t*-butyldimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-3'-deoxyadenosine (**8a**)

To compound **7a** (380 mg, 0.78 mmol), co-evaporated twice with anhydrous pyridine and redissolved in 20 ml pyridine, trimethylsilyl chloride (0.5 ml, 4 mmol) was added and the solution was stirred at

room temperature for 2 h. Then 4-methoxytriphenylmethyl chloride (310 mg, 1 mmol) and *N*-methylimidazole (0.08 ml, 1 mmol) were added. The solution was stirred at  $38^\circ\text{C}$  for 48 h and then 25 ml saturated aqueous  $\text{NaHCO}_3$  were added and the solution extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 100$  ml). The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated. The residue was dissolved in 50 ml methanol and 5 ml 25% aqueous  $\text{NH}_3$  were added. The solution was stirred at room temperature for 15 min, concentrated *in vacuo* and the residue was purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (95:5 v/v) to yield **8a** as a white foam (345 mg, 58.0%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  -0.45 (s, 3H), -0.10 (s, 3H), 0.85 (s, 9H), 3.26–3.33 (m, 3H), 3.71 (m, 1H), 3.87 (m, 1H), 3.78 (m, 3H), 4.56 (dd,  $J = 5.3$  Hz and  $J = 5.5$  Hz, 1H), 6.21 (d,  $J = 5.6$  Hz, 1H), 6.80–8.07 (m, 19H), 8.26 (s, 1H), 8.76 (s, 1H), 9.18 (s, 1H). LSIMS ( $\text{MH}^+$ )  $m/z$  found 757.3; calculated for  $\text{C}_{43}\text{H}_{48}\text{N}_6\text{O}_5\text{Si}$  756.38.

### *N*<sup>6</sup>-Benzoyl-2'-*O*-(*t*-butyldimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-3'-deoxyadenosine 5'-(2-cyanoethyl-*N,N*-diisopropyl)phosphoramidite (**2a**)

Compound **8a** (230 mg, 0.31 mmol) was dissolved in 5 ml anhydrous  $\text{CH}_2\text{Cl}_2$ . Diisopropylammonium tetrazolide (52.7 mg, 0.32 mmol) and 2-(cyanoethyl)-*N,N,N',N'*-tetraisopropylphosphorodiamidite (0.32 ml, 0.93 mmol) were added and the solution was stirred at room temperature for 2 h. Saturated aqueous  $\text{NaHCO}_3$  (5 ml),  $\text{H}_2\text{O}$  (10 ml) and  $\text{CH}_2\text{Cl}_2$  (30 ml) were added and, following extraction, the organic phase was separated, dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated. The residue was purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ : $\text{Et}_3\text{N}$  (99:1 v/v) to yield a white foam (205 mg, 77.6%).  $^{31}\text{P}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  149.60, 149.75.

### 3'-Azido-3'-deoxyuracil (**5u**)

Compound **5u** was synthesized as previously described (24).  $^{13}\text{C}$  NMR: ( $\text{D}_2\text{O}$ )  $\delta$  61.54, 61.89, 74.82, 82.75, 88.61, 102.77, 141.30, 151.56, 163.94.

### 3'-Azido-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-3'-deoxyuracil (**6u**)

To compound **5u** (800 mg, 20.97 mmol), co-evaporated twice with anhydrous pyridine and redissolved in 20 ml pyridine, 4,4'-dimethoxytriphenylmethyl chloride (1.92 g, 5.65 mmol) and 4-dimethylaminopyridine (181 mg, 1.47 mmol) were added. The reaction mixture was stirred for 24 h at room temperature. To this was added 5% aqueous  $\text{NaHCO}_3$  (100 ml) and the reaction mixture was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 100$  ml). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated. The residue was co-evaporated twice with toluene and then purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ :MeOH (95:5 v/v) to yield **6u** as a white foam (1.21 g, 71.3%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  3.48 (m, 1H), 3.65 (m, 1H), 3.79 (s, 6H), 4.14 (m, 1H), 4.34 (m, 1H), 4.66 (dd,  $J = 2.2$  Hz and  $J = 5.0$  Hz, 1H), 5.40 (d,  $J = 8.1$  Hz, 1H), 5.94 (d,  $J = 2.2$  Hz, 1H), 6.87–7.44 (m, 13H), 8.03 (d,  $J = 8.1$  Hz, 1H), 8.65 (s, 1H).

### 3'-Amino-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-3'-deoxyuracil (**1u**)

Compound **6u** (1.20 g, 2.10 mmol) was dissolved in 30 ml pyridine:triethylamine (85:15 v/v). The solution was cooled to

0°C and H<sub>2</sub>S was bubbled through the reaction mixture for 30 min. Stirring was continued for another 60 min at 0°C. The solvent was evaporated and the residue co-evaporated with toluene and subsequently dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 ml). The organic phase was washed with 5% NaHCO<sub>3</sub> (100 ml) and H<sub>2</sub>O (50 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The residue was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>:MeOH (97:3 v/v) to yield **1u** as a white foam (825 mg, 71.9%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 3.41 (m, 1H), 3.68 (m, 2H), 3.79 (s, 6H), 3.98 (m, 1H), 4.18 (d, *J* = 3.9 Hz, 1H), 5.35 (d, *J* = 7.9 Hz, 1H), 5.80 (s, 1H, H-1'), 6.85–7.44 (m, 13H), 8.15 (d, *J* = 7.9 Hz, 1H).

### 3'-Amino-5'-O-(4,4'-dimethoxytriphenylmethyl)-2'-O-(t-butyl-dimethylsilyl)-3'-deoxyuracil (**9u**)

To compound **1u** (130 mg, 0.24 mmol), co-evaporated twice with anhydrous pyridine and redissolved in 10 ml pyridine, t-butyl-dimethylsilyl chloride (72 mg, 0.48 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. Saturated aqueous NaHCO<sub>3</sub> (25 ml) was added and the reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 ml). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The residue was co-evaporated twice with toluene and then purified by silica gel column chromatography eluting with *n*-hexane:EtOAc (1:1 v/v) to yield **9u** as a white foam (115 mg, 73.3%). <sup>1</sup>H NMR: (CDCl<sub>3</sub>) δ 0.18 (s, 3H), 0.29 (s, 3H), 0.95 (s, 9H), 3.53–3.94 (3 m, 4H), 3.81 (s, 6H), 4.14 (d, *J* = 3.9 Hz, 1H), 5.32 (d, *J* = 8.1 Hz, 1H), 5.78 (s, 1H, H-1'), 6.86–7.44 (m, 13H), 8.21 (d, *J* = 8.1 Hz, 1H).

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