

7-DEAZAGUANOSINE: PHOSPHoramidite AND PHOSPHONATE BUILDING BLOCKS  
FOR SOLID-PHASE OLIGORIBONUCLEOTIDE SYNTHESIS

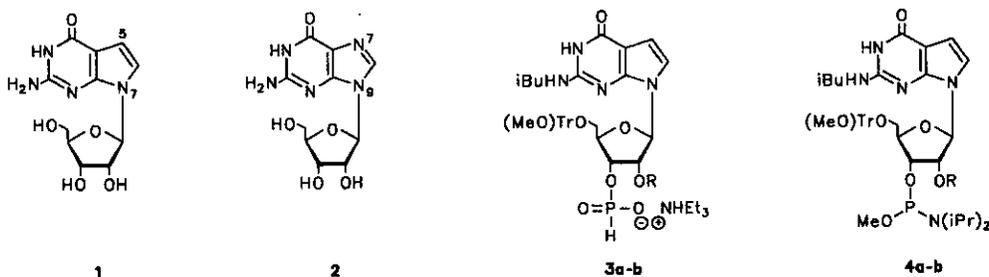
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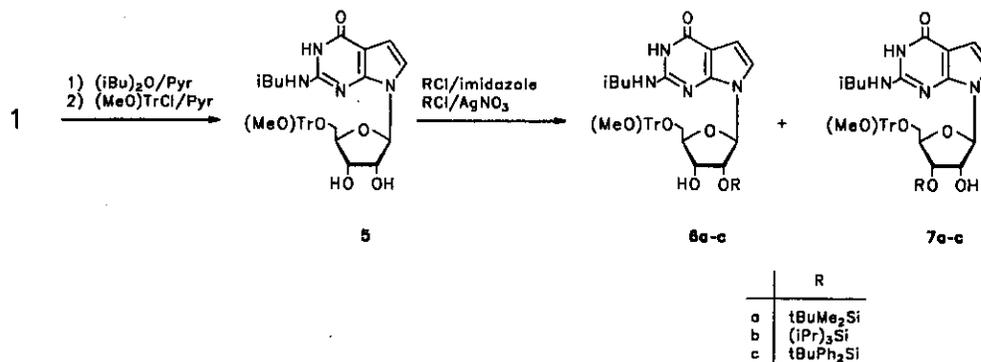
**Abstract**—The synthesis of oligoribonucleotide building blocks (phosphonates and phosphoramidites) of 7-deazaguanosine (2-amino-7-( $\beta$ -D-ribofuranosyl)-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one, **1**) is described. Silylation of the 2'-hydroxy group of 7-deaza-N<sup>2</sup>-isobutyryl-5'-O-(4-monomethoxytrityl)guanosine (**5**) was studied with regard to the 2'-selectivity. Best results were obtained with triisopropylsilyl chloride/AgNO<sub>3</sub>. The 2'-O-protected intermediates (**6a** and **6b**) were converted into the phosphonates (**3a**, **b**) or methylphosphoramidites (**4a**, **b**), which were successfully used for solid-phase oligoribonucleotide synthesis of (c<sup>7</sup>G-C)<sub>3</sub> (**9**).

Guanine-rich regions of nucleic acids can associate to form multi-stranded structures. Enzymatically prepared poly(G) can aggregate to four-stranded helices.<sup>1</sup> Tetramerization of short tracts of G-residues in naturally occurring RNA has also been reported.<sup>2</sup> In the case of 2'-deoxyguanosine containing nucleic acids, so-called G4-DNA<sup>3</sup> is formed.

Earlier investigations have shown that N-7 participates in hydrogen bonding of such structures.<sup>4</sup> As a consequence, we have reported on oligodeoxyribonucleotide building blocks of 7-deaza-2'-deoxyguanosine for solid-phase synthesis<sup>5</sup> as well as di- and triphosphates<sup>6,7</sup> for enzymatic polymerization. In the following we present the synthesis of phosphoramidites and phosphonates of suitably protected 7-deazaguanosine (*c*<sup>7</sup>G, **1**) and their use in solid-phase oligoribonucleotide synthesis.



The starting material, compound (**1**), was synthesized as described earlier.<sup>8,9</sup> For the protection of the exocyclic amino group, the isobutyryl residue was chosen as it was already successfully used for the 2'-deoxy compound.<sup>10</sup> The tetraisobutyrylated derivative was prepared as intermediate according to Khorana<sup>11</sup> and the sugar protecting groups were selectively removed with 2 N aq. NaOH. With a  $\tau_{1/2}$  of 115 min (25 %  $\text{NH}_4\text{OH}$  at 40°C) for 7-deaza-N<sup>2</sup>-isobutyrylguanosine, a similar rate of deprotection was observed as for the 2'-deoxy derivative. As 5'-OH-protecting group, the 4-methoxytrityl residue was introduced by the standard protocol,<sup>12</sup> resulting in **5**.



Ogilvie and Usman<sup>13,14</sup> described RNA-building blocks (cyanoethylphosphoramidites) containing the *t*-butyldimethylsilyl group for 2'-OH-protection. Other authors demonstrated the use of this protecting group in the phosphonate approach.<sup>15</sup> Although silyl residues show several disadvantages - they can isomerize and are difficult to remove from oligomers, their selective deprotection with fluoride secures their current use in oligoribonucleotide synthesis. Three different trialkylsilyl chlorides (*t*BuMe<sub>2</sub>SiCl, *i*Pr<sub>3</sub>SiCl, *t*BuPh<sub>2</sub>SiCl) were used in the case of **5**, and either AgNO<sub>3</sub> or imidazole was employed as a catalyst. As Table 1 indicates, the highest 2'-selectivity was found in the case of triisopropylsilyl chloride and AgNO<sub>3</sub>. In no case, formation of the 2',3'-bis-silylated compound was observed.

The position of the silyl residues was confirmed by <sup>1</sup>H-, <sup>13</sup>C-nmr- and <sup>1</sup>H-nmr NOE difference spectroscopy. Table 2 summarizes <sup>13</sup>C-nmr data. It can be seen that 2'-O-silylation leads to a downfield shift of the C-2' signal; 3'-O-silylation to a deshielding of C-3'. Furthermore, NOE experiments on compound (**6a**) show an NOE of 5% on 3'-OH after irradiation of H-4', while saturation of H-1' gives no effect.<sup>16</sup> On the other hand, irradiation of H-1' of the 3'-O-silylated derivative (**7a**) gives an NOE at 2'-OH (1%), while saturation of H-4' gives no effect.

Compounds (**6a**) and (**6b**) were converted into their methylphosphoramidites<sup>18</sup> [**4a**, **b** (<sup>31</sup>P-nmr (CDCl<sub>3</sub>): δ 151.9 and 150.2 ppm (**4a**); 153.0 and 149.6 ppm (**4b**))] as well as into phosphonates<sup>19</sup> [**3a**, **b** (<sup>31</sup>P-nmr (DMSO-d<sub>6</sub>): δ 2.20 ppm (**3a**) and 2.35 ppm (**3b**))].

As phosphonates are easier to handle than phosphoramidites and are highly stable against oxidation, we decided to use compounds (**3a**, **b**) in automated solid-phase synthesis. Two hexamers, (GC)<sub>3</sub> (**8**) and (c<sup>7</sup>GC)<sub>3</sub> (**9**), were synthesized on an Applied Biosystems 380 B synthesizer. The cycle times for detritylation, coupling, and activation followed the protocol of Stawinski *et al.*<sup>20</sup> The 5'-O-detritylated oligomers were removed from solid support and deprotected at the base with ammonia/EtOH (3 : 1) at 55°C for 16 h. 2'-O-Deprotection was carried out with 1 M Bu<sub>4</sub>NF in THF over 24 h at room temperature. After

desalting (Sephadex G25 F column; Pharmacia, Germany) under sterile conditions with 0.05 M ammonium acetate, 20% polyacrylamide gel electrophoresis (25 cm x 16 cm x 1.5 mm) under denaturing conditions was used for purification.<sup>21</sup> After elution from the gel with 0.1 M ammonium acetate, the oligomers were again desalted on a Sephadex column.

**Table 1.** Formation of 2'- and 3'-Isomers upon Silylation of 5.

Silylating Agent <sup>a)</sup> [mmol]	Catalyst [mmol]		Yield of Isomers <sup>b)</sup> [%] (R <sub>f</sub> ) <sup>c)</sup>		Reaction Time <sup>a)</sup> [h]
	AgNO <sub>3</sub>	imidazole	2'-Isomer	3'-Isomer	
<b>tBuPh<sub>2</sub>SiCl</b>					
2.0	2.0	-	42 (0.65)	30 (0.55)	30
1.5	-	2.0	38	46	30
<b>tBuMe<sub>2</sub>SiCl</b>					
1.5	1.5	-	56 (0.75)	21 (0.7)	24
1.5	-	1.5	25	63	19
<b>iPr<sub>3</sub>SiCl</b>					
2.5	2.5	-	63 (0.7)	16 (0.6)	48
2.5	-	2.5	54	29	48

<sup>a)</sup> In all the cases, THF was used as a solvent; the reaction was started with 0.4 mmol of 5 and 1.0 eq. of silylating agent (1.5 eq. in the case of iPr<sub>3</sub>SiCl) and after 12 h (24 h) the remainder was added to the total amount. <sup>b)</sup> Individual isomers were obtained by silica gel column chromatography (silica gel 60 H, Merck) using CH<sub>2</sub>Cl<sub>2</sub>/light petroleum ether/EtOAc, 1:1:1 as the eluent. <sup>c)</sup> Tlc on silica gel glass plates (Sil G-25 UV<sub>254</sub>, Merck), solvent: CH<sub>2</sub>Cl<sub>2</sub>/light petroleum ether/EtOAc, 1:1:1.

**Table 2.**  $^{13}\text{C}$ -Nmr Chemical Shifts of 7-Deazaguanosine Derivatives<sup>a)</sup>

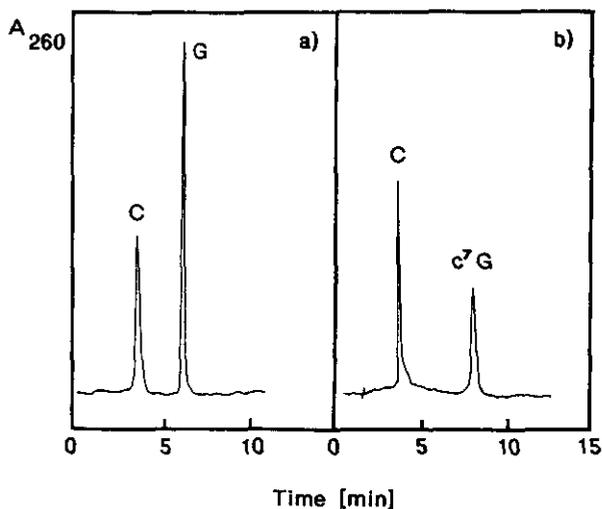
compd. <sup>b)</sup>	C-4	C-2	C-7a	C-6	C-5	C-4a	CHiBu	CH <sub>3</sub> iBu	CO
3a	156.6	148.8	147.2	118.8	103.7	104.0	34.9	18.9	180.1
3b	156.7	148.8	147.2	118.8	103.9	104.1	34.9	18.9	180.1
5 <sup>c)</sup>	156.7	148.2	147.0	119.7	103.1	104.3	34.8	19.0	180.1
6a <sup>d)</sup>	156.6	148.4	146.9	118.9	103.4	104.0	34.7	18.8	180.0
7a <sup>d)</sup>	156.6	148.4	146.9	119.3	103.2	104.3	34.7	18.9	180.0
6b	156.7	148.6	147.0	119.0	103.7	104.2	34.8	18.9	180.1
7b	156.7	148.5	147.0	119.3	103.3	104.4	34.8	19.0	180.1
6c	156.7	148.4	146.9	119.0	103.5	104.3	34.8	18.9	180.1
7c	156.7	148.5	147.0	119.4	103.1	104.4	34.8	19.0	180.1

	C-1'	C-4'	C-2'	C-3'	C-5'	OCH <sub>3</sub>	C-MMT <sub>r</sub>	Me <sub>3</sub> C-Si/Me <sub>2</sub> CH-Si
3a	84.9	83.7	75.0	73.2	63.9	55.1	86.1	17.7
3b	85.2	83.3	74.8	73.6	63.7	55.1	86.2	11.7
5	86.7	82.8	73.7	70.5	64.2	55.1	85.9	-
6a <sup>d)</sup>	85.6	83.9	76.1	70.7	64.0	55.0	86.0	17.7
7a <sup>d)</sup>	85.9	83.4	72.9 <sup>e)</sup>	72.6 <sup>e)</sup>	63.6	55.0	86.1	18.0
6b	86.1	84.1	75.9	71.1	64.0	55.2	86.1	11.8
7b	86.1	84.0	73.4 <sup>e)</sup>	72.5 <sup>e)</sup>	63.7	55.1	86.2	11.8
6c	86.0	84.4	76.4	70.7	63.9	55.2	86.0	18.8
7c	85.9	83.8	73.3 <sup>e)</sup>	73.1 <sup>e)</sup>	63.5	55.1	85.9	19.0

a) Spectra were measured in DMSO- $d_6$  rel. to TMS. b) Systematic numbering. c) Assignment of aglycone signals according to the 2'-deoxyribonucleosides.<sup>10,17</sup> d) Assignment from gated-decoupled spectra. e) Tentative assignment.

The composition of the oligomers (8) and (9) was derived from enzymatic digestion using snake venom phosphodiesterase followed by alkaline phosphatase and analysis by reverse phase RP-18 hplc (Figure). Quantification was made on the basis of peak areas and the extinction coefficients of the nucleosides at 260 nm and shows equivalent amounts of G and C for 8, and  $c^7G$  and C for 9, respectively. The identity of compound (1) was proved by addition of synthetic 1. The successful incorporation of  $c^7G$  (1) into an oligoribonucleotide opens a wide range of experiments to elucidate changes in secondary and tertiary structure caused by replacement of nitrogen-7 by carbon as well as to study the interaction with RNA-processing enzymes or other proteins.



**Figure.** Hplc profiles of enzymatically hydrolyzed hexamers a) 8 and b) 9 carried out on RP-18 column (LiChrosorp (7  $\mu$ m), 250 x 4.9 mm) using 5% MeCN in 0.1 N ammonium acetate; digestion was performed with snake venom phosphodiesterase followed by alkaline phosphatase; retention times were 3.6 min (cytidine), 6.3 min (guanosine) and 7.5 min (7-deazaguanosine).

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