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**A REMARKABLE STABILIZATION OF COMPLEXES FORMED BY 2,6-DIAMINOPURINE  
OLIGONUCLEOTIDE N3'→P5' PHOSPHORAMIDATES.**

Tracy Matray, Soya Gamsey, Krisztina Pongracz and Sergei Gryaznov\*

*Geron Corporation, 230 Constitution Drive, Menlo Park, CA 94025, U.S.A.*

*E.mail: [sgryaznov@geron.com](mailto:sgryaznov@geron.com)*

**ABSTRACT:** 2'-Deoxyribo- and ribo-oligonucleotide N3'→P5'phosphoramidates containing 2,6-diaminopurine nucleosides were synthesized. Thermal denaturation experiments demonstrated a significant stabilization of the complexes formed by these compounds with DNA and RNA complementary strands, relative to adenosine-containing phosphoramidate counterparts. The increase in melting temperature of the complexes reached up to 6.9 °C per substitution. The observed stabilization was attributed to the apparent synergistic effects of N-type sugar puckering of the oligonucleotide N3'→P5' phosphoramidate backbone, and the ability of 2,6-diaminopurine bases to form three hydrogen bonds.

### **Introduction**

Synthetic oligonucleotides have drawn significant attention due to their unique properties, primarily their ability to recognize specifically and efficiently the natural carriers of genetic information - DNA and RNA molecules. The well-known and established Watson-Crick base pairing rules govern this recognition. This unique feature of oligonucleotides led to the development of these compounds as rationally designed therapeutic and diagnostic agents, as well as making them essential tools for molecular biological and biochemical research.<sup>1</sup> Meanwhile, research efforts continue, which are

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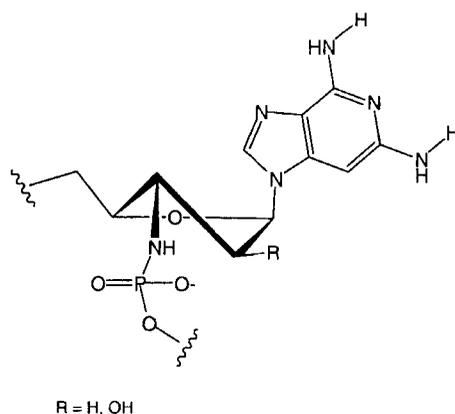
This work is dedicated to the memory of Alexander Antonovich Krayevsky

aimed at the preparation of modified oligonucleotides with enhanced nucleic acid binding characteristics, resistance to nucleases, and with improved pharmacological properties.<sup>2</sup> A significant increase of thermal stability per composing nucleoside unit may allow for the construction of relatively short - 6-8-mer oligonucleotides, which could be used as sequence specific and efficient DNA and RNA recognizing agents.<sup>3</sup>

We would like to report the synthesis and hybridization properties of 2'-deoxy- and 2'-ribo-oligonucleotide analogues containing N3'→P5' phosphoramidate sugar-phosphate linkages and 2,6-diaminopurine bases (FIG. 1).<sup>4</sup> These compounds form exceptionally stable duplexes with complementary DNA and RNA strands, where the increase in melting temperature reached up to 6.9 °C per substituted nucleoside base.

### Results and Discussions

Oligonucleotides with natural phosphodiester groups containing 2,6-diaminopurine (DAP) bases have been previously reported.<sup>5</sup> It was predicted that these compounds would form noticeably more stable duplexes than adenine containing counterparts, since 2,6-diaminopurine can potentially form three hydrogen bonds with a complementary thymine or uracil base, unlike adenine, which forms only two hydrogen bonds. However, the reported experimental results indicated that, in some disagreement with the predictions, the duplexes of DAP-containing phosphodiester oligonucleotides with DNA were only marginally, if at all, more stable than those formed by the adenine containing counterparts. Increase of the duplexes melting temperature,  $\Delta T_m$ , was only 0-0.5 °C per substituted base,<sup>5d,f</sup> which indicates that formation of a third hydrogen bond between the 2-amino group of the purine bases and oxygen-2 of the complementary pyrimidine bases was very inefficient at best. Loss of the B-DNA helix hydration spine, caused by incorporation of 2,6-DAP into DNA oligomers, was also implicated in the lack of the net stabilization of the formed duplexes.<sup>5c</sup> A stronger stabilization was observed for the complexes with RNA strands, where  $\Delta T_m$  reached up to 2°C per substitution.<sup>5c</sup> We rationalized that a C3'-endo conformation of the nucleoside furanose rings would dictate an A-form of the helix, which may be a crucial factor for the proper positioning of the 2,6-diaminopurine base in the duplex and, allow for formation of a third stable hydrogen bond. Consequently,



**FIGURE 1.** Structure of an oligonucleotide N3' → P5' phosphoramidate with a C3'-endo or N-type sugar conformation containing a 2,6-diaminopurine base.

incorporation of 2,6-DAP bases into oligonucleotide N3' → P5' phosphoramidates, which adopt C3'-endo sugar pucker,<sup>6</sup> should result in a significant duplex stabilizing effect.

Thus, model pentamers **2** and **4**, **Table 1**, containing four 2,6-diaminopurines and a 5'-terminal 2'-deoxyadenosine, were synthesized to investigate the binding properties of this base in the context of the N3' → P5' phosphoramidate sugar-phosphate backbone. We found that the 2'-deoxy phosphoramidate pentamer **2** binds unusually well to complementary DNA and especially well to complementary RNA strands. The complexes melting temperature was increased by ~25 °C, or by ~6.2°C per substituted nucleobase - compare experiments 1 and 2, **Table 1**. A similar stabilization effect was observed for 2,6-DAP- containing ribo-N3' → P5' phosphoramidate pentamer **4**, where the increase in the complex melting temperature with RNA reached approximately 28°C. This corresponds to a remarkable ~7°C per residue increase in complex  $T_m$ , when compared to the adenine-containing RNA phosphoramidate pentamer **3**. It is important to note that the 2'-deoxyribo-N3' → P5' phosphoramidate pentamers **1** and **2**, **Table 1**, containing either adenine or 2,6-diaminopurine bases may form triple stranded 2Py : 1Pu complexes with poly d(T) or poly (U) strands under the experimental conditions employed, as was judged by mixing curve experiments. However, in these triple-stranded complexes the 2-amino group of 2,6-diaminopurine is not involved in formation of the Hoogsteen-type hydrogen bonds with the second polypyrimidine strand, which

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

Expt.	5'-Oligonucleotide-3' <sup>a</sup>	$T_m$ , °C <sup>b,c</sup>		$\Delta T_m$ °C/substitution <sup>d</sup>	
		DNA <sup>e</sup>	RNA <sup>e</sup>	DNA	RNA
1.	d-AAAAA, <b>1</b>	22.7; 30.3 <sup>c</sup>	29.8; 44.5 <sup>c</sup>		
2.	d-ADDDD, <b>2</b>	25.1; 34.3	54.2; 69.4	0.6; 1.0 <sup>c</sup>	6.1; 6.2 <sup>c</sup>
3.	d-A-r-AAAA, <b>3</b>	no; no	19.3; 32.3		
4.	d-A-r-DDDD, <b>4</b>	no; <15	47.0; 57.5		6.9; 6.3
5.	d-AAAAAAAAAA, <b>5</b>	30.0	48.0		
6.	d-TAG <sub>3</sub> T <sub>2</sub> AGACA <sub>2</sub> , <b>6</b>	nd	71.7		
7.	d-TDG <sub>3</sub> T <sub>2</sub> DGDCD <sub>2</sub> , <b>7</b>	nd	78.1		1.3
8.	d-TD <sub>4</sub> T <sub>2</sub> D <sub>3</sub> CD <sub>2</sub> , <b>8</b>	nd	38.0 <sup>f</sup>		-10.0
9.	d-TAG <sub>3</sub> T <sub>2</sub> AGACA <sub>2</sub> , <b>9</b>	nd	45.2		

<sup>a</sup> All oligonucleotides contain 3'-NHP(O)(O)O-5' internucleoside phosphoramidate linkages except **9**, expt. 9, which contains natural phosphodiester groups; D corresponds to 2,6-diaminopurine nucleotides;

<sup>b</sup> Melting temperature,  $T_m$ , ( $\pm 0.3^\circ\text{C}$ ) in 150 mM sodium chloride, 10 mM sodium phosphate buffer, pH 7.4, and <sup>c</sup> $T_m$  in the same buffer containing additional 10 mM magnesium chloride - second column of numbers; no -  $T_m$  was not observed; nd -  $T_m$  was not determined; concentration of oligonucleotides  $\sim 2\mu\text{M}$  in 0.5 ml of the buffers; temperature gradient  $1^\circ\text{C}/\text{minute}$ ; all melting curves were reversible upon heating and cooling.

<sup>d</sup> Increase of  $T_m$  per each adenosine substituted by 2,6-diaminopurine; for expt 8 -- change of  $T_m$  per each guanosine substituted by 2,6-diaminopurine relative to oligomer **7**, expt 7;

<sup>e</sup> poly (dT) and poly (U) were used as complementary DNA and RNA in expts 1-5; natural phosphodiester RNA oligomer r-U<sub>2</sub>GUCUA<sub>2</sub>C<sub>3</sub>UA<sub>2</sub>CUC was used as the complement strand in expts 6-8;

<sup>f</sup> broad melting curve.

utilized only N-7 and 6-aminogroup of either adenine or 2,6-diaminopurine bases. This observation is in a good concordance with the reported triplex formation properties of adenosine and 2,6-diaminopurine containing di-nucleotides.<sup>12</sup> It is also important to note that the stoichiometry of the complexes formed by pentamers **1** and **2** is the same. Thus, the observed difference in thermal stability of these complexes is determined solely by substitution of adenosine by 2,6-diaminopurine nucleosides.

In a similar manner, other sugar-phosphate backbone modified oligonucleotide complexes were stabilized by replacement of adenine by 2,6-DAP. Thus, it was recently reported that oligomers

containing an anhydrohexitol sugar-phosphate backbone and a single 2,6-DAP base, result in an increase of duplex  $T_m$  by  $\sim 6^\circ\text{C}$ , or by  $9.5^\circ\text{C}$  for three 2,6-DAP bases.<sup>7,8</sup> Also, introduction of three 2,6-DAP bases into a 2'-O-allyl RNA oligonucleotide resulted in an increase of  $T_m$  for the duplex with RNA by  $\sim 9^\circ\text{C}$ .<sup>9</sup>

Interestingly, stabilization of a complex formed by 2'-deoxy phosphoramidate **2** with a DNA complement was only by  $2.4\text{--}4.0^\circ\text{C}$ , or  $0.6\text{--}1.0^\circ\text{C}$  per substitution. No duplex formation was observed for adenine or 2,6-diaminopurine containing ribo-N3' $\rightarrow$ P5' phosphoramidates **3** and **4** with DNA – experiments 3 and 4, **Table 1**. The observed difference in thermal stability of complexes formed by 2,6-DAP-containing phosphoramidates correlates well with the anticipated structures: A-type complexes stabilized to a much greater extent, than B-type structures. An additional comparison was made to a 2'-deoxy decaadenylate N3' $\rightarrow$ P5' phosphoramidate **5**, experiment 5, **Table 1**. The data revealed that the 10-mer duplex with RNA, where the oligomer forms twenty A-to-U hydrogen bonds, has a lower  $T_m$  (by  $\sim 6^\circ\text{C}$ ), than the duplex formed by the 2,6-diaminopurine containing pentamer, which theoretically has only fourteen Watson-Crick type hydrogen bonds - compare experiments 2 and 5, **Table 1**.

The enhanced binding properties of 2,6-diaminopurine-containing oligonucleotides to RNA complements is probably not solely a result of the additional hydrogen bonding capabilities of the base. In aqueous solutions an additional hydrogen bond contributes approximately  $1^\circ\text{C}$  or  $0.4\text{--}1.3$  kcal/mole to the duplexes' melting temperature.<sup>10</sup> Thus, taking only hydrogen bonding between bases into account, the net increase in melting temperature for oligomers **2** and **4** relative to **1** and **3** should be approximately  $5^\circ\text{C}$ , and yet we have observed an approximately  $25\text{--}28^\circ\text{C}$  increase. It is possible that a synergistic interaction between the N3' $\rightarrow$ P5' phosphoramidate sugar phosphate backbone and the 2,6-diaminopurine bases, results in a complex structure, which allows formation of additional (3'-NH-to-phosphate backbone) inter strand hydrogen bonds, most likely mediated by surrounding water molecules. Extensive hydration of the N3' $\rightarrow$ P5' phosphoramidate duplex's major groove, associated with the presence of 3'-aminogroups (as donors and acceptors of hydrogen bonds), was demonstrated previously through crystallographic analysis.<sup>11</sup>

Additionally, increased  $\pi$ - $\pi$  stacking interactions of adjacent DAP bases may account for a substantial fraction of the enhanced binding of 2,6-diaminopurine-containing oligomers. To evaluate the stacking contributions of 2,6-DAP, the mixed base oligonucleotide **7**, **Table 1**, containing non-contiguous 2,6-diaminopurines (a model compound where 2,6-DAP stacking interactions are interrupted) was prepared and shown to form a more stable duplex than either the adenine-containing oligonucleotide **6** –  $T_m$  of 78.1 °C, or the parent phosphodiester **9** –  $T_m$  of 45.2 °C. The increase in the duplex's  $T_m$  for oligonucleotide **7** was only 6.4 °C or 1.3 °C per 2,6-DAP base substitution. This indicates that the base stacking could be an important factor contributing to the complexes' overall thermal stability. However, it may also be unreasonable to expect a more significant change in melting temperature of an already very stable complex, formed by adenine-containing phosphoramidate **6**,  $T_m$  above 71 °C, expt.6, **Table 1**.

Base pairing of 2,6-diaminopurine is highly specific with complementary uracil or thymine pyrimidines, but not with cytidine bases. Substitution of guanine - cytidine by 2,6-diaminopurine-cytidine base pairs as in oligonucleotide **8**, **Table 1**, resulted in a dramatic destabilization of the duplex by -40 °C, or by 10 °C per each 2,6-diaminopurine - cytidine mismatch – compare experiments 7 and 8, **Table 1**. Further investigations including NMR, X-ray, and molecular modeling studies will be needed to evaluate the structural features of the 2,6-diaminopurine containing phosphoramidate duplexes, which result in the observed stabilization effects.

In summary, oligonucleotide N3'→P5' phosphoramidates with 2,6-diaminopurine replacing adenine offer a high affinity structural motif for specific recognition of nucleic acids, especially RNA molecules.

### Experimental Section

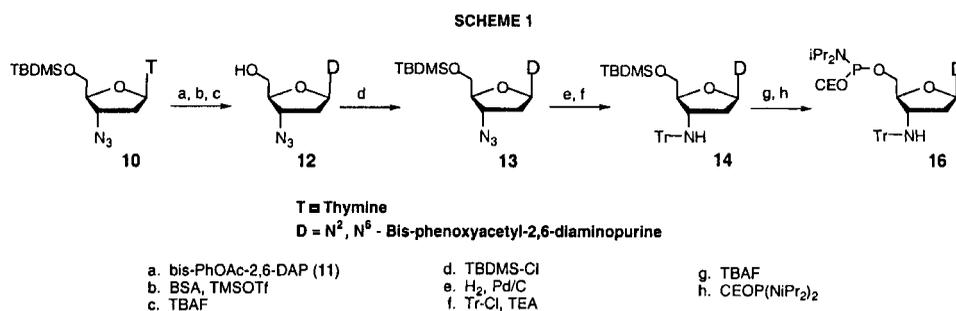
Oligonucleotide N3'→P5' phosphoramidates were prepared on an ABI 394 DNA/RNA synthesizer using 5'-phosphoramidites of fully protected 3'-aminonucleosides using CPG-based solid phase supports as described before.<sup>4</sup> The manufacturer recommended DNA and RNA 1  $\mu$ mole scale synthesis protocols were used for the synthesis of DNA and RNA phosphoramidate oligomers

respectively, with all the standard reagents purchased from Cruachem or Glen Research. At the end of the synthesis (for 2'-deoxy oligonucleotides) the protecting groups were removed in ammonia at 55 °C, 6 hours, and the product was desalted on NAP-5 gel filtration columns (from Pharmacia) and lyophilized *in vacuo*. Oligonucleotide ribo-N3'→P5'phosphoramidate were additionally (after deprotection with ammonia/ethanol, 3/1, v/v, 55 °C, 6-8 hours) treated with 1.0 M tetrabutylammonium fluoride (TBAF) solution in tetrahydrofuran for 16 hours at room temperature, followed by gel filtration. Ion exchange (IE) HPLC analysis and purification of oligonucleotides were performed on a DIONEX DX 500 system using Pharmacia MonoQ 10/10 ion exchange column at pH 12 (10 mM NaOH) with a 1%/min linear gradient of 1.5 M NaCl in 10 mM NaOH, flow rate of 2 ml/min. After purification the oligonucleotides were desalted by gel filtration on NAP-5 columns. <sup>1</sup>H NMR and <sup>31</sup>P NMR experiments were performed on a VARIAN Unity Plus 400 MHz instrument. Thermal dissociation experiments were performed on a CARY 100 Bio UV/VIS spectrophotometer equipped with a temperature controller and data processor. Absorbency values at 260 nm were collected at 1 minute intervals at a heating rate of 1.0°C/min.

**Preparation of 5'-O- cyanoethyl-N,N-diisopropylamino phosphoramidite-3'-aminotriyl - 2'-deoxy - (or 2'- O-TBDMS) - ribonucleoside building blocks:**

Chemical transformations used for preparation of these phosphoramidite building blocks are outlined in **SCHEMES 1** and **2**.

**A) Synthesis of 2'-deoxyribo-2,6-diaminopurine monomer building blocks ( SCHEME 1 ) :**



**5'-O-(*t*-Butyldimethylsilyl)-3'-azido-3'-deoxy-thymidine, 10**

5'-Hydroxy-3'-azido-thymidine (16 g, 60 mmole) was dried by co-evaporation with anhydrous pyridine and then dissolved in anhydrous pyridine (200 ml). *Tert*-butyldimethylsilyl chloride (18 g, 120 mmole) and 4-(dimethylamino)-pyridine (730 mg, 6 mmol) were added, and the reaction mixture was stirred overnight at room temperature. The reaction was quenched with methanol (15ml), and the mixture evaporated *in vacuo* to dryness. The oily residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (200 ml) and washed with sat. NaHCO<sub>3</sub> (2 x 100 ml), and with brine (2 x 100 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to an oil. The product was purified by silica gel flash column chromatography using a 0-2% gradient of methanol in CH<sub>2</sub>Cl<sub>2</sub> to yield 15 g (75%) of pure **1** as a foam. <sup>1</sup>H NMR, (CDCl<sub>3</sub>): δ 9.36 (br s, 1H NH), 7.40 (s, 1H, H-6), 6.19 (t, 1H, H-1', J=6.6 Hz) 4.19 (m, 1H H-3'), 3.93-3.74 (m, 3H, H-4', H-5'), 2.37 (m, 1H, H-2'), 2.19 (m, 1H, H-1'), 1.87 (s, 3H, -CH<sub>3</sub>), 0.89 (s, 9H, -C(CH<sub>3</sub>)<sub>3</sub>), 0.09 (s, 6H, Si-(CH<sub>3</sub>)<sub>2</sub>).

**N<sup>2</sup>,N<sup>6</sup>-Bis-phenoxyacetyl-2,6-diaminopurine, 11**

2,6-Diaminopurine (15 g, 100 mmol) was suspended in anhydrous pyridine (200 ml), cooled to 0°C, and phenoxyacetic anhydride (86 g, 300 mmol) was added. The mixture was heated for 3h at 85°C. Upon cooling, the product precipitated, after which the reaction mixture was evaporated *in vacuo* to a 10 ml volume. Water (1000 ml) was added and the mixture was stirred overnight at room temperature. The light brown crystalline material was filtered, suspended in sat. NaHCO<sub>3</sub> (500 ml) for 20 minutes, and then filtered again. The resulting product (40 g, 95%) was dried in a dessicator over P<sub>2</sub>O<sub>5</sub>. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 10.48 (br s, 1H NH), 8.24 (s, 1H, H-8), 7.24 (m, 4H, aromatic protons), 6.91 (m, 6H, aromatic protons), 5.04 (s, 2H -CH<sub>2</sub>), 5.00 (s, 2H, -CH<sub>2</sub>). ES MS m/z (M+H)<sup>+</sup> 419, (M-H)<sup>-</sup> 417.

**N<sup>2</sup>,N<sup>6</sup>- Bis-phenoxyacetyl-3'-azido-2',3'-dideoxy-β-2,6-diaminopurine, 12**

Compounds **10** (10 g, 26.2 mmole) and **11** (44g, 105 mmole) were suspended in anhydrous acetonitrile (260 ml) and *N,O*-bis-(trimethylsilyl)acetamide (BSA) (51.8 ml, 209 mmole) was added. The reaction mixture was heated at 85°C for 45 minutes. Upon addition of 1 more equivalent of BSA a clear brown solution was obtained. After 15 minutes of additional stirring the reaction mixture was cooled to room temperature and trimethylsilyl trifluoromethanesulfonate (9.5 ml, 52.4

mmole) was added. The reaction mixture was refluxed for 2 hours. After cooling the reaction mixture to 0°C, a cold saturated solution of ammonia in methanol (260 ml) was added to precipitate excess heterocyclic base **11**. After stirring for an additional 15 minutes at 0°C the methanolic ammonia was removed *in vacuo*. The remaining suspension was filtered, and the filtrate was evaporated to an oil and subjected to flash column chromatography using a 0-2% gradient of methanol in CH<sub>2</sub>Cl<sub>2</sub> to yield 8 g of crude N<sup>2</sup>,N<sup>6</sup>-bis-phenoxyacetyl-5'-*O*-TBDMS-3'-azido-2',3'-dideoxy- $\alpha,\beta$ -2,6-diaminopurine as an orange foam. This mixture of  $\alpha,\beta$ -anomers (8 g, 12 mmole) was dissolved in anhydrous THF (100 ml), and tetrabutylammonium fluoride (1M in THF, 18 mmol) was added. The reaction was stirred for 3.5 hours, and then evaporated to dryness. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated NaHCO<sub>3</sub> and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The resulting oil was subjected to silica gel flash column chromatography using a 1.5-2 % gradient of methanol in CH<sub>2</sub>Cl<sub>2</sub>. Fractions containing the faster eluting product ( $\beta$ -isomer) were combined and evaporated to yield 1.2 g (4.5% yield based on nucleoside **10**) of pure **12** as a white foam. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  9.84 (br s, 1H, imino), 9.527 (s, 1H, NH), 7.972 (s, 1H, H-8), 7.309-7.245 (m, 4H, aromatic protons), 7.01-6.96 (m, 6H, aromatic protons), 6.13 (dd, 1H, H-1', J H1'-H2'=7.0 Hz, J H1'-H2''=5.0 Hz), 5.27 (br s, 1H, OH), 4.79 (t, 1H, H-3'), 4.73 (d, 2H, -CH<sub>2</sub>), 4.58 (d, 2H, -CH<sub>2</sub>), 4.01-3.79 (m, 3H, H-4', H-5'), 3.31 (m, 1H, H-2''), 2.47 (m, 1H, H-2'). ES MS m/z, (M+Na)<sup>+</sup> 582, (M-H)<sup>-</sup> 558.

**N<sup>2</sup>,N<sup>6</sup>-Bis-phenoxyacetyl-5'-*O*-(*tert*-butyldimethylsilyl)-3'-azido-2',3'-dideoxy- $\beta$ -2,6-diaminopurine, **13****

Compound **12** (1.2 g, 2 mmol) was dried by co-evaporation with anhydrous pyridine and then dissolved in anhydrous pyridine (100 ml). *Tert*-butyldimethylsilyl chloride (0.75 g, 5 mmole) and DMAP (20 mg, 0.16 mmol) were added and the reaction mixture was stirred overnight. The solvent was removed *in vacuo*, and the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (150 mL). The organic material was washed with saturated NaHCO<sub>3</sub> (100mL) and brine (100 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. The organic layer was evaporated *in vacuo* to yield compound **13** as oil (1.3 g, 95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  9.29 (br s 1H, NH), 9.04 (br s, 1H, NH), 8.15 (s, 1H, H-8), 7.31 (m, 4H, aromatic protons), 7.02 (m, 6H, aromatic protons), 6.30 (dd, 1H, H-1' J H1'-H2'=6.6 Hz, J H1'-H2''=5.0 Hz), 4.90 (m, 2H, -CH<sub>2</sub>),

4.70 (m, 3H, H-3', -CH<sub>2</sub>), 4.01 (m, 1H, H-4'), 3.91-3.77 (m, 2H, H-5'), 2.90 (m, 1H, H-2''), 2.55 (m, 1H, H-2'), 0.85 (s, 9H, -C(CH<sub>3</sub>)<sub>3</sub>) 0.04 (s, 3H, Si-CH<sub>3</sub>), 0.03 (s, 3H, Si-CH<sub>3</sub>). ES MS m/z, (M+H)<sup>+</sup> 674, (M-H)<sup>-</sup> 672.

**N<sup>2</sup>,N<sup>6</sup>-Bis-phenoxyacetyl-5'-O-(tert-butylidimethylsilyl)-3'-aminotriptyl-2',3'-dideoxy-β-2,6-diaminopurine, 14**

Compound **13** (1.3 g, 1.9 mmol) was dissolved in an ethanol/dichloromethane solution (1/1, v/v, 200 ml), and 10% palladium on carbon (300 mg) was added. The system was evacuated and purged with hydrogen several times. The reaction mixture was kept overnight under a hydrogen atmosphere with vigorous stirring. The reaction mixture was filtered, after which the filtrate was evaporated to dryness *in vacuo*, and then co-evaporated with pyridine (10 ml). The resulting material was dissolved in dry pyridine (100 ml), and triphenylmethyl chloride (1.55 g, 5.6 mmol), DMAP (20 mg), and triethylamine (0.8 ml, 5.6 mmol) were added. The reaction mixture was stirred overnight at 40 C°. The reaction was quenched by addition of water (100 ml) after which the product was extracted with diethylether (3 x 300 ml). The combined organic phases were evaporated to dryness and the resulting oil was precipitated from CH<sub>2</sub>Cl<sub>2</sub>/hexane, resulting in 1.5 g of crude **14**. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.93 (s, 1H, H-8), 7.50-6.99 (m, 25H, aromatic protons), 6.22 (t, 1H, H-1'), 4.92 (m, 2H, -CH<sub>2</sub>), 4.73 (m, 2H, -CH<sub>2</sub>), 3.86 (m, 2H, H-3', H-4'), 3.75 (m, 1H, H-5'), 3.69 (m, 1H, H-5'), 1.69 (m, 1H, H-2''), 1.59 (m, 1H, H-2'), 0.76 (s, 9H, -C(CH<sub>3</sub>)<sub>3</sub>), -0.07 (s, 3H, Si-CH<sub>3</sub>), -0.08 (s, 3H, Si-CH<sub>3</sub>). ES MS m/z, (M+H)<sup>+</sup> 890, (M-H)<sup>-</sup> 888.

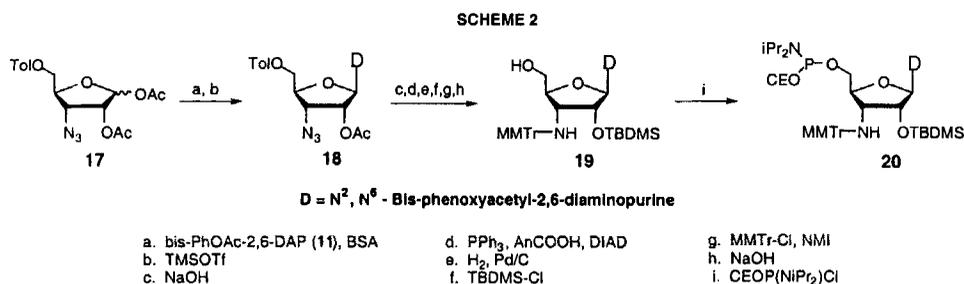
**N<sup>2</sup>,N<sup>6</sup>-Bis-phenoxyacetyl-3'-aminotriptyl-2',3'-dideoxy-β-2,6-diaminopurine, 15**

Compound **14** (1.5 g, 1.7 mmol) was dissolved in anhydrous THF (20 ml) and TBAF (1.0 M in THF, 3.4 mmol) was added. The reaction mixture was stirred for 3h and then diluted with CH<sub>2</sub>Cl<sub>2</sub> (150 ml), washed with saturated NaHCO<sub>3</sub> and brine. The organic material was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to yield 1.3 g of crude **15** as foam. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.87 (s, 1H, H-8), 7.52-6.98 (m, 25H, aromatic protons), 6.11 (t, 1H, H-1' J=6.4 Hz), 4.89 (m, 2H, -CH<sub>2</sub>), 4.63 (m, 2H, -CH<sub>2</sub>), 3.80 (m, 2H, H-3', H-4'), 3.71 (m, 1H, H-5'), 3.59 (m, 1H, H-5'), 1.82 (m, 1H, H-2''), 1.62 (m, 1H, H-2'). ES MS m/z, (M+Na)<sup>+</sup> 798, (M-H)<sup>-</sup> 774.

***N*<sup>2</sup>,*N*<sup>6</sup>-Bis-phenoxyacetyl-3'-aminotriyl-2',3'-dideoxy-β-2,6-diaminopurine-5'-(2-cyanoethyl-*N,N*-diisopropylamino)phosphoramidite, 16**

To compound **15** (1.2 g, 1.55 mmol) dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 ml) was added diisopropylammonium tetrazolide (0.53 g, 3.1 mmol) and 2-cyanoethyl-*N,N,N,N*-tetraisopropylamino-phosphorodiamidite (1 ml, 3.1 mmol), and the reaction mixture was stirred at room temperature for 2.5 hours. The mixture was then diluted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated NaHCO<sub>3</sub> and brine. After drying over Na<sub>2</sub>SO<sub>4</sub> the organic layer was evaporated *in vacuo*. The oily residue was subjected to silica gel flash column chromatography using ethylacetate : hexane : triethylamine, (70/30/1, v/v/v) as eluent to yield **16** (500 mg, 33.1% from **14**). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ 149.34, 149.18. ES MS *m/z* (M+Na)<sup>+</sup> 998, (M-H)<sup>-</sup> 974.

**B) Synthesis of *ribo*-2,6-diaminopurine monomer building blocks (SCHEME 2) :**



**3'-Azido-2'-*O*-acetyl-5'-*O*-toluoyl-*N*<sup>2</sup>,*N*<sup>6</sup>-phenoxyacetyl-2,6-diaminopurine, 18**

*N*<sup>2</sup>,*N*<sup>6</sup>-phenoxyacetyl-*N*<sup>2</sup>,*N*<sup>6</sup>,9-tris(trimethylsilyl)-2,6-diaminopurine was prepared *in situ* from 5.0 g (11.9 mmol) of *N*<sup>2</sup>,*N*<sup>6</sup>-bis(phenoxyacetyl)-2,6-diaminopurine **11** and BSA (41.6 mmol) as described before in the section A. This compound was then combined with 3-azido-1,2-bis-*O*-acetyl-5-*O*-toluoyl-3-deoxy-D-ribofuranose **17**<sup>13</sup> (3.0 g, 8.0 mmol) in dry toluene (100 ml), and trimethylsilyl triflate (2.4 ml, 12.0 mmol) was added. The reaction mixture was heated to 80°C and stirred for 4 h, cooled to room temperature and diluted with ethyl acetate (400 ml). The resulting solution was washed with sat. NaHCO<sub>3</sub> (200 ml) and sat. NaCl (200 ml). The aqueous phases were back-extracted with ethyl acetate (100 ml), and the combined organic phases were filtered through celite

to remove unreacted  $N^2,N^6$ -phenoxyacetyl-2,6-diaminopurine. The filtrate was dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo* to give a yellow foam. The residue was purified by silica gel chromatography eluting with a gradient of EtOAc : hexanes (65:35, v/v to 80:20, v/v) to afford 3'-azido-2'-*O*-acetyl-5'-*O*-toluoyl- $N^2,N^6$ -phenoxyacetyl-2,6-diaminopurine as a yellow foam (3.52 g, 60%).  $^1\text{H NMR}$ : ( $\text{CDCl}_3$ )  $\delta$  2.19 (s, 3H), 2.28 (s, 3H), 4.35-4.40 (m, 1H), 4.45-4.76 (m, 4H), 4.80 (s, 2H), 6.89 (s, 1H), 6.95 (s, 2H), 6.98-7.11(m, 8H), 7.26-7.38 (m, 4H), 7.78 (d,  $J = 8.8$  Hz, 2H), 7.91 (s, 1H), 9.15, (br s, 1H), 9.28 (br s, 1H). Exact mass (HR FAB $^+$ ) calculated for  $\text{C}_{36}\text{H}_{33}\text{N}_9\text{O}_9$  ( $\text{M}+\text{Na}^+$ ) 758.2299, found 758.2314.

**$N^2,N^6$ -phenoxyacetyl-2'-*O*-(*t*-butyldimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-2,6-diaminopurine, 19**

3'-Azido-2'-*O*-acetyl-5'-*O*-toluoyl- $N^2,N^6$ -phenoxyacetyl-2,6-diaminopurine **18** (2.8 g, 3.8 mmol) was dissolved in a 1.0 M NaOH solution (65/30/5 pyridine/MeOH/ $\text{H}_2\text{O}$ , v/v/v, (60 ml)) at  $0^\circ\text{C}$ . The mixture was stirred for 7 min and quenched by addition of sat.  $\text{NH}_4\text{Cl}$  (500 ml). The solution was extracted with  $\text{CH}_2\text{Cl}_2$  (4 x 200 ml) and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo*. The resulting material was dissolved in dimethylformamide (50 mL) and  $\text{PPh}_3$  (1.5 g, 5.7 mmol) was added. *p*-Anisic acid (0.87 g, 5.7 mmol) and diisopropyl azodicarboxylate (1.12 mL, 5.7 mmol) were dissolved in dimethylformamide (5 ml) and added dropwise to the reaction mixture. The solution was stirred at room temperature for 3 h and was then quenched by pouring into sat.  $\text{NaHCO}_3$  (500 ml). The mixture was extracted with ethyl acetate (2 x 200 ml) after which the combined organic phases were washed with sat. NaCl (150 ml). The organic phase was then dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was passed through a short pad of silica gel eluting with EtOAc:Hexanes:MeOH (47.5:47.5:5, v/v/v).

The crude material then was dissolved in a mixture of EtOH (100 ml) and  $\text{CH}_2\text{Cl}_2$  (25 ml), 10% Pd/C (300 mg) was added, and the reaction mixture was hydrogenated at room temperature for 15 h. The catalyst was removed by filtration and washed well with pyridine (100 ml). The filtrate was concentrated *in vacuo* and then resuspended in dry pyridine (40 mL). Imidazole (0.52 g, 7.6 mmol) and *t*-butyldimethylsilyl chloride (0.86 g, 5.7 mmol) were added to the mixture which was then stirred at room temperature for 15 h. The reaction was then diluted with  $\text{CH}_2\text{Cl}_2$  (200 ml) and washed with sat.  $\text{NaHCO}_3$  (200 ml) and sat. NaCl (50 ml). The combined aqueous phases were

back-extracted with additional  $\text{CH}_2\text{Cl}_2$  (50 ml). The organic layers were dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated *in vacuo* to give a viscous material. This material was subsequently dissolved in pyridine (40 ml) and 4-methoxytriphenylmethyl chloride (4.7 g, 15.2 mmol) and *N*-methylimidazole (1.2 ml, 15.2 mmol) were added. The reaction mixture was warmed to  $40^\circ\text{C}$  for 60 h and then quenched by MeOH (3 ml). The mixture was concentrated *in vacuo* to give a brown viscous residue which was purified by silica gel chromatography eluting with EtOAc:hexanes (50:50, v/v) to yield a white foam (0.96 g, 24% for 5 steps).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  -0.09 (s, 3H), 0.11 (s, 3H), 0.87 (s, 9H), 2.81-2.88 (m, 2H), 3.23-3.35 (m, 1H), 3.52 (s, 3H), 3.83 (s, 3H), 4.39-4.42 (m, 1H), 4.67-4.91 (m, 4H), 5.05 (br, s, 2H), 5.61 (s, 1H), 6.57 (d,  $J = 8.8$  Hz, 2H), 6.81-6.89 (m, 4 H), 6.89-7.13 (m, 10H), 7.22-7.38 (m, 10H), 7.62 (d,  $J = 8.8$  Hz, 2H), 8.13 (s, 1H), 8.78 (br s, 1H), 9.39 (br s, 1H). Molecular mass (ESI<sup>+</sup>) calculated for  $N^2,N^6$ -phenoxyacetyl - 2' - O - (t-butyldimethylsilyl) - 3' - (4-methoxytriphenylmethylamino)-5'-*O*-anisoyl-2,6-diaminopurine -  $\text{C}_{60}\text{H}_{63}\text{N}_7\text{O}_{10}\text{Si}$  (M+Na<sup>+</sup>) 1092, found 1092.

The isolated  $N^2,N^6$ -phenoxyacetyl - 2' - O - (t-butyldimethylsilyl) - 3' - (4-methoxytriphenylmethylamino)-5'-*O*-anisoyl-2,6-diaminopurine (0.95 g, 0.90 mmol) was treated with 1.0 M NaOH in mixture of pyridine/MeOH/ $\text{H}_2\text{O}$ , 65/30/5, v/v/v (20 ml) at  $0^\circ\text{C}$ . The mixture was stirred for 5 min, and quenched by addition of sat.  $\text{NH}_4\text{Cl}$  (250 ml). The solution was extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 100 mL) and the organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography eluting with EtOAc:hexanes:MeOH (47.5:47.5:5, v/v/v) to give  $N^2,N^6$ -phenoxyacetyl-2'-*O*-(t-butyldimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-2,6-diaminopurine **19** (0.5 g, 60%) as a white foam.  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ )  $\delta$  -0.34(s, 3H), -0.12 (s, 3H), 0.80 (s, 3H), 3.07-3.11 (m, 1H), 3.21-3.24 (m, 1H), 3.48-3.54 (m, 2H), 3.71 (s, 3H), 3.86-3.88 (m, 1H), 4.71 (br s, 2H), 4.93 (br s, 2H), 6.02 (d,  $J = 5.2$  Hz, 1H), 6.72 (d,  $J = 8.4$  Hz, 2H) 6.97-7.46 (m, 22H), 8.19 (s, 1H), 8.93 (br s, 1H), 9.39 (br s, 1H). Exact mass (HR FAB<sup>+</sup>) calculated for  $\text{C}_{52}\text{H}_{57}\text{N}_7\text{O}_8\text{Si}$  (M+H<sup>+</sup>) 936.4114, found 936.4148.

**$N^2,N^6$ -phenoxyacetyl-2'-*O*-(t-butyldimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-2,6-diaminopurine-5'-(2-cyanoethyl-*N,N*-diisopropylamino) phosphoramidite, **20****

$N^2,N^6$ -phenoxyacetyl-2'-*O*-(t-butyldimethylsilyl)-3'-(4-methoxytriphenylmethylamino)-2,6-

diaminopurine **19** (0.50 g, 0.53 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 ml), 2-cyanoethyl-N,N-diisopropylamino-chlorophosphoramidite (0.15 ml, 0.70 mmol) and N,N-diisopropylethylamine (0.37 ml, 2.1 mmol) were added. The solution was stirred at room temperature for 3 h and then diluted with CH<sub>2</sub>Cl<sub>2</sub> (150 ml). The resulting solution was washed with sat. NaHCO<sub>3</sub> (150 mL) and sat. NaCl (100 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and then concentrated *in vacuo*. The residue was purified by silica gel chromatography eluting with EtOAc:hexanes (50:50, v/v) containing 1% triethylamine to yield **20** as a white foam (0.45 g, 75%). <sup>31</sup>P NMR: (CDCl<sub>3</sub>) δ 149.41, 149.46.

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