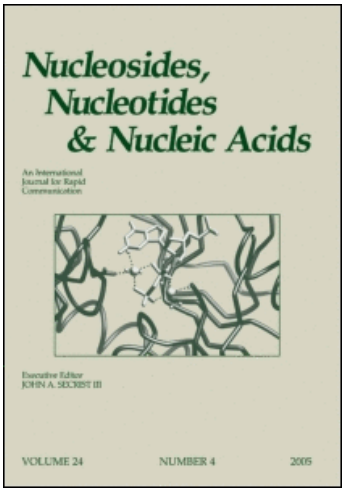

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SYNTHETIC OLIGONUCLEOTIDE COMBINATORIAL LIBRARIES. 3. SYNTHESIS OF POLYAMINONUCLEOSIDES

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ABSTRACT: Synthesis of polyamino-2'-deoxynucleosides was studied. A synthesis of 2'-deoxyadenosine and 2'-deoxyguanosine derivatives carrying a protected spermine moiety at *N*-6 and *N*-2 positions respectively is described using unprotected polyamines as substrates. The question of reactivity of primary and secondary amino groups present in polyamines was studied. The evidence that only primary amino groups react with the synthesised precursors was accomplished from experiments with a secondary amine (di-*n*-butylamine). An approach to analyse properties of polyaminooligonucleotides using their synthetic combinatorial libraries is discussed.

Introduction

Combinatorial chemistry allows the study of the properties of many classes of compounds much faster than a classical serial analysis. Synthetic combinatorial libraries of many different compounds were prepared and used to reveal their properties. Exploring of molecular diversity of organic compounds facilitates finding of new leads in drug design as well as optimising their structures.[1-3]

Main factors governing a structure and biological functions of nucleic acids are known. However, we are still missing their full understanding due to a very complex and flexible network of their interactions. One should point out that modification of a nucleic acid structure results in changes of conformation and a network of intra– and intermolecular interactions which, in turn, may change their biological properties. This fact is very well known from the studies of RNAs and especially tRNAs structures that contain modified nucleotides in high extent. Thus, one has to rely on experimental data

for each specific modified oligonucleotide structure and sequence. Studies performed on model modified oligonucleotides can be used only as a starting point for further, more detailed research. A combinatorial approach to study properties of compounds should be very useful for modified oligonucleotides.

Peptides and oligonucleotides are among the first compounds studied by a combinatorial approach.[1-3] Synthetic oligonucleotides find various applications in molecular biology and numerous applications in diagnostics and therapy including antisense and antigene therapy.[4] Many different types of analogues of nucleic acids were studied including modifications in internucleotide bonds as well as modifications of sugar and nucleobase residues.[4] Among these oligonucleotide analogues are conjugates with biogenic polyamines and their analogues.

For many years the influence of natural polyamines on stabilisation of nucleic acid tertiary structures and their complexes is well known.[5-8] In recent years oligonucleotide derivatives bearing various polyamine residues attached at different positions in nucleic acids were synthesised and their properties were investigated. Thus, oligonucleotides derived both through 5'- and 2'-hydroxyl function were obtained.[9-12] Another group of oligonucleotides modified with polyamines contains heterocyclic bases with polyamine moieties attached at different positions.[13-17] In all cases a stabilising effect of polyamines on complexes of nucleic acids was observed.[13-17]

We have undertaken systematic studies of oligonucleotides modified in the base moieties.[18,19] Moreover, we would like to apply a combinatorial approach in order to investigate their properties more effectively. Studies on polyaminonucleoside building blocks for polyaminooligonucleotide synthesis are described in this paper.

Results and Discussion

Polyamine moieties can be introduced into oligonucleotides either on a level of nucleoside building blocks (a direct synthesis) or post–synthetically by reaction of appropriate polyaminonucleosides' precursors present in oligonucleotide chains. The direct approach was applied in cases reported in the literature for 5'- and 2'-hydroxyl groups of an oligonucleotide sugar ring[12] and oligonucleotides containing thymidine, deoxyuridine and deoxycytidine units modified with polyamine group *via* methylene

group of thymine,[13] at position *C*-5 of uracil,[15] and *N*-4 of cytosine.[14,18] The post–synthetic introduction of polyamine residues into oligonucleotides was applied at their 5'-end[9-11] as well as for deoxyguanosine,[16,17] deoxycytidine,[20] and deoxyadenosine[21,22] units modified at positions *N*-2, *N*-4, and *N*-6 respectively.

The introduction of the polyamino group during the final deprotection step is more difficult to control due to analytical and purification problems encountered for these polar compounds. This might become a more serious obstacle when many modification sites are considered in a single oligonucleotide chain. A post–synthetic introduction of various polyamines seems to be very complicated. On the contrary, an approach based on using appropriately protected nucleoside building blocks appears to be free of the above restraints. In general, this approach is more flexible and allows for a better control of purity of modified oligonucleotide analogues derived from chemical synthesis.

Appropriate precursors should be used in a case of the direct approach to prepare oligonucleotides carrying polyamine residues at nucleobases. Then, polyaminonucleosides after introduction of suitable protecting groups, are transformed into their nucleotide building blocks, *e.g.* 3'-phosphoramidites or *H*-phosphonates.

Many different nucleoside intermediates could be used to obtain polyamino-2'-deoxynucleosides. The use of polyamines as unprotected substrates would be the most convenient approach avoiding preparation of partially protected polyamines. Such an approach seems a reasonable one, especially if the use of symmetrical polyamines is considered at the initial stage of studies. Moreover, if the participation of secondary amino groups of polyamines in nucleobase modifications could be excluded, then using an excess of polyamine would practically suppress formation of α,ω-disubstituted polyamine derivatives.

Thus, it was necessary to choose proper reactive nucleosides, which might react with polyamines. The reactive nucleoside derivatives should be also 5'-*O*-protected with 4,4'-dimethoxytrityl group as this functionality would add lipophilicity to polyaminonucleoside products and thus, significantly ease the workup procedure and removal of the excess of polyamine.

Polyamine derivativse of 2'-deoxyadenosine : Recently a synthesis of 9-(2-deoxy-*β*-D-*erythro*-pentofuranose)-6-(1,2,4-triazol-4-yl)purine (**2**) was reported.[23,24] It was also

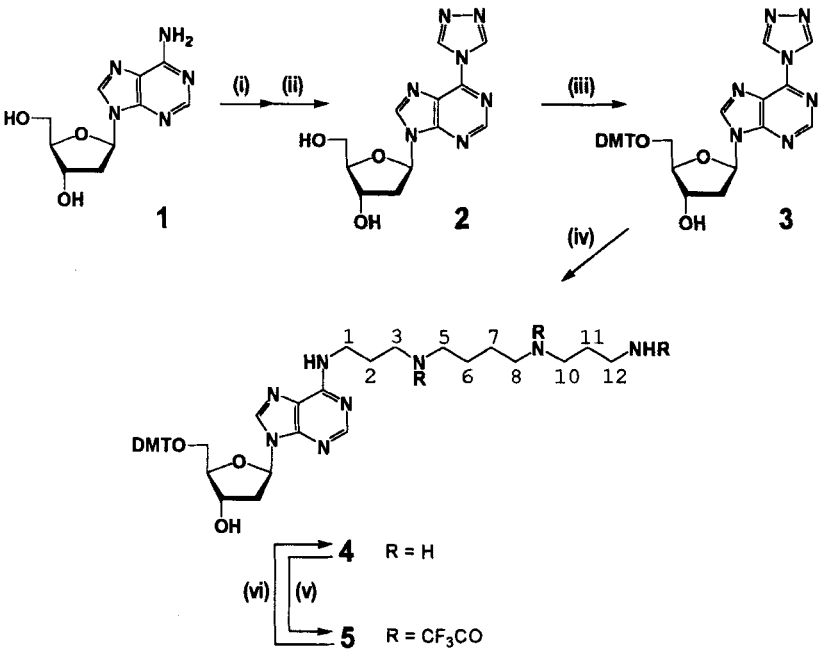

Figure. 1. The synthetic route to obtain the spermine derivative of 2'-deoxyadenosine: (i) TMSCl (2.0 eq.), 1,2-bis[(dimethylamino)methylene]-hydrazine (3.9 eq.), pyridine, 100 °C, 24 h; (ii) MeOH, overnight, overall yield 70 %; (iii) DMTCl (1.3 eq.), pyridine, 6 h, yield 89 %; (iv) spermine (10 eq.), pyridine, 70 °C, 8 h, yield 97 %; (v) $(CF_3CO)_2O$ (5 eq.), pyridine, 5 min., yield 87 %; (vi) 32 % NH_3 – pyridine (1:1), 50 °C, overnight. Numbering of atoms in amino side chains is used to ease description of NMR spectra.

shown that **2** reacts with ammonia and dimethylamine to give deoxyadenosine and 6-*N*,*N*-dimethyl-2'-deoxyadenosine respectively.[23,24] We have taken the advantage of 9-(5-*O*-dimethoxytrityl-2-deoxy-*β*-D-*erythro*-pentofuranose)-6-(1,2,4-triazol-4-yl)purine (**3**) in preparation of spermine derived deoxyadenosine analogue, namely 5'-*O*-dimethoxytrityl-6-*N*-(4,9,13-triazatridecane-1-yl)-2'-deoxyadenosine (**4**) (FIG. 1).[19] *N*-Substituted deoxyadenosine analogues can be obtained from deoxyadenosine in higher overall yields *via* the triazole route than from deoxyinosine *via* its 6-*O*-arylsulphonyl derivatives. Synthesis of 6-*O*-arylsulphonyl-2'-deoxyinosine is accompanied by almost the same yield of 1-*N*-arylsulphonyl-2'-deoxyinosine.[25] Compound **2** was obtained by the reported procedure,[23,24] with some minor modifications, and transformed in high yield into its 5'-*O*-dimethoxytrityl derivative **3**. Then, **3** was reacted with tenfold excess of

spermine in pyridine at 70 °C during 8 h to give **4** as single product in 97 % yield. Crude **4** was then reacted during 5 min. with trifluoroacetic anhydride in pyridine at room temperature to give **5** in 87 % yield.

Polyamine derivative of 2'-deoxyguanosine : Several procedures to obtain 2-fluoro-6-*O*-(4-nitrophenylethyl)-2'-deoxyinosine (**8**) have been reported.[26-30] A mechanism of nucleophilic substitution is based on replacement of the guanine amino group by a fluorine atom *via* a diazonium salt intermediate. In most cases, a significant excess of hydrogen fluoride, used as a source of fluoride ions, is applied. Those experiments were repeated in our laboratory with rather low yields.[19] Adib *et al.*[30] have published a procedure for obtaining the 2-fluoro derivative of deoxyinosine in which polyvinylpyridinium polyhydrogenfluoride (PVPHF)[31] – a notably milder source of fluoride anions – was used. It has allowed the generation of the desired product in high yield. In our approach the 5'- and 3'-hydroxyl functions of 6-*O*-(4-nitrophenylethyl)-2'-deoxyguanosine (**6**) were protected with 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDSi) group[32] yielding 98 % of **7**. Compound **7** was converted into the corresponding 2-fluoro deoxyinosine derivative using *tert*-butyl nitrite and PVPHF, using the reported reaction conditions (Fig. 2).[27] Thus, 2-fluoro-6-*O*-(4-nitrophenylethyl)-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyinosine was formed as a main product as was confirmed by its NMR spectra. Some minor compounds of lower chromatographic mobility were also formed, presumably 2-fluoro-6-*O*-(4-nitrophenylethyl)-2'-deoxyinosine derivatives protected either at 3'- or 5'-hydroxyl with 3-hydroxy- or 3-fluorotetraisopropyldisiloxane-1-yl group[32] (a partially cleaved TIPDSi group). Hence, the silylated intermediates were not isolated and removal of the protecting group from the ribose ring was carried out on crude products. 2-Fluoro-6-*O*-(4-nitrophenylethyl)-2'-deoxyinosine (**8**) was obtained in a good overall yield (58 %) and was then transformed into its 5'-*O*-dimethoxytrityl derivative **9**. Introduction of a spermine moiety was performed in pyridine at room temperature with tenfold excess of the polyamine. It was observed that after approximately 1 h, besides the main product **10**, the undesired spermine derivative of 2'-deoxyguanosine (**14**) began to form, so after 4 h, the reaction was stopped. The TLC analysis indicated that about 5 % of the substrate **9** had not reacted, and roughly 10 % of **14** was produced as well. Column chromatography gave

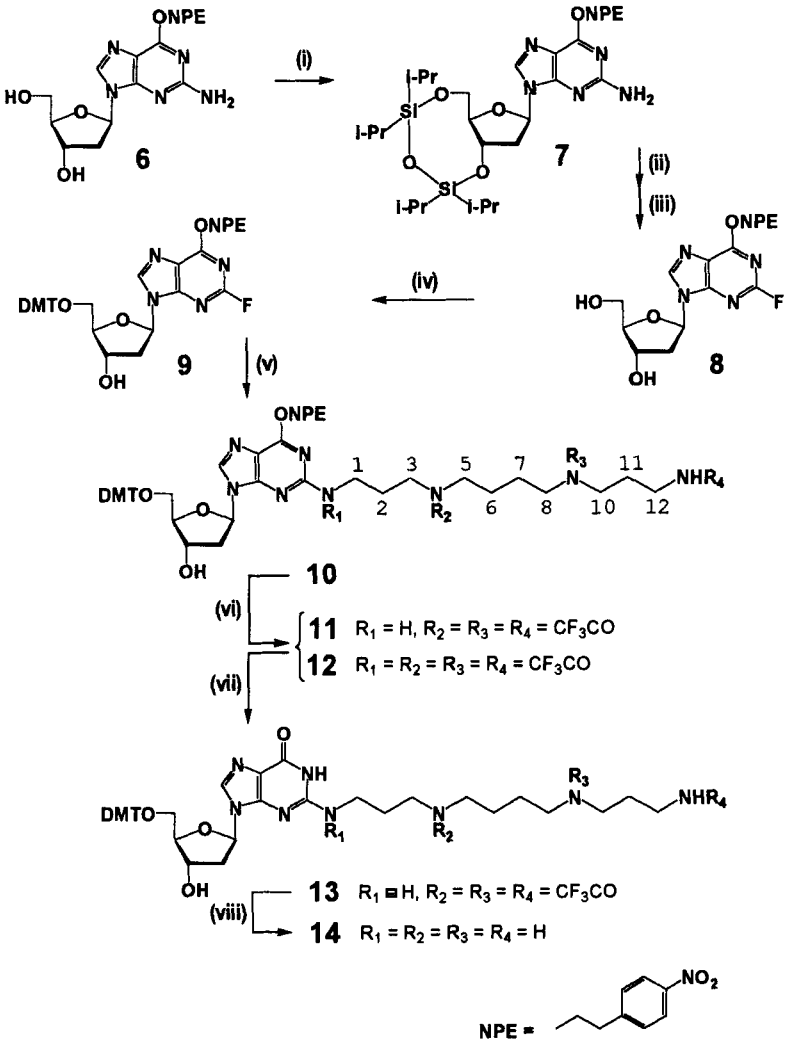

Figure 2. The synthetic route to obtain the spermine derivative of 2'-deoxyguanosine: (i) $TIPDSiCl_2$ (1.5 eq.), pyridine, 2.5 h, yield 98 %; (ii) PVPHF, t-BuONO (2 eq.), toluene, 2 h, -5 to 0 °C; (iii) aq. HF in pyridine, 2 h 40 min., yield 58 %; (iv) DMTCl (1.2 eq.), pyridine, 3 h, yield 94 %; (v) spermine (10 eq.), pyridine, room temp., 1.5 h, yield 80 %; (vi) $(CF_3CO)_2O$ (5 eq.), pyridine, 30 min., 0 °C to room temp., yield 58 %; (vii) 0.5 M DBU in pyridine, 2 h; (viii) 32 % NH_3 – pyridine (1:1), overnight, 50 °C.

almost pure **10** in 80 % yield. The next stage was to protect amino groups of the spermine residue using 5 eq. of trifluoroacetic anhydride and pyridine as a solvent. The initially formed tetrakis(trifluoroacetyl) derivative **12** was not sufficiently stable during chromatography and could not be obtained as the pure compound. The MS analysis of the obtained mixture of **11** and **12** confirmed the structures of both compounds as the tris- and tetrakis(trifluoroacetyl) derivatives respectively. The mixture of compounds **11** and **12** gave quantitatively the tris(trifluoroacetyl) derivative **11** after 4 h treatment with *ca.* 65% methanol in dichloromethane at room temperature. The structure of the stable derivative **11** was confirmed by NMR and MS spectra. In order to simplify synthetic procedures we decided to use the mixture of trifluoroacetyl derivatives in further experiments.

Reactivity of polyaminonucleoside precursors towards secondary amines : Due to the presence of primary and secondary amino groups in spermine molecule and their different nucleophilicity it was necessary to test the reactivity of polyaminonucleoside precursors with a secondary amine upon the same reaction conditions as applied in analogous set of experiments with spermine. Under these reaction conditions no reaction with di-*n*-butylamine was observed for **3**, **9**, **15** and **16** (FIG. 3). However, if a mixture of pyridine and water (1:1) was used as a solvent the reaction of **3** with di-*n*-butylamine gave **17** in 30 % yield. Some unknown minor products were also observed.

In a case of the precursor **9** increasing the reaction temperature to 70 °C resulted in the formation of **19** in 60 % yield. It should be pointed out that in the above reaction conditions presence of an amine causes complete removal of a protecting 4-nitro-phenylethyl group from 6-*O* position of guanine moiety.[19]

Also some interesting observations were made when working with 5-bromo-5'-*O*-dimethoxytrityl derivative of deoxyuridine (**16**). The compound **16** did not to react with di-*n*-butylamine, so it was subjected to tenfold excess of putrescine at room temperature. At the beginning of the reaction, an expected product started to appear, but after 6 to 8 h its concentration started to diminish and a mixture of unknown by–products was produced. Therefore, we applied the polyaminonucleosides functionalised *via* methylene group of thymine.[33]

Removal of protecting groups from polyaminonucleosides : Removal of protecting groups from **5** and the mixture of **11** and **12** to obtain **4** and **14** respectively (FIG. 1 and

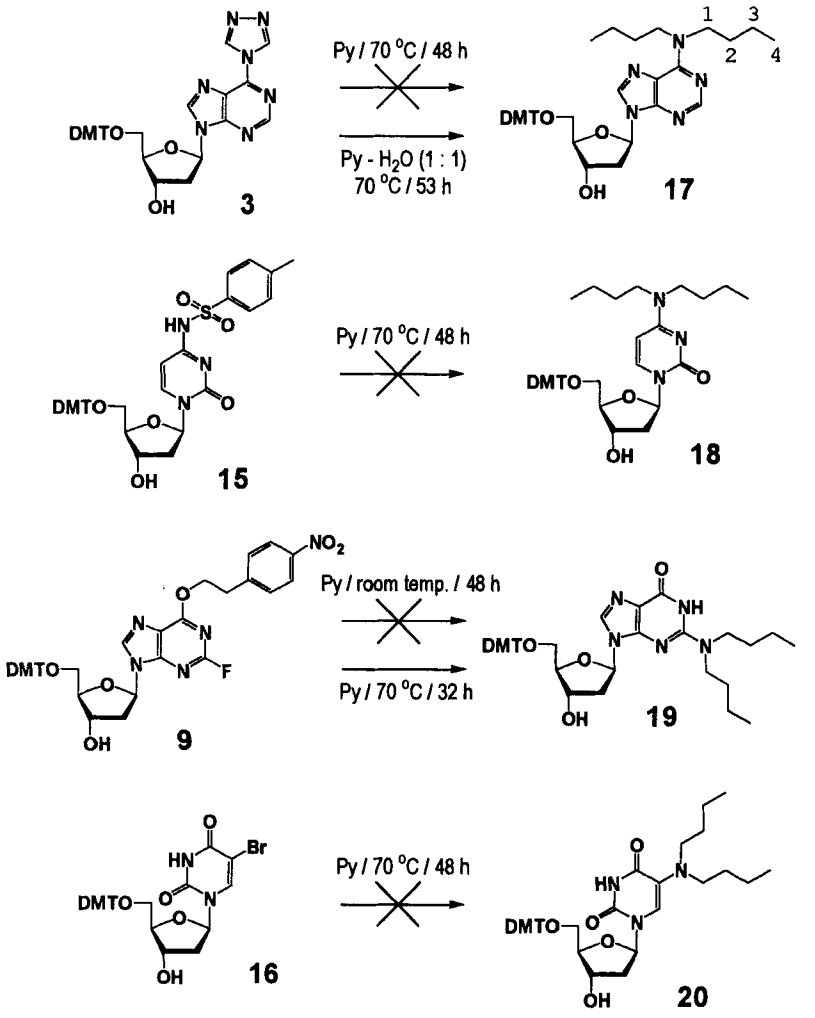

Figure. 3. Reactions of polyaminonucleosides' precursors with di-*n*-butylamine. In all experiments tenfold excess of amine was used.

2) would be another proof, besides NMR analysis of the final products, that trifluoroacetylation reactions have occurred correctly. Trifluoroacetyl protecting groups of the amino functions of spermine residues would be removed from polyaminooligonucleotides post–synthetically. So, conditions for deprotection of exo-amino groups at heterocyclic moieties were applied with one modification, *e.g.* a mixture of pyridine and 32 % ammonium (1:1) was used. The presence of pyridine was

necessary to dissolve **5, 11** and **12**. This change of conditions could only slow down removal of trifluoroacetyls. The reactions were carried out overnight at 50 °C. The TLC analysis has shown quantitative removal of the protecting groups. In a case of spermine derivatives of 2'-deoxyguanosine **11** and **12**, first 6-*O* position was deprotected applying 0.5 M solution of DBU in pyridine. The reaction went to completion after 2 h and **13** was obtained without formation of any side products. As the 4-nitrophenylethyl group was removed from 2-fluoro-5'-*O*-dimethoxytrityl derivative of deoxyinosine **9** a mixture of unknown side products was formed and the corresponding 5'-*O*-dimethoxytrityl-2-fluoro-2'-deoxyinosine[19] was obtained in a low yield (*ca.* 35 %) after chromatographic purification.

Experimental

All the solvents used in the reactions were purified and dried according to procedures published earlier. The organic extracts were dried over anhydrous sodium sulphate. *tert*-Butyl nitrite was prepared based on the standard procedure.[34] 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[32] ($TIPDSiCl_2$) was provided by Ifotam Ltd. (Poland). POCh (Poland) supplied hexamethyldisilazane. Diethyl azodicarboxylate – DEAD, poly[4-vinylpyridiniumpoly(hydrogen fluoride)] – PVPHF, and 2-(4-nitrophenyl)-ethanol were purchased from Aldrich. Spermine and trifluoroacetic anhydride were supplied by Fluka. Triphenylphosphine was purchased from Organica (Belgium).

Thin layer chromatography (TLC) was performed on E. Merck pre–coated plates: (i) silica gel 60 HF_{254} in the following solvent systems: (A) dichloromethane – methanol (9:1, by volume), (B) dichloromethane – methanol (95:5, by volume), (ii) silica gel 60 HF_{254} silanised (RP) in the solvent system (C) methanol – 40 % aqueous methylamine – water (7:1:2, by volume). Short column chromatography was performed on silica gel Merck H 60 in dichloromethane containing methanol or in a mixture of methanol, methylamine, and water.

^1H NMR data were obtained with a 300 MHz Varian Unity 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. ^{19}F NMR spectra were recorded on the same apparatus operating at 282.2 MHz using trifluoroacetic acid

as external standard. Centrifugation was performed using BECKMAN centrifuge model J2-21.

9-(2-deoxy-β-D-*erythro*-pentofuranose)-6-(1,2,4-triazol-4-yl)purine (2) 2'-Deoxyadenosine (251 mg, 1 mmol) was evaporated with anhydrous pyridine (4×3 ml). Then, it was dissolved in pyridine (2.5 ml) and 1,2-bis[(dimethylamino)methylene]-hydrazine (560 mg, 3.9 mmol, 3.9 eq.) and TMSCl (250 µl, 2 mmol, 2.0 eq.) was poured. The reaction was carried out under reflux at 100 °C. After 24 h, the reaction was checked by the TLC which revealed that most of silyl were hydrolysed. Then, an extra silylation step was carried out with 0.5 eq. of TMSCl. Dichloromethane was added (20 ml) and extracted with 1 M aq. HCl (3×20 ml) and subsequently was evaporated under reduced pressure. The oily crude was dissolved in 4 ml of MeOH and left for several hours at room temperature. Compound **2** crystallised from methanol and was filtered. The crystals were washed with hexane and ethyl ether to give 212 mg of the product, 70 % yield. R_f(A) = 0.11. ^{1}H NMR (DMSO-d_6): δ 9.65 (s, 2H, H-3, 5 of triazole), 9.02 (s, 1H, H-8), 8.94 (s, 1H, H-2), 6.54 (t, 1H, *J* 6.3 Hz, H-1'), 5.43 (d, 1H, *J* 4.5 Hz, OH-3'), 5.03 (t, 1H, *J* 5.4 Hz, OH-5'), 4.51-4.45 (m, 1H, H-3'), 3.95-3.91 (m, 1H, H-4'), 3.69-3.52 (m, 2H, H-5', 5''), 2.85-2.76 (m, 1H, H-2'), 2.46-2.38 (m, 1H, H-2''). ^{13}C NMR (DMSO-d_6/D_2O): δ 153.28 (C-4), 151.99 (C-2), 146.00 (C-8), 142.69 (C-6), 141.03 (2C of triazole), 122.67 (C-5), 88.09 (C-4'), 84.27 (C-1'), 70.42 (C-3'), 61.31 (C-5'), 39.49 (C-2').

9-(5-*O*-Dimethoxytrityl-2-deoxy-β-D-*erythro*-pentofuranose)-6-(1,2,4-triazol-4-yl)purine (3) Compound **2** (1.0 g, 3.3 mmol) was evaporated with anhydrous pyridine (4×30 ml), then the substrate **2** was dissolved in 60 ml of anhydrous pyridine and DMTCl (1.23 g, 3.63 mmol, 1.1 eq.) was added. After 3 h and then, 5 h 45 min. two portions of DMTCl (2×112 mg, 0.66 mmol, 0.2 eq.) were added. The reaction went to completion after 6 hours. The reaction mixture was partitioned between saturated $NaHCO_3$ (100 ml) and CH_2Cl_2 (100 ml). The aqueous phase was extracted with dichloromethane (3×100 ml). The organic extracts were evaporated under reduced pressure and the residue was subjected to short column chromatography. The product **3** eluted in CH_2Cl_2/MeOH (98:2 – 96:4). Fractions were evaporated and the product was lyophilised from dioxane to give 1.785 g of yellowish–white solid, 89 % yield. R_f(B) = 0.23. ^{1}H NMR (DMSO-

d_6): δ 9.65 (s, 2H, H-3, 5 of triazole), 8.91 (s, 1H, H-8), 8.84 (s, 1H, H-2), 6.69-7.31 (m, 13H, DMT aromatic), 6.56 (t, 1H, *J* 6.6 Hz, H-1'), 5.46 (d, 1H, *J* 4.5 Hz, OH-3'), 4.49-4.57 (m, 1H, H-3'), 4.05-4.1 (m, 1H, H-4'), 3.68 (2×s, 6H, 2×OCH_3 of DMT), 3.14-3.27 (m, 2H, H-5', 5"), 2.95-3.03 (m, 1H, H-2'), 2.4-2.46 (m, 1H, H-2").

5'-*O*-Dimethoxytrityl-6-*N*-[tris(*N*,*N'*,*N''*-trifluoroacetyl)-4,9,13-triaza-tridecane-1-yl]-2'-deoxyadenosine (5) Compound **3** (1.5 g, 2.47 mmol) was dissolved in pyridine (25 ml). After adding spermine (4.5 g, 24.7 mmol, 10 eq.) the reaction flask was tightly closed and placed in an oven at 70 °C. The reaction was finished after 8 h and the solution was partitioned between water (100 ml) and dichloromethane (100 ml). The organic phase was extracted twice with water (2×100 ml). Each time emulsion was appearing, so the mixture was centrifuged (20 min. and 40 min., speed: 16 000 RPM). The organic layer was evaporated under diminished pressure to obtain 1.78 g of **4** as white foam, 97 % yield, R_f(C, RP) = 0.61. ^{1}H NMR (DMSO-d_6): δ 8.23 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.92 (s, 1H, NH), 6.77-7.35 (m, 13H, DMT aromatic), 6.36 (t, 1H, *J* 6.6 Hz, H-1'), 4.48 (m, 1H, H-3'), 3.96-4.0 (m, 1H, H-4'), 3.71-3.72 (2×s, 6H, 2×OCH_3 of DMT), 2.28-3.5 (m, 22H, H-2', 2", 5', 5", H-1, 3, 5, 8, 10, 12 of spermine residue), 1.4-1.75 (m, 8H, H-2, 6, 7, 11 of spermine residue). R_f(C, RP) = 0.66.

The product was dried in a desiccator overnight. Then, **4** was dissolved in 25 ml of anhydrous pyridine. The reaction vessel was closed with a rubber stopper and trifluoroacetic anhydride (1.629 ml, 12.05 mmol, 5 eq.) was poured using a syringe. After 5 min. the reaction was worked–up with saturated $NaHCO_3$ (100 ml) and CH_2Cl_2 (3×100 ml). The extracted organic fractions were taken to dryness evaporated and purified by short column chromatography and eluted with CH_2Cl_2/MeOH (98:2 – 97:3) to give 2.207 g of **5** as a yellowish–white foam, 87 % yield. R_f(A) = 0.5. ^{1}H NMR (DMSO-d_6): δ 9.45-9.53 (m, 1H, NH-13 of spermine residue), 8.25 (2×s, 1H, H-8), 8.16 (s, 1H, H-2), 7.86-7.93 (m, 1H, C(6)-NH), 6.77-7.35 (m, 13H, DMT aromatic), 6.37 (t, 1H, 6.0 Hz, H-1'), 5.37 (d, 1H, *J* 4.5 Hz, OH-3'), 4.45-4.52 (m, 1H, H-3'), 3.98 (q, 1H, H-4'), 3.71-3.72 (2×s, 6H, 2×OCH_3 of DMT), 3.16-3.5 (m, 14H, H-5', 5", H-1, 3, 5, 8, 10, 12 of spermine residue), 2.84-2.92 (m, 1H, H-2'), 2.3-2.37 (m, 1H, H-2"), 1.53-1.96 (m, 8H, H-2, 6, 7, 11 of spermine residue). FAB-MS: $[MH]^+$ 1027.3 calc. for $C_{47}H_{52}F_9N_8O_8$ 1027.95.

6-*O*-(4-Nitrophenylethyl)-2'-deoxyguanosine (6) 2'-Deoxyguanosine (5.34 g, 20 mmol) was suspended in dry DMF (30 ml) and hexamethyldisilazane (8 ml, 80 mmol, 4 eq.) was added. After 10 min., the reaction mixture became almost limpid and then immediately a white solid precipitated. After a further 5 min. period, the silylation reaction was complete and the excess of hexamethyldisilazane and the solvent were evaporated. The crude 3',5'-bis-O-trimethylsilyl-2'-deoxyguanosine was suspended in dry 1,4-dioxane (100 ml) and 2-(4-nitrophenyl)-ethanol (5.432 g, 32.5 mmol, 1.6 eq.), triphenylphosphine (12.648 g, 48.2 mmol, 2.4 eq.), and DEAD (7.686 ml, 48.8 mmol, 2.4 eq.) were added. After 4 h of stirring, the reaction was finished and dioxane was evaporated to give red oil. The last step of the synthesis was removal of trimethylsilyl groups from a sugar residue using HF in pyridine, which was prepared by mixing 10.76 ml of 40 % aqueous HF with 200 ml of pyridine and evaporating the resulting solution to a volume of approximately 110 ml. In this reagent the oily crude from the previous step was dissolved. The reaction was carried out for 1.5 hour. The solution was partitioned between saturated $NaHCO_3$ (150 ml) and CH_2Cl_2 (150 ml). The aqueous phase was extracted with dichloromethane (2×150 ml). The organic extracts were evaporated under reduced pressure and the residue was subjected to short column chromatography (eluting in CH_2Cl_2/MeOH, 95:5) to obtain the title product as pale–yellow foam: 5.879 g, 68 % overall yield. R_f(A) = 0.41, R_f(B) = 0.1. ^{1}H NMR ($CDCl_3$): δ 8.17-8.21 (m, 2H, 2×CH aromatic of 4-NPE), 8.09 (s, 1H, H-8), 7.62-7.65 (m, 2H, 2×CH aromatic of 4-NPE), 6.47 (s, 2H, NH_2), 6.19-6.23 (dd, 1H, $J_{1'-2',\,2''}$ 9.0 Hz, 7.8 Hz, H-1'), 5.28 (br, 1H, OH-3'), 4.97 (br, 1H, OH-5'), 4.65-4.7 (t, 2H, *J* 6.9 Hz, CH_2 aliphatic of 4-NPE), 4.34-4.36 (m, 1H, H-3'), 3.81-3.84 (m, 1H, H-4'), 3.47-3.6 (m, 2H, H-5', 5''), 3.23-3.28 (t, 2H, *J* 6.6 Hz, CH_2 aliphatic of 4-NPE), 2.54-2.62 (m, 1H, H-2'), 2.17-2.24 (m, 1H, H-2'').

6-*O*-(4-Nitrophenylethyl)-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2'-deoxyguanosine (7) 6-*O*-(4-nitrophenylethyl)-2'-deoxyguanosine (**6**) (1.173 g, 2.7 mmol) was evaporated with anhydrous pyridine (4×15 ml). Then, it was dissolved in anhydrous pyridine (11 ml) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.289 ml, 4.05 mmol, 1.5 eq.) was added. After 2.5 h, the reaction went to completion. The solution was partitioned between 0.1 M $NaHCO_3$ (70 ml) and CH_2Cl_2 (70 ml). The aqueous phase was extracted with dichloromethane (2×70 ml). The organic extracts were evaporated

under reduced pressure and the residue was purified by short column chromatography (eluting in CH_2Cl_2/MeOH, 98:2) to obtain the title product 7 as pale–yellow foam: 1.8 g, 98 % yield. R_f(B) = 0.51. ^{1}H NMR (DMSO-d_6): δ 8.17-8.2 (m, 2H, 2×CH aromatic of 4-NPE), 7.98 (s, 1H, H-8), 7.62-7.65 (m, 2H, 2×CH aromatic of 4-NPE), 6.44 (s, 2H, NH_2), 6.13-6.17 (dd, 1H, $J_{1'-2',\,2''}$ 7.5 Hz, 3.6 Hz, H-1'), 4.72-4.77 (m, 1H, H-3'), 4.65-4.69 (t, 2H, *J* 6.9 Hz, CH_2 aliphatic of 4-NPE), 3.76-3.96 (m, 3H, H-5', 5", 4'), 3.23-3.28 (t, 2H, *J* 6.9 Hz, CH_2 aliphatic of 4-NPE), 2.7-2.79 (m, 1H, H-2'), 2.45-2.54 (m, 1H, H-2"), 0.94-1.1 (m, 28H, 4×$CH(CH_3)_2$ of TIPDSi group).

2-Fluoro-6-*O*-(4-nitrophenylethyl)-2'-deoxyinosine (8) To a polypropylene bottle filled with argon, PVPHF (1.345 g) and anhydrous toluene (8 ml) were added. The bottle was closed tightly and placed in a cool bath (-10°C). Then, compound 7 (500 mg, 0.74 mmol) in anhydrous toluene (5 ml), and *tert*-butyl nitrite (315 ml, 1.48 mmol, 2 eq.) were added using syringes. After 2 h of stirring at -5 to 0 °C, the reaction mixture was filtered to remove PVPHF. Fluorination reagent was washed twice with toluene and twice with ethyl acetate. The mixture was partitioned between 0.1 M $NaHCO_3$ (150 ml) and ethyl acetate (3×150 ml). The organic layers were collected and evaporated under reduced pressure to give 533 mg of oily crude consisting mainly of 2-fluoro-6-*O*-(4-nitrophenylethyl)-3',5'-*O*-(tetra-isopropyldisiloxane-1,3-diyl)-2'-deoxyinosine: ^{1}H NMR (DMSO-d_6): δ 8.44 (s, 1H, H-8), 8.17-8.2 (m, 2H, 2×CH aromatic of 4-NPE), 7.63-7.66 (m, 2H, 2×CH aromatic of 4-NPE), 6.27-6.31 (dd, 1H, $J_{1'-2',\,2''}$ 8.1 Hz, 2.4 Hz, H-1'), 5.06-5.13 (m, 1H, H-3'), 4.81-4.85 (t, 2H, *J* 6.3 Hz, CH_2 aliphatic of 4-NPE), 3.77-3.93 (m, 3H ,H- 5', 5", 4'), 3.28-3.33 (t, 2H, *J* 6.3 Hz, CH_2 aliphatic of 4-NPE), 2.82-2.9 (m, 1H, H-2'), 2.54-2.64 (m, 1H, H-2"), 0.97 (m., 28H, 4×$CH(CH_3)_2$ of TIPDSi group). ^{19}F NMR (DMSO-d_6): δ 24.971 (s, C(2)-F). The above crude contained as well presumably 2-fluoro-6-*O*-(4-nitrophenylethyl)-2'-deoxyinosine derivatives protected either at 3'- or 5'-hydroxyl with 3-hydroxy-or /and 3-fluorotetraisopropyldisiloxane-1-yl group (a partially cleaved TIPDSi group).

In the next step, the crude was reacted with HF in pyridine to remove TIPDSi group. For this purpose a mixture of aqueous HF in pyridine was prepared, (*i.e.* 4.165 ml of 40 % HF in 16.66 ml of pyridine). The crude was dissolved in 5.253 ml of aq. HF in pyridine. After 2 h 40 min. the reaction was finished and worked–up with 0.1 M

$NaHCO_3$ (210 ml) and CH_2Cl_2 (3×200 ml). The organic phases were evaporated under reduced pressure and the resulting brownish oil was subjected to a short column chromatography to give 189 mg of the desired product **8**, 58 % total yield. R_f(A) = 0.48. ^{1}H NMR (DMSO-d_6): δ 8.57 (s, 1H, H-8), 8.17-8.2 (m, 2H, 2×CH aromatic of 4-NPE), 7.63-7.66 (m, 2H, 2×CH aromatic of 4-NPE), 6.31 (t, 1H, *J* 6.9 Hz, H-1'), 5.35 (d, 1H, *J* 4.2 Hz, OH-3'), 4.94 (t, 1H, *J* 5.7 Hz, OH-5'), 4.83 (t, 2H, *J* 6.3 Hz, CH_2 aliphatic of 4-NPE), 4.37-4.43 (m, 1H, H-3'), 3.84- 3.88 (m, 1H, H-4'), 3.47-3.64 (m, 2H, H-5', 5"), 3.31 (t, 2H, *J* 6.6 Hz, CH_2 aliphatic of 4-NPE), 2.62-2.71 (m, 1H, H-2'), 2.28-2.35 (m, 1H, H-2"). ^{19}F NMR (DMSO-d_6): δ 25.231 (s, C(2)-F).

5'-*O*-(4,4'-Dimethoxytrityl)-2-fluoro-6-*O*-(4-nitrophenylethyl)-2'-deoxyinosine (9). Compound **8** (182 mg, 0.42 mmol) was evaporated with anhydrous pyridine (3×5 ml) and then it was dissolved in 3 ml of pyridine. Dimethoxytrityl chloride (157 mg, 0,46 mmol, 1.1 eq.) was added. After 2 h and 40 min DMTCl (14 mg, 42 mmol, 0.1 eq.) was added again. The reaction went to completion after 3 hours. The standard work–up procedure was applied, *e.g.* saturated $NaHCO_3$ (20 ml) and CH_2Cl_2 (3×20 ml). Crude **9** as a yellow oil was purified by short column chromatography (eluting in CH_2Cl_2/MeOH 98:2) to yield 290 mg (94 %) of a pale–yellow foam. R_f(B) = 0.46. ^{1}H NMR (DMSO-d_6): δ 8.15-8.19 (m, 2H, 2×CH aromatic of 4-NPE), 8.03 (s, 1H, H-8), 7.48-7.51 (m, 2H, 2×CH aromatic of 4-NPE), 6.78-7.3 (m, 13H, DMT aromatic), 6.35-6.39 (t, 1H, *J* 6.6 Hz, H-1'), 4.81-4.85 (t, 2H, *J* 6,9 Hz, CH_2 aliphatic of 4-NPE), 4.64-4.7 (m, 1H, H-3'), 4.08-4.14 (m, 1H, H- 4'), 3.78 (s, 6H, 2×OCH_3 of DMT), 3.35-3.49 (m, 2H, H-5', 5"), 3.29-3.34 (t, 2H, *J* 6.6 Hz, CH_2 aliphatic of 4-NPE), 2.7-2.82 (m, 1H, H-2'), 2.47-2.57 (m, 1H, H-2"). ^{19}F NMR (DMSO-d_6): δ 24.754 (m, C(2)-F).

5'-*O*-(4,4'-Dimethoxytrityl)-6-*O*-(4-nitrophenylethyl)-2-*N*-(4,9,13-triazatridecane-1-yl)-2'-deoxyguanosine (10) Compound **9** (1.0 g, 1.39 mmol) was dissolved in pyridine (14 ml) and spermine (2.813 g, 13.9 mmol, 10 eq.) was added. The reaction was carried out at ambient temperature during 4 hours. Then, pyridine was removed on a rotary evaporator and the oily residue was partitioned between water (100 ml) and CH_2Cl_2 (100 ml). The organic layer was extracted with water (2×100 ml) to separate excess of spermine. Each time after shaking two phases emulsion was appearing, so the mixture was centrifuged (2×20 min., speed: 16 000 RPM). Subsequently, the organic

layer was evaporated under diminished pressure and the crude was subjected to short column chromatography yielding almost pure **10** as yellowish foam (1.008 g, 80 %). **10** eluted in MeOH / 40 % aq. $MeNH_2$ / H_2O (7.8–7.7/0.2–0.3/2). R_f(C, RP) = 0.48. ^1H NMR (DMSO-d_6): δ 8.13 (m, 2H, 2×CH aromatic of 4-NPE), 7.71 (s, 1H, H-8), 7.45 (m, 2H, 2×CH aromatic of 4-NPE), 6.75-7.38 (m, 13H, DMT aromatic), 6.27 (t, 1H, *J* 5.4 Hz, H-1'), 4.81 (m, 1H, H-3'), 4.7 (t, 2H, *J* 6.6 Hz, CH_2 aliphatic of 4-NPE), 3.99-4.16 (m, 1H, H-4'), 3.75 (s, 6H, 2×OCH_3 of DMT), 2.46-3.4 (m, 18H, H-5', 5", 2', 2", CH_2 aliphatic of 4-NPE, H-1, 3, 5, 8, 10, 12 of spermine residue), 1.51-1.8 (m, 8H, H-2, 6, 7, 11 of spermine residue).

5'-*O*-(4,4'-Dimethoxytrityl)-6-*O*-(4-nitrophenylethyl)-2-*N*-[tris(*N*,*N'*,*N''*-trifluoroacetyl)-4,9,13-triazatridecane-1-yl]-2'-deoxyguanosine (11) Compound **10** (306 mg, 0.34 mmol) was dissolved in pyridine (3.4 ml). The reaction flask was placed into an ice bath at 0 °C and trifluoroacetic anhydride was added drop wise. After 15 min. the flask was taken out from the cold bath and left at room temperature for the additional 15 minutes. Then, the solution was partitioned between saturated $NaHCO_3$ (50 ml) and CH_2Cl_2 (50 ml). The aqueous phase was extracted with CH_2Cl_2 (3×50 ml), and then was evaporated under reduced pressure. The oily residue containing two major compounds *i.e.* **11** and 5'-*O*-(4,4'-dimethoxytrityl)-6-*O*-(4-nitrophenylethyl)-2-*N*-trifluoroacetyl-[tris-(*N*,*N'*,*N''*-trifluoroacetyl)-4,9,13-triazatridecane-1-yl]-2'-deoxyguanosine **(12)** was purified by gel chromatography (eluent CH_2Cl_2 / MeOH) to give the mixture of **11** and **12** (250 mg) with R_f(B) values 0.20 and 0.35 respectively. The products were dissolved in the mixture of CH_2Cl_2 / MeOH (1:2) and after 4 h the TLC analysis showed the presence **11** only. The pure **11** was obtained after chromatography (CH_2Cl_2 / MeOH) as a yellowish foam (222 mg, 55% yield). ^1H NMR ($CDCl_3$): δ 9.43-9.55 (m, 1H, NH-13 of spermine residue), 8.54 (s, 1H, H-8), 8.15-8.19 (m, 2H, 2×CH aromatic of 4-NPE), 7.6-7.64 (m, 2H, 2×CH aromatic of 4-NPE), 6.74-7.32 (m, 14H, DMT aromatic, C(2)-NH), 6.38 (t, 1H, *J* 6.6 Hz, H-1'), 5.41-5.43 (m, 1H, 3'-OH), 4.77-4.84 (m, 2H, CH_2 aliphatic of 4-NPE), 4.4 (m, 1H, H-3'), 3.97-4.02 (m, 1H, H-4'), 3.84-3.95 (m, 2H, H-5', 5"), 3.7-3.71 (2×s, 6H, 2×OCH_3 of DMT), 3.09-3.42 (m, 14H, CH_2 aliphatic of 4-NPE, H-1, 3, 5, 8, 10, 12 of spermine residue), 2.73-2.83 (m, 1H, H-2'), 2.33-2.42 (m, 1H, H-2"), 1.48-1.92 (m, 8H, H-2, 6, 7, 11). FAB-MS: **11** $[MH]^+$ 1192.7 calc. for $C_{55}H_{59}F_9N_9O_{11}$ 1192.42; **12** $[MH]^+$ 1288.6 calc. for $C_{57}H_{58}F_{12}N_9O_{12}$ 1192.42.

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*-[tris(*N*,*N'*,*N''*-trifluoroacetyl)-4,9,13-triazatridecane-1-yl]-2'-deoxyguanosine (13) A mixture of **11** and **12** (138 mg, 0.12 mmol) was dissolved in 700 μl of 0.5 M solution of DBU in pyridine. After 2 h the reaction went to completion. Then, the standard workup procedure was applied (15 ml $NaHCO_3$ / 15 ml CH_2Cl_2). The aqueous phase was extracted with CH_2Cl_2 (3×15 ml). The combined organic layers were evaporated and the resulting crude product was purified by short column chromatography. **13** eluted in CH_2Cl_2 / MeOH (96:4) to yield 102 mg (84 %). R_f(A) = 0.43. ^{1}H NMR (DMSO-d_6): δ 10.72-10.75 (m, 1H, NH-1), 9.47-9.54 (m, 1H, NH-13 of spermine residue), 7.8 (s, 1H, H-8), 6.78-7.35 (m, 13H, DMT aromatic), 6.58 (m, 1H, C(2)-NH), 6.19 (t, 1H, *J* 6.3 Hz, H-1'), 5.34 (d, 1H, *J* 4.8 Hz, OH-3'), 4.35-4.44 (m, 1H, H-3'), 3.91-3.95 (m, 1H, H-4'), 3.72 (2×s, 6H, 2×OCH_3 of DMT), 3.16-3.35 (m, 14H, H-1, 3, 5, 8, 10, 12 CH_2 of spermine residue, H-5', 5"), 2.69-2.74 (m, 1H, H-2'), 2.24-2.28 (m, 1H, H-2"), 1.5-1.84 (m, 8H, H-2, 6, 7, 11 CH_2 of spermine residue).

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*-(4,9,13-triazatridecane-1-yl)-2'-deoxyguanosine (14) Compound **13** (86 mg, 0.08 mmol) was dissolved in pyridine (500 μl) and 500 μl of concentrated ammonia was added. The reaction vessel was tightly closed and kept in an oven at 50 °C overnight. The title product 13 was generated quantitatively. R_f(C, RP) = 0.86. ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H, H-8), 6.79-7.36 (m, 13H, DMT aromatic), 6.18-6.22 (t, 1H, *J* 6.3 Hz, H-1'), 4.44-4.49 (m, 1H, H-3'), 3.91-3.96 (m, 1H, H-4'), 3.71-3.72 (2×s, 6H, 2×OCH_3 DMT), 3.18-3.27 (m, 2H, H-5', 5"), 2.74-2.82 (m, 1H, H-2'), 2.47-2.62 (m, 12H, H-1, 3, 5, 8, 10, 12 CH_2 of spermine residue), 2.25-2.33 (m, 1H, H-2"), 1.42-1.64 (m, 8H, H-2, 6, 7, 11 CH_2 of spermine residue).

5'-*O*-(4,4'-Dimethoxytrityl)-6-*N*,*N*-di-*n*-butyl-2'-deoxyadenosine (17) Compound **3** (100 mg, 0.17 mmol) was dissolved in a mixture of pyridine (850 μl) and water (850 μl). Next, di-*n*-butylamine (286 μl, 1.7 mmol, 10 eq.) was added. The flask was tightly closed and put into an oven at 70 °C for 53 hours. Then, the reaction was partitioned between water (15 ml) and CH_2Cl_2 (3×15 ml). The organic layers were evaporated. The resulting crude was subjected to short column chromatography to obtain 16 with approximately 40 % of impurity by an unknown side product. The fractions were collected and evaporated, and the residue was dissolved in dichloromethane. Immediately 16 precipitated as a white solid (33 mg, 30 %). R_f(A) = 0.35. ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H, H-8), 7.99 (s, 1H, H-2), 6.78-7.33 (m, 13H,

DMT aromatic), 6.32-6.36 (t, 1H, *J* 6.6 Hz, H-1'), 5.4 (m, 1H, OH-3'), 4.43 (m, 1H, H-3'), 3.95-4.0 (m, 1H, H-4'), 3.72-3.73 (2×s, 6H, 2×OCH_3 DMT), 3.11-3.2 (m, 2H, H-5', 5"), 2.73-2.82 (m, 1H, H-2'), 2.54-2.59 (t, 4H, *J* 7.2 Hz, H-1 CH_2 of butyl chains), 2.3-2.39 (m, 1H, H-2"), 1.23-1.46 (m, 8H, H-2, 3, CH_2 of butyl chains), 0.85-0.89 (t, 6H, *J* 7.2 Hz, H-4 CH_2 of butyl chains).

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*,*N*-di-*n*-butyl-2'-deoxyguanosine (19)

Compound **9** (100 mg, 0.14 mmol) was dissolved in pyridine (1.4 ml) and di-*n*-butylamine (236 µl, 1.4 mmol, 10 eq.) was added. The flask was tightly closed and kept in an oven at 70 °C for 32 hours. The reaction mixture was partitioned between water (15 ml) and CH_2Cl_2. (15 ml). The aqueous phase was extracted with CH_2Cl_2 (3×15 ml) and the organic layers were evaporated. The resulting crude product was purified by short column chromatography with CH_2Cl_2/MeOH as an eluent to give 18 as yellowish foam (57 mg, 60 %). R_f(B) = 0.23. ^{1}H NMR ($CDCl_3$): δ 9.97 (br, 1H, NH-1), 7.58 (s, 1H, H-8), 6.77-7.42 (m, 13H, DMT aromatic), 6.22-6.27 (t, 1H, *J* 6.6 Hz, H-1'), 4.54-4.59 (m, 1H, H-3'), 4.07-4.12 (m, 1H, H-4'), 3.77 (s, 6H, 2×OCH_3 DMT), 3.41-3.5 (m, 5H, H-1 CH_2 of butyl, H-5'), 3.25-3.3 (m, 1H, H-5"), 2.61-2.7 (m, 1H, H-2'), 2.4-2.48 (m, 1H, H-2"), 1.52-1.62 (m, 4H, H-2 CH_2 of butyl chains), 1.29-1.41 (m, 4H, H-3 CH_2 of butyl chains), 0.89-0.94 (t, 6H, *J* 7.5 Hz, H-4 CH_3 of butyl chains).

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