1*H*-Benzotriazole as Synthetic Auxiliary in a Facile Route to N^6-(Arylmethyl)-2′-deoxyadenosines: DNA Intercalators Inserted into Three-Way Junctions

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

The 2′-deoxy-N^6-(naphthalen-1-ylmethyl)- (**5a**) and N^6-(pyren-1-ylmethyl)adenosine (**5b**) were synthesized in two steps from 2′-deoxyadenosine and the adequate arenecarbaldehyde with 1*H*-benzotriazole as a synthetic auxiliary (*Scheme*). When the N^6-(arylmethyl)-2′-deoxyadenosines were inserted into the junction region of a DNA three-way junction, its thermal stability increased.

1. Introduction. – Carcinogenic polycyclic aromatic hydrocarbons are known to undergo metabolic activation to reactive diol epoxide intermediates that bind covalently to DNA *in vivo* [1–3]. Moreover, the presence of bulky polycyclic aromatic residues derived from the binding of stereoisomeric mutagenic and tumorigenic metabolites of benzo[*a*]pyrene to 2′-deoxyguanosine residues in DNA is known to block replication [4] and to induce mutations [5], and is believed to constitute the critical initial step in carcinogenesis [6]. To gain greater insight into polyaromatic hydrocarbon (PAH) adducts to DNA and the biological consequences of their replication, efficient methods for the site-specific synthesis of PAH-oligodeoxynucleotides, with the PAH attached covalently to the exocyclic amino groups of specific dA and dG bases, are required.

Oligonucleotides complementary to strategic regions of viral or messenger RNAs were first shown by *Zamecnik* and *Stephenson* [7] to inhibit viral replication. The factors that are usually assumed to limit the activity of antisense oligonucleotides are cellular uptake, resistance to nuclease, and the stability of the hybrid formed. Modifications are usually chosen to improve one or more of these properties. However, a steady improvement in activity has been achieved by this rational approach that is encouraging for the design of conjugates to meet specific requirements. The manipulation of sequence-specific protein binding and gene expression with antigene or antisense technology requires oligonucleotides that bind DNA or RNA with high affinity and specificity [8–11]. Oligonucleotides capable of selective recognition of RNA must accommodate, in addition, both the kinetic [12][13] and thermodynamic [13][14] consequences of nonuniform RNA conformation. The structural complexity inherent in RNA complicates recognition because it limits the accessibility of single-stranded regions that must be differentiated for specific binding. It is estimated that

11 – 15 nucleotides are necessary to define a unique mRNA sequence in eukaryotic cell [15], yet few well-characterized RNA secondary structures contain contiguous single-stranded regions of this size.

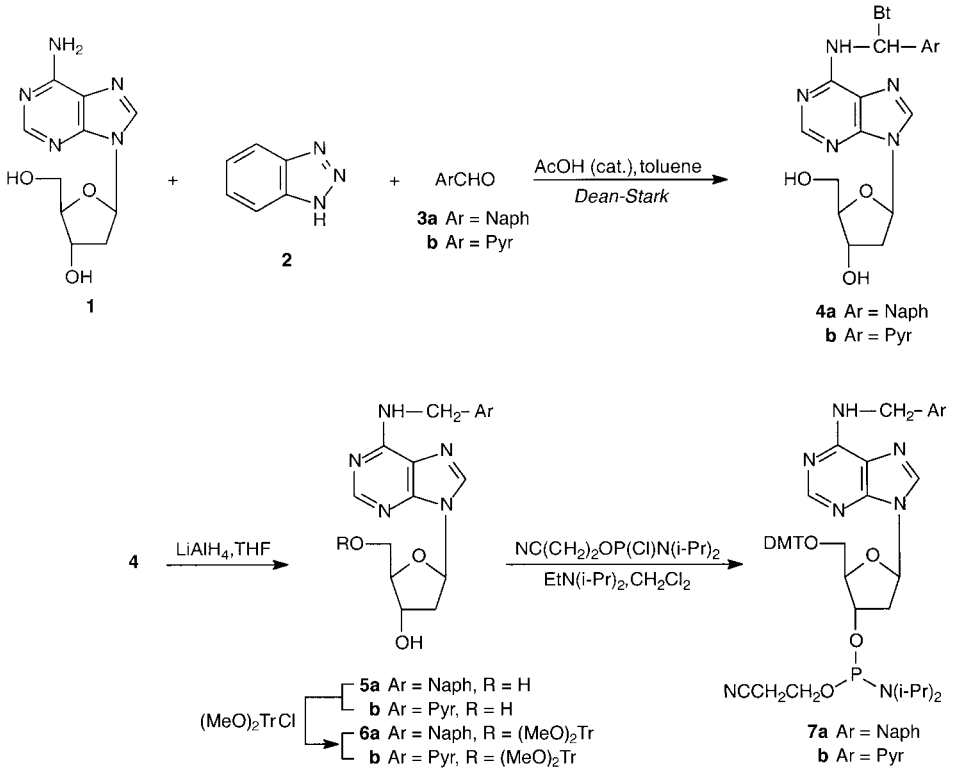
The object of this investigation will be focused on the effect of intercalators as a chemical modification on strengthening the binding force in an oligonucleotide three-way junction (TWJ). It is believed that three-way junctions with two short single-stranded arms are interesting target sites for antisense oligonucleotides when contiguous single-stranded regions are not available for binding. However, the stability of the hybridization of oligodeoxynucleotides to TWJ single-stranded arms is lower compared to the corresponding duplex with the same number of base pairs [16][17].

2. Synthesis. – In continuation of our previous work [18], we now report a facile and short direct route to synthesize 2′-deoxy-N^6-(naphthalen-1-ylmethyl)adenosine (**5a**) and 2′-deoxy-N^6-(pyren-1-ylmethyl)adenosine (**5b**) by means of 1*H*-benzotriazole [19] as a facile and versatile reagent, and this in good yield and without any by-products [20][21] arising from direct alkylation at other sites, *e.g.*, at N(1), N(3) or N(7). *Harvey* and co-workers [22] reported recently the synthesis of 2′-deoxy-N^6-(pyren-1-ylmethyl)-adenosine (**5b**), which involves in the key step coupling of an appropriately protected halopurine derivative with the amino derivative of the polyaromatic hydrocarbon, in this case pyrene-1-methanamine. Our synthetic strategy entailed in the key step the condensation of 1*H*-benzotriazole (**2**) with the appropriate polyaromatic aldehydes **3a**,**b** and with 2′-deoxyadenosine (**1**) by refluxing this mixture in toluene with azeotropic removal of H_2O (*Dean-Stark*) in an acid-catalyzed reaction, *i.e.*, the reaction took place in the presence of a few drops of AcOH (*Scheme*). After *ca.* 6 h, the solvent was evaporated, and the 1*H*-benzotriazole adducts **4a** or **4b** were obtained from the residue after flash-chromatographic (FC) purification in 85 and 80% yield, respectively. The 1*H*-benzotriazole adducts **4a** and **4b** were then reduced by $LiAlH_4$ in dry THF to give **5a** or **5b** in 80 and 70% yield, respectively, after FC purification. The structures of the adducts **4a**,**b** were elucidated from their ^{1}H- and ^{13}C-NMR and FAB mass spectra. The former spectra were complex due to the presence of the stereogenic C-atom Bt-*C*H-Ar. The diastereomer mixtures could not be separated by column chromatography.

In our previous study [18], we had the problem of very bad yields, or no reaction at all, when the reaction between 1*H*-benzotriazole (**2**), 2′-deoxyadenosine (**1**), and polycyclic aromatic aldehyde **3** was carried out by reflux in EtOH in the presence of a catalytic amount of AcOH. We believed that this may be due to the steric hindrance around the stereogenic C-atom (N^6-CH) generated by the presence of the 1*H*-benzotriazol-1-yl and the bulky polycyclic aromatic group at the same C-atom. We also thought that the H_2O liberated during the condensation reaction could retard the reaction progress. Therefore, we now modified the reaction conditions by carrying out the reaction in refluxing toluene, using the *Dean-Stark* trap to remove azeotropically the H_2O formed, to force the reaction to proceed to completion.

The reduction of the adducts **4a**,**b** was performed with $LiAlH_4$ in THF at room temperature to give the N^6-(arylmethyl)deoxyadenosines **5a**,**b** in 70 – 80% yield. Attempts to carry out the reduction with $NaBH_4$ resulted in poor yields and required long reaction times. The spectra of 2′-deoxy-N^6-(pyren-1-ylmethyl)adenosine (**5b**) were in good agreement with those reported [24].

Scheme



Naph = naphthalen-1-yl, Pyr = pyren-1-yl, Bt = 1*H*-benzotriazol-1-yl, DMT = 4,4′-dimethoxytrityl = $(MeO)_2Tr$

The 4,4′-dimethoxytrityl ($(MeO)_2Tr$), and the phosphoramidite derivatives of **5a**,**b**, *i.e.*, **6a**,**b** and **7a**,**b**, respectively, were prepared according to *Caruthers*' methodology [23]. For the preparation of **7a**,**b** the $(MeO)_2Tr$ derivatives **6a**,**b** were treated with 2-cyanoethyl diisopropylphosphoramidochlorite in the presence of $^iPr_2EtN$. The purity of the obtained phosphoramidites **7a**,**b** (yield 83 and 85%, resp.) was 94 and 99%, respectively, according to ^{31}P-NMR, the remaining 1–6% being non-phosphoramidite by-products.

The modified and unmodified oligodeoxynucleotides (ODNs) were synthesized on a *Pharmacia Gene-Assembler-Special* synthesizer on a 0.2-μmol scale according to the standard phosphoramidite method [23] with **7** and commercial phosphoramidites. Phosphorothioates were prepared with the *Beaucage* reagent [25][26] (3*H*-1,2-benzodithiol-3-one 1,1-dioxide) on a 0.2-μmol scale. The ability of ODNs to hybridize to their complementary DNA strand was examined by UV melting measurements. The melting points T_m were determined as the maxima of the first derivative of the melting curves. DNA Three-way junctions were formed from equimolar amounts (3 μM) in each strand at pH 7.0 in a 1 mM EDTA, 10 mM Na_2HPO_4, and 140 mM NaCl buffer.

3. Stability of Three-Way Junction. – To study the intercalating ability of the prepared modified nucleosides **5**, we chose a three-way junction composed of two dangling single strands and a double-stranded DNA stem rich in C · G base pairs to shift the melting temperature of the stem to a higher temperature to avoid its interference with those obtained when hybridizing the dangling strands (*Fig. 1*).

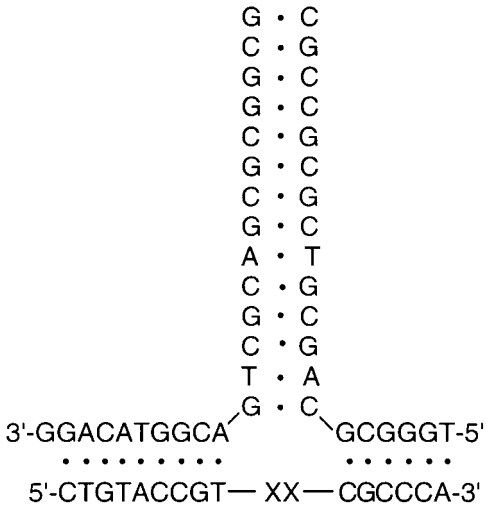


Fig. 1. *Three-way junction I*. XX = Site of insertion. The nucleoside symbols represent 2′-deoxynucleosides.

On inserting compound **5a** and an extra dG as a bulge in the junction region of the three-way junction I (*Table 1*, *Entry 3*), an increase in T_m of 6.4° was observed compared to the insertion of dA and dG as a reference (*Entry 2*). Insertion of **5a** in both the 5′-end and the junction region had a small further effect (*Entry 7*, $\Delta T_m = 6.8°$) when compared to the reference with dA instead of **5a** (*Entry 4*). When the reference ODN had **5a** added at the 5′-end and dG and dA in the junction region (*Entry 6*), a $\Delta T_m = 3.2°$ was observed when **5a** was inserted instead of dA in the junction region (*Entry 7*). Mismatches in the targeting ODN resulted in remarkable decreases in the

Table 1. *Hybridization Data for the Three-Way Junction I with Inserted dG.* **X** = **5a**.

Entry	Targeting ODN	T_m [°C]
1	5′-d(CTGTACCGT CGCCCA)-3′	41.2
2	5′-d(CTGTACCGT AG CGCCCA)-3′	44.8
3	5′-d(CTGTACCGT **X**G CGCCCA)-3′	51.2
4	5′-d(ACTGTACCGT AG CGCCCA)-3′	44.0
5	5′-d(ACTGTACCGT **X**G CGCCCA)-3′	48.4
6	5′-d(**X**CTGTACCGT AG CGCCCA)-3′	47.6
7	5′-d(**X**CTGTACCGT **X**G CGCCCA)-3′	50.8
8	5′-d(CT<u>TG</u>ACCGT **X**G CGCCCA)-3′[a])	31.2
9	5′-d(CTGTACCGT **X**G C<u>CG</u>CCA)-3′[a])	40.0

[a]) Mismatch site in the targeting ODN is underlined.

stabilization of the three-way junction (*Entries 8* and *9*). This confirms hybridization in both arms of TWJ.

Insertion of a dG in the junction region could lead to base pairing with the first dC in the stem, leading to the disruption of the corresponding first base pair in the stem. In fact, DNA folding calculations [27] show that inserted d(AG) in the junction region leads to disruption of the first two base pairs in the stem (d(GC) and d(AT)). To overcome this problem, another antisense ODN containing dT and dA and dT and **5a** insertions in the junction region was used. The hybridization data shown in *Table 2* showed a $\Delta T_m = 6.0°$ when inserting dT and **5a** in the junction region (*Entry 11*) compared to the reference with d(TA) in the same region (*Entry 13*). A more pronounced effect ($\Delta T_m = 13.6°$) was achieved when two modified nucleosides **5a** (*Entry 12*) were inserted in the junction region compared to the insertion of two dA (*Entry 14*) in the same region. As a preliminary test for the increased binding properties of **5b** over **5a**, a single trial was made with the insertion of dT and **5b** in the junction region, and a $\Delta T_m = 10.4°$ was observed (*Entry 11 vs. 13*). We have recently suggested that intercalators inserted at TWJ junctions stabilize the TWJ by stacking on top of the deflecting arm [28]. Further stabilization by insertion of two intercalators (*Entry 12*) could then be due to stacking of both intercalators at the top of the deflecting arm.

Table 2. *Hybridization Data for the Three-Way Junction I Avoiding Inserted dG.* **X** = **5a**.

Entry	Targeting ODN	T_m [°C]
10	5′-d(CTGTACCGT CGCCCA)-3′	41.2
11	5′-d(CTGTACCGT T**X** CGCCCA)-3′	44.8 (49.2) [a]
12	5′-d(CTGTACCGT **XX** CGCCCA)-3′	51.2
13	5′-d(CTGTACCGT TA CGCCCA)-3′	38.8
14	5′-d(CTGTACCGT AA CGCCCA)-3′	37.6

[a]) Value in parentheses for **X** = **5b**.

The human immunodeficiency virus (HIV) contains at least two structured RNAs that interact with viral protein and mediate novel genetic regularity pathways. One of these is a 234-nucleotide sequence located within the *env* coding region called the Rev response element (RRE). Interaction of the RRE with viral protein Rev [29][30] regulates the appearance of unspliced or singly spliced viral mRNAs in the cytoplasm of infected cells [31–40]. *Cload* and *Schepartz* [41] studied the *in vitro* inhibitory effect of some tethered oligonucleotide probes (TOPs) on the function of RRE. We chose the same antisense sequence (*Fig. 2*) as that reported by *Cload* and *Schepartz* to have potent concentration-dependent inhibition of RRE function *in vitro*. Only the tethering propane-1,3-diol moiety was replaced with an intercalating nucleoside.

Thus, a ΔT_m of 12.2° was observed when inserting **5b** and dG in the junction region (*Table 3*, *Entry 16*) compared to *Entry 15*. Also mismatching by interchanging two nucleosides in the antisense ODN decreased the stability of the system (*Entries 17* and *18*), indicating that full complementarity took place between the three strands of the system.

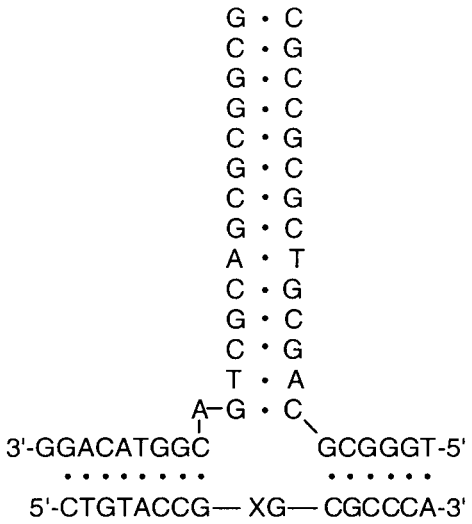


Fig. 2. *Three-way junction II*. XG = Site of insertion. The nucleoside symbols represent 2′-deoxynucleosides.

Considering the stabilizing effect of the pyrenyl intercalator on DNA TWJ, it was found interesting to test the activity of the corresponding phosphorothioates against HIV-1 in MT-4 cells. The ODNs given in *Table 3* were also synthesized as their phosphorothioate analogues, as well as additional examples with dA, d(AA), or d(XX) insertions in the middle of the oligonucleotide. The HIV-1 strain HTLV-IIIB was propagated in MT-4 cells at 37° with RPMI-1640, 10% heat-inactivated fetal-calf serum (FCS), and antibiotics (growth medium). The cells were preincubated with lipofectin to improve their uptake of the oligonucleotide. Unfortunately, none of the prepared phosphorothioates showed any *anti*-HIV activity. Moreover, they were cytotoxic at 100 μM.

Table 3. *Hybridization Data for the Three-Way Junction II.* **X** = **5b**.

Entry	Targeting ODN	T_m [°C]
15	5′-d(CTGTACCG CGCCCA)-3′	40.8
16	5′-d(CTGTACCG **X**G CGCCCA)-3′	53.0
17	5′-d(CTGTACCG **X**G C<u>CG</u>CCA)-3′ (m)[a])	33.2
18	5′-d(CT<u>TG</u>ACCG **X**G CGCCCA)-3′ (m)[a])	40.4

[a]) Mismatch site in the targeting ODN is underlined.

Experimental Part

General. Anal. TLC: *Merck* precoated silica gel *60 F_{254}* plates. Flash column chromatography (FC): silica gel (0.040 – 0.063 mm) from *Merck*. NMR Spectra: at 300 (^{1}H), 75.5 (^{13}C), and 121.5 MHz (^{31}P); *Varian-Gemini 2000* 300-MHz spectrometer; δ values in ppm rel. to $SiMe_4$ as an internal standard (^{1}H, ^{13}C) and rel. to 85% H_3PO_4 as external standard (^{31}P). FAB-MS: positive mode; *Kratos MS-50-RF* spectrometer.

Oligonucleotides. Melting experiments were carried out on a *Perkin-Elmer* UV/VIS spectrometer *Lambda 2* fitted with a *PTP-6-Peltier* temp.-programming element. The UV absorbance at 260 nm as a function of time was recorded while the temp. was raised gradually (1°/min) in a 1-cm cuvette, at a 3 μM concentration of each component. DNA Syntheses were performed on a *Pharmacia Gene-Assembler-Special* DNA synthesizer, on a

0.2-μmol scale. The coupling efficiencies for the modified amidites (20-min couplings) **7a**,**b** were *ca.* 85% compared to 99% for the commercial ones (2-min coupling). The efficiency of each coupling step was monitored by the release of dimethoxytrityl cation after each step. Removal from solid support and deprotection was carried out at r.t. for 4 days in 25% ammonia and 0.01M EDTA in 25% ammonia for the phosphorothioates. Desalting of the oligonuclotides was accomplished with disposable *NAP-10* columns (*Pharmacia*).

N^6-*[(1*H*-Benzotriazol-1-yl)(naphthalen-1-yl)methyl]-2′-deoxyadenosine* (**4a**). A soln. of 2′-deoxyadenosine (**1**; 2.51 g, 10 mmol), 1*H*-benzotriazole (**2**; 1.43 g, 12 mmol), and naphthalene-1-carboxaldehyde (**3a**; 1.6 ml, 12 mmol) was refluxed in toluene in the presence of 5 drops of AcOH as a catalyst with azeotropic removal of H_2O in a *Dean-Stark* apparatus. After 6 h, the solvent was evaporated and the residue submitted to FC ($CHCl_3$/MeOH 99:1): **4a** (4.3 g, 85%). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 509 ($[M+H]^+$).

N^6-*[(1*H*-Benzotriazol-1-yl)pyren-1-yl)methyl]-2′-deoxyadenosine* (**4b**): As described for **4a**, with **1** (2.51 g, 10 mmol), **2** (1.43 g, 12 mmol), pyrene-1-carboxaldehyde (**3b**; 2.76 g, 12 mmol), toluene and 5 drops of AcOH: **4b** (4.6 g, 80%). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 583 ($[M+H]^+$).

2′-Deoxy-N^6*-(naphthalen-1-ylmethyl)adenosine* (**5a**). A soln. of **4a** (2.55 g, 5 mmol) was stirred with a suspension of $LiAlH_4$ (1.14 g, 30 mmol) in dry THF at r.t. for 2 h. The mixture was then poured into ice-water, neutralized with AcOH and extracted with $CHCl_3$. The crude mixture was purified by FC ($CHCl_3$/MeOH 97:3): **5a** (1.56 g, 80%). ^{1}H-NMR ($CDCl_3$): 8.39 (*s*, H–C(8)); 8.06 (*s*, H–C(2)); 7.86–7.36 (*m*, Naph.); 6.12 (*m*, H–C(1′)); 5.25 (br., NHCH_2Ar); 4.69 (*d*, 1 OH); 4.07 (*m*, H–C(3′)); 3.92 (*m*, H–C(4′)); 3.77 (*m*, 2 H–C(5′)); 2.94 (*m*, 1 H–C(2′)); 2.17 (*m*, 1 H–C(2′)). ^{13}C-NMR ($CDCl_3$): 154.86 (C(6)); 152.71 (C(2)); 147.86 (C(4)); 139.47 (C(8)); 133.88, 133.36, 131.53, 128.83, 128.70, 126.63, 126.49, 126.03, 125.42, 123.48 (Naph); 121.17 (C(5)); 89.54 (C(4′); 87.56 (C(1′)); 73.06 (C(3′)); 63.25 (C(5′)); 42.57 (NH*C*H$_2$Ar); 40.67 (C(2′)). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 392 ($[M+H]^+$).

2′-Deoxy-N^6*-(pyren-1-ylmethyl)adenosine* (**5b**). As described for **5a**, with **4b** (1.16 g, 2 mmol) and $LiAlH_4$ (0.456 g, 12 mmol) in dry THF: **5b** (0.65 g, 70%). ^{1}H-NMR ((D$_6$)DMSO): 8.60 (*s*, H–(C8)); 8.56 (*s*, H–C(2)); 8.42–8.05 (*m*, Pyr); 6.41 (*m*, H–C(1′)); 5.49 (br., NHCH_2Ar); 5.35 (*d*, 1 OH); 4.61 (*t*, 1 OH); 4.46 (*m*, H–C(3′)); 3.94 (*m*, H–C(4′)); 3.61 (*m*, 2 H–C(5′)); 2.78 (*m*, 1 H–C(2′)); 2.32 (*m*, 1 H–C(2′)). ^{13}C-NMR ($CDCl_3$): 154.30 (C(6)); 152.36 (C(2)); 147.79 (C(4)); 139.03 (C(8)); 131.19, 130.51, 130.15, 130.00, 128.10, 127.23, 126.76, 126.62, 125.93, 25.41, 124.62, 124.55, 124.10, 123.90, 122.32 (Pyr); 120.48 (C(5)); 88.74 (C(4′)); 86.33 (C(1′)); 71.61 (C(3′)); 62.50 (C(5′)); 41.58 (NH*C*H$_2$Ar), 40.03 (C(2′)). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 466 ($[M+H]^+$).

4,4′-Dimethoxytrityl Derivatives **6**: *General Procedure.* Compound **5a** or **5b** (2 mmol) was stirred in pyridine at r.t. with 2.2 mmol of 4,4′-dimethoxytrityl chloride for 3 h. The pyridine was evaporated and the residue submitted to FC ($CHCl_3$): yield 90%.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^6*-(naphthalen-1-ylmethyl)adenosine* (**6a**): ^{1}H-NMR ($CDCl_3$): 8.42 (*s*, H–C(8)); 8.06 (*s*, H–C(2)); 7.84–7.13 (*m*, arom. H); 6.42 (*m*, H–C(1′), NH); 5.30 (br., NHCH_2Ar); 4.62 (*m*, OH); 4.10 (*m*, H–C(3′)); 3.80–3.69 (*m*, MeO, H–C(4′)); 3.37 (*m*, 2 H–C(5′)); 2.72 (*m*, 1 H–C(2′)); 2.47 (*m*, 1 H–C(2′)). ^{13}C-NMR ($CDCl_3$): 154.95 (C(6)); 152.25 (C(2)); 149.63 (C(4)); 138.25 (C(8)); 136.37–123.52 (arom. C), 120.04 (C(5)); 113.20 ((MeO)$_2$*Tr*); 86.01 (C(4′)); 84.20 (C(1′)); 72.22 (C(3′)); 63.64 (C(5′)); 55.11 (MeO); 42.80 (NHCH$_2$); 40.22 (C(2′)). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 694 ($[M+H]^+$).

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^6*-(pyren-1-ylmethyl)adenosine* (**6b**): ^{1}H-NMR ($CDCl_3$): 8.33 (*s*, H–C(8)); 8.30 (*s*, H–C(2)); 8.17–7.95 (*m*, Pyr); 7.37–7.16 (*m*, (MeO)$_2$*Tr*); 6.25 (*t*, H–C(1′)); 5.53 (br., NHCH_2Ar), 4.55 (*m*, OH); 4.03 (*m*, H–C(3′)); 3.70 (*s*, MeO); 3.30 (*m*, H–C(5′)); 2.60 (*m*, 1 H–C(2′)); 2.33 (*m*, 1 H–C(2′)). ^{13}C-NMR ($CDCl_3$): 154.45 (C(6)); 153.23 (C(2)); 144.59 (C(4)); 138.13 (C(8)); 135.72–122.93 (arom. C); 120.08 (C(5)); 113.16 ((MeO)$_2$*Tr*); 85.47 (C(4′)); 84.02 (C(1′)); 72.19 (C(3′)); 65.80 (C(5′)); 55.07 (MeO); 42.82 (NH*C*H$_2$Ar); 40.00 (C(2′)). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 768 ($[M+H]^+$).

Phosphoramidite Derivatives **7**: *General Procedure.* Compound **6a** or **6b** (0.5 mmol) was co-evaporated with dry MeCN and dissolved in a mixture of ^{i}Pr$_2$EtN (0.4 ml) and dry CH_2Cl_2 (2.5 ml). Then, 2-cyanoethyl diisopropylphosphoramidochlorite (0.8 mmol) was added dropwise under Ar. The mixture was stirred at r.t. for 1 h. Then the reaction was quenched by addition of MeOH (0.2 ml). After addition of AcOEt and CH_2Cl_2, the org. phase was washed with sat. $NaHCO_3$ soln. (3 × 30 ml), dried (Na_2SO_4), and evaporated. The product was purified by CC (AcOEt/CH_2Cl_2/Et$_3$N 45:45:10). The resulting gum was dissolved in 2 ml of dry toluene, and the soln. was added dropwise to stirred cold petroleum ether (100 ml). The solid product was filtered off: **7a**,**b**.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)*-N^6*-(naphthalen-1-ylmethyl)adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**7a**): ^{31}P-NMR ($CDCl_3$): 149.951 (94% pure). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 894 ($[M+H]^+$).

*2′-Deoxyy-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{6}-(pyren-1-ylmethyl)adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**7b**): ^{31}P-NMR ($CDCl_3$): 149.98 (99% pure). FAB-MS ($CHCl_3$ + 3-nitrobenzyl alcohol): 968 ($[M+H]^+$).

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