On the Distance-Independent Hole Transfer over Long $(A \cdot T)_n$-Sequences in DNA

by **Ernst Furrer** and **Bernd Giese***

Department of Chemistry, University of Basel, St. Johanns-Ring 19, CH-4056 Basel
(fax: +41 61 267 11 05; e-mail: bernd.giese@unibas.ch)

Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

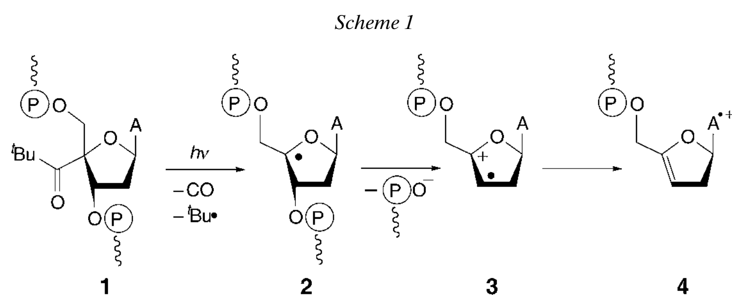
Electron holes can travel through DNA double strands over long distances in a multistep 'hopping' process. But the influence of the DNA sequence on this process is still not understood in all details. We have carried out new experiments to understand the recent observation that the efficiency of the hole transport between guanines (G), which are separated from each other by long adenine · thymine (A · T) sequences, is nearly independent of the length of the $(A \cdot T)_n$ sequence for $n \geq 4$. For this purpose, a new synthesis of the modified adenosine **16** and its incorporation into a DNA double strand was worked out. Subsequent experiments demonstrated that the hole transport between GGG units and the H_2O trapping of the guanine radical cation display similar rates. We conclude that the charge must be already partially equilibrated before being trapped by H_2O. Thus, the weak distance effect is caused not only by the rate of the hole transport, but also by its equilibration over the $(A \cdot T)_n$ sequence.

Introduction. – The question raised already 40 years ago [1][2] of whether and how electrons migrate through DNA over long distances has been a matter of controversial discussion during the last decade [3–8]. Today, there is no doubt that both electrons and electron holes can migrate through double-stranded DNA over long distances [9–12] and that this process occurs in a multistep 'hopping' mechanism. Nevertheless, there are still some puzzling observations. For example, recent experiments have shown that the efficiency of the hole transport between guanines (G) separated from each other by long adenine · thymine (A · T) sequences, only slightly depend upon the length of the $(A \cdot T)_n$ sequence for $n \geq 4$ [13–16]. This surprising result is difficult to explain. Therefore, we have carried out new experiments to shed light on this phenomenon.

Results and Discussion. – *Assay.* Recently, we described an assay for direct charge 'injection' into A with the modified adenosine **1** [16]. Photolysis of **1** leads to the radical **2**, which undergoes a heterolytic bond cleavage to the radical cation **3**. The latter then oxidizes A, presumably *via* through-bond electron transfer (*Scheme 1*).

In double stranded DNA, the hole migrates from the adenine radical cation **4** to Gs, which stabilize a positive charge much better than As [17][18]. The charge is detected by reaction of H_2O with the newly formed guanine radical cation, leading to oxidized guanine derivatives. At this site, the DNA strand can be cleaved selectively with piperidine or ammonia under aerobic conditions [9][19]. With this assay, we have recently shown [16] that, in the double strand **5**, the ratio of the cleavage products

Scheme 1



($P_{5'}/P_{3'}$) was nearly independent of the length of the $(A \cdot T)_n$ sequence (*Fig. 1*). Thus, the length of the $(A \cdot T)_n$ sequence between the first formed adenine radical cation and the GGG units plays only a minor role regarding the efficiency of this charge-transport process.

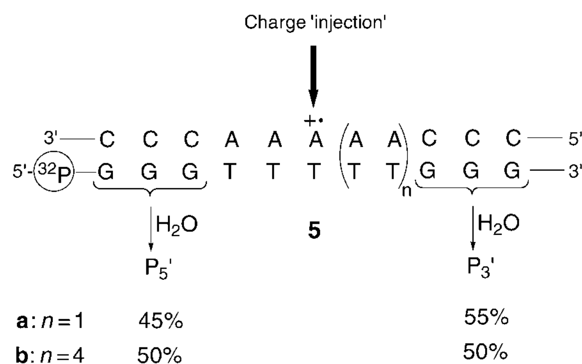


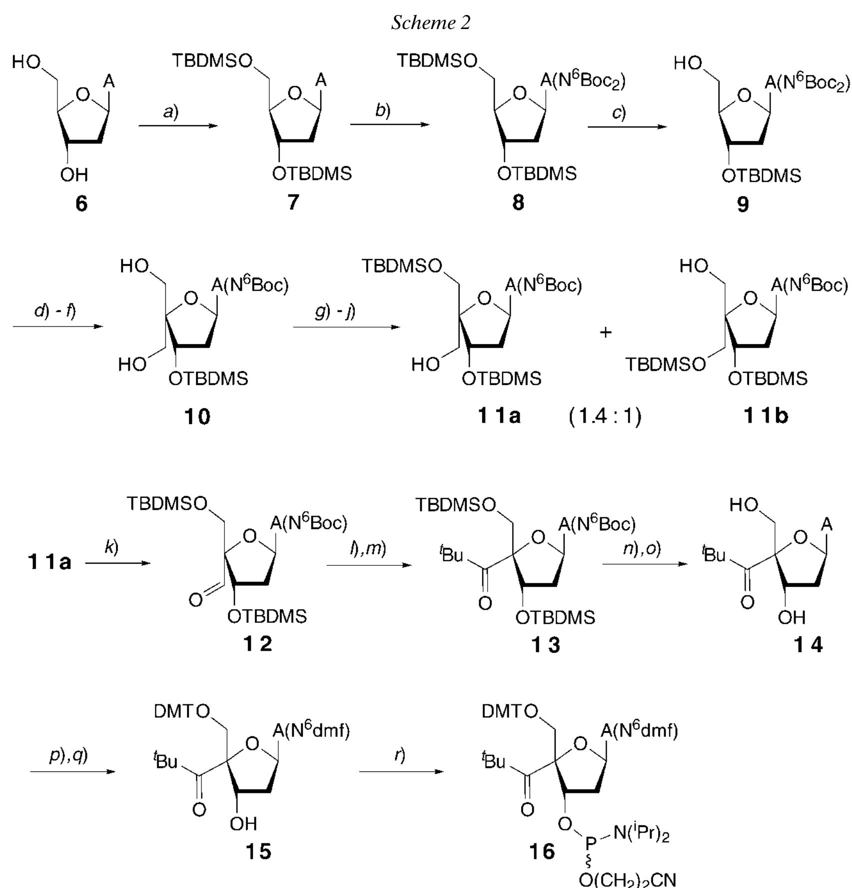
Fig. 1. '*Charge injection' into adenines* (A) *and H_2O-trapping at 5'- and 3'-GGG sites, leading to cleavage products $P_{5'}$ and $P_{3'}$, resp., upon treatment with piperidine*

A possible explanation for this observation is that the trapping of the positive charge at a GGG unit by H_2O is slower than the back transfer of the charge from Gs to As. In this case, the charge equilibrates over the strand before the product-forming, irreversible H_2O trapping occurs. The efficiency of the process would, thus, be given by the thermodynamic distribution of the charge over the DNA and the rate of the H_2O-trapping reaction. The charge should be trapped by H_2O with nearly the same rates because GGG units are used on both sites in **5**. Thus, the efficiency of the charge-transport experiment might be determined by the thermodynamic stabilization of the charge, which might explain the *ca.* 1:1 ratio of the products $P_{5'}/P_{3'}$ (*Fig. 1*).

Synthesis of the 'Charge-Injection' System. To introduce a 4'-pivaloyl-substituted deoxyadenosine into duplex DNA, we worked out a new synthesis of the building block **16** in a reaction sequence similar to that for the synthesis of the corresponding pivaloyl-modified guanosine [20]. However, it turned out that the preparation of the adenosine derivative required different protecting groups[1]) (*Scheme 2*).

[1]) For abbreviations, see the *General* section in the *Exper. Part*.

Scheme 2



a) TBDMSCl, imidazole, DMF; 95%; *b*) $(Boc)_2O$, DMAP, DMF; 94%; *c*) TBAF, AcOH, THF; 54%; *d*) CMC, DMSO, py·TFA; *e*) aq. CH_2O, $Ba(OH)_2$, dioxane, H_2O; *f*) $NaBH_4$, EtOH; 48% (3 steps); *g*) $MeC(OMe)_3$, CSA, CH_2Cl_2; *h*) 20% aq. AcOH; *i*) TBDMSCl, imidazole, DMF; *j*) MeONa, MeOH; **6a**: 49%, **6b**: 36% (4 steps each); *k*) *Dess–Martin* periodinane, CH_2Cl_2; 81%; *l*) ^{t}BuLi, THF, $-78°$; *m*) *Dess–Martin* periodinane, CH_2Cl_2; 25% (2 steps); *n*) Bu_4NF, THF; *o*) SiO_2, high vacuum, 80°; 37% (2 steps); *p*) *N*-(dimethoxymethyl)-*N*,*N*-dimethylamine, MeOH; *q*) DMTCl, collidine, DMF; 55% (2 steps); *r*) 2-cyanoethyl *N*,*N*-(diisopropyl)-chlorophosphoramidite, $^iPr_2NEt$; 78%. Overall yield: 0.50% (18 steps). For abbrev., see the *Exper. Part.*

The crucial steps in the synthesis of **16** are the introduction of the hydroxymethyl group (**9** → **10**), its oxidation (**11a** → **12**), and the introduction of the ^{t}Bu group (**12** → **13**). The synthetic problems consisted of finding the right protecting groups. In *Scheme 2*, an optimized sequence is shown.

DNA Oligomers containing 4′-pivaloyladenosine in one of the strands were prepared with a DNA synthesizer, but the coupling of the modified adenosine **16** was carried out manually by pumping solutions of **16** and the coupling reagent through the column with two syringes (see the *Exper. Part*).

Charge-Transport Experiments. To determine how fast the H_2O trapping of the guanine radical cation is compared to the endothermic oxidation of an A by the

guanine radical cation, we have synthesized a modified double strand, which, by photolysis, generates the adenine radical cation **17** (*Fig. 2*). The hole then migrates to the first GGG site, where it is trapped by H_2O. If the trapping process is much faster than the endothermic oxidation of an adjacent A by the guanine radical cation, all the charge should be trapped at the 5′-GGG site, but no charge should arrive at the 3′-GGG site. If the H_2O trapping is much slower than both this endothermic oxidation and the charge transport over the $(A \cdot T)_n$ sequence, then the two GGG sites should give rise to H_2O trapping in a 1:1 ratio.

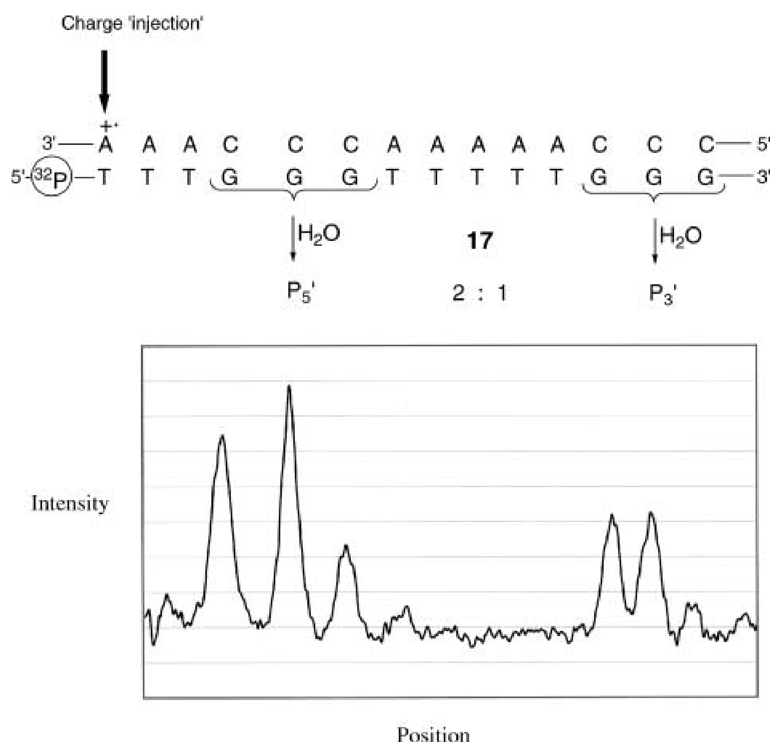


Fig. 2. *Histogram of denaturating polyacrylamide gels* (after substracting control experiments, *i.e.*, irradiation of unmodified strand) *from irradiation experiments with the modified strand, leading to* **17**, *and relative yields of the strand-cleavage products at the 5′- and 3′-GGG units* ($P_{5'}$ and $P_{3'}$).

The above experiments showed that the positive charge detected by H_2O trapping was, indeed, found both at the 5′-GGG unit (leading to cleavage product $P_{5'}$) *and* at the 3′-GGG unit (leading to $P_{3'}$). However, the ratio was not 1:1 – which would have indicated complete charge equilibration – it was actually *ca.* 2:1. This means that the H_2O trapping of the GGG radical cation is aproximately as fast as both the endothermic oxidation and charge transport over the $(A \cdot T)_n$ sequence. Thus, the almost distance-independent efficiency of the hole transport between Gs, separated from each other by long $(A \cdot T)_n$ sequences, as shown in *Fig. 1*, is not caused by a complete equilibration of the charge before the H_2O trapping occurs. In order to rationalize this distance-independent efficiency, one has to assume that the rate of the

'charge hopping' over the $(A \cdot T)_n$ sequence depends only weakly on n. To determine the influence of the $(A \cdot T)_n$ sequence on the hole transfer rate alone, a new assay has to be worked out in which the trapping of the radical cation is faster than the hole transfer between Gs.

Experimental Part

General. Abbreviations: A = adenine, ATP = adenosine triphosphate, Boc = (*tert*-butoxy)carbonyl, C = cytosine, CMC = 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide, CSA = (±)-camphorsulfonic acid, DMAP = (4-dimethylamino)pyridine, dmf = (dimethylamino)methylidene, DMT = 4,4′-dimethoxytrityl (= bis(4-methoxyphenyl)(phenyl)methyl), EDTA = ethylenediamine tetraacetate, G = guanine, PAGE = polyacrylamide-gel electrophoresis, PMMA = polymethyl methacrylate, py = pyridine, RP = reversed-phase, T = thymine, TBAF = Bu_4NF, TBDMS = $^tBuMe_2Si$, TBE = Tris-borate-EDTA buffer, TEAA = triethylammonium acetate, TFA = trifluoroacetic acid, TMEDA = *N,N,N′,N′*-tetramethylethylene-1,2-diamine, *Tris* = tris(hydroxymethyl)aminomethane (= 2-amino-2-(hydroxymethyl)propane-1,3-diol).

2′-Deoxyadenosine was purchased from *Pharma Waldhof*. Chemicals for DNA synthesis were purchased from *Glen Research*. Sodium citrate (20 mM, pH 5.0) and sodium phosphate (20 mM, pH 7.0) buffer solns. were obtained from *Fluka* (HPCE quality). TEAA (1M) Stock solns. for RP-HPLC were purchased from *Fluka*. Disposable PMMA cuvettes for photolyses and optical-density measurements were purchased from *Semadeni*. T_4 Polynucleotide kinase (10000 units/ml) was purchased from *New England Biolabs*. *Mini-QuickSpin* columns for oligonucleotide purification were provided by *F. Hoffmann–LaRoche*. All reactions were carried out in vacuum-dried standard glassware under Ar atmosphere. Solvents for extraction and prep. chromatography were of technical grade and distilled prior to use. Thin-layer chromatography (TLC): silica gel *60 F_{254}* on aluminum (*Merck*); visualization by UV and/or by dipping into a soln. made of *a*) cerium(IV)sulfate tetrahydrate (10 g), *b*) ammonium heptamolybdate tetrahydrate (25 g), *c*) conc. H_2SO_4 (100 ml), and *d*) H_2O (900 ml), followed by heating. Flash chromatography (FC): silica gel *C560D* (40–63 μm, 230–400 mesh; *Uetikon*) at low pressure, unless indicated otherwise. High-performance liquid chromatography (HPLC): *Hewlett-Packard 1050* chromatograph, *Merck LiChroSpher* (RP-18) column, UV detection at 260 nm. UV/VIS (for determination of optical densities and DNA melting curves): *Perkin-Elmer Bio-Lambda II* spectrophotometer, featuring a *PTP-6* peltier unit, extinction coefficients of oligonucleotides calc. by standard incremental method [21]. ^1H- and ^{13}C-NMR: *Varian Gemini-300* and *Bruker DPX-400*, coupling constants *J* in Hz, chemical shifts δ in ppm rel. to $SiMe_4$ (δ 0 ppm) based on solvent signals; designation of NOEs (irradiated H → affected H): ++ = strong, + = medium, (+) = weak. ^{31}P-NMR: triphenyl phosphate as an external standard. Mass spectra: FAB (fast-atom-bombardment): *MAT 312* spectrometer, matrix: 3-nitrobenzyl alcohol; ESI (electrospray ionization): *Finnigan MAT LCQ* spectrometer, in MeOH; MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight): *Vestec Benchtop II* apparatus, matrix: 2,4-dihydroxyacetophenone, laser wavelength: 337 nm, acceleration voltage: 25 kV. The syntheses of oligonucleotides were performed on a *Perseptive Biosystems Expedite 8909* synthesizer applying standard phosphoramidite chemistry. Photolyses were performed on an *Oriel 68810* photolysis stand with an *Osram* 500-W high-pressure Hg lamp equipped with a *Schott* 320-nm cut-off filter. DNA-Containing probes were dried on a *Savant Speed Vac Plus*. Polyacrylamide-gel electrophoreses (PAGE) were performed in TBE buffer (0.1M Tris-borate, pH 8.3; 2 mM EDTA) on a *Life Technologies Model 2* apparatus equipped with a *Pharmacia* potentiostatic power unit; acrylamide/bisacrylamide solns. (*AccuGel 19:1*) for PAGE analyses were provided by *National Diagnostics*. Gels were quantized by means of a phosphorimager using the *ImageQuant* software by *Molecular Dynamics*.

*3′,5′-Bis-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxyadenosine* (**7**). A soln. of 2′-deoxyadenosine (**6**; 10.0 g, 39.8 mmol), 1*H*-imidazole (17.9 g, 263 mmol, 6.6 equiv.), and TBDMSCl[1]) (19.1 g, 127 mmol, 3.2 equiv.) in anh. DMF (80 ml) was stirred overnight at r.t. The reaction was quenched by addition of MeOH (20 ml), and the solvent was removed *in vacuo*. The residue was dissolved in a mixture of CH_2Cl_2 (400 ml) and 0.3M aq. tartaric-acid soln. (600 ml), and extracted with CH_2Cl_2 (2 × 300 ml). The combined org. phases were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue (colorless oil that tends to crystallize) was co-evaporated with toluene (2 × 15 ml) to yield crude **7** (18.9 g, 99%). Recrystallization from hexane afforded pure **7** (14.0 g, 74%). White solid. M.p. 135°. R_f 0.42 (AcOEt). IR (KBr): 3316, 3151, 2930, 2857, 1666, 1601, 1254, 1111, 837, 777. ^1H-NMR (300 MHz, $CDCl_3$): 8.35 (*s*, H–C(2)); 8.15 (*s*, H–C(8)); 6.45 (*t*-like, *J* = 6.4, H–C(1′)); 5.81 (br. *s*, NH_2); 4.63–4.59 (*m*, H–C(3′)); 4.01 (*q*-like, *J* = 3.4, H–C(4′)); 3.87 (*dd*, *J* = 4.1, 11.2, H_a–C(5′));

3.77 (*dd*, *J* = 3.2, 11.3, $H_b-C(5')$); 2.66 – 2.59 (*m*, $H_a-C(2')$); 2.47 – 2.40 (*m*, $H_b-C(2')$); 0.91 (*s*, ᵗBuSi); 0.10, 0.09 (2*s*, 2 Me_2Si). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 155.3 (C(6)); 152.8 (C(2)); 149.6 (C(4)); 139.1 (C(8)); 87.9 (C(1')); 84.3 (C(4')); 71.9 (C(3')); 62.6 (C(5')); 41.3 (C(2')); 26.0, 25.8 (2 Me_3CSi); 18.4, 18.0 (2 CSi); −4.6, −4.8, −5.3, −5.5 (4 MeSi). FAB-MS: 480 ($[M+H]^+$). Anal. calc. for $C_{22}H_{41}N_5O_3Si_2$ (479.77): C 55.08, H 8.61, N 14.60; found C 55.20, H 8.58, N 14.60.

N^6,N^6-*Bis[(*tert-*butoxy)carbonyl]-3',5'-bis-*O*-[(*tert-*butyl)dimethylsilyl]-2'-deoxyadenosine* (**8**). A soln. of **7** (38.1 g, 79.5 mmol), Boc_2O (52.1 g, 239 mmol, 3.0 equiv.), and DMAP (29.2 g, 239 mmol, 3.0 equiv.) in anh. DMF (100 ml) was stirred overnight at r.t. The solvent was removed *in vacuo*, the residue was dissolved in CH_2Cl_2 (700 ml), extracted with 0.3M aq. tartaric-acid soln. (2 × 700 ml), and the org. phase was re-extracted with CH_2Cl_2 (700 ml). The combined org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. FC of the crude material (hexane/AcOEt 3:1 → 2:1) afforded pure **8** (49.1 g, 91%). Colorless oil. R_f 0.40 (hexane/AcOEt 2:1). IR ($CHCl_3$): 3019, 2956, 2951, 1791, 1758, 1601, 1370, 1255, 1215, 1110, 838. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.87 (*s*, H−C(2)); 8.45 (*s*, H−C(8)); 6.56 (*t*-like, *J* = 6.3, H−C(1')); 4.65 (*dd*-like, *J* = 1.9, 3.6, H−C(3')); 4.09 – 4.04 (*m*, H−C(4')); 3.92 (*dd*, *J* = 4.6, 11.3, $H_a-C(5')$); 3.81 (*dd*, *J* = 3.2, 11.2, $H_b-C(5')$); 2.72 – 2.62 (*m*, $H_a-C(2')$); 2.54 – 2.46 (*m*, $H_b-C(2')$); 1.48 (*s*, ᵗBuO); 0.95 (*s*, ᵗBuSi); 0.14, 0.12 (2*s*, 2 Me_2Si). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 151.9 (C(6)); 150.4 (C(2)); 143.2 (C(8)); 88.0 (CO); 84.5 (C(1')); 83.7 (C(4')); 71.7 (C(3')); 62.7 (C(5')); 41.4 (C(2')); 27.8 (*Me*C); 26.0, 25.7 (2 Me_3CSi); 18.4, 18.0 (2 CSi); −4.6, −4.8, −5.4, −5.5 (4 MeSi). FAB-MS: 680 ($[M+H]^+$). Anal. calc. for $C_{32}H_{57}N_5O_7Si_2$ (680.01): C 56.52, H 8.45, N 10.30; found C 56.60, H 8.49, N 10.26.

N^6,N^6-*Bis[(*tert-*butoxy)carbonyl]-3'-*O*-[(*tert-*butyl)dimethylsilyl]-2'-deoxyadenosine* (**9**). To a stirred soln. of **8** (37.9 g, 55.7 mmol) in THF (108 ml), a mixture of TBAF (1M in THF, 55.7 ml, 55.7 mmol, 1.0 equiv.) and glacial AcOH (12 ml) were slowly added over 15 min at 0°. Stirring was continued for 40 min at 0°, before the soln. was warmed to r.t. After 3 h, another 0.1 equiv. of TBAF/AcOH was added, and the reaction was allowed to go to completion (5 h). SiO_2 (25 g) was added, and the solvent was removed *in vacuo*. FC (SiO_2, hexane/AcOEt 2:1) yielded pure **9** (17.0 g, 54%). White solid. M.p. 156 – 158°. R_f 0.24 (hexane/AcOEt 2:1). IR (KBr): 3308, 2933, 2858, 1745, 1708, 1607, 1464, 1355, 1270, 1163, 1121, 1025, 930, 836, 788. ^{1}H-NMR (300 MHz, (D_6)DMSO): 8.86 (*s*, H−C(2)); 8.85 (*s*, H−C(8)); 6.48 (*t*-like, *J* = 6.8, H−C(1')); 5.02 (*t*-like, *J* = 5.6, OH); 4.64 (*quint.*-like, *J* = 2.8, H−C(3')); 3.92 – 3.86 (*m*, H−C(4')); 3.66 – 3.57 (*m*, $H_a-C(5')$); 3.56 – 3.47 (*m*, $H_b-C(5')$); 2.91 (*ddd*, *J* = 6.0, 7.2, 13.3, $H_a-C(2')$); 2.38 (*ddd*, *J* = 3.5, 6.2, 13.3, $H_b-C(2')$); 1.39 (*s*, ᵗBuO); 0.91 (*s*, ᵗBuSi); 0.12 (*s*, Me_2Si). ^{13}C-NMR (75.5 MHz, (D_6)DMSO): 151.4 (C(6)); 150.4 (C(2)); 144.3 (C(8)); 90.3 (CO); 87.9 (C(1')); 84.0 (C(4')); 73.8 (C(3')); 63.2 (C(5')); 41.3 (C(2')); 27.8 (*Me*C); 25.8 (Me_3CSi); 18.0 (CSi); −4.7, −4.8 (2 MeSi). FAB-MS: 604 ($[M+K]^+$); 566 ($[M+H]^+$). Anal. calc. for $C_{26}H_{43}N_5O_7Si$ (565.75): C 55.20, H 7.66, N 12.38; found C 55.49, H 7.73, N 12.05.

N^6-*[(*tert-*Butoxy)carbonyl]-3'-*O*-[(*tert-*butyl)dimethylsilyl]-4'-(hydroxymethyl)-2'-deoxyadenosine* (**10**). Compound **9** (18.6 g, 32.9 mmol) and CMC (48.7 g, 115 mmol, 3.5 equiv.) were co-evaporated with toluene (2×), added to a soln. of pyridinium trifluoroacetate (3.50 g, 18.1 mmol, 0.55 equiv.) in anh. DMSO (210 ml), and stirred for 18 h at r.t. The yellow mixture was cooled in an ice-bath, a soln. of oxalic acid (1.63 g, 18.1 mmol, 0.55 equiv.) in MeOH (30 ml) was added, and the soln. was stirred for another 75 min. The colorless precipitate was filtered off and washed with cold CH_2Cl_2 (250 ml). The org. phase was washed with sat. aq. $NaHCO_3$ soln. (500 ml), and the aq. phase was re-extracted with CH_2Cl_2 (4 × 300 ml). The combined org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield a yellow oil, which was dissolved in a mixture of 1,4-dioxane (237 ml), H_2O (107 ml), and 36% aq. HCOH (27.1 ml). Then, $Ba(OH)_2 \cdot 8\,H_2O$ (14.8 g, 47.0 mmol, 1.43 equiv.) was added, and the mixture was sonicated for 5 min under vigorous shaking, stirred for 19 h at r.t., and poured into aq. sat. NH_4Cl soln. (500 ml). The aq. phase was extracted with CH_2Cl_2 (4 × 400 ml), the combined org. phase was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and co-evaporated with toluene. The residue was dissolved in anh. EtOH (120 ml), cooled to 0°, and $NaBH_4$ (1.87 g, 49.4 mmol, 1.50 equiv.) was slowly added. After stirring for 75 min at r.t., the mixture was cooled to 0°, AcOH (6 ml) was added, and the soln. was concentrated. The green residue was dissolved in CH_2Cl_2 (500 ml), and the org. layer was washed with brine (400 ml). After re-extracting the aq. phase with CH_2Cl_2 (4 × 300 ml), the combined org. phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo* for 10 min. The residue was dissolved in AcOEt (23 ml) and kept at r.t. until no more precipitate was obtained (48 – 72 h). The off-white precipitate was filtered off, washed with cold AcOEt (10 ml) and hexane (20 ml), and dried *in vacuo* to yield pure **10** (7.82 g, 48%). White solid. M.p. 208 – 210°. R_f 0.39 (AcOEt). IR (KBr): 3414, 3354, 2933, 2857, 1754, 1619, 1585, 1470, 1403, 1369, 1331, 1232, 1146, 1116, 1057, 1017, 950, 873, 838. ^{1}H-NMR (300 MHz, (D_6)DMSO): 8.65 (*s*, NH, H−C(2)); 8.59 (*s*, H−C(8)); 6.45 (*t*-like, *J* = 6.6, H−C(1')); 4.99 (*t*-like, *J* = 5.5, OH); 4.70 (*quint.*-like, *J* = 3.8, H−C(3')); 4.46 (*t*-like, *J* = 5.5, OH); 3.65 – 3.49 (*m*, $CH_2(5')$, C*H*$_2$OH); 2.99 – 2.89 (*m*, $H_a-C(2')$); 2.48 – 2.38

(m, H_b–C(2′)); 1.48 (s, ^{t}BuO); 0.91 (s, ^{t}BuSi); 0.11 (s, Me_2Si). ^{13}C-NMR (75.5 MHz, (D_6)DMSO): 151.3 (C(6)); 151.0 (C(2)); 150.0 (C(4)); 142.5 (C(8)); 123.8 (C(5)); 89.6 (CO); 83.0 (C(1′)); 80.1 (C(4′)); 72.7 (C(3′)); 61.9, 61.0 (CH_2OH, C(5′)); 40.4 (C(2′)); 28.7 (MeC); 25.7 (Me_3CSi); 17.7 (CSi); −4.9, −5.2 (2 MeSi). ESI-MS: 518 ($[M+Na]^+$). Anal. calc. for $C_{22}H_{37}N_5O_6Si$ (495.66): C 53.31, H 7.52, N 14.13; found C 53.13, H 7.54, N 14.13.

N^6-*[(*tert*-Butoxy)carbonyl]-3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-4′-(hydroxymethyl)-2′-deoxyadenosine* (**11**). After co-evaporation with toluene (2 × 10 ml), **10** (6.18 g, 12.5 mmol) was dissolved in anh. CH_2Cl_2 (60 ml) and cooled to −78°. Trimethyl orthoacetate (7.85 ml, 62.5 mmol, 5.0 equiv.) and racemic CSA (232 mg, 1.00 mmol, 0.080 equiv.) were added. After 10 min, the cooling bath was removed, and the soln. was stirred for 3 h at r.t. The soln. was cooled to −20°, 20% aq. AcOH (83 ml) was added, and the biphasic mixture was stirred at −20 to 0° for 17 h. Then, CH_2Cl_2 (500 ml) and 1% aq. NaOH soln. (500 ml) were added, followed by sat. aq. $NaHCO_3$ soln. (*ca.* 600 ml) to adjust the pH to 8–9 (caution: evolution of CO_2 requires slow addition). The resulting mixture was extracted with CH_2Cl_2 (3 × 400 ml). The combined org. phase was dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure. After co-evaporation with toluene (2 × 10 ml), the colorless residue was dissolved in anh. DMF (75 ml). 1*H*-Imidazole (2.55 g, 37.5 mmol, 3.0 equiv.) and TBDMSCl (2.83 g, 18.8 mmol, 1.5 equiv.) were added, and the soln. was stirred at. r.t. for 15 h. The reaction was quenched by addition of MeOH (30 ml), and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (500 ml) and 0.3M aq. tartaric acid soln. (1000 ml), and extracted with CH_2Cl_2 (3 × 300 ml). The combined org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was dissolved in MeOH (60 ml), chilled to 0°, and MeONa (10.3 g) was added. After stirring the white suspension for 30 min at 0°, CH_2Cl_2 (500 ml) and sat. aq. NH_4Cl soln. (850 ml) were added. Then, the soln. was adjusted to pH ≈ 5 by addition of glacial AcOH. The mixture was extracted with CH_2Cl_2 (4 × 300 ml), the combined org. phase was dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure. FC (SiO_2; hexane/AcOEt 2:1 → AcOEt) yielded the pure diastereoisomers **11a** (2.77 g, 36%) and **11b** (3.74 g, 49%) in a ratio of **11a/11b** 10:13.

Data of **11a**: White foam. M.p. 109–111° (liquid crystal). R_f 0.27 (hexane/AcOEt 1:1). IR (KBr): 3419, 2955, 2930, 2857, 1753, 1612, 1524, 1467, 1393, 1367, 1330, 1255, 1147, 1082, 953, 837. ^{1}H-NMR (300 MHz, (D_6)DMSO): 8.65 (s, NH, H–C(2)); 8.58 (s, H–C(8)); 6.43 (t-like, J = 6.6, H–C(1′)); 5.02 (t-like, J = 5.5, OH); 4.75 (t-like, J = 5.8, H–C(3′)); 3.75 (d, J = 10.7, H_a–C(5′)); 3.69 (d, J = 10.7, H_b–C(5′)); 3.57–3.46 (m, CH_2OH); 2.90–2.81 (m, H_a–C(2′)); 2.52–2.43 (m, H_b–C(2′)); 1.47 (s, ^{t}BuO); 0.89, 0.88 (2s, 2 ^{t}BuSi); 0.09, 0.08 (2s, 2 MeSi); 0.05, 0.04 (2s, 2 MeSi). ^{13}C-NMR (75.5 MHz, (D_6)DMSO): 151.6 (C(6)); 151.4 (C(2)); 150.0 (C(4)); 142.6 (C(8)); 123.8 (C(5)); 89.1 (CO); 83.1 (C(1′)); 80.1 (C(4′)); 72.2 (C(3′)); 63.1, 61.8 (CH_2OH), C(5′)); 40.4 (C(2′)); 27.8 (MeC); 25.8, 25.6 (2 Me_3CSi); 18.1, 17.7 (2 CSi); −4.8, −5.3, −5.5, −5.6 (4 MeSi). FAB-MS: 610 ($[M+H]^+$). Anal. calc. for $C_{28}H_{51}N_5O_6Si_2$ (609.92): C 55.14, H 8.43, N 11.48; found C 55.17, H 8.29, N 11.41.

Data of **11b**: White foam. M.p. 61–62°. R_f 0.10 (hexane/AcOEt 1:1). IR (KBr): 3420, 3256, 2955, 2931, 2858, 1753, 1613, 1584, 1525, 1470, 1392, 1368, 1329, 1255, 1231, 1147, 1083, 951, 837. ^{1}H-NMR (300 MHz, (D_6)DMSO): 8.59, 8.58 (2s, NH, H–C(2), H–C(8)); 6.44 (t-like, J = 6.5, H–C(1′)); 4.75 (t-like, J = 5.3, OH); 4.50 (t-like, J = 5.2, H–C(3′)); 3.69 (s, CH_2(5′)); 3.66 (dd, J = 4.3, 11.5, CH_a–C(4′)); 3.51 (dd, J = 6.2, 11.5, CH_b–C(4′)); 3.07–2.98 (m, H_a–C(2′)); 2.51–2.40 (m, H_b–C(2′)); 1.47 (s, ^{t}BuO); 0.90, 0.84 (2s, 2 ^{t}BuSi); 0.11, 0.10 (2s, 2 MeSi); 0.01, −0.01 (2s, 2 MeSi). ^{13}C-NMR (75.5 MHz, (D_6)DMSO): 151.5 (C(6)); 151.3 (C(2)); 150.0 (C(4)); 142.5 (C(8)); 123.8 (C(5)); 88.9 (CO); 82.7 (C(1′)); 80.1 (C(4′)); 72.3 (C(3′)); 62.6, 60.5 (CH_2OH, C(5′)); 39.0 (C(2′)); 27.8 (MeC); 25.7, 25.6 (2 Me_3CSi); 18.0, 17.7 (2 CSi); −4.8, −5.3, −5.5, −5.6 (4 MeSi). FAB-MS: 610 ($[M+H]^+$). Anal. calc. for $C_{28}H_{51}N_5O_6Si_2$ (609.92): C 55.14, H 8.43, N 11.48; found C 55.17, H 8.36, N 11.31.

N^6-*[(*tert*-Butoxy)carbonyl]-3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-4′-formyl-2′-deoxyadenosine* (**12**). A soln. of **11a** (9.70 g, 15.9 mmol) and 1,1,1-triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1*H*)-one (*Dess–Martin* reagent; 16.9 g, 39.8 mmol, 2.5 equiv.) in anh. CH_2Cl_2 (200 ml) was stirred at r.t. for 6 h. ^{t}BuOMe (500 ml) was added, and the mixture was extracted with 0.3M aq. NaOH soln. (3 × 300 ml) containing $Na_2S_2O_3$ (15 g each). The org. phase was washed with brine (300 ml). The aq. phase was re-extracted with ^{t}BuOMe (300 ml), the combined org. phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. FC (SiO_2, hexane/AcOEt 3:1 → 1:1) yielded pure **12** (7.84 g, 81%). White foam. M.p. 64°. R_f 0.32 (hexane/AcOEt 1:1). IR (KBr): 3421, 3178, 2955, 2858, 1742, 1610, 1465, 1329, 1257, 1229, 1145, 1096, 945, 838. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.68 (s, CHO); 8.76 (s, H–C(2)); 8.29 (s, H–C(8)); 8.01 (br. s, NH); 6.78 (t-like, J = 6.9, H–C(1′)); 4.88 (t-like, J = 4.2, H–C(3′)); 4.06 (d, J = 11.3, H_a–C(5′)); 3.93 (d, J = 11.3, H_b–C(5′)); 2.90–2.83 (m, H_a–C(2′)); 2.57–2.52 (m, H_b–C(2′)); 1.57 (s, ^{t}BuO); 0.90, 0.89 (2s, 2 ^{t}BuSi); 0.11, 0.10 (2s, 2 MeSi); 0.08, 0.07 (2s, 2 MeSi). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 200.9 (CHO); 153.0 (C(6)); 149.9 (C(2)); 149.6 (C(4)); 141.0 (C(8)); 121.8 (C(5)); 93.3 (CO); 85.5 (C(4′)); 82.3 (C(1′)); 75.9 (C(3′)); 63.9 (C(5′)); 41.6 (C(2′)); 28.1 (MeC);

25.9, 25.6 (2 *Me*$_3$CSi); 18.3, 17.9 (2 CSi); −4.7, −5.3, −5.5, −5.6 (4 MeSi). FAB-MS: 646 ([*M* + K]$^+$); 608 ([*M* + H]$^+$). Anal. calc. for $C_{28}H_{49}N_5O_6Si_2$ (607.90): C 55.32, H 8.12, N 11.52; found C 55.33, H 8.07, N 11.36.

N^6-*[(*tert*-Butoxy)carbonyl]-3′,5′-bis-*O*-[(*tert*-butyl)dimethylsilyl]-4′-(2,2-dimethylpropanoyl)-2′-deoxyadenosine* (**13**). Compound **12** (9.56 g, 15.7 mmol) was co-evaporated with toluene (2 × 10 ml), dissolved in anh. Et_2O (200 ml), and cooled to −78°. Cold ^{t}BuLi (1.5M in pentane, 52.4 ml, 78.6 mmol, 5.0 equiv.) was added within 3 min, and the brown mixture was stirred for 10 min. Then, the reaction was quenched by addition of sat. aq. NH_4Cl soln. (95 ml), the pale yellow mixture was warmed to r.t., treated with H_2O (400 ml), and extracted with CH_2Cl_2 (5 × 300 ml). The combined org. phase was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and co-evaporated with toluene (2 × 10 ml). The resulting yellow foam was dissolved in anh. CH_2Cl_2 (150 ml), treated with 1,1,1-triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1*H*)-one (*Dess–Martin* reagent; 16.6 g, 39.3 mmol, 2.5 equiv.), and stirred at r.t. for 25 h. Then ^{t}BuOMe (500 ml) was added, and the mixture was extracted with 0.3M aq. NaOH soln. (3 × 300 ml) containing $Na_2S_2O_3$ (15 g each). The org. phase was washed with brine (300 ml), and the aq. phase was re-extracted with ^{t}BuOMe (300 ml). The combined org. phase was dried ($MgSO_4$), filtered, concentrated under reduced pressure, and co-evaporated with toluene. FC (SiO_2; hexane/AcOEt 7:2 → 2:1) yielded pure **13** (2.62 g, 25%). White foam. M.p. 70–72°. R_f 0.27 (hexane/AcOEt 2:1). IR (KBr): 3422, 3246, 3181, 2957, 2931, 2859, 1758, 1721, 1703, 1610, 1463, 1366, 1329, 1257, 1225, 1144, 941, 836. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.76 (*s*, H−C(2)); 8.42 (*s*, H−C(8)); 8.11 (br. *s*, NH); 6.76 (*dd*, *J* = 5.4, 9.5, H−C(1′)); 4.56 (*d*-like, *J* = 4.6, H−C(3′)); 3.99 (*d*, *J* = 10.5, H_a−C(5′)); 3.87 (*d*, *J* = 10.5, H_b−C(5′)); 2.70 (*ddd*, *J* = 4.7, 9.4, 13.0, H_a−C(2′)); 2.48 (*dd*-like, *J* = 5.5, 12.9, H_b−C(2′)); 1.54 (*s*, ^{t}BuO); 1.22 (*s*, ^{t}Bu−CO); 0.88, 0.87 (2*s*, 2 ^{t}BuSi); 0.11, 0.08, 0.07, 0.06 (4*s*, 4 MeSi). NOE: ^{t}BuC=O → H−C(8): (+); H−C(1′): ++; H−C(3′): (+); H_b−C(5′): ++; H_a−C(2′): (+). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 213.5 (^{t}Bu*C*O); 153.1 (C(6)); 149.7 (C(2)); 149.6 (C(4)); 140.9 (C(8)); 121.7 (C(5)); 100.4 (NCO); 85.5 (C(4′)); 82.2 (C(1′)); 75.6 (C(3′)); 69.2 (C(5′)); 45.1 (Me_3*C*−CO); 42.9 (C(2′)); 28.3, 28.2, 28.1 (*Me*$_3$C−O); 26.3, 25.9, 25.8, 25.7, 25.5 (2 *Me*$_3$CSi, *Me*$_3$C−CO); 18.4, 18.0 (2 CSi); −4.9, −5.2, −5.4, −5.5 (4 MeSi). FAB-MS: 702 ([*M* + K]$^+$); 664 ([*M* + H]$^+$). Anal. calc. for $C_{32}H_{57}N_5O_6Si_2$ (664.01): C 57.88, H 8.65, N 10.55; found C 58.01, H 8.67, N 10.18.

4′-(2,2-Dimethylpropanoyl)-2′-deoxyadenosine (**14**). A mixture of **13** (2.27 g, 3.42 mmol) and TBAF (1M in THF, 34.2 ml, 34.2 mmol, 10 equiv.) was stirred for 1 h at 0° and for 80 min at r.t. SiO_2 (11 g) was added, and the solvent was removed under reduced pressure. The reagent was removed by passing the mixture through a short column of SiO_2, eluting with AcOEt. Toluene (10 ml) and SiO_2 gel (4.0 g, activated at 80° *in vacuo* for 24 h) were added, and the mixture was concentrated under reduced pressure (26 mbar). The mixture was heated to 70° *in vacuo* (*ca.* 4×10^{-2} mbar) for 65 h, then, the temp. was raised to 80° for another 72 h. The mixture was cooled to r.t., extracted with DMF (30 ml) and MeOH (30 ml), filtered, and concentrated *in vacuo*. Purification by FC (SiO_2; AcOEt → acetone) yielded pure **14** (75.0 mg, 37%). Clear, light-amber crystals. M.p. 87–90° (liquid crystal). R_f 0.21 (AcOEt/MeOH 9:1). IR (KBr): 3342, 3189, 2962, 2869, 1691, 1647, 1601, 1481, 1371, 1256, 1212, 1103, 944, 907. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.30 (*s*, H−C(2)); 7.94 (*s*, H−C(8)); 7.18–7.13 (*m*, NH_2); 6.42 (*dd*, *J* = 5.1, 10.3, H−C(1′)); 5.96 (br. *s*, 2 OH); 4.88 (*d*-like, *J* = 4.9, H−C(3′)); 3.97 (*d*, *J* = 11.4, H_a−C(5′)); 3.89 (*d*, *J* = 11.4, H_b−C(5′)); 3.22 (*ddd*, *J* = 4.9, 10.3, 13.0, H_a−C(2′)); 2.36 (*dd*-like, *J* = 5.2, 12.9, H_b−C(2′)); 1.21 (*s*, ^{t}BuC). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 217.5 (^{t}Bu*C*O); 156.3 (C(6)); 152.3 (C(2)); 148.4 (C(4)); 140.3 (C(8)); 121.2 (C(5)); 102.5 (C(4′)); 88.4 (C(1′)); 75.7 (C(3′)); 68.2 (C(5′)); 45.5 (Me_3*C*−CO); 39.6 (C(2′)); 25.7 (*Me*$_3$C−CO). FAB-MS: 374 ([*M* + K]$^+$); 336 ([*M* + H]$^+$). Anal. calc. for $C_{15}H_{21}N_5O_4$ (335.37): C 53.72, H 6.31, N 20.88; found C 53.45, H 6.50, N 20.75.

*5′-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-*N^6*-[(dimethylamino)methylidene]-4′-(2,2-dimethylpropanoyl)-2′-deoxyadenosine* (**15**). After co-evaporation with toluene (2 × 2 ml), compound **14** (20 mg, 59.6 μmol) was suspended in anh. MeOH (5 ml), *N*-(dimethoxymethyl)-*N,N*-dimethylamine (= dimethyl acetal of DMF; 39.7 μl, 298 μmol, 5.0 equiv.) was added, and the mixture was stirred at r.t for 16 h. The clear soln. was concentrated under reduced pressure, co-evaporated with MeOH/toluene 1:1 (3 × 3 ml), and dried *in vacuo*. DMF (3 ml), DMTCl (30.3 mg, 89.4 μmol, 1.5 equiv.), and collidine (79.0 μl, 596 μmol, 10 equiv.) were added, and the mixture was stirred at 30° for 17 h. MeOH (2 ml) was added, and the soln. was concentrated under reduced pressure. The residue was co-evaporated with toluene (2 × 2 ml) and subjected to FC (SiO_2; AcOEt → acetone/ Et_3N 99:1) to yield **15** (12.8 mg, 55%). Off-white foam. M.p. 125–127°. R_f 0.36 (acetone/Et_3N 99:1). IR (KBr): 3424, 2959, 2925, 2854, 1702, 1637, 1561, 1509, 1459, 1420, 1352, 1250, 1177, 1112, 1033, 937, 829. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.92 (*s*, H−C(2)); 8.46 (*s*, H−C(8)); 7.96 (*s*, *H*−CNMe$_2$); 7.36–7.19, 6.80–6.77 (2*m*, arom. H); 6.72 (*dd*, *J* = 5.3, 9.7, H−C(1′)); 4.71 (*d*-like, *J* = 4.6, H−C(3′)); 3.77 (*s*, MeO); 3.55 (*d*, *J* = 9.8, H_a−C(5′)); 3.42 (*d*, *J* = 9.6, H_b−C(5′)); 3.26, 3.21 (2*s*, Me_2N); 2.92 (*ddd*, *J* = 5.1, 9.6, 13.5, H_a−C(2′)); 2.53 (*dd*, *J* = 5.3, 13.2, H_b−C(2′)); 1.21 (*s*, ^{t}BuC). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 217.0 (^{t}Bu*C*O); 158.6 (*C*NMe$_2$); 158.0, 152.7, 151.6, 143.9, 140.1, 135.1, 130.1, 128.3, 128.2, 127.9, 127.0, 113.1 (arom. C); 98.6 (C(4′)); 87.2 (Ar_3*C*);

85.2 (C(1′)); 76.0 (C(3′)); 67.8 (C(5′)); 55.2 (MeO); 45.7 (Me_3*C*–CO); 41.2 (C(2′)); 38.7, 35.2 (2 MeN); 26.1 (*Me*$_3$C–CO). ESI-MS: 715 ([*M* + Na]$^+$); 693 (*M*$^+$). An accurate combustion analysis could not be obtained.

*5′-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-*N*^{6}-[(dimethylamino)methylidene]-4′-(2,2-dimethylpropanoyl)-2′-deoxyadenosine 3′-O-[(2-Cyanoethyl)-N,N-diisopropylphosphoramidite]* (**16**). Well-dried **15** (56.7 mg, 81.7 µmol) was dissolved in anh. CH_2Cl_2 (2.0 ml), $^iPr_2NEt$ (*Hünig* base; 75.5 µl, 441 µmol, 5.4 equiv.) and 2-cyanoethyl *N*,*N*-(diisopropyl)chlorophosphoramidite (54.7 µl, 245 µmol, 3.0 equiv.) were added, and the soln. was stirred at r.t. for 3.5 h. The mixture was poured into sat. aq. $NaHCO_3$ soln. (20 ml), which was extracted with CH_2Cl_2 (3 × 20 ml). The combined org. phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by FC (SiO_2; hexane/acetone/Et_3N 49.5 : 49.5 : 1) to afford **16** (57.1 mg, 78%), which was used without further purification for oligonucleotide synthesis. Colorless solid (mixture of two diastereoisomers). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.91, 8.90 (*s*, H–C(2)); 8.48, 8.44 (*s*, H–C(8)); 7.92, 7.91 (*s*, *H*C–NMe_2); 7.32–7.19, 6.76–6.73 (2*m*, arom. H); 6.71–6.65 (*m*, H–C(1′)); 4.23–4.07 (*m*, H–C(3′), CH_2OP); 3.75 (*s*, MeO); 3.55–3.44 (*m*, H_a–C(5′), H_b–C(5′), Me_2*CH*); 3.24, 3.19 (2*s*, Me_2N); 2.76–2.72 (*m*, H_a–C(2′), CH_2CN); 2.60–2.57 (*m*, H_b–C(2′)); 1.27–1.14 (*m*, *Me*$_2$CH), Me_3C–CO). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 218.1 (^{t}Bu*C*O); 158.7 (*C*NMe_2); 157.3, 153.2, 152.0, 144.8, 140.1, 135.1, 130.2, 129.0, 128.1, 127.7, 127.0, 116.8, 113.1 (arom. C, CN); 97.0 (C(4′)); 87.1 (Ar_3*C*); 85.9 (C(1′)); 77.2 (C(3′)); 70.8 (C(5′)); 58.1, 58.0 (MeO); 55.1 (CH_2OP); 46.9 (Me_3*C*–CO); 45.6 ((Me_2*CH*)$_2$NH); 41.3 (C(2′)); 37.2, 35.1 (Me_2N); 26.0 (*Me*$_3$C–CO); 22.9, 22.8, 22.7, 22.6 ((*Me*$_2$CH)$_2$NH); 20.0, 19.1 (*C*H_2CN). ^{31}P-NMR (121 MHz, $CDCl_3$): 149.1, 147.4. ESI-MS: 915 ([*M* + Na]$^+$); 893 ([*M* + H]$^+$). An accurate combustion analysis could not be obtained.

General Procedure for the Solid-Phase Synthesis of 4′-Modified Oligonucleotides. All syntheses were carried out on a 0.2-µmol scale (500-Å controlled-pore-glass support) applying standard methodology for 2-cyanoethyl phosphoramidites, except that coupling of the modified nucleotide **16** was carried out manually by pumping solns. of **16** in MeCN and the coupling reagent through the column by means of two syringes for 20 min. The crude oligonucleotides were purified by RP-HPLC, first 'trityl-on' mode (linear gradient of 15–40% MeCN (25 min) in 0.1% TEAA soln., pH 7.0), followed by detritylation, then 'trityl-off' mode (linear gradient of 6–13% MeCN (20 min) in 0.1% TEAA soln., pH 7.0). The purity and identity of the oligonucleotides were checked by RP-HPLC and MALDI-TOF MS, resp. The modified single strand 3′-$A_3GA_2A^*A_2C_3A_5C_3A_5GA_3$-5′, in which A* is the pivaloyl-substituted adenoside (as in **1**; *cf. Scheme 1*), was synthesized by this procedure.

General Procedure for ^{32}P-Labeling of Oligonucleotides. To a soln. of oligonucleotide (20 pmol) in kinase buffer (4 µl; 70 mM Tris · HCl pH 7.6, 10 mM $MgCl_2$, 5 mm dithiothreitol) were added [γ-^{32}P]-ATP (2 pmol), T_4 polynucleotide kinase (10 units), and nanopure H_2O (total volume: 40 µl). After incubation (45 min at 37°), the labeled oligonucleotide was purified by centrifugation *via* a *mini-QuickSpin* column. All oligonucleotides were used for experiments within 14 h after labeling to minimize decomposition caused by radiation and radioactive decay.

General Procedure for Irradiation of Oligonucleotides Followed by PAGE Analysis. Double-stranded DNA was generated by mixing the 4′-modified oligonucleotide **17** (6.7 pmol) with 1.5 equiv. of the corresponding ^{32}P-labelled complementary strand in citrate buffer (1.0 ml; pH 5, 20 mM citrate, 100 mM NaCl) and heating to 80° for 5 min, followed by cooling slowly to r.t. Ar gas was bubbled through the soln. in the cuvette for 6 min before irradiation. Double-stranded DNA solns. (200 µl) were irradiated for 6 min at 15° (thermostated cuvette) with a 500-W Hg high-pressure lamp, using a 320-nm cut-off filter, to determine cleavage yields at the modified position (typically 38–45%). A portion of the irradiated soln. (40 µl) was mixed with 1M piperidine soln. (200 µl) and heated to 90° for 30 min, then evaporated. After dissolving the residue in a mixture of loading buffer (20 µl; 90% formamide, 10% TBE buffer, some bromophenol blue) and H_2O (20 µl) at 40° for 20 min, β-radiation intensity was standardized by liquid scintillation of the samples (5.0 µl) from every probe to ensure that every lane on the gel contained an identical amount of radioactivity. The 12% denaturing polyacrylamide gels were cast according to standard procedures with urea (50 g), nanopure H_2O (20 ml), 10 × TBE buffer (10 ml), and *AccuGel 19:1* (30 ml). The gel mixture was degassed by a water-jet vacuum pump for 12 min, then polymerization was initiated by addition of TMEDA and 10% ammonium persulfate (80 µl each), and the gel cast at a thickness of 0.4 mm. Gels were processed at 1500 V for 130 min, then transferred onto filter paper (*Whatman 3 MM Chr*), wrapped into *Saran* foil, and exposed to a storage phosphor screen for 14–17 h. Relative yields were calculated by volume integration of single spots, and histograms were obtained by line integration along the lanes. The results correspond to the calculated differences between experiments containing 4′-modified oligonucleotides and blind probes (containing unmodified strands). From five experiments, the average ratio of P'_5/P'_3 was 2 : 1 (± 20%).

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