Building-Block Approach for the Straightforward Incorporation of a New FRET (Fluorescence-Resonance-Energy Transfer) System into Synthetic DNA

by **Lilia Clima** and **Willi Bannwarth***

Institut für Organische Chemie und Biochemie, Albert-Ludwigs Universität Freiburg, Albertstr. 21, D-79104 Freiburg
(phone: +497612036073; fax: +497612038705; e-mail: willi.bannwarth@organik.chemie.uni-freiburg.de)

The synthesis of the two new phosphoramidites **5** and **8** bearing a carbostyril (=quinolin-2(1*H*)-one) chromophore used as donor entity in our recently developed new FRET (fluorescence-resonance-energy transfer) system is described (*Schemes 1* and *2*) The high stability of the chromophore to basic conditions enables the incorporation of the phosphoramidites directly into DNA during solid-phase synthesis (*Schemes 3* and *4*). Since this is also possible for the (bathophenanthroline)ruthenium(II) complex used as acceptor (*Scheme 4*, *Steps d* and *e*), the whole labelling procedure to insert the FRET system into synthetic DNA is straightforward and represents a major improvement to our previous strategy.

Introduction. – Fluorescently labelled nucleic acids are particularly important tools in molecular biology, diagnostics, and structural studies [1][2]. In this context, DNA with incorporated dyes representing fluorescence-resonance-energy transfer (FRET) systems have proven to be important tools for the elucidation of distance-dependent interactions on the molecular level and in real time mode. Any changes in donor–acceptor distance will affect the rate of energy transfer which can be measured either by steady-state or time-resolved fluorescence studies. The latter approach requires labels with a long lifetime of their fluorescence, and this is usually achieved *via* the use of lanthanide chelates as acceptor in FRET systems [3][4]. As alternative labels, we have developed, some time ago, (bathophenanthroline)ruthenium(II) complexes (bathophenanthroline = 4,7-diphenyl-1,10-phenanthroline) which show, after excitation of their metal-to-ligand charge transfer, a fluorescence lifetime in the low μsec range [5]. In combination with a quinolinone derivative as donor, this yielded a highly efficient and robust new FRET system, of which the feasibility was first demonstrated in peptides in the course of the development of a homogeneous assay for the serine protease thrombin [6][7].

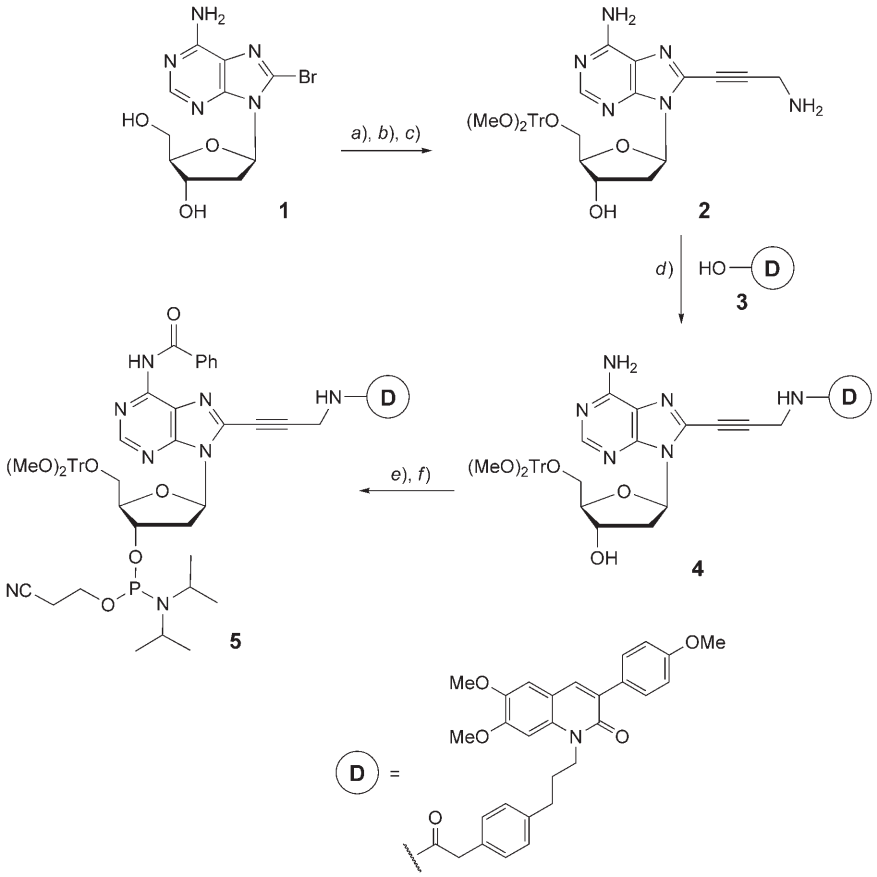
Later, we were also able to demonstrate the efficiency of the system in DNA [8]. In this study, both constituents, the (bathophenanthroline)ruthenium(II) complex as well as the carbostyril (=quinolin-2(1*H*)-one) chromophore were attached to amino-modified DNA after deprotection following solid-phase assembly of the oligonucleotide chain. The described method was time-consuming. Hence, we sought for possibilities to improve this labelling procedure.

In preliminary evaluations, we noted the outstanding chemical stability of both dyes; concentrated ammonia at elevated temperature did not cause any harm to them. Therefore, we surmized that their introduction into synthetic DNA should be possible *via* the pertinent phosphoramidite building blocks, and the final deprotection with conc. ammonia should have no influence on either chromophore.

Results and Discussions. – As possible attachment points for the carbostyril donor, we envisaged the 8-position of adenine leading to the building block **5** and the 5-position of uracil (=α-position of thymine) leading to phosphoramidite **8**.

For the synthesis of adenosine phosphoramidite **5** bearing the carbostyril moiety, 2′-deoxyadenosine was first converted to 8-bromo-2′-deoxyadenosine (**1**) [9] (*Scheme 1*).

Scheme 1. *Synthesis of Phosphoramidite* **5**

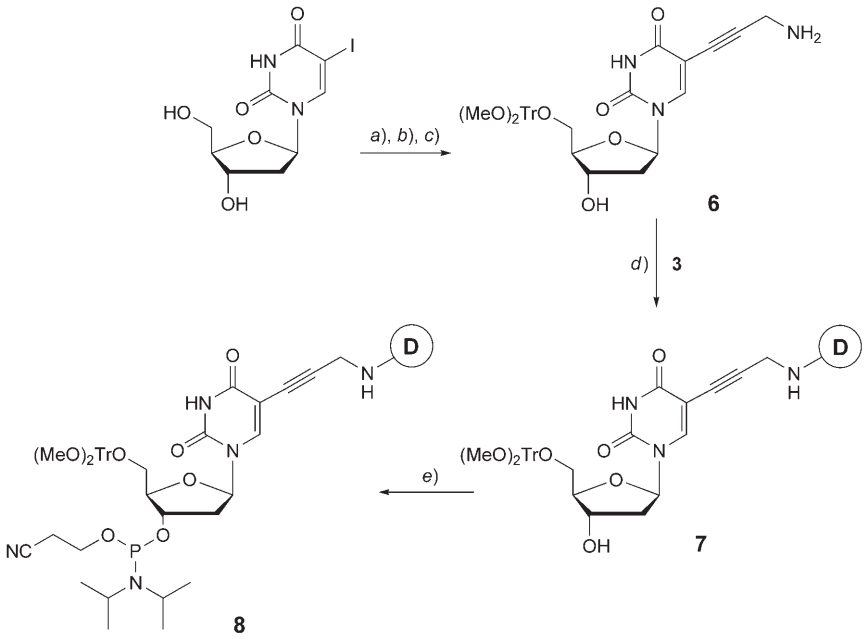


a) $(MeO)_2TrCl$, pyridine, *N,N*-dimethylpyridin-4-amine (DMAP), Et_3N, r.t., 4 h; 85%. *b*) $[Pd(PPh_3)_2Cl_2]$, CuI, Et_3N, 2,2,2-trifluoro-*N*-propynylacetamide, 50°, 5 h; 85%. *c*) 25% aq. NH_3 soln., MeOH, r.t., 16 h; quant. *d*) **3**, TBTU, $^iPr_2EtN$, DMF, r.t, 16 h; 67% (over 2 steps). *e*) Me_3SiCl, pyridine, BzCl, 25% aq. NH_3 soln.; 72%. *f*) $(NCCH_2CH_2O)P(N^iPr_2)_2$, diisopropylammonium 1*H*-tetrazolide, CH_2Cl_2, r.t., 2.5 h; 51%.

After regioselective introduction of the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) group at the 5′-position, a *Sonogashira* coupling with 2,2,2-trifluoro-*N*-(prop-2-yn-1-yl)acetamide) in the presence of $[Pd(PPh_3)_2Cl_2]$ as catalyst [10] and deprotection of the trifluoroacetyl group under basic conditions (aq. ammonia/MeOH) afforded compound **2** in 58% yield over 3 steps starting from 2′-deoxyadenosine. The chromophore **3**, activated with TBTU (TBTU = *O*-(1*H*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate [11]), was then coupled to the amino group of **2**, which led to **4** in 67% yield. This step was followed by protection of the 6-amino function *via* a three-step protocol [12]. Finally, conversion to the pertinent phosphoramidite at the 3′-position was performed with 2-cyanoethyl tetraisopropylphosphorodiamidite in the presence of diisopropylammonium 1*H*-tetrazolide to yield the desired pure phosphoramidite **5** (51%) after purification by short-column chromatography, of which the ^{31}P-NMR revealed exclusively P^{III} signals.

For the synthesis of the building block **8**, 2′-deoxy-5-iodouridine and 2,2,2-trifluoro-*N*-(prop-2-yn-1-yl)acetamide) [13] were subjected to a *Sonogashira* reaction [14][15] in the presence of Pd/C and *Amberlite-IRA-67* resin which afforded the coupling product in 76% yield (*Scheme 2*). This step was followed by regioselective introduction of the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) group at the 5′-position. The trifluoroacetyl group was cleaved off with aq. NH_3 solution/MeOH to give amine **6**. The reaction between **6** and **3** was this time performed with TSTU (TSTU = *N*,*N*,*N*′,*N*′-tetramethyl-

Scheme 2. *Synthesis of Phosphoramidite* **8**

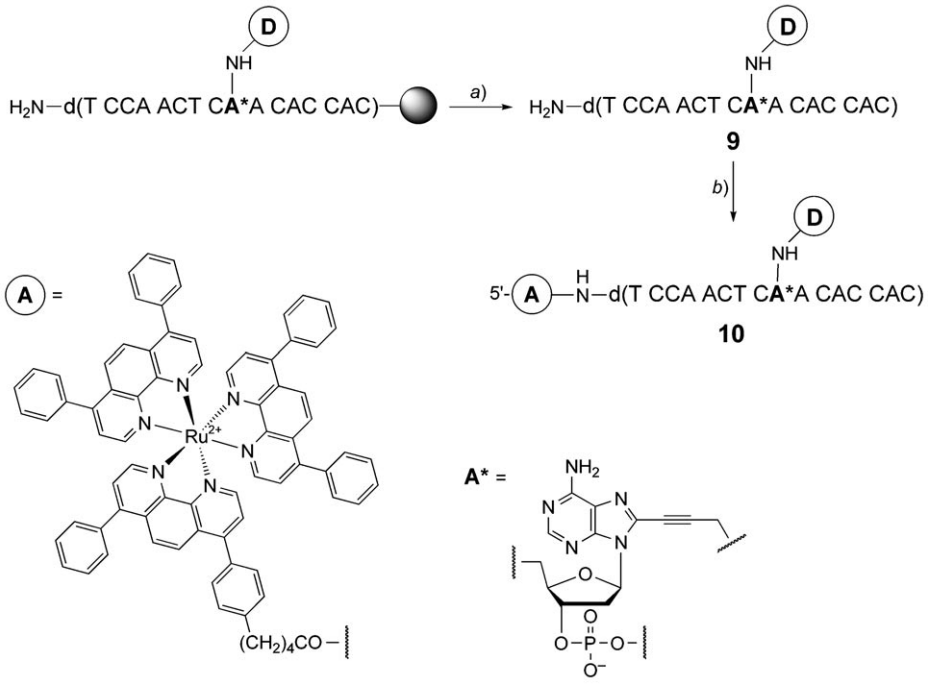


a) Pd/C, *Amberlite IRA-67*, CuI, DMF, 50°, 16 h; 76%. *b*) $(MeO)_2TrCl$, DMAP, Et_3N, pyridine, 4 h; 81%. *c*) 25% aq. NH_3 soln./MeOH; 92%. *d*) **3**, TSTU, $^iPr_2EtN$, DMF, r.t., 16 h; 98%. *e*) $(NCCH_2CH_2O)P(N^iPr_2)_2$, diisopropylammonium 1*H*-tetrazolide, CH_2Cl_2, r.t., 1.5 h; 54%.

O-succinimidouronium tetrafluoroborate) [11]) as coupling reagent in DMF to give product **7** in 98% yield. Compound **7** was converted to the pertinent phosphoramidite **8** at the 3′-position by phosphitylation with 2-cyanoethyl tetraisopropylphosphorodiamidite in the presence of diisopropylammonium 1*H*-tetrazolide (54%).

For the evaluation of the incorporation of the donor building block **5** during solid-phase synthesis, DNA sequence **10** (*Scheme 3*) was chosen. The donor phosphoramidite **5** was inserted during a 1 μmol scale synthesis as base number eight. Then, the synthesis was continued with standard phosphoramidite building blocks. As the last building block, 5′-deoxy-5′-[(4-methoxytrityl)amino]thymidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) was coupled, after which the (MeO)Tr was removed by 3% dichloroacetic acid to yield the 5′-unprotected amino terminus. Ammonia treatment for the removal from the support and deprotection gave the crude DNA fragment **9**. After replacement of the NH_4^+ ions by K^+ and desalting, a small amount was analyzed by polyacrylamide-gel electrophoresis (PAGE) (*Fig. 1*, *Lanes 4* and *8*). The rest was dissolved in a mixture of DMF/1,4-dioxane/H_2O 1:1:1, and then $^i Pr_2EtN$ together with an excess of (bathophenanthroline)ruthenium(II) complex activated as ester with hydroxysuccinimide [5] were added and incubated for 24 h to yield fragment **10** having now both dyes of the FRET system incorporated. After removal of the

Scheme 3. *Synthesis of* **10**



a) 25% aq. NH_3 soln., 55°, 4 h. *b*) (Bathophenanthroline)ruthenium(II) complex acceptor (as ester with hydroxysuccinimide), DMF/1,4-dioxane/water 1:1:1, $^i Pr_2EtN$, 24 h.

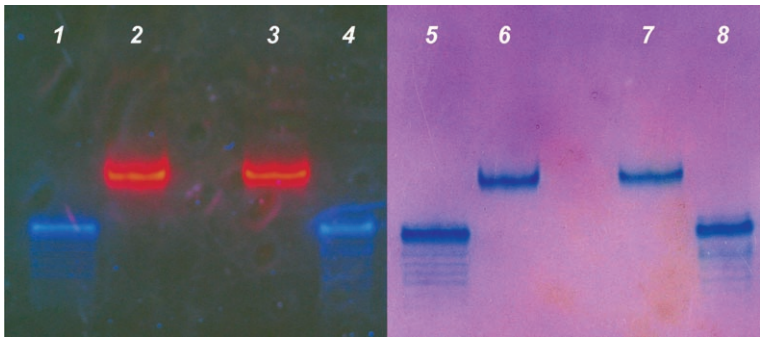


Fig. 1. *Polyacrylamide-gel electrophoresis* (PAGE; 20%) *of* **9**, **10**, **12**, *and* **13**. At 366 nm: *Lane 1*, **12**; *Lane 2*, **13**; *Lane 3*, **10**; *Lane 4*, **9**. Stained with 3,3′-diethyl-9-methyl-4,5,4′,5′-dibenzothiacarbocyanine bromide soln.: *Lane 5*, **12**; *Lane 6*, **13**; *Lane 7*, **10**; *Lane 8*, **9**.

solvents, the excess Ru-complex was removed by $CHCl_3$ extraction, and the crude fragment **10** was purified by HPLC and analyzed by PAGE (*Fig. 1*, *Lanes 3* and *7*).

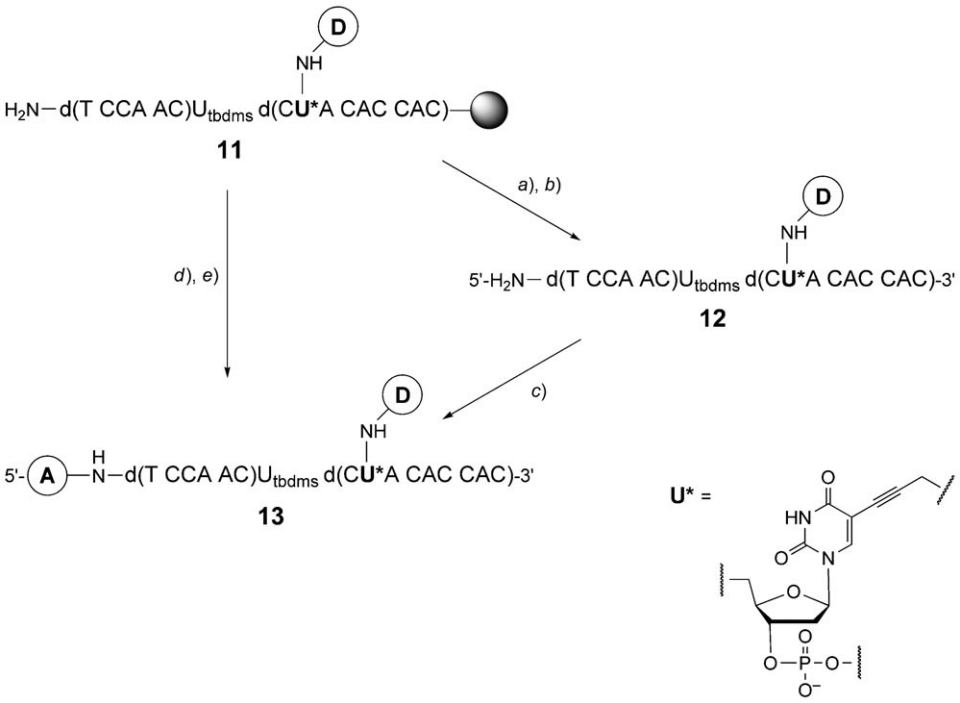
For the evaluation of donor-building block **8**, we have chosen virtually the same oligonucleotide sequence as for the incorporation of donor building block **5**, except that we inserted now building block **8** instead of **5** in position eight from the 3′-end (*Scheme 4*). On the one hand, the continuation of the synthesis as well as the attachment of the Ru-complex as acceptor to the crude fragment **12** yielding sequence **13** were performed in the same way as described for the preparation of sequence **10** (*Scheme 4*, *a* – *c*).

On the other hand, we tried as an alternative for the attachment of the (bathophenanthroline)ruthenium(II) complex its solid-phase incorporation (*Scheme 4*, *d*), according to a procedure described by *Richert* and co-workers [16]. If successful, this would be a significant improvement compared to the tedious procedure for the attachment of the Ru-complex in solution. For this purpose, a mixture of the (bathophenanthroline)ruthenium(II) complex (as free acid), HBTU (HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), HOBt (HOBt = 1-hydroxy-1*H*-benzotriazole), and $^{i}Pr_2EtN$ in DMF were added to the solid-phase-bound sequence **11** (*Scheme 4*, *d*). After coupling, the supernatant was removed and the support washed several times. Cleavage with conc. ammonia (*Scheme 4*, *e*) yielded then directly the desired fragment **13** with the incorporated FRET system.

A HPLC comparison of the crude DNA **13** obtained by the Ru-complex attachment in solution (*Fig. 2*, *a*) and by the solid-phase coupling of the Ru-complex (*Fig. 2*, *b*)) revealed that high efficiency was achieved in both couplings but the latter approach turned out to be far easier to perform.

To evaluate whether the type of base carrying the donor of the FRET system would have any influence on the intensity of the FRET, sequences **10** and **13** were hybridized to their pertinent complementary sequence according to *Fig. 3*, *a* and *b*. As can be seen from *Fig. 3*, *c*, both FRET systems lead to a strong emission at 620 nm upon excitation at 350 nm. There is no significant difference in the intensity of this emission, indicating that both building blocks **5** and **8** carrying the donor are equally suited to our FRET system in DNA.

Scheme 4. *Synthesis of* **13**



a) 2M NH_4OH in EtOH, 30 min, 65°. *b*) 7M $MeNH_2$ in EtOH, 30 min, 65°. *c*) (Bathophenanthroline)-ruthenium(II) complex (as ester with hydroxysuccinimide), DMF/1,4-dioxane/H_2O 1:1:1, $^iPr_2EtN$, 25°, 24 h. *d*) (Bathophenanthroline)ruthenium(II) complex (as free acid), HOBt, HBTU, $^iPr_2EtN$, 24 h. *e*) 25% aq. NH_3 soln., 40°, 2 h.

Conclusions. – In summary, we have synthesized two new phosphoramidite building blocks carrying the donor chromophore of our newly established FRET system that allows their insertion into DNA during synthesis on solid support. Together with the solid-phase coupling of the (bathophenanthroline)ruthenium(II) complex as acceptor, this represents an easy and straightforward way to establish the FRET system in DNA. Such labelled DNA is a valuable tool to monitor structural changes caused either by the formation of secondary structures or by supramolecular interactions in real-time mode.

The authors would like to thank Prof. *P. Gräber* and *R. Bienert* for fluorescence-spectra measurements and Dr. *Moneesha D'Costa* for help in the preparation of the manuscript.

Experimental Part

General. Oligonucleotide synthesis was carried out on an *Expedite*-6800 DNA synthesizer on a 1 μmol scale by means of standard phosphoramidite technique. Replacement of NH_4^+ with K^+ was done by co-evaporation (3×) of the oligonucleotide with KCl (10 mg) in H_2O (200 μl). Oligonucleotide samples were desalted on *NAP-10* columns. Column chromatography (CC): short column; *Merck* silica gel *60*. HPLC: *Merck*/*Hitachi* system; reversed phase: *SP-250/10-Nucleosil-100-5-C18*, $A = 0.1$M $Et_3N \cdot$ AcOH buffer at pH 7.0, $B =$ MeCN. UV Spectra: *Perkin-Elmer Lambda-35* UV/VIS spectrometer.

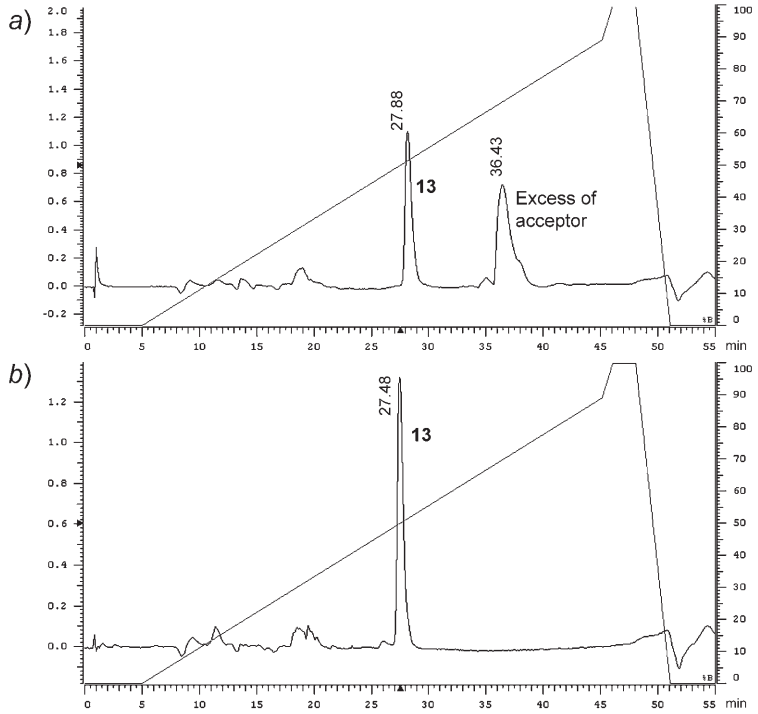


Fig. 2. *HPLC Plots after the synthesis of* **13**: a) *Crude* **13** *synthesized by the* Steps a – c *of* Scheme 4), *and* b) *crude* **13** *synthesized by the* Steps d *and* e *of* Scheme 4. HPLC conditions: reversed phase; 0 → 90% *B*/*A* within 40 min; *A* = 0.1M $Et_3N \cdot AcOH$ (pH 7), *B* = MeCN.

Fluorescence spectra: *Perkin-Elmer LS45* spectrometer. ^{1}H-(400 MHz) and ^{13}C-NMR (100 MHz) Spectra: *Bruker DRX-500* spectrometers; chemical shifts δ in ppm rel. to Me_4Si, referenced to residual solvent signals; *J* in Hz. MS: *LCQ Advantage* (ESI) mass spectrometer.

Analytical Polyacrylamide-Gel Electrophoresis (PAGE). Polyacrylamide gels (20%) of 0.4 mm thickness were used. Pre-electrophoresis was performed for 2 h at 500 V with *Tris* · borate running buffer. Oligonucleotide (1 µl, 0.1 *OD* units) and bromophenol blue/xylenecyanol soln. (3 µl) were heated to 90° for 2 min and rapidly cooled to 0°. Electrophoretic separation was performed for 2 h at 500 V and 4 mA. Oligonucleotide bands were visualized at 366 nm or stained with a soln. of 3,3′-diethyl-9-methyl-4,5,4′,5′-dibenzothiacarbocyanine bromide (= 'Stains-All'; *Fluka*).

8-Bromo-2′-deoxyadenosine (**1**) [9]. To a soln. of 2′-deoxyadenosine (1 g, 3.72 mmol) in 1,4-dioxane (10 ml) and 0.5M aq. AcONa buffer (pH 4.7) (16 ml), a soln. of Br_2 (0.24 ml, 4.64 mmol) in 1,4-dioxane (20 ml) was added dropwise while stirring. The mixture was stirred overnight at r.t. To the mixture, conc. $Na_2S_2O_5$ soln. was added until the red color vanished. The soln. was neutralized to pH 7.0 with 0.5M NaOH. The 1,4-dioxane was evaporated, when a white solid precipitated. The solid was filtered off, washed with cold 1,4-dioxane, and dried under high vacuum: 1 g (82%) of **1**. ^{1}H-NMR (400 MHz, (D_6)DMSO): 2.15 – 2.21 (*m*, 1 H–C(2′)); 3.20 – 3.27 (*m*, 1 H–C(2′)); 3.46 – 3.49 (*m*, 1 H–C(5′)); 3.62 – 3.66 (*m*, 1 H–C(5′)); 3.86 – 3.89 (*m*, H–C(4′)); 4.46 – 4.49 (*m*, H–C(3′)); 5.28 (br. *s*, OH–C(3′)); 5.35 (br. *s*, OH–C(5′)); 6.28 (*t*, *J* = 6.44, H–C(1′)); 7.48 (br. *s*, NH_2); 8.10 (*s*, H–C(2)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 36.97; 62.10; 71.14; 86.34; 88.30; 119.68; 126.61; 149.91; 152.35; 155.06. ESI-MS: 214.2 (92, $C_5H_3BrN_5^+$), 216.2 (90, $C_5H_3BrN_5^+$), 254.4 (40), 256.4 (43), 329.7 (100, $[M+1]^+$), 331.7 (97.5, $[M+1]^+$). Anal. calc. for $C_{10}H_{12}BrN_5O_3$: C 36.38, H 3.66, N 21.21, found: C 36.38, H 3.73, N 21.07.

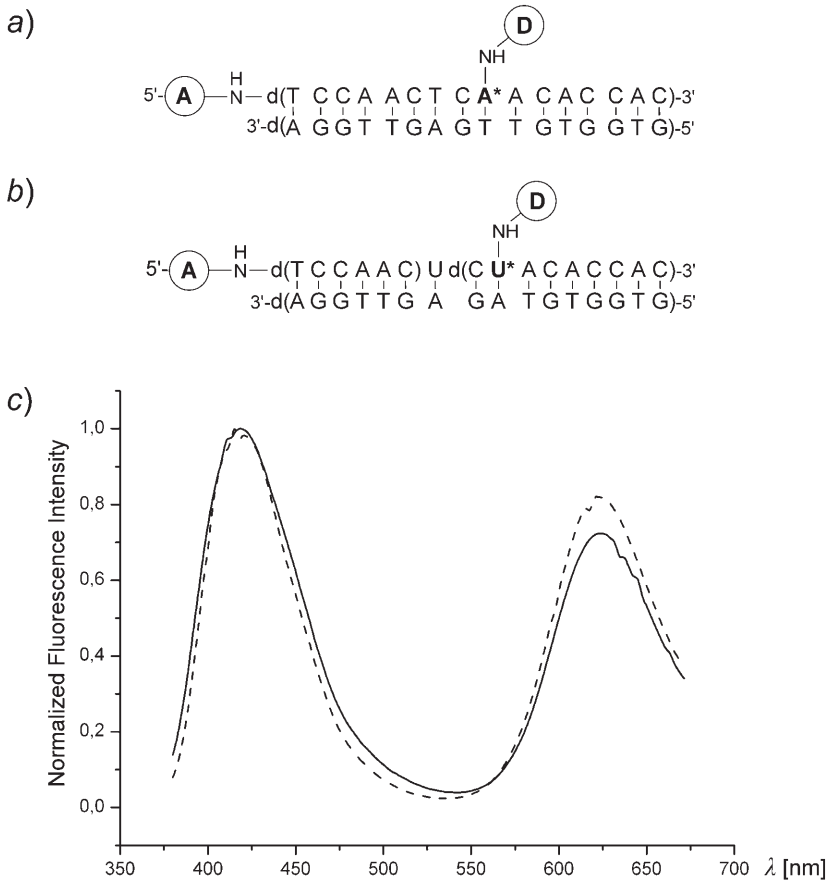


Fig. 3. a) b) *Hybridization of* **10** *and* **13**, *respectively, to their complementary sequences* (hybridization conditions: 1:3 ratio of **10** or **13** to complementary sequence, 70°, slow cooling to r.t.). c) *Fluorescence emission spectra of hybridized* **10** (—) *and hybridized* **13** (---) (excitation at 350 nm)

(MeO)$_2$Tr Protection of **1**: *8-Bromo-5′*-O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxyadenosine.* The 8-bromo-2′-deoxyadenosine (**1**) [9] (2 g, 6 mmol) was co-evaporated with anh. pyridine and then dissolved in anh. pyridine (30 ml). Et_3N (1.7 ml, 12 mmol), DMAP (180 mg, 1.5 mmol), and $(MeO)_2TrCl$ (3.3 g, 9.6 mmol) were added under Ar, and the mixture was stirred at r.t. for 4 h. Then, MeOH (5 ml) was added and the mixture extracted with CH_2Cl_2. The org. phase was washed with aq. $NaHCO_3$ soln., the combined org. layer dried (Na_2SO_4) and concentrated, and the residue purified by CC (silica gel, 2 → 5% MeOH/CH_2Cl_2): 3.14 g (85%) of product. Slightly yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.24–2.31 (*m*, 1 H–C(2′)); 3.32 (*m*, CH_2(5′)); 3.44–3.51 (*m*, 1 H–C(2′)); 3.68 (*s*, MeO); 3.68 (*s*, MeO); 4.03–4.07 (*m*, H–C(4′)); 4.85–4.89 (*m*, H–C(3′)); 5.74 (*s*, NH_2); 6.32 (*t*, *J* = 6.19, H–C(1′)); 6.65–6.70 (*m*, 4 arom. H); 7.06–7.30 (*m*, 9 arom. H); 7.98 (*s*, H–C(2)). ^{13}C-NMR (100 MHz, $CDCl_3$): 36.85; 45.87; 55.25 (2 C); 63.84; 72.94; 86.07; 86.06; 86.34; 113.10 (2 C); 113.22; 120.44; 126.81; 127.79 (2 C); 127.89; 128.19 (2 C); 129.22; 130.06 (2 C); 130.09 (2 C); 135.99; 136.05; 144.8; 150.83; 152.61; 154.21; 158.50. ESI-MS: 289.2 (18), 303.2 (100, $[(MeO)_2Tr]^+$), 631.6 (37, M^+), 633.7 (38, M^+).

5′-O*-[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-[(2,2,2-trifluoro-1-oxoethyl)amino]prop-1-yn-1-yl}adenosine.* Through the soln. of 5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-8-bromo-2′-de-

oxyadenosine (510 mg, 2.82 mmol) in dry DMF (7 ml), Ar was bubbled for 10 min. $[Pd(Ph_3P)_2Cl_2]$ (57 mg, 0.08 mmol) was added, and Ar was bubbled through the soln. for another 5 min. Et_3N (230 µl, 1.62 mmol) was added *via* syringe, followed by 2,2,2-trifluoro-*N*-(prop-2-yn-1-yl)acetamide (245 mg, 1.62 mmol) and CuI (31 mg, 0.16 mmol). The mixture was stirred at 50° for 5 h, the solvent evaporated, and the residue purified by CC (silica gel, 2 → 5% MeOH/CH_2Cl_2): 480 mg (85%) of product. Slightly yellow foam. ^{1}H-NMR (400 MHz, (D_6)DMSO): 2.19 – 2.25 (*m*, 1 H–C(2′)); 3.16 (*m*, CH_2(5′)); 3.26 – 3.32 (*m*, 1 H–C(2′)); 3.69 (*s*, MeO); 3.70 (*s*, MeO); 3.94 – 3.98 (*m*, H–C(4′)); 4.36 (*d*, *J* = 5.3, C*H*$_2$NH); 4.61 – 4.67 (*m*, H–C(3′)); 5.32 (br. *s*, OH); 6.40 (*t*, *J* = 6.1, H–C(1′)); 6.72 – 6.79 (*m*, 4 arom. H); 7.13 – 7.19 (*m*, 9 arom. H); 7.45 (br. *s*, NH_2); 7.98 (*s*, H–C(2)); 10.20 (*t*, *J* = 5.4 Hz, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 29.37; 36.71; 54.84 (2 C); 63.75; 70.75; 72.16; 84.50; 85.17; 85.84; 90.92; 112.88 (2 C); 112.93 (2 C); 114.27; 117.13; 126.40; 127.54 (2 C); 127.60 (2 C); 129.50; 129.63 (2 C); 132.88; 36.65 (2 C); 144.93; 148.39; 153.38; 155.86; 156.11; 156.48; 157.86; 157.92. ESI-MS: 303.2 (32, $[(MeO)_2Tr]^+$), 587.1 (100, $[M+1]^+$).

*8-(3-Aminoprop-1-yn-1-yl)-5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxyadenosine* (**2**). To a soln. of 5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-[(2,2,2-trifluoro-1-oxoethyl)amino]-prop-1-yn-1-yl}adenosine (320 mg, 0.45 mmol) in MeOH (3 ml), 25% aq. NH_4OH soln. (9 ml) was added, and the mixture was stirred overnight at r.t. The mixture was extracted with CH_2Cl_2/sat. $NaHCO_3$ soln. and the org. layer dried (Na_2SO_4), and evaporated: **2** (275 mg, quant.). ^{1}H-NMR (400 MHz, (D_6)DMSO): 2.19 – 2.26 (*m*, 1 H–C(2′)); 3.18 (*m*, CH_2(5′)); 3.23 – 3.29 (*m*, 1 H–C(2′)); 3.62 (*m*, CH_2); 3.70 (*s*, MeO); 3.71 (*s*, MeO); 3.95 – 3.99 (*m*, H–C(4′)); 4.63 – 4.67 (*m*, H–C(3′)); 5.33 (br. *s*, OH); 6.44 (*t*, *J* = 6.9 Hz, H–C(1′)); 6.73 – 7.79 (*m*, 4 arom. H); 7.12 – 7.38 (*m*, 9 arom. H); 7.95 (*s*, H–C(2)). ^{13}C-NMR (100 MHz, (D_6)DMSO): 36.75; 40.17; 54.93 (2 C); 54.95 (2 C); 63.88; 70.87; 84.27; 85.19; 85.77; 112.90 (2 C); 112.95 (2 C); 119.11; 126.43; 127.56 (2 C); 127.61 (2 C); 129.55 (2 C); 129.64 (2 C); 133.67; 135.63; 135.69; 144.97; 148.48; 153.05; 155.73; 157.88; 157.93. LC/ESI-MS: 303.2 (9, $[(MeO)_2Tr]^+$), 702.8 (100, $[M+97\ (CF_3CO)]^+$). APCI-MS (+ 90 V): 289.2 (47), 303.2 (100, $[(MeO)_2Tr]^+$), 606.9 (37, M^+), 702.6 (56, $[M+97(CF_3CO)]^+$), 853.7(28).

*5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-{{2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}prop-1-yn-1-yl}adenosine* (**4**). To a soln. of 4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}benzeneacetic acid (**3**; 100 mg, 0.20 mmol) in DMF (4 ml), TBTU (72 mg, 0.22 mmol) and ^{i}Pr$_2$EtN (100 µl, 0.61 mmol) were added under Ar. The mixture was stirred for 1 h, and **2** (150 mg, 0.24 mmol) was added, and the mixture stirred overnight. After removal of DMF, the residue was purified by short CC (silica gel, 2 → 10% MeOH/CH_2Cl_2): **4** (145 mg, 67%). Slightly yellow foam. ^{1}H-NMR (400 MHz, (D_6)DMSO): 1.92 – 1.96 (*m*, CH_2); 2.18 – 2.25 (*m*, 1 H–C(2′)); 2.71 (*t*, *J* = 7.2, $C_6H_4CH_2$); 3.23 – 3.38 (*m*, CH_2); 3.32 – 3.40 (*m*, 1 H–C(2′)); 3.77 (*s*, MeO); 3.81 (*s*, MeO); 3.93 – 3.98 (*m*, H–C(4′)); 4.20 (*d*, *J* = 5.4 Hz, C*H*$_2$NH); 4.25 – 4.28 (*m*, C*H*$_2$N); 4.61 – 4.66 (*m*, CH_2); 5.30 – 5.33 (*m*, 1 H); 6.39 (*t*, *J* = 6.2, H–C(1′)); 6.64 (*s*, 1 H); 6.71 – 7.12 (*m*, 24 arom. H); 7.66 (*s*, 1 H); 7.68 (*s*, 1 H); 8.16 (br. *s*, N*H*$_2$); 8.68 (*t*, *J* = 5.5, NH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 12.43 (2 C); 16.69 (2 C); 18.04 (2 C); 32.02; 39.75; 39.96; 40.17 (2 C); 53.55 (2 C); 54.90; 55.07; 55.68; 55.71; 85.17; 85.19; 93.22; 112.88; 112.93; 113.24 (2 C); 113.63; 119.16; 126.21; 126.41; 127.27; 127.55; 127.58; 127.60; 128.22; 128.26; 128.98; 129.03; 129.33; 129.49; 129.52; 129.58; 129.63; 129.80 (2 C); 133.14; 133.45; 133.62; 135.39; 135.55; 135.57; 135.64; 135.67; 139.39; 144.58; 144.95; 148.43; 151.62; 155.47; 155.81; 157.87; 157.92; 158.68; 160.06; 170.22. LC/ESI-MS: 1098.3 (100, $[M+22]^+$). Anal. calc. for $C_{63}H_{61}N_7O_{10} \cdot 4\ H_2O$: C 65.90, H 6.06, N 8.54, found: C 65.83, H 6.05, N 8.77.

N^6*-Benzoyl Protection of* **4**: N^6*-Benzoyl-5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-{{2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}prop-1-yn-1-yl}adenosine.* Compound **4** (760 mg, 0.7 mmol) was dried 3 × by co-evaporation with pyridine and suspended in dry pyridine (6 ml). Trimethylchlorosilane (450 µl, 3.5 mmol) was added to the ice-cooled mixture and stirred for 1.5 h at r.t. The mixture was cooled to 0°, benzoyl chloride (410 µl, 3.5 mmol) added, and the mixture stirred at r.t. for another 3.5 h. It was then cooled in an ice bath, and H_2O (3 ml) was added. After 5 min, 25% aq. NH_3 (1.5 ml) was added, and the mixture was stirred at r.t. for 30 min. The mixture was concentrated and the residue purified by short CC (silica gel, 2 → 5% MeOH/CH_2Cl_2): 590 g (72%) of slightly yellow foam. ^{1}H-NMR (400 MHz, (D_6)DMSO): 1.90 – 1.96 (*m*, CH_2); 2.16 – 2.25 (*m*, 1 H–C(2′)); 2.71 (*t*, *J* = 7.0 Hz, $C_6H_4CH_2$); 3.20 – 3.36 (*m*, CH_2); 3.32 – 3.39 (*m*,

CH_2); 3.75 (*s*, 2 MeO); 3.80 (*s*, 3 MeO); 3.92–3.98 (*m*, H–C(4′)); 4.10 (*d*, *J* = 5.4 Hz, C*H*$_2$NH); 4.24–4.27 (*m*, CH_2N); 4.61–4.66 (*m*, CH_2); 5.30–5.33 (*m*, 1 H); 6.40 (*t*, *J* = 6.18, H–C(1′)); 6.64 (*s*, 1 H); 6.71–7.12 (*m*, 28 arom. H); 7.64 (*s*, 1 H); 7.67 (*s*, 1 H); 8.66 (*t*, *J* = 5.5, NH). LC/ESI-MS: 303.3 (18, $[(MeO)_2Tr]^+$), 796 (33), 1098.3 (23, $[M-106\ (PhCO)+22]^+$), 1202.3 (100, $[M+22]^+$).

N^6-*Benzoyl-5′*-O-*[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-{[2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}prop-1-yn-1-yl}adenosine 3′-[2-Cyanoethyl Bis(1-methylethyl)phosphoramidite]* (**5**). The *N*^{6}-benzoyl-protected adenosine obtained from **4** (350 mg, 0.33 mmol) and diisopropylammonium 1*H*-tetrazolide (42 mg, 0.24 mmol) were co-evaporated with anh. MeCN (3 × 5 ml). They were then dissolved in dry CH_2Cl_2 (5 ml) and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphordiamidite (217 µl, 0.72 mmol) was added under Ar. The mixture was stirred for 2.5 h under Ar at r.t. Then, the mixture was poured into sat. $NaHCO_3$ soln. and extracted with CH_2Cl_2. The combined org. phase was dried (Na_2SO_4) and concentrated and the residue purified by short CC (deactivated silica gel (0.1% Et_3N), 2% MeOH/CH_2Cl_2: **5** (230 mg, 51%). Colorless foam. ^{31}P-NMR (120 MHz, $CDCl_3$): 149.80; 150.00.

5′-O-*[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-{[2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}prop-1-yn-1-yl}uridine* (**7**). As described for **4**, with **3** (100 mg, 0.20 mmol), DMF (6 ml), TSTU (76 mg, 0.26 mmol), ^{i}Pr$_2$EtN (100 µl, 0.6 mmol), and amine **6** [17] (180 mg, 0.3 mmol): **7** (210 mg, 98%). Colorless foam. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.05–2.13 (*m*, $C_6H_4CH_2$); 2.19–2.28 (*m*, 1 H–C(2′)); 2.39–2.43 (*m*, 1 H–C(2′)); 2.68–2.69 (*m*, 1 H); 2.76–2.77 (*m*, CH_2); 3.28–3.29 (*m*, CH_2); 3.30–3.36 (*m*, CH_2(5′)); 3.73 (*s*, MeO); 3.75 (*s*, MeO); 3.85–3.88 (*m*, CH_2) 3.90 (*s*, MeO); 4.04–4.05 (*m*, H–C(4′)); 4.3–4.31 (*m*, CH_2N); 4.48–4.50 (*m*, H–C(3′)); 5.53 (*t*, *J* = 5, 1 H); 6.25 (*t*, *J* = 6, H–C(1′)); 6.48 (*s*, 1 H); 6.76–7.78 (*m*, 4 arom. H); 6.86–6.89 (*m*, 3 arom. H); 7.15 (*s*, 4 arom. H); 7.20–7.37 (*m*, 9 arom. H.); 7.57–7.61 (*m*, 3 arom. H); 8.10 (*s*, H–C(6)); 8.76 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 28.60; 30.15; 33.00; 38.69; 41.66; 42.73; 42.83; 55.33; 55.37; 55.42; 56.11; 56.27; 63.59; 72.20; 74.24; 77.30; 85.82; 86.59; 87.13; 89.33; 97.02; 99.43; 109.47; 113.25; 113.47 (2 C); 113.65 (2 C); 114.50; 127.09; 127.85; 127.92; 127.96; 128.16; 129.03; 129.21; 129.54; 129.60; 130.05; 130.10; 130.20 (2 C); 132.53; 134.09; 135.54; 135.59; 140.35; 143.04; 144.65; 145.17; 148.98; 151.86; 158.73; 158.75; 159.39; 161.36; 161.55; 162.62; 170.29. ESI-MS: 773 (12), 1075 (100, $[M+22]^+$). Anal. calc. for $C_{62}H_{60}N_4O_{12}\cdot MeOH\cdot H_2O$: C 68.59, H 6.03, N 5.08, found: C 68.48, H 6.05, N 4.90.

5′-O-*[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-8-{3-{[2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}prop-1-yn-1-yl}uridine 3′-[2-Cyanoethyl Bis(1-methylethyl)phosphoramidite]* (**8**). As described for **5**, with **7** (500 mg, 0.47 mmol), diisopropylammonium 1*H*-tetrazolide (60 mg, 0.35 mmol), MeCN (3 × 5 ml), CH_2Cl_2 (5 ml), and 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphordiamidite (390 µl, 1.22 mmol); reaction time 1.5 h: **8** (595 mg, 54%). ^{31}P-NMR (120 MHz, $CDCl_3$): 149.6; 150.1.

*DNA d{(5′-Amino-5′-deoxy)T-C-C-A-A-C-T-C-{8-{3-{[2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}prop-1-yn-1-yl}A-A-C-A-C-C-A-C} 5′-Terminal Amide with Bis (4,7-diphenyl-1,10-phenanthroline-*κN^1,*κ*N^{10})[4-(7-phenyl-1,10-phenanthrolin-4-yl-*κN^1,*κ*N^{10})benzenepentanoic Acid]ruthenium (II)* (**10**). The synthesis of **9** was carried out on a 1 µmol scale. Modified building blocks **5** (100 mg) and 5′-deoxy-5′-{[(4-methoxyphenyl)diphenylmethyl]amino}thymidine 3′-[2-cyanoethyl bis(1-methylethyl)phosphoramidite] [18] (60 mg) were incorporated manually at specific sites. In the last cycle, the (MeO)Tr-protecting group at the 5′-end was cleaved off. Cleavage from solid support was performed with 25% aq. NH_3 at 55° for 4 h. After replacement of NH_4^+ with K^+ and desalting with a *NAP-10* column, **9** (26 *OD*, 157 nmol) was dissolved in DMF/1,4-dioxane/H_2O 1:1:1 (470 µl), and ^{i}Pr$_2$EtN (5 µl, 32 µmol) as well as (bathophenanthroline)ruthenium(II) complex (as ester with hydroxysuccinimide) [5] (5 mg, 4 µmol) were added. The mixture was incubated at 25° in the dark for 24 h. After removal of the solvents, the residue was washed with EtOH (3 × 700 µl) to remove the excess of the acceptor. The crude **10** was purified by reversed-phase HPLC *EC-125/4-Nucleosil-100-5 C18* column, 0 → 90% *B* in 40 min (t_R 27.23 min). After purification, **10** was analyzed by PAGE (*Fig. 1*, *Lanes 3* and *7*).

*DNA d{(5′-Amino-5′-deoxy)T-C-C-A-A-C-{2′-{[(1,1-dimethylethyl)dimethylsilyl]oxy}U-C-{α-{2-{{2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2*H*)-yl]propyl}phenyl}acetyl}amino}-ethylidyne}}T-A-C-A-C-C-A-C}* (**12**). The synthesis of **12** was carried out on a 1 µmol scale. Modified

building blocks **8** (100 mg) and 5′-deoxy-5′-{[(4-methoxyphenyl)diphenylmethyl]amino}thymidine 3′-[2-cyanoethyl bis(1-methylethyl)phosphoramidite] [18] (60 mg) were incorporated manually at specific sites, by using 0.3M BMT (BMT = 5-(benzylmercapto)tetrazole = 5-(benzylthio)-1*H*-tetrazole) as activation reagent. In the last cycle, the (MeO)Tr-protecting group at the 5′-end was cleaved off **11**. For solid-phase coupling of the (bathophenanthroline)ruthenium(II) complex (see below), 10 mg of **11** (on solid support) was set aside. The rest of the material (**11**) was cleaved from the solid support with 2M NH_4OH in EtOH (500 µl) for 30 min at 65° and then treated with 7M $MeNH_2$ in EtOH (500 µl) for 30 min at 65°. After replacement of NH_4^+ with K^+ and desalting with a *NAP-10* column, 37 *OD* of **12** were obtained.

*DNA d{(5′-Amino-5′-deoxy)T-C-C-A-A-C-{2′-{[(1,1-dimethylethyl)dimetylsilyl]oxy}U-C-[α-{2-{{2-{4-{3-[6,7-dimethoxy-3-(4-methoxyphenyl)-2-oxoquinolin-1(2H)-yl]propyl}phenyl}acetyl}amino}ethyl-idyne}}T-A-C-A-C-C-A-C} 5′-Terminal Amide with Bis(4,7-diphenyl-1,10-phenanthroline-*κ*N^{1},*κ*N^{10})[4-(7-phenyl-1,10-phenanthrolin-4-yl-*κ*N^{1},*κ*N^{10})benzenepentanoic Acid]ruthenium(II)* (**13**). a) *Coupling of the (Bathophenanthroline)ruthenium(II) Complex as Ester to* **12**, *in Solution.* To soln. of **12** (28 *OD*, 1.72 nmol) in DMF/1,4-dioxane/H_2O 1:1:1 (520 µl), $^{i}Pr_2EtN$ (6 µl, 34.5 µmol) and (bathophenanthroline)ruthenium(II) complex (as ester with hydroxysuccinimide); (6 mg, 4.3 µmol) were added. The mixture was incubated at 25° in the dark for 24. After removal of the solvents, the residue was washed with $CHCl_3$ (3 × 700 µl) to remove the excess of the acceptor. The crude **13** was purified by reversed-phase HPLC (*EC-125/4-Nucleosil-100-5- C18* column, 0 → 90% *B* in 40 min; t_R 27.88).

b) *Coupling of (Bathophenanthroline)ruthenium(II) Complex as Free Acid to* **11**, *on Solid Support.* A mixture of HOBt (15 mg, 100 µmol), HBTU (34 mg, 90 µmol), and (bathophenanthroline)ruthenium(II) complex (as free acid; 119 mg, 100 µmol) was dissolved in dry DMF (600 µl). $^{i}Pr_2EtN$ (41 µl, 232 µmol) was added to this soln, followed by vortexing for 10 min. This resulting soln. was added to the NH_2-bearing solid support **11** (10 mg, *ca.* 0.35 µmol loading) in an *Eppendorf* tube. The soln. was slowly vortexed for 24 h. After removal of the supernatant, the solid support was washed with DMF (2 × 1 ml) and MeCN (2 × 1 ml). Cleavage from the solid support was performed with 25% aq. NH_3 (500 µl) for 2 h at 40°: **13**. The crude **13** was purified by reversed-phase HPLC (*EC-125/4-Nucleosil-100-5-C18* column, with a 0 → 90% *B* in 40 min; t_R 27.48 min).

REFERENCES

[1] K. E. Sapsford, L. Berti, I. L. Medintz, *Minerva Biotecnol.* **2004**, *16*, 247.
[2] K. E. Sapsford, L. Berti, I. L. Medintz, *Angew. Chem.* **2006**, *118*, 4676.
[3] M. Li, P. R. Selvin, *J. Am. Chem. Soc.* **1995**, *117*, 8132.
[4] M. Li, P. R. Selvin, *Bioconjugate Chem.* **1997**, *8*, 127.
[5] W. Bannwarth, D. Schmidt, R. L. Stallard, C. Hornung, R. Knorr, *Helv. Chim. Acta* **1988**, *71*, 2085.
[6] E. K. Kainmüller, E. Pinyol Ollé, W. Bannwarth, *Chem. Commun.* **2005**, 5459.
[7] E. K. Kainmüller, W. Bannwarth, *Helv. Chim. Acta* **2006**, *89*, 3056.
[8] L. Clima, C. Hirtz-Haag, A. Kienzler, W. Bannwarth, *Helv. Chim. Acta* **2007**, *90*, 1082.
[9] Z. Kazimierczuk, J. A. Vilpo, F. Seela, *Helv. Chim. Acta* **1992**, *75*, 2289.
[10] L. A. Agrofoglio, I. Gillaizeau, Y. Saito, *Chem. Rev.* **2003**, *103*, 1875.
[11] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, *Tetrahedron Lett.* **1989**, *30*, 1927.
[12] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 1316.
[13] K. A. Cruickshank, D. L. Stockwell, *Tetrahedron Lett.* **1988**, *29*, 5221.
[14] N. K. Garg, C. C. Woodroofe, C. J. Lacenere, S. R. Quake, B. M. Stoltz, *Chem. Commun.* **2005**, 4551.
[15] l. Yin, J. Libscher, *Chem. Rev.* **2007**, *107*, 133.
[16] S. Narayanan, J. Gall, C. Richert, *Nucleic Acids Res.* **2004**, *9*, 2901.
[17] S. L. Beaucage, D. E. Bergstrom, G. D. Glick, R. A. Jones, 'Current Protocols in Nucleic Acid Chemistry', John Wiley & Sons, New York, 2000.
[18] W. Bannwarth, *Helv. Chim. Acta* **1988**, *71*, 1517.

Received September 17, 2007