83. Nucleotide Coupling in Reverse Micelles

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Nucleotide coupling was investigated in reverse micelles formed by (cetyl)trimethylammonium bromide (CTAB), in hexane/pentan-1-ol. In particular, the coupling of 2′-deoxy-5′-*O*-methylcytidine 3′-*O*-phosphate, prepared by phosphoramidite chemistry, with 5′-amino-5′-deoxythymidine was studied in the presence of a H_2O-soluble carbodiimide at $w_0 = 11$ and 22 ($w_0 = [H_2O]/[CTAB]$). The effect of w_0 on the reaction rate was investigated. A solid-phase strategy was developed for the synthesis of 2′-deoxy-5′-*O*-methyl-cytidyl-(3′–5′)-5′-amino-5′-deoxythymidine. The nucleotide coupling yielding the expected product occurred readily in reverse micelles. Nucleotide coupling is thus possible in reverse micelles, and this is discussed in connection with the micellar self-replication program.

1. Introduction. – The main aim of the present paper is to investigate whether, and to what extent, nucleotide coupling can occur in reverse micelles, whether the reaction is affected by the micellar properties, and to assess a comparison between reverse micelle and bulk H_2O concerning rate and yield. This investigation was prompted also by the development of the work on self-replicating micelles [1–3]. The interest in the self-replication of micelles would be significantly greater if the increase of the number of micelles would be accompanied by a corresponding self-replication of nucleic-acid material contained in the micellar core. Recently, self-replication of nucleotides or nucleotide analogs in bulk solution was described by *von Kiedrowski* and coworkers [4] [5] and independently by *Rebek* and coworkers [6] [7].

The most important parameter which affects the properties of reverse micelles is the molar ratio H_2O to surfactant, usually indicated as $w_o = [H_2O]/[SURF]$. The dimensions of the micelles, the amount of H_2O bound to the surfactant, and all corresponding microscopic physicochemical properties are directly dependent on w_o [8–10].

In this work, we have chosen a reverse-micellar system similar to that proposed by *Hilhorst* and coworkers [11], containing the cationic surfactant CTAB (= (cetyl)-trimethylammonium bromide = (hexadecyl)trimethylammonium bromide) in the solvent mixture 9.9% (*v*/*v*) pentan-1-ol/hexane, using w_o values of 11 and 22, which correspond unter our conditions to 1 and 2% of H_2O (*v*/*v*). The amount of H_2O present in the micellar core was determined by near-infrared (NIR) spectroscopy (*ca.* 90% of the total added H_2O). Furthermore, the size of reverse-micellar system was characterized by dynamic light scattering.

2. Results and Discussion. – 2.1. *Physicochemical Characterization of the CTAB Reverse-Micellar System.* We first determined the H_2O distribution within the micellar system by NIR spectroscopy. The NIR spectral region is generally defined as the wavelength range from 700 to *ca.* 2500 nm (this range is depending on different instrument types). The absorption bands in this region are due to overtones and combination bands of the fundamental mid-IR molecular-vibration bands. The energy transitions are between the ground state and the second or third excited vibrational states [12].

H_2O has an important combination band at *ca.* 1940 nm (due to the combination of scissoring and asymmetric stretching vibrations), an OH stretch first overtone at 1440 nm, a weaker combination band near 1200 nm, and the OH stretch second overtone at 960 nm. The combination band at *ca.* 1940 nm was extensively used to characterize H_2O in reverse-micellar systems [13–15].

We used an approach similar to that of *Seno* and coworkers [15] to describe the H_2O distribution in our system. As it clearly can be seen from *Fig. 1*, the amount of H_2O dispersed in the bulk organic phase seems to increase constantly with the increase of the total H_2O concentration, such H_2O being always in equilibrium with the H_2O solubilized in the micellar core, as indicated by the presence of the isosbestic point at *ca.* 1890 nm.

The high solubility of H_2O in the organic phase is a consequence of the presence of pentan-1-ol as co-surfactant (H_2O is practically insoluble in neat hexane), which can act as a real surfactant, as was observed for other alkanols [16] (*Fig. 1*). An experimental evidence supports this hypothesis: H_2O added to the organic solvent mixture alone shows a NIR spectrum characterized by two well defined signals, one from H_2O dispersed in the

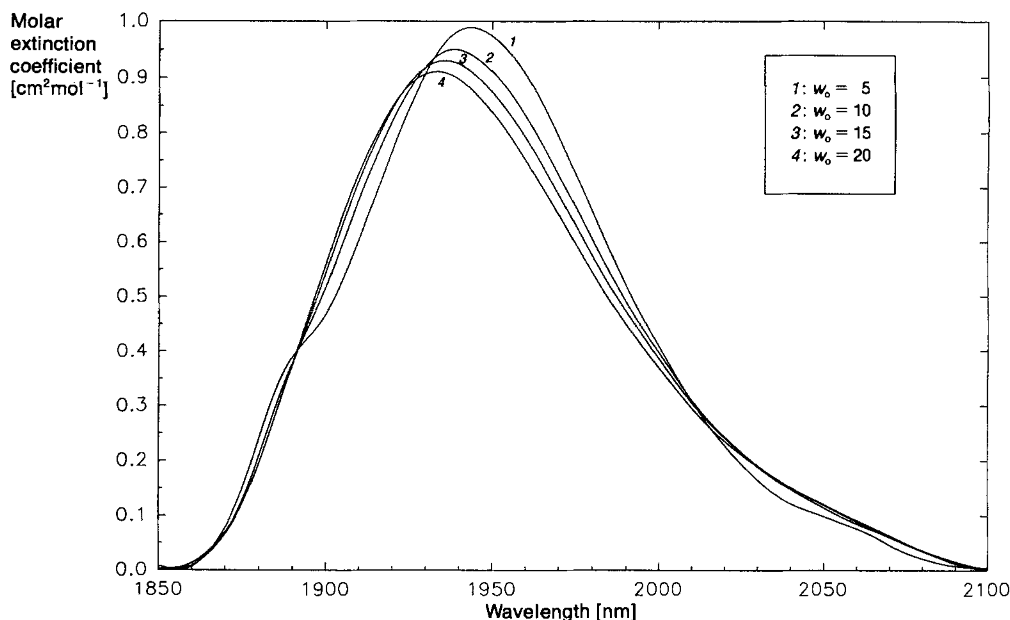


Fig. 1. *NIR Spectra of 50* mM *CTAB reverse-micellar solutions* (9.9% pentan-1-ol/hexane) *containing variable amounts* (0.25–0.99M) *of* Hepes *buffer* (100 mM in H_2O, pH 7.5), *normalized for the H_2O concentration in the system.* Cells, 1 cm; reference, 9.9% pentan-1-ol/hexane; $T = 25 \pm 0.1°$.

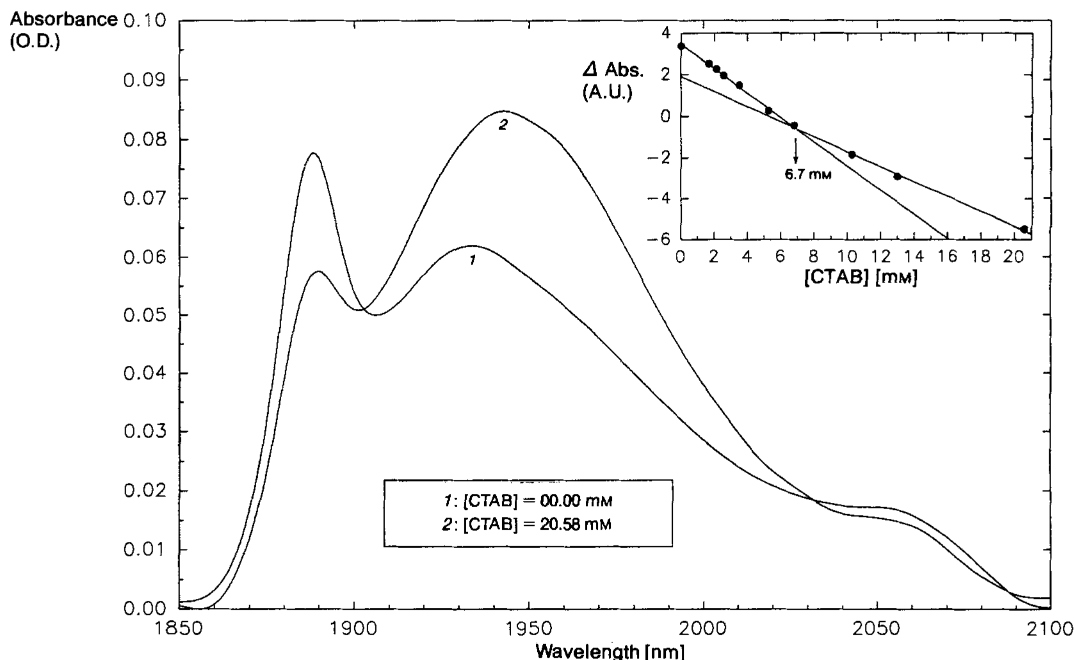


Fig. 2. *NIR Spectra of H_2O-saturated 9.9% pentan-1-ol/hexane solutions with (2) and without (1) added surfactant* (for sake of clarity, only the extreme data points of this series are represented). *Inset: absorbance difference of the two peaks as a function of the CTAB concentration in the system* (the correlation coefficient of each line is higher than 0.997).

bulk organic phase (with a peak at 1887 nm), the other arising from the H_2O molecules interacting with themselves and with the OH group of the co-surfactant (*Fig. 2*). Also the addition of a small amount of CTAB does not affect the shape of the NIR spectra. Only when the concentration of CTAB approaches 20 mM, a spectrum of similar shape than the spectra reported in *Fig. 1* is obtained, however, with a still well defined peak at *ca.* 1890 nm (*Fig. 2*). A computer-assisted deconvolution [17] of all these spectra was used to evaluate quantitatively the amount of H_2O dispersed in the organic phase and also the critical micellar concentration for CTAB in the solvent mixture. The former was found to be less than 10% of the total added H_2O (*Fig. 3*), while the latter was *ca.* 6.7 mM.

This analysis of the H_2O distribution permits us to conclude, that under our conditions (w_o 11 and 22), the largest part of H_2O (more than 90%) is localized in the H_2O-pools of the CTAB reverse micelles. This is the location of the H_2O-soluble reagents added to the micellar system. In this system, we will perform the nucleotide coupling reactions as described below.

In addition, the micellar system was characterized by dynamic light scattering. In particular, we investigated the reverse micellar system at three different w_o values (w_o = 11, 22, and 27), establishing the presence of monodisperse, spherical aggregates. The experimentally obtained hydrodynamic radius (R_h) was transformed into a H_2O-pool radius (R_w), taking 20.6 Å for the extended length of one CTAB molecule [18] (*Table*).

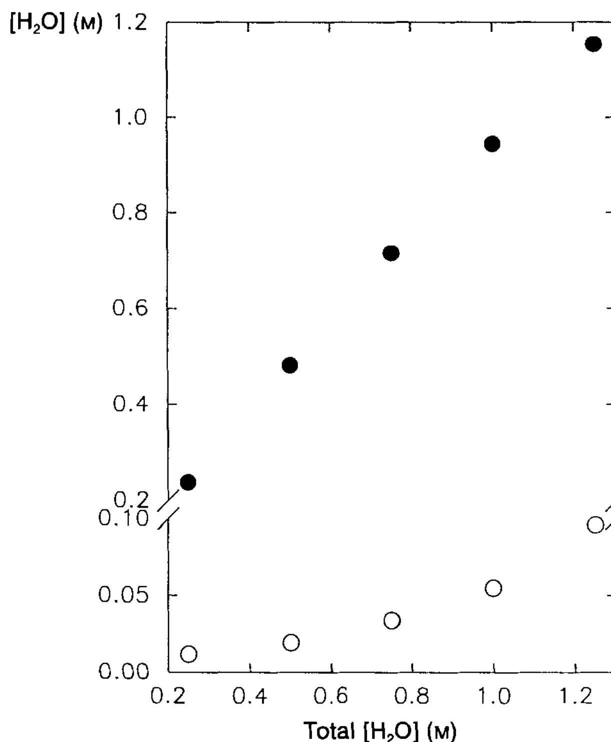


Fig. 3. *Molar concentration of H_2O dispersed in the organic bulk phase* (o) *and in the micellar core* (●) *as a function of the total H_2O concentration, as determined by computer-assisted deconvolution of the NIR spectra of 50 mM CTAB reverse-micellar solutions containing variable amounts of* Hepes *buffer* (100 mM in H_2O, pH 7.5)

Table. *Light-Scattering Data of the System CTAB* (50 mM) *in 9.9% Pentan-1-ol/Hexane at* w_0 = *11, 22, and 27* (by solubilizing the corresponding amounts of 0.1M *Hepes* buffer (pH 7.5). $R_w = R_h - 20.6$ (see *Exper. Part*).

w_0	11	22	27
R_h/Å	30.4 ± 0.05	50.3 ± 0.08	55.4 ± 0.04
R_w/Å	9.8	29.7	34.8

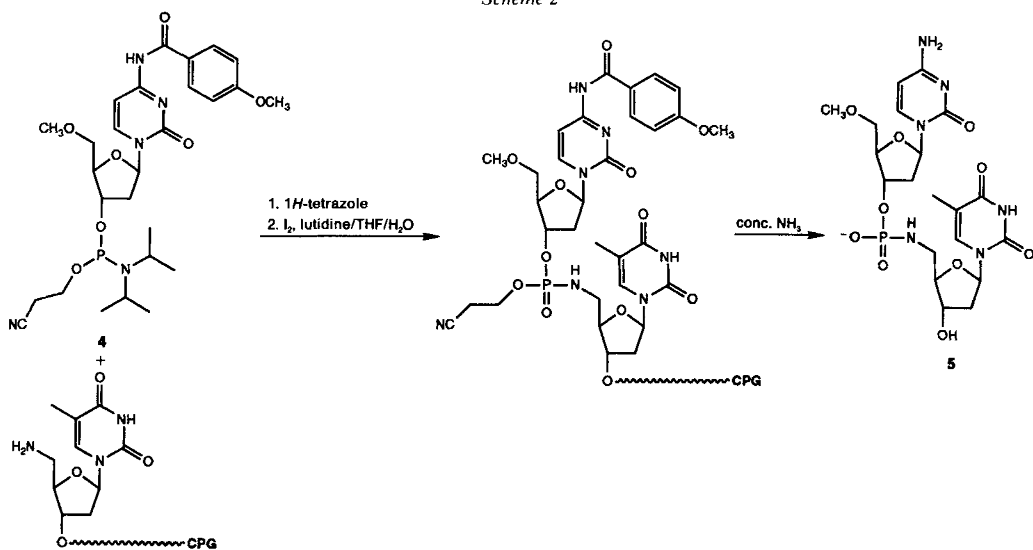
2.2. *Synthesis.* Compound **3** was synthesized starting from N^4-anisoyl-2′-deoxy-5′-*O*-methyl-cytidine [19] (**1**) by phosphinylation with bis(2-cyanoethoxy)(diisopropylamino)phosphine in the presence of 1*H*-tetrazole followed by oxidation with 3-chloroperbenzoic acid (→**2**) and deprotection with conc. ammonia (*Scheme 1*).

The dinucleotide **5** corresponding to the condensation product of **3** with 5′-amino-5′-deoxythymidine was synthesized independently by a solid-phase strategy on controlled-pore glass (CPG) modified with 5′-amino-5′-deoxythymidine. The latter was prepared according to [20] and linked *via* the 3′-succinate to CPG support (*Scheme 2*). The coupling of the phosphoramidite **4** with this modified support proceeded with high efficiency [20]. Subsequent oxidation with I_2 and removal of the protecting groups and the

Scheme 1

O
HN
N
OCH_3
CH_3O
N
O
O
OH
+
N
P
O
O
NC
CN
1. 1*H*-tetrazole
2. 3-$ClC_6H_4CO_3H$
1
2
conc. NH_3
NH_2
3

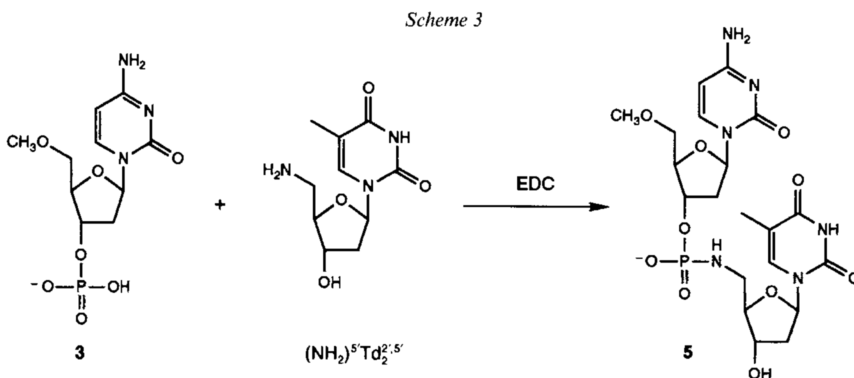
Scheme 2



support with conc. ammonia gave pure **5** in 44% yield, after chromatography on *DEAE-Sephadex* (linear gradient of 0.05→0.5M (Et_3NH)HCO_3).

2.3. *Nucleotide Coupling in* H_2O *and in CTAB-Reverse Micelles.* The basic aim of this work was to establish whether it is possible to find conditions under which a condensation according to *Scheme 3* ist possible in reverse micelles. *Von Kiedrowski* and coworkers showed that in *Hepes* buffer (0.1M, pH 7.5), the condensation between an oligotrinucleotide 3′-phosphate activated by a H_2O-soluble carbodiimide and an oligotrinucleotide bearing an NH_2 group instead of the natural OH function at its 5′-end is much faster [5], due to the increased nucleophilicity of the NH_2 group. We carried out the same type of condensation in reverse micelles.

Scheme 3



First the reaction of **3** with $(NH_2)^{5'}Td_2^{2',5'}$ was optimized in an aqueous buffer. A nucleotide concentration of *ca.* 36 mM and a 16.5-fold excess (600 mM) of *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) was used for the studies. The first reverse-micellar system tested for the condensation was the well investigated, negatively charged AOT/isooctane system (AOT = Aerosol OT; sodium bis(2-ethylhexyl)sulfosuccinate) [21]. Solubilisation of all compounds was achieved very easily, but no reaction occurred. This is possibly due to the negatively charged surfactant interfering unfavorably with the negatively charged reagents.

By changing the system to a positively charged surfactant like (cetyl)trimethylammonium bromide (CTAB) in pentan-1-ol/hexane, successful condensation to yield **5** was observed. The existence of the expected product was proved by HPLC coinjection with the independently synthesized dimer. It is important to notice that the reagents used in our work (nucleotides and activator) were practically insoluble in this organic mixture in the absence of micelles.

This consideration is particularly important if one wishes to compare reactions in micelles with bulk H_2O. Our first experiments were carried out with a reverse-micellar system having the same H_2O-pool concentration as the bulk aqueous reference system. This was achieved with an aqueous overall concentration of 36 mM and a reverse-micellar system of $w_o = 22$ (*i.e.* 2% of H_2O(*v*/*v*)), calculated, assuming that all reagents, as stated above, are localized in the H_2O pool. Under those conditions, the virtual overall micellar concentration is 50 times smaller than the local (or H_2O-pool) concentration. Results are

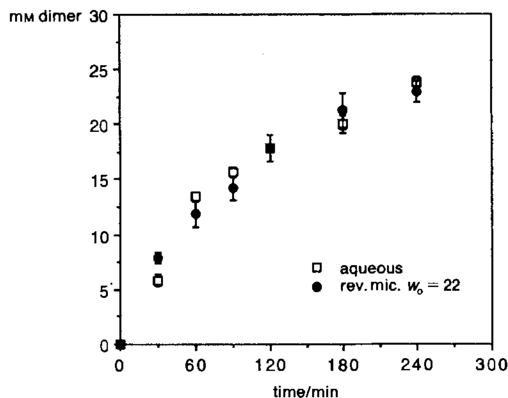


Fig. 4. *Time progress of nucleotide coupling* (see *Scheme 3*) *in H_2O and in the reverse-micellar system CTAB/pentan-1-ol/hexane* ($w_0 = 22$) *as determined by HPLC* (linear gradient 1→15% MeCN in $(Et_3NH)OAc$ buffer (pH 7) within 16 min; UV detection at 254 nm). The local H_2O concentrations of reactants are the same in both systems. Initial concentrations in 0.1M *Hepes* buffer (pH 7.5): [3] = $[(NH_2)^{5'}Td_2^{3',5'}]$ = 36.32 mM, [EDC] = 600 mM; $T = 25°$. Error bars represent standard deviations of four independent runs. The yield of **5** after 4 h is 64%.

shown in *Fig. 4*. The time course of the reaction is very similar in H_2O and reverse micelles. This, among other, suggests that the physically meaningful concentration in reverse micelles is the local concentration. If the reaction is carried out in H_2O at a concentration corresponding to the virtual micellar overall concentration (*i.e. ca.* 50 times smaller than in the experiment of *Fig. 4*), no reaction occurs (data not shown).

In view of the relevance of the H_2O-pool concentration for the micellar reaction, we thought interesting to run the micellar reaction also at $w_o = 11$, *i.e.* with the half of the total amount of H_2O, but with the same overall micellar concentration. At $w_o = 11$, then, the local concentration will be twice as in the experiment with $w_o = 22$. Results are shown in *Fig. 5*. The reaction is faster in the first phase (*ca.* 90 min), and saturation is reached earlier than at $w_o = 22$.

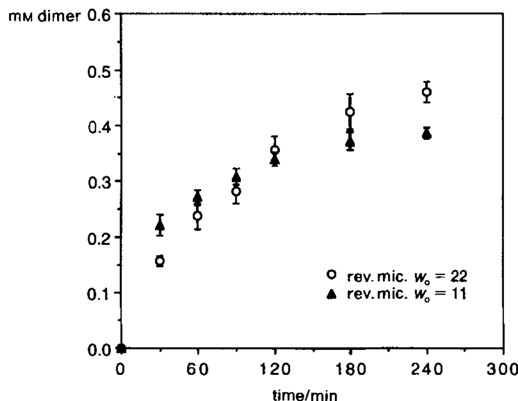


Fig. 5. *Progress of nucleotide coupling* (see *Scheme 3*) *as a function of time in the reverse-micellar systems CTAB/pentanol/hexane of* $w_0 = 11$ *and 22, as determined by HPLC* (see *Fig. 4*). Initial overall concentrations: [3] = $[(NH_2)^{5'}Td_2^{3',5'}]$ = 0.73 mM, [EDC] = 12 mM; $T = 25°$. Error bars represent standard deviations of 4 independent runs.

The faster reaction in the earlier phase is in keeping with the obervation that the local concentration of the H_2O pool is determining the rate. It does not appear, however, easy to rationalize the earlier saturation obtained in the second part of the reaction. It is known that, by reducing w_o, the micelles become smaller and increase in number. This was verified by dynamic light scattering experiments (see above). Also, based on the

analogous case of AOT reverse micelles, a decrease in radius is accompanied by a change in the physical properties of H_2O [21]. All these factors may affect the equilibrium of the reaction at an extent which cannot be rationalized on the basis of our present knowledge in the field of reverse micelles.

The course of the reaction was followed by HPLC with external standard calibration. The concentration of the components was determined by UV detection at 254 nm. For more details on the analytical procedures, see *Exper. Part.*

3. Conclusion. – This work has shown that it is possible to find conditions for the coupling of nucleotides in reverse micelles with basically the same rate and yield as in aqueous buffer. The correspondence of rate and yield between micelles and aqueous system is found when the micellar system has the same H_2O-pool concentration than the bulk overall aqueous solution. In principle, a different result might have been expected. For example, in enzymatic reactions, a good correspondence between reverse micelles and H_2O solution is only found when the overall enzyme concentration is the same in the two systems [21] [22]. Apparently, in the present case, it is the actual local concentration that determines the outcome of the reaction.

We have now a micellar system which is well characterized from the physical point of view and which is also a suitable medium for the coupling of nucleotides. It seems, therefore, possible to perform in such a system nucleotide coupling in the presence of a template, in order to achieve self replication. This will be described in a forthcoming paper using as a basic reaction the coupling of two trinucleotides in the presence of a hexanucleotide template.

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Experimental Part

1. *General.* All solvents were of highest purity available from *Fluka* and *Merck*. *N*-[3-(Dimethylamino)propyl]-*N'*-ethyl-carbodiimide hydrochloride (EDC) was from *Fluka*. The 1*H*-tetrazole (*Fluka*) was purified by sublimation. Bis(2-cyanoethoxy)(diisopropylamino)phosphine, (2-cyanoethoxy)bis(diisopropylamino)phosphine, and diisopropylammonium tetrazolide were prepared according to [23] and [24], 5'-amino-5'-deoxythymidine ($(NH_2)^{5'}Td_2^{2',5'}$) according to [20], and N_4-anisoyl-2'-deoxy-5'-*O*-methyl-cytidine (**1**) from 2'-deoxycytidine according to [19]. High-precision glass-capillary tubes were from *Assistent* ('*Assistent*-Einmal-Volumenkapillaren'; 1 ± 0.03 µl tolerance). Short-column chromatography (CC) [25]: silica gel *60* (0.063–0.04 mm, *Merck*). TLC: HPTLC silica-gel plates (*Merck*): detection by UV at 254 nm, P-containing compounds also by *Zinzadze* reagent [26]. Anion-exchange chromatography: *Sephadex A25* (*Pharmacia*): $(Et_3NH)HCO_3$ buffer (pH 7.5) as eluent. HPLC: *Perkin-Elmer*-series-*4* chromatograph with a *LCI-100* chromato-integrator; linear gradient of 1→15% MeCN in $(Et_3NH)OAc$ (0.1M, pH 7) within 16 min. ^1H-NMR (250 MHz): chemical shifts in ppm rel. to TMS. ^{31}P-NMR (101.2 MHz): chemical shifts rel. to H_3PO_4 (external).

Calibration of the HPLC and Quantitation of the Reaction. Five different concentrations of 5'-amino-5'-deoxythymidine were taken as standards (0.01–0.05 mM), and a calibration curve was determined by linear regression of peak area *vs.* concentration. The concentration of the product **5** was calculated by a decrease of the 5'-amino-5'-deoxythymidine, concentration by substracting each value from the reference concentration (c_0). In aq. systems, c_0 was determined immediately after addition of the EDC-containing buffer to the nucleotide mixture, making sure

that no reaction had occurred so far. In reverse-micellar systems, c_0 was determined before addition of EDC. The reaction was followed for 4 h, and HPLC injections were carried out at 1 and 30 min and at 1, 1.5, 2, 3, and 4 h. Three or four independent reactions under identical conditions for each aq. system and reverse-micellar systems at $w_0 = 11$ and 22 were analyzed and used for statistical data analysis represented by error bars in *Figs. 4* and *5*; the accuracy lies around 5–7% reproducibility).

2. *Synthesis*. 2.1. N^4*-Anisoyl-2′-deoxy-5′-*O*-methyl-cytidine 3′-*O*-[Bis(2-cyanoethyl)phosphate]* (**2**). A mixture of 1.5 mmol (563 mg) of N^4-anisoyl-2′deoxy-5′-*O*-methyl-cytidine and 2.25 mmol (158 mg) of 1*H*-tetrazole was evaporated 3 times with anh. MeCN and dried under vacuum overnight. By syringe, 70 ml of anh. MeCN and 2.25 mmol (610 mg) of bis(2-cyanoethoxy)(diisopropylamino)phosphine were added, and the mixture was stirred at r. t. under Ar. TLC (CH_2Cl_2/MeOH 9:1, R_f 0.63) after 1 h showed complete reaction. Oxidation was performed with 1.6 mmol (275 mg) of 3-chloroperbenzoic acid. TLC (R_f 0.47) after 2 h showed complete reaction. The mixture was taken up in 100 ml of H_2O containing 0.5% of AcOH and extracted 3 times with CH_2Cl_2. The org. layer was washed twice with sat. $NaHCO_3$ soln. dried (Na_2SO_4), and evaporated: 943 mg of a yellowish oil. The crude product was purified by CC (gradient 5→7% MeOH in CH_2Cl_2/AcOH 99.5:0.5). The pure fractions (TLC (CH_2Cl_2/MeOH/AcOH 95:5:0.5): R_f 0.26) gave after evaporation and co-evaporation with anh. MeCN (3×), 649 mg (77%) of **2**. Colorless powder. ^{1}H-NMR (250 MHz, $CDCl_3$): 2.24–2.33 (*m*, 1 H–C(2′)); 2.81–2.90 (*m*, 1 H–C(2′), 2 OCH_2CH_2CN); 3.41 (*s*, MeO–C(5′)); 3.64–3.75 (*AB* of *ABX*, 2 H–C(5′)); 3.89 (*s*, *Me*OC_6H_4); 4.30–4.38 (*m*, 2OCH_2 CH_2CN); 4.47 (*q*, H–C(4′)); 5.13 (*dt*, H–C(3′)); 6.36–6.41 (*m*, H–C(1′)); 7.00 (*d*, 2 H_m); 7.54 (br. *d*, H–C(5)); 7.87 (*d*, 2 H_o); 8.18 (*d*, H–C(6)); 8.7 (br. *s*, NH). ^{31}P-NMR (101.2 MHz, $CDCl_3$): −2.56. MS (ion spray): 562.0 ([*M* + H]$^+$).

2.2. *2′-Deoxy-5′-*O*-methyl-cytidine 3′-*O*-(Triethylammonium Phosphate)* (**3**·(Et_3NH)). A suspension of 1.16 mmol (649 mg) of **2** in 20 ml of conc. NH_3 soln. was stirred for 1 day at r. t. in a tightly closed flask (rubber stopper). The soln. was transferred into 15 *Eppendorf* tubes with screw caps and kept for 3 h at 70°. The conc. NH_3 soln. was evaporated in a speed-vac concentrator. The crude mixture was taken up in 7 ml of doubly distilled H_2O, and insoluble compounds were removed by filtration. The remaining soln. was applied onto a *DEAE-Sephadex-A25* column and the product eluted by an increasing (Et_3NH) HCO_3 gradient (0.05→1.0M, 600 ml each). The product (TLC monitoring (PrOH/NH_3/H_2O 7:1:2): R_f 0.22, blue with *Zinzadze* reagent) was eluted at 0.75M (Et_3NH)HCO_3. The product fractions were evaporated and co-evaporated with for EtOH several times to remove (Et_3NH)HCO_3. The residue was taken up in H_2O/EtOH 3:2, transferred into *Eppendorf* tubes, and finally evaporated 3 more times in a speed-vac concentrator: 225 mg of **3**·(Et_3NH). ^{1}H-NMR (250 MHz, (D_6)DMSO): 1.13–1.19 (*t*, (CH_3CH_2)$_3$N); 1.96, 2.35 (2*m*, 2 H–C(2′)); 2.92–3.01 (*q*, (CH_3CH_2)$_3$N); 3.29 (*s*, MeO); 3.49–3.54 (*AB* of *ABX*, 2 H–C(5′)); 4.09 (*q*, H–C(4′)); 4.56 (*dt*, H–C(3′)); 5.73–5.76 (*d*, H–C(5)); 6.16 (*t*, H–C(1′)); 7.2 (br. *s*), NH_2); 7.63–7.66 (*d*, H–C(6)). ^{31}P-NMR (101.2 MHz, (D_6)DMSO): 0.301. MS (ion spray): 320.1 (100, [M^-]).

2.3. N^4*-Anisoyl-2′-deoxy-5′-*O*-methyl-cytidine 3′-*O*-[(2-cyanoethyl)* N,N*-Diisopropylphosphoramidite]* (**4**). A mixture of 1.3 mmol (488 mg) of N^4-anisoyl-2′-deoxy-5′-*O*-methyl-cytidine and 0.65 mmol (112 mg) of diisopropylammonium tetrazolide was evaporated **3** times from anhy. MeCN. CH_2Cl_2 (40 ml) from a freshly opened bottle was dried overnight with $CaCl_2$ and then added to the reactants with a syringe. In the some way, (2-cyanoethoxy)bis(diisopropylamino)phosphine (1.43 mmol, 431 mg) was added and the mixture stirred at r.t. (TLC monitoring). After 3 subsequent additions of each 0.5 mmol (50 mg) of phosphinylation agent, the reaction was complete after 4 h (TLC (CH_2Cl_2/AcOEt/Et_3N 45:45:10; the plates were in touch with the eluent before applying the mixture): R_f 0.52, 0.59, as diastereoisomers). The mixture was poured onto 60 ml of sat. $NaHCO_3$ soln. and extracted 3 times with 50 ml of CH_2Cl_2 each, the combined org. layer dried (Na_2SO_4), and evaporated after addition of a few droplets of Et_3N, and the crude product (diastereoisomer mixture) dissolved in CH_2Cl_2/MeOH and purified by CC (CH_2Cl_2/AcOEt/Et_3N 45:45:10). Pure fractions (TLC) were evaporated and coevaporated several times with anh. MeCN. The residue was taken up in a few ml of Et_2O and precipitated from pentane yielding 526 mg (70%) of **4**. ^{1}H-NMR (250 MHz, (D_6)DMSO): 1.15–1.20 (*d*, 2 *Me*$_2$CH); 2.23 (*m*, H–C(2′)); 2.49–2.51 (*m*, H′–C(2′), DMSO); 2.77–2.82 (*t*, OCH_2CH_2CN); 3.32–3.34 (*s*, MeO–C(5′), H_2O); 3.57–3.78 (*m*, 2 Me_2CH, OCH_2CH_2CN, 2 H–C(5′)); 3.84 (*s*, $MeOC_6H_4$); 4.21 (2*q*, H–C(4′), 2 diastereoisomers); 4.50 (*m*, H–C(3′)); 6.16 (*t*, H–C(1′)); 7.02–7.05 (*d*, 2 H_m); 7.39 (br. *d*, H–C(5)); 8.03 (*d*, 2 H_o); 8.17 (*d*, H–C(6)); 11.1 (br. *s*, NH). ^{31}P-NMR (101.2 MHz, (D_6)DMSO): 148.94 (P^{III}). MS: 576.2(0.05, M + H]$^+$).

2.4. *2′-Deoxy-5′-*O*-methylcytidyl-(3′–5′)-5′-amino-5′-deoxythymidine* (**5**). The synthesis was performed on controlled-pore glass (CPG) modified with 5′-amino-5′-deoxythymidine using a simple device for manual synthesis of DNA fragments [27]. To 500 mg (13.15 μmol) of this support, **4** was coupled in the presence of 1*H*-tetrazole. The condensation step was carried out twice for 10 min with a 5-fold excess of **4** each. The support material was distributed among 6 *Eppendorf* tubes with screw caps, and deprotection was carried out with 700 μl of conc. NH_3

soln. for 4 h at 70°. After removal of the support, the NH_3 soln. was evaporated on a speed-vac concentrator. The residue was taken up in 300 µl of H_2O/dioxane and precipitated by addition of 600 µl of THF. After centrifugation (15 min, 0°), the supernatant was removed, and the pellets were dissolved in $(Et_3NH)HCO_3$ soln. to be purified on a *DEAE-Sephadex-A-25* column (linear gradient 0.05→0.5M $(Et_3NH)HCO_3$). Pure fractions (checked by HPLC) were collected and evaporated: pure **5** (44%; checked by UV).

3. *Coupling Reactions in Aqueous and Reverse-Micellar Phase.* 3.1. *Stock Solutions of* **3** *and* $(NH_2)^{5'}Td_2^{2',5'}$. They were prepared by dissolving the compounds in H_2O in *Eppendorf* tubes (the concentration of **3** was determined spectrophotometrically (ε_{254} = 6541 M^{-1} cm^{-1}) [24], the concentration of $(NH_2)^{5'}Td_2^{2',5'}$ was obtained by weighing).

3.2. *Reaction Stock Solutions.* They were prepared by co-evaporating aliquots of both **3** and $(NH_2)^{5'}Td_2^{2',5'}$ stock solns. and dissolving the residue in a certain volume of 0.1M *Hepes* buffer (pH 7.5).

3.3. *Aqueous Reaction Solutions.* They were prepared by first dissolving the activator molecule EDC in a certain volume of 0.1M *Hepes* buffer (pH 7.5) to obtain a final concentration of 600 mM (11.5 mg/100 µl; 191.7). From this soln., 40 µl were transferred into an *Eppendorf* tube containing both monomers evaporated, which resulted in a nucleotide concentration of 36.32 mM. The reaction soln. was sonicated for 5–10 s and then transferred into 1-µl-end-to-end high-precision capillary tubes. The capillary tubes were put into a glass vessel with a screw cap, containing a H_2O layer on the bottom to ensure a constant H_2O amtosphere [28]. The capillary tubes containing glass vessel was thermostated at 25°. All these experimental steps had to be carried out as fast as possible because of hydrolysis of EDC.

3.4. *Aqueous Reaction Solution* ([**3**] = $[(NH_2)^{5'}Td_2^{2',5'}]$ = 0.73 mM; [EDC] = 12 mM). No reaction occurred.

Preparation of Samples for HPLC Analysis. At exact times the capillary tubes were taken out of the glass vessel and washed out into 499 µl of starting HPLC eluting buffer (0.1M $(Et_3NH)OAc$, pH 7) by applying a slight overpressure on the top of the capillary tube.

3.5. *Reverse-Micellar Reaction Solutions.* Preparation of the reverse-micellar system (50 mM CTAB, 9.9% (*v*/*v*) pentan-1-ol, 0.45% H_2O in hexane): 911 mg of CTAB were suspended in 4.95 ml of pentan-1-ol and 225 µl of H_2O. This mixture was dissolved in hexane by sonification to a total volume of 50 ml. A transparent soln. (w_o = 5) was obtained (CTAB *System 1*).

All reactions were carried out in 1-ml glass vessels with screw caps. In contrary to the aq. reaction systems, the activator (EDC) was added to the soln. as a solid.

Reverse-Micellar System (w_o = 22): Into 1 ml of CTAB *System 1*, 15.5 µl of a 46.86 mM stock soln. of $(NH_2)^{5'}Td_2^{2',5'}$ and **3** in *Hepes* buffer was injected. After sonicating for 20 s, the turbid soln. became transparent. The reaction was started by addition of 2.3 mg of EDC (overall concentration of 12 mM). After sonicating for 5 s, the reaction vial was shaken at 150 rpm on a minishaker at 25°.

Reverse-Micellar System (w_o = 11): Into 1 ml of CTAB *System 1*, 5.5 µl of a 132.1 mM stock soln. of **3** and $(NH_2)^{5'}Td_2^{2',5'}$ in *Hepes* buffer was injected. The mixture was treated in the same way as the system of w_o = 22.

HPLC Analysis of Reverse-Micellar Reaction Solutions: The reaction soln. (20 µl) was diluted with $(Et_3NH)OAc$ (280 µl). After slight shaking, the sample was injected into the HPLC.

3.6. *Co-injection Experiments.* A soln. of 0.05 mM **5** in *Hepes* buffer was prepared. The concentration of **5** was determined spectrophotometrically (ε_{260} 15200 M^{-1} cm^{-1} [29]). An aq. reaction soln. after 1 h reacting time was diluted by a factor of 250. A mixture of 25 µl of this sample and 25 µl of the dimer soln. (0.05 mM **5**) were submitted to HPLC. Only one peak in the region of 14 min was detected.

4. *NIR Spectroscopy.* NIR Spectra were recorded on a *Perkin-Elmer-330* spectrophotometer, equipped with a digital temp.-control unit. The analogic spectra were digitalized with a *Hitachi-HDG-1217D* tablet digitizer using the 'Sigmascan' program as software interface with a *PC/AT-386/387* computer. The spectra were smoothed with the *Fourier* algorithm (to reduce the noise arising from digitalization) before any computer-assisted deconvolution.

5. *Dynamic Light Scattering.* All experiments were performed with a *Malvern-4700-PS/MW* spectrometer and an Ar ion laser (coherent; *Innova*, model *200-10*, λ_o = 488 nm).

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