Oligonucleotide Analogues with a Nucleobase-Including Backbone

Part 5

2′-Deoxy-8-(hydroxymethyl)adenosine- and 2′-Deoxy-6-(hydroxymethyl)uridine-Derived Phosphoramidites: Synthesis and Incorporation into 14-Mer DNA Strands

by **Werngard Czechtizky** and **Andrea Vasella***

Laboratorium für Organische Chemie, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

The pairing propensity of new DNA analogues with a phosphinato group between O–C(3′) and a newly introduced OCH_2 group at C(8) and C(6) of 2′-deoxyadenosine and 2′-deoxyuridine, respectively, was evaluated by force-field calculations and *Maruzen* model studies. These studies suggest that these analogues may form autonomous pairing systems, and that the incorporation of single modified units into DNA 14mers is compatible with duplex formation. To evaluate the incorporation, we prepared the required phosphoramidites **3** and **4** from 2′-deoxyadenosine and 2′-deoxyuridine, respectively. The phosphoramidite **5** was similarly prepared to estimate the influence of a CH_2OH group at C(8) on the duplex stability. The modified 14-mers **6**–**9** were prepared by solid-phase synthesis. Pairing studies show a decrease of the melting temperature by 2.5° for the duplex **13**·**9**, and of 6–8° for the duplexes **10**·**6**, **11**·**6**, **13**·**7**, and **14**·**8**, as compared to the unmodified duplexes.

1. Introduction. – We have conceived oligonucleotide analogues with a nucleobase-including backbone (*Fig. 1*, **B**); these compounds should allow us to answer the question of whether the structural differentiation between nucleobase and backbone in DNA, RNA, and their analogues[1]) is a prerequisite for the formation of stable homo- and/or heteroduplexes [6][7].

We have so far described oligouridine and oligoadenosine analogues that are linked by ethynediyl groups between C(5′) and C(6) of uridines, or C(5′) and C(8) of adenosines [6–9]. In addition to these uncharged analogues that require a stepwise synthesis in solution, we have considered phosphodiester analogues that should be accessible by conventional solid-phase synthesis [10]. Similarly to DNA, they should be water-soluble. These analogues possess phosphinato groups between O–C(3′) and a oxymethyl substituent at C(8) or C(6) of neighbouring 2′-deoxyadenosyl or 2′-deoxyuridyl units, respectively.

Fig. 2 shows the fully modified analogues (3′-10)dA^*_n (**1**) and (3′-7)dU^*_n (**2**), incorporating the repeating units dA* and dU*[2]), and the phosphoramidites **3** and **4**

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[1]) For DNA and RNA analogues that have been prepared in search of antisense-compounds, to investigate autonomous pairing-systems, protein/DNA-interactions, electron-transfer in DNA, and DNA-damage, see [1–5].

[2]) The repeating units of B-DNA are denoted as dA and dT; units that are linked *via* O(3′) and O(10), or O(7), respectively, are denoted as dA* and dU*, and the dA units CH_2OH-substituted at C(8) as dA** (*Fig. 2*). The linking mode is indicated by two numerals in parenthesis. A dash, as in (3′-10), indicates a phosphinato group between O(3′) and O(10), and indicates the (3′ → 10) direction of the link. In fully modified analogues, all dA and dT repeating units of DNA are replaced by dA* and dU*, while partially modified analogues correspond to DNA strands in which part of the dA or dT units are replaced by dA* or dU*.

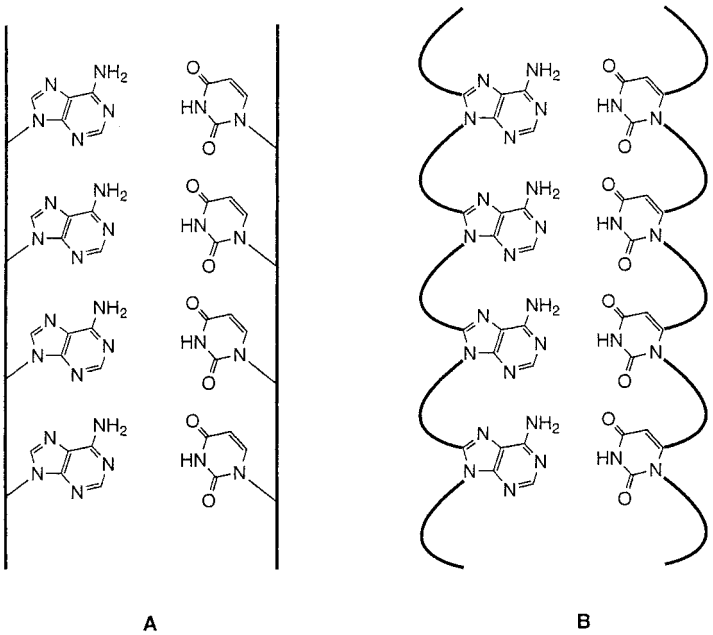


Fig. 1. *Conventional oligonucleotides with structural separation of the nucleobases and the backbone* (**A**) *and oligonucleotides with a nucleobase-including backbone* (**B**)

derived from dA* and dU*, respectively. In this context, we also prepared the phosphoramidite **5** to study the influence of a *C*(8)-hydroxymethylated dA on the pairing behaviour of DNA oligomers. To avoid unfavourable steric interactions between the Me group at C(5) of thymidine and the backbone connected to C(6), we have replaced thymidine by 2′-deoxyuridine analogues.

We report on force-field calculations of the homoduplex (3′-10)$dA^*_{14}\cdot$(7-3′)dU^*_{14}, the heteroduplex (3′-10)$dA^*_{14}\cdot$(5′-3′)dT_{14}, and partially modified duplexes, and describe the synthesis of singly modified tetradecamers containing one dA*, dU*, or dA** unit.

2. Results and Discussion. – 1. *Model Studies and Calculations.* The conformations and the pairing propensity of the above described oligonucleotide analogues (*Fig. 2*) were first evaluated with the help of *Maruzen* models. Models of the fully modified homoduplex (3′-10)$dA^*_{14}\cdot$(7-3′)dU^*_{14}, and heteroduplexes (3′-10)$dA^*_{14}\cdot$(5′-3′)dT_{14} and (3′-7)$dU^*_{14}\cdot$(5′-3′)dA_{14}, respectively, were constructed on the basis of the results of a conformational analysis of DNA [11], *i.e.*, by assuming a ^2E pucker of the carbohydrate moiety, a $\pm$sc conformation around the P–O bonds, a 'zigzag' conformation of the backbone-chain, and minimal 1,5-repulsions in the single-strands. This led to two plausible helical models for the homoduplex (3′-10)$dA^*_{14}\cdot$(7-3′)dU^*_{14}, both characterized by antiparallel strands, but differing in the dihedral angles for the nucleosidic and for the P–O bonds.

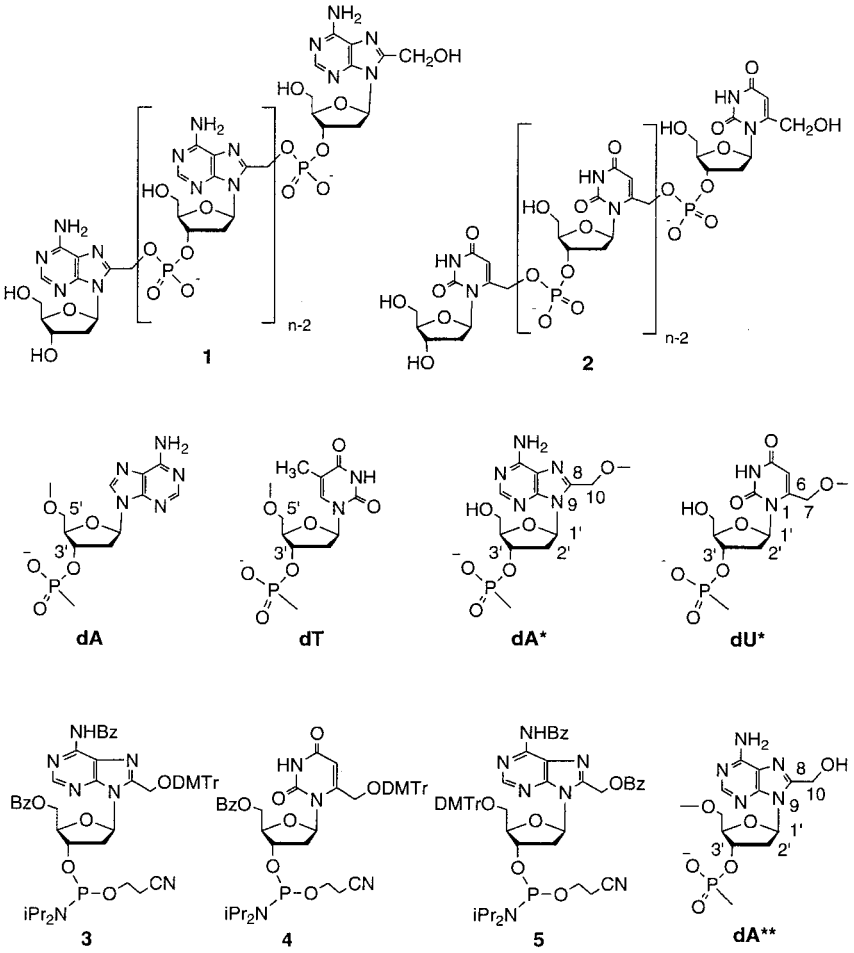


Fig. 2. *Proposed adenosine and uridine oligomers* dA_n^* *and* dU_n^*, *DNA repeating units dA and dT, modified repeating units dA*, dU*, and dA**, and phosphoramidites* **3**, **4**, *and* **5** *derived from dA*, dU*, and dA**, respectively*

In the first model, the nucleobases adopt the *anti*-conformation with dihedral angles O(4′)–C(1′)–N(9)–C(4) of *ca.* −100° in the dA* units and dihedral angles O(4′)–C(1′)–N(1)–C(2) of *ca.* −130° in the dU* units, comparable to the corresponding dihedral angles in B-DNA [12]. In the second model, the nucleobases adopt a *syn*-conformation with dihedral angles O(4′)–C(1′)–N(9)–C(4) and O(4′)–C(1′)–N(1)–C(2) of *ca.* 70–80°. Depending on the *syn/anti*-conformation, the dihedral angles for the P–O bonds are −*sc* in the first model and +*sc* in the second one. In both duplexes, the dihedral angles characterizing the torsion about the C(3′)–O(3′) bond are beween *ap* and −*ac*. The angles for the C(8)–C(10) and the C(6)–C(7) bonds are *ca.* 180°, corresponding to an antiperiplanar orientation of N(9) and O(10), and of N(1) and O(7). Both models show a parallel orientation of the

Watson-Crick base pairs at a distance of *ca.* 3.3–3.5 Å, comparable to the distance between base pairs in B-DNA (3.3–3.4 Å) [13], indicating that these analogues may indeed form stable homoduplexes.

Helical models of the heteroduplexes (3′-10)$dA^*_{14}\cdot$(5′-3′)dT_{14} and (3′-7)$dU^*_{14}\cdot$(5′-3′)dA_{14} with antiparallel strands could be constructed only if the nucleobases in the modified strands adopt the *anti*-conformation. The conformations of the dA* and dU* units correspond to those of the homoduplexes with an *anti*-conformation (see above). In the complementary DNA strand, the dA and dT units adopt conformations similar to those described for dA and dT units in B-DNA [14].

We have also evaluated partially modified analogues. The incorporation of a single dA* or dU* unit into a B-DNA duplex does not considerably change the *Watson-Crick* base pairing and base stacking, irrespectively of the specific sequence, as long as the nucleobases in the modified units adopt an *anti*-conformation. Again, the conformations of the modified nucleotides are similar to those described for dA* and dU* in the models of the homoduplexes characterized by an *anti*-conformation.

This qualitative analysis formed the basis of force-field calculations (Macromodel V. 4.5, Amber* force-field, gas-phase [15]) to evaluate the potential of the fully and partially modified analogues to form homo- and heteroduplexes similar to DNA. Since the starting geometries were derived from B-DNA, only duplexes with an *anti*-conformation of the nucleobases were investigated. The starting geometry of the antiparallel, fully and partially modified duplexes were generated by building the B-helix of $dA_{14}\cdot dT_{14}$ with the grow-tool of Macromodel, and then replacing some or all nucleotides by the modified nucleotides. The starting conformations of the repeating units dA* and dU* were similar to those derived from the *Maruzen* models. After energy minimization (2000 to 3000 iterations), both the homoduplex (3′-10)$dA^*_{14}\cdot$(7-3′)dU^*_{14} (*Fig. 3*,*a*) and the heteroduplex (3′-10)$dA^*_{14}\cdot$(5′-3′)dT_{14} (*Fig. 3*,*b*) retained a conformation similar to the one initially chosen.

The helical models show an almost parallel arrangement of the nucleobases at a distance of around 3.3 Å, suitable for *Watson-Crick* base pairing and base stacking. Several DNA duplexes partially modified in one strand were similarly investigated. The partially modified B-DNA duplexes containing one, two, or three dA* or dU* units with an *anti*-conformation also showed base pairing and base stacking similar to B-DNA. The values of the propeller- and buckle-twists as well as the length of the H-bonds within the *Watson-Crick* base pairs [16][17] showed no significant deviation from those in B-DNA.

Neither the position of the modified units in the singly modified strands nor the sequence of these strands appeared (at this level of calculation) to significantly affect the pairing propensity. Among the possible singly modified tetradecamers, we chose to synthesize **6**–**9** (*Table 1*), which possess a modified unit in the center of the chain, flanked by dC and dG units. The choice of **6** was facilitated by an ample supply of the complementary RNA **10**[3]) that allowed to also study the pairing of **6** with RNA.

2. *Synthesis of Oligomers.* For the synthesis of the oligomers **6**–**9** and **11**–**14** (*Table 1*), we intended to use the standard phosphoramidite chemistry; the (benzoyloxy)methyl group of the required phosphoramidites **3**–**5** should not interfere with the

[3]) We thank *S. Pitsch* for a sample of 5′-r(GAUAGCUCGGAUGC)-3′ (**10**) [18].

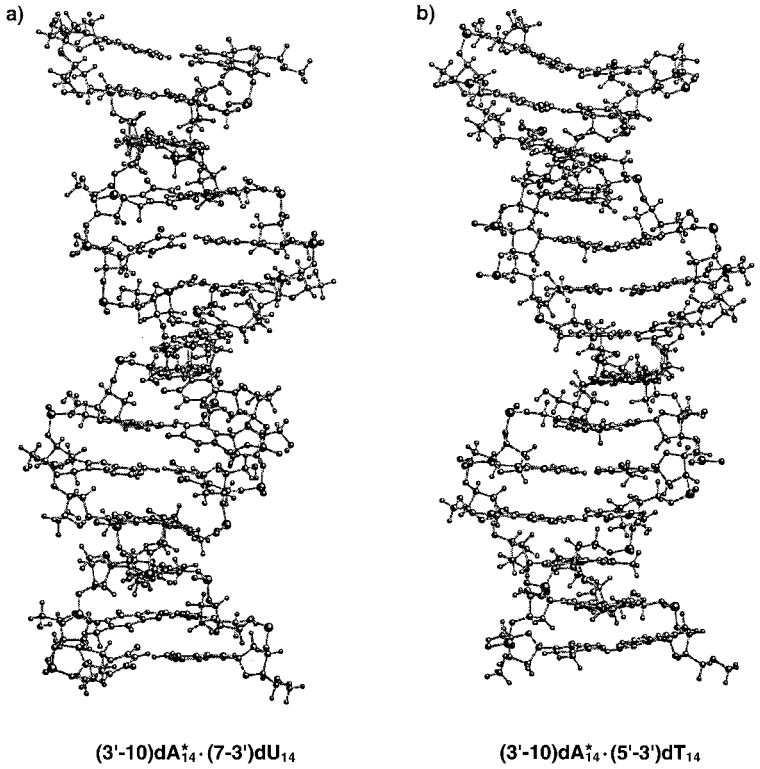


Fig. 3. *Amber*-calculated structures of the fully and the semi-modified duplexes (3′-10)dA^*_{14} · (7-3′)dU^*_{14} and (3′-10)dA^*_{14} · (5′-3′)dT_{14}*

Table 1. *Oligodeoxynucleotides* **6**–**9** *and* **11**–**14**, *Total Yields, and Molecular Masses*

Sequence		Total yield[a])	Calculated mass	MALDI-TOF
5′-d(GCATCCG**A***GCTATC)-3′	**6**	87%	4253.8	[b])
5′-d(CTATCG**A***GCTTACG)-3′	**7**	87%	4268.8	4268.6
5′-d(CGTAAGC**U***CGATAG)-3′	**8**	80%	4303.8	4308.2
5′-d(CTATCG**A****GCTTACG)-3′	**9**	74%	4268.8	4268.3
5′-d(GATAGCTCGGATGC)-3′	**11**	91%	4303.8	[b])
5′-d(GCATCCGAGCTATC)-3′	**12**	93%	4223.8	4226.7
5′-d(CGTAAGCTCGATAG)-3′	**13**	94%	4287.8	4287.4
5′-d(CTATCGAGCTTACG)-3′	**14**	99%	4238.8	4239.1

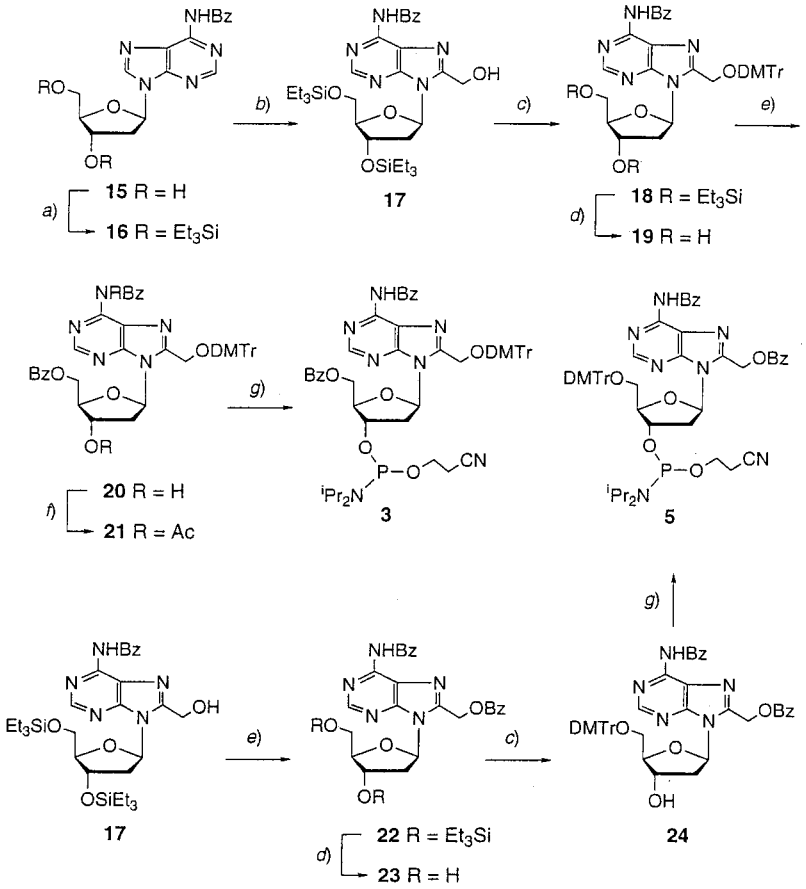
[a]) Calculated from the yield of the coupling-steps, as determined by the detritylation assay. [b]) Not determined.

coupling and be readily cleaved under the ammonolytic conditions applied for the deprotection of the oligomers.

To prepare **3**, 2′-deoxyadenosine was monobenzoylated in 90% yield by treatment with Me_3SiCl and benzoyl chloride (BzCl) [19] (*Scheme 1*). Disilylation of the resulting crystalline diol **15** with Et_3SiCl and imidazole in DMF [20] gave 88% of the Et_3Si-

protected nucleoside **16**. Adenosine derivatives have been C(8)-hydroxymethylated by deprotonation with LDA (= lithium diisopropylamide) or lithium-2,2,6,6-tetramethylpiperidide, addition of ethyl formiate, and reduction with $NaBH_4$ [21]. Subjecting **16** to this protocol yielded only 30% of the desired alcohol **17**, while replacing ethyl formiate by DMF increased the yield to *ca.* 70%. 4,4′-Dimethoxytritylation of **17** gave **18** (89%), which was desilylated to **19** (Bu_4NF in THF; 95%). The regioselective benzoylation of the diol **19** with BzCl and *Hünig*'s base in CH_2Cl_2, at $-10°$, as described for 2′-deoxyuridine derivatives [22], gave 75% of the monobenzoate **20** that was converted to the phosphoramidite **3** in the usual way [23].

Scheme 1



a) Et_3SiCl, imidazole, DMF, 23°; 88%. *b*) 1. LDA/THF, −78°; 2. DMF, −78°; 3. AcOH, 23°; 4. $NaBH_4$/EtOH, 23°; 70%. *c*) 4,4′-Dimethoxytrityl chloride (= bis(4-methoxyphenyl)phenylmethyl chloride; DMTrCl), CH_2Cl_2, $^iPr_2NEt$, 4-(dimethyamino)pyridine (DMAP), 23°; 89% (**18**), 72% (**24**). *d*) Bu_4NF, THF, 23°; 95% (**19**), 83% (**23**). *e*) BzCl, CH_2Cl_2, $^iPr_2NEt$, −10°; 75% (**20**), 73% (**22**). *f*) Ac_2O, Py, 23°; 53%. *g*) $(NCCH_2CH_2O)$-$P(^iPr_2N)Cl$, CH_2Cl_2, $^iPr_2NEt$, 23°; 82% (**3**), 72% (**5**).

The phosphoramidite **5**, which corresponds to a 3′,5′-linked repeating unit dA**, was prepared in an overall yield of 31% by benzoylation of the alcohol **17**, followed by desilylation, 4,4′-dimethoxytritylation at O(5′) (72%), and phosphitylation.

The phosphoramidites **3** and **5** are *ca.* 3:2 to 1:1 mixtures of diastereoisomers. At $-10°$ under Ar, they are stable for several months.

The regioselectivity of the benzoylation of **19** was confirmed by *N,O*-acetylation of the monobenzoate **20**. The signals of H–C(3′) and both H–C(5′) of **20** and of the acetylated product **21** were assigned on the basis of their multiplicity and coupling constants (see *Table 5*). Compared to H–C(3′) of **20** (δ 5.05 ppm), H–C(3′) of **21** (*Scheme 1*) is shifted downfield by 0.57 ppm, while the chemical shift for H–C(5′) is hardly affected. The broad *d* of HO–C(3′) at 2.70 ppm (J(H,OH) $\approx$ 1.0 Hz) disappeared upon H/D exchange of **20**, while the broad *m* of H–C(3′) collapsed to a *dt*.

As a rule, the shift from the *anti*- to the *syn*-conformation is indicated by a downfield shift of $H_{pro\text{-}S}$–C(2′) and H–C(3′), and an upfield shift of C(2′) [24]. The significant downfield shifts of the signals of $H_{pro\text{-}S}$–C(2′) ($\Delta\delta = 0.29-0.86$ ppm) and H–C(3′) ($\Delta\delta = 0.08-0.54$ ppm) and the upfield shift of C(2′) ($\Delta\delta = -0.8$ to -4.3 ppm) for the nucleosides **17**–**20**, **22**–**24**, and for the phosphoramidites **3** and **5**, as compared to the corresponding signals of **16**, evidence that all *C(8)*-substituted nucleosides adopt predominantly the *syn*-conformation (*Table 2*).

Table 2. *^{1}H- and ^{13}C-NMR Chemical-Shift Differences $\Delta\delta$(H–C(2′)), $\Delta\delta$(H–C(3′)), and $\Delta\delta$(C(2′))* [ppm] *for the Nucleosides* **3**, **5**, *and* **17**–**24** *as Compared to* **16**, *and Ratio of* S/N*-Conformers of these Nucleosides, as Deduced from* $J(1',2')/J(3',4')$ [Hz]

Nucleoside	$\Delta\delta$(H–C($2'_{pro\text{-}S}$))	$\Delta\delta$(H–C(3′))	$\Delta\delta$(C(2′))	J(1′,2′)	J(3′,4′)	Ratio *S*/*N*
16	–	–	–	6.4	3.8	63/37
17	0.55	0.08	−2.5	6.6	3.7	64/36
18	0.84	0.10	−4.1	6.8	2.8	71/29
19	0.29	0.13	−0.8	9.5	< 1.0	> 90/10
20	0.71	0.42	−3.5	5.6	5.0	53/47
21	0.96	0.99[a])	−6.6	8.1	3.0	73/27
3	0.86/0.81	0.54/0.45	−2.1	7.4/6.2	5.3/4.5	58/42, 58/42
22	0.84	0.13	−3.0	6.6	3.3	67/33
23	0.39	0.17	−0.8	9.5	< 1.0	> 90/10
24	0.81	0.27	−3.6	6.6	4.1	62/38
5	0.73/0.63	0.35/0.27	−4.3	6.5	[b])	[b])

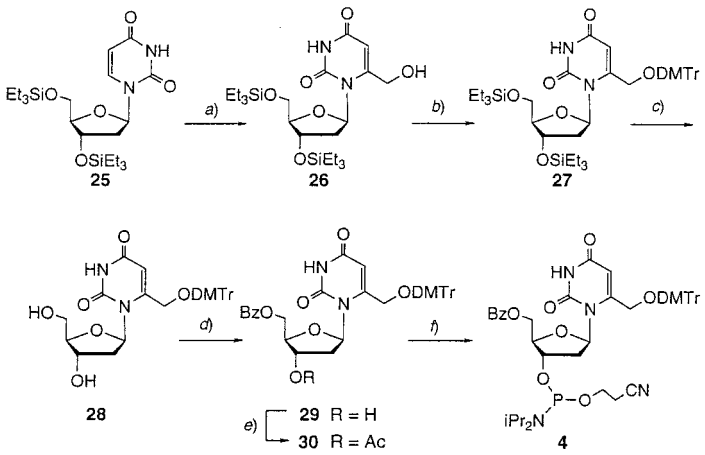
[a]) Partially due to acetylation at O(3′). [b]) J(1′,2′) and J(3′,4′) only partially determined.

The ratio $J(1',2'_{pro\text{-}S})/J(3',4')$ is considered a measure of the position of the equilibrium between the *S*- (sugar-pucker $_3E-{}^2T_1$) and *N*-conformers (sugar-pucker $^3T_2-{}^3T_4$) [25]. As expected, the *S*-conformation is slightly preferred in the O(5′)-protected nucleosides **16**–**18**, **20**–**22**, and **24**, as indicated by the $J(1',2'_{pro\text{-}S})$ (6.2–8.1 Hz) and J(3′,4′) values (2.8–5.0 Hz) (*Table 2*). In the diols **19** and **23**, $J(1',2'_{pro\text{-}S})$ and J(3′,4′) differ considerably from these values: $J(1',2'_{pro\text{-}S}) = 9.5$ and $J(3',4') \leq 1.0$ Hz show a clear predominance of the *S*-conformer of **19** and **23**. The strong downfield shift for HO–C(5′) of **19** and **23** in $CDCl_3$ (δ 6.23 and 6.06 ppm, resp.) evidences an intramolecular O(5′)H···N(3) H-bond. Such H-bonds are well-documented [7][26–31]. The nine-membered ring formed by the H-bond is rigid [7][26][27][32], as

evidenced by the characteristic $J(5'_{pro\text{-}S}, OH) \leq 1.0$, and $J(5'_{pro\text{-}R}, OH) = 11.0-11.2$ Hz. Both $J(4',5')$ of **19** and **23** are ≤ 1.5 Hz, indicating a *sc* arrangement of H–C(4′) and both H–C(5′), again in agreement with the proposed H-bond. The intramolecular H-bonds in **19** and **23** are confirmed by broad, intensive IR absorptions at 3289 cm^{-1} and 3286 cm^{-1} of the diols **19** and **23**.

The phosphoramidite **4** was synthesized from 2′-deoxyuridine by silylation to **25** [20], followed by treatment with LDA, DMF, and $NaBH_4$ (*Scheme* 2). This yielded only 30–35% of the alcohol **26** besides 45% of starting material; **25** and **26** were readily separated by flash chromatography. Increasing or decreasing the amount of LDA and/or DMF lowered the yield of **26**. The unsatisfactory yield is in agreement with results of *Miyasaka* and co-workers [33], who showed that deprotonation of 2′-deoxyuridine derivatives with LDA occurs in a maximum yield of 52%. BuLi and BuLi/TMEDA (= *N*,*N*,*N*′,*N*′-tetramethylethylenediamine) preferentially deprotonate uridine derivatives at C(5) [33]. Lithium 2,2,6,6-tetramethylpiperidide led to similar results as LDA [21], while the use of LHMDS (= lithium hexamethyldisilazane) did not lead to **26**. 4,4′-Dimethoxytritylation of the alcohol **26** to **27**, desilylation to the diol **28**, and monobenzoylation led to **29**, which was converted to the phosphoramidite **4** in the usual way; again, **4** was obtained as a *ca.* 1:1 mixture of diastereoisomers that proved stable at $-10°$ under Ar for several months.

Scheme 2



a) 1. LDA/THF, $-78°$; 2. DMF, $-78°$; 3. AcOH, 23°; 4. $NaBH_4$/EtOH, 23°; 30% (**26**), 45% (**25**). *b*) DMTrCl, CH_2Cl_2, $^iPr_2NEt$, DMAP, 23°; 63%. *c*) Bu_4NF, THF, 23°; 87%. *d*) BzCl, CH_2Cl_2, $^iPr_2NEt$, $-10°$; 65%. *e*) Ac_2O, Py, 23°; 75%. *f*) $(NCCH_2CH_2O)P(^iPr_2N)Cl$, CH_2Cl_2, $^iPr_2NEt$, 23°; 72%.

The regioselectivity of the benzoylation was confirmed by H/D exchange and acetylation of **29**. The signal of the H–C(3′) of **30** (see *Table 7*) is shifted downfield by 0.69 ppm, while the chemical shift for the two H–C(5′) remained practically unaltered. Again, the downfield shifts for $H_{pro\text{-}S}$–C(2′) and H–C(3′), and the upfield shift for C(2′) of both diastereoisomers **4** and of **26**–**30** (as compared to the corresponding signals of **25**) show that the *C(6)*-substituted nucleosides adopt predominantly the *syn*-conformation (*Table 3*).

Table 3. *^{1}H- and ^{13}C-NMR Chemical-Shift Differences $\Delta\delta(H-C(2'))$, $\Delta\delta(H-C(3'))$, and $\Delta\delta(C(2'))$* [ppm] *for the Nucleosides* **26**–**30** *and* **4** *as Compared to* **25**, *and Ratio of* S/N-*Conformers of these Nucleosides, as Deduced from* $J(1',2')/J(3',4')$ [Hz]

Nucleoside	$\Delta\delta$(H–C($2'_{pro\text{-}S}$))	$\Delta\delta$(H–C(3'))	$\Delta\delta$(C(2'))	J(1′,2′)	J(3′,4′)	Ratio S/N
25	–	–	–	6.2	3.7	63/37
26	0.50	0.05	– 2.8	7.2	4.0	64/36
27	0.82	–	– 3.6	6.2	4.7	57/43
28	0.86	0.27	– 3.3	7.5	4.4	63/37
29	0.84	0.33	– 3.1	4.1	6.5	39/61
30	1.08	1.02[a])	– 6.3	5.6	5.0	53/47
4	0.84	0.37	– 3.9	[b])	[b])	[b])

[a]) Partially due to acetylation at O(3′). [b]) J(1′,2′) and J(3′,4′) only partially determined.

The position of the equilibrium between the *S*- and *N*-conformers of **25**–**28** and **30** (*ca.* 60 : 40) was deduced from the $J(1',2'_{pro\text{-}S})$ and J(3′,4′) values (*Table 3*); it is slightly in favour of the *S*-conformer, while the *N*-conformer of **29** is slightly preferred. There is no NMR evidence for an intramolecular O(5′)H···O(2) H-bond of the diol **28** in $CDCl_3$. The J(4′,5′) values (4.1 and 2.8 Hz) of **28** are similar to those of the *O(5′)*-protected alcohol **26**. These coupling constants evidence that the *gauche,gauche*-conformation about the C(4′)–C(5′) bond (essential for an intramolecular H-bond between HO–C(5′) and O(2)) is less populated in **28** than in the 2′-deoxyadenosine-derived diols **19** and **23** ($J(4',5') \leq 1.5$ Hz) that possess the intramolecular O(5′)H··· N(3) H-bond.

3. *Solid-Phase Synthesis of 14-Mer DNA Strands Containing One dA*, dU*, or dA** Unit, and of the Complementary DNA Strands. Table 1* shows the 14mer DNA strands **11**–**14** that possess an internal dA or dT unit. Replacement of the internal dA of the unmodified 14mers **12** and **14** by dA* (derived from **3**) led to the modified strands **6** and **7**, respectively, while replacement of dA of **14** by dA** (derived from **5**) led to **9**. The exchange of the internal dT of the 14mer **13** by dU* (derived from **4**) yielded the modified strand **8**.

The solid-phase syntheses of the oligomers **6**–**9** and **11**–**14** were carried out on a DNA synthesizer and were based on a protocol for the synthesis of pRNA [10]. The coupling time for the attachment of **3**–**5** and of the DNA phosphoramidites immediately following the incorporation of **3**–**5** was arbitrarily set to 30 min; it led to coupling yields between 80–92%[4]) (99% for all other couplings). Longer coupling times did not improve the yields.The 14-mers **6**, **7**, **9**, and **11**–**14** were deprotected and cleaved off the solid support by ammonolysis with sat. aqueous NH_3 solution/MeOH 1 : 1 at 50° within 20 h. Prolonged heating of the modified DNA strand **8** (incorporating dU*) at 50° led to degradation; this strand was deprotected at 35°. The crude products were analyzed by RP-HPLC and showed a composition in agreement with the detritylation assay. The products were purified by RP-HPLC, desalted, and analyzed by RP-HPLC and MALDI-TOF mass spectroscopy. The total yield of the oligomers, as

[4]) Each chain-elongation cycle began with an acid-promoted cleavage of the 4,4′-dimethoxytrityloxy group. The efficiency of each coupling step was calculated by UV determination of the amount of $DMTr^+$ ($\varepsilon = 70000\ l \cdot mol^{-1} \cdot cm^{-1}$).

determined from the detritylation assay, and their molecular masses are shown in *Table 1*.

4. *Pairing Studies.* In a first set of measurements, we examined the influence of the incorporation of one dA* unit into a DNA · DNA duplex or into the DNA strand of a DNA · RNA duplex. The modified 14-mer **6** (*Table 1*) was hybridized with the complementary 14-mer **11**, or its RNA analogue **10**, at a concentration of (2 + 2) μM and a pH of 7 in 0.15M aqueous NaCl (*Table 4*).

Table 4. *Melting Points* T_m [°C] *of* **11** · **12**, **10** · **12**, **11** · **6**, **10** · **6**, **13** · **14**, **13** · **7**, **13** · **9**, *and* **14** · **8** *Obtained from Temperature-Dependent UV Spectroscopy*

Duplex		T_m	ΔT_m	Strand conc.	Conc. NaCl
5′-d(GATAGCTCGGATGC)-3′ 3′-d(CTATCGAGCCTACG)-5′	**11** · **12**	57°	–	(2 + 2) μM	0.15M
5′-r(GAUAGCUCGGAUGC)-3′ 3′-d(CTATCGAGCCTACG)-5′	**10** · **12**	53°	–	(2 + 2) μM	0.15M
5′-d(GATAGCTCGGATGC)-3′ 3′-d(CTATCG**A***GCCTACG)-5′	**11** · **6**	49.5°	− 7.5°	(2 + 2) μM	0.15M
5′-r(GAUAGCUCGGAUGC)-3′ 3′-d(CTATCG**A***GCCTACG)-5′	**10** · **6**	45°	− 8.0°	(2 + 2) μM	0.15M
5′-d(CGTAAGCTCGATAG)-3′ 3′-d(GCATTCGAGCTATC)-5′	**13** · **14**	50°	–	(2 + 2) μM	0.10M
5′-d(CGTAAGCTCGATAG)-3′ 3′-d(GCATTCG**A***GCTATC)-5′	**13** · **7**	43°	− 7.0°	(2 + 2) μM	0.10M
5′-d(CGTAAGCTCGATAG)-3′ 3′-d(GCATTCG**A****GCTATC)-5′	**13** · **9**	47.5°	− 2.5°	(2 + 2) μM	0.10M
5′-d(CTATCGAGCTTACG)-3′ 3′-d(GATAGC**U***CGAATGC)-5′	**14** · **8**	44°	− 6.0°	(2 + 2) μM	0.10M

The modified duplexes show a decrease in melting temperature by 7.5° (**11** · **6**) and 8.0° (**10** · **6**) relative to the corresponding non-modified duplexes **11** · **12** and **10** · **12** (see *Fig. 4* for the melting curves).

Next, the 14-mer DNA strands **7** – **9** incorporating one dA*, dU*, or dA** unit were hybridized with the complementary DNA strands **13** or **14** at a concentration of (2 + 2) μM and a pH of 7 in 0.1M aqueous NaCl (*Table 4*). The melting curves of the non-modified duplex **13** · **14**, and the modified duplexes **13** · **7** and **14** · **8** are shown in *Fig. 5*, *a*.

Again, the duplex **13** · **7** (containing dA*) melted 7° lower than the DNA duplex **13** · **14**. The duplex **14** · **8** (containing dU*) showed a depression of the melting temperature by 6°, as compared to the non-modified DNA duplex **13** · **14**.

Pairing of the modified 14mer **9**, containing one dA** unit, and the complementary DNA strand **13** yielded a duplex that melted only 2.5° lower than the non-modified DNA duplex **13** · **14** (*Table 4* and *Fig. 5*, *b*).

A decrease in melting temperature of 6 – 8° for an incorporated dA* or dU* unit indicates a strong destabilization of the DNA duplex. The degree of duplex destabilization caused by dA* and dU* is comparable to the degree of duplex destabilization imposed by mismatched base pairs. In the 13mer DNA duplex GCGTACACATGCG · CGCATG**X**GTACGC, for example, the exchange of the matching **X** = thymidine by adenosine, guanosine, or cytosine leads to a decrease in melting temperature of 5.4 – 13.7° at a concentration of (1.5 + 1.5) μM and a pH of 7 in

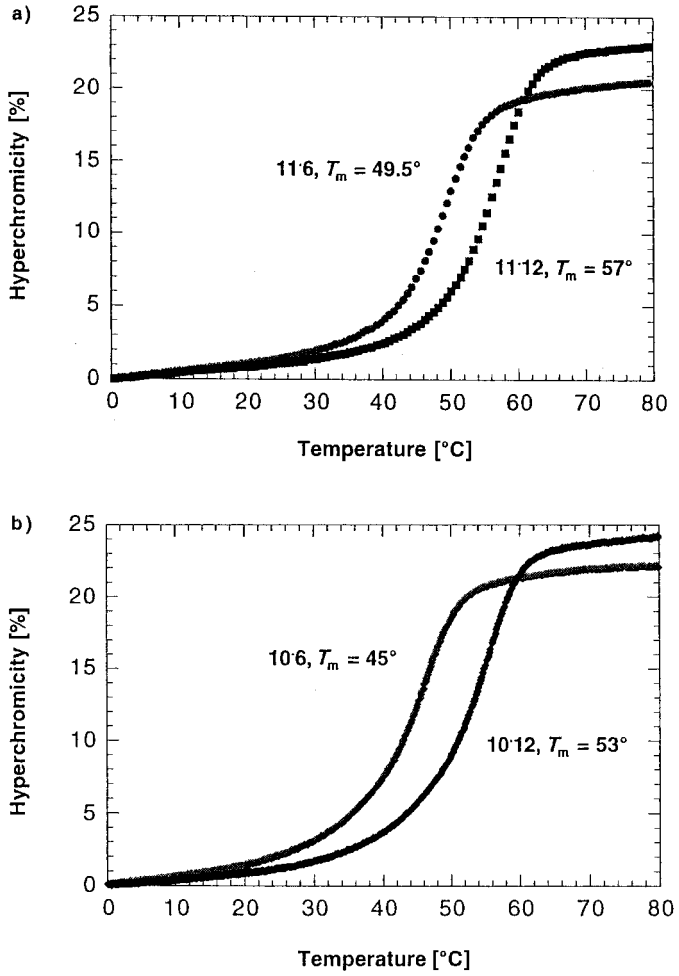


Fig. 4. a) *Temperature-dependent UV spectra* ('melting curves') *of the non-modified and modified DNA · DNA duplexes* **11 · 12** *and* **11 · 6** *and* b) *the non-modified and modified DNA · RNA duplexes* **10 · 12** *and* **10 · 6**

0.1M aqueous NaCl [34]. The decrease by 2.5° of the melting temperature for the duplex **13 · 9**, as compared to 6–8° for the other modified duplexes, evidences the strong influence of the linking mode, rather than just of the introduction of a CH_2OH group, on the duplex destabilisation.

Experimental Part

General. Solvents were distilled before use: THF from K/benzophenone, CH_2Cl_2 from CaH_2. Reactions were run under Ar. Qual. TLC: precoated silica-gel plates (*Merck* silica gel *60* F_{254}); detection by spraying with 'mostain' (400 ml of 10% aq. H_2SO_4, 20 g of $(NH_4)_6Mo_7O_{24} \cdot H_2O$, 0.4 g of $Ce(SO_4)_2$) and heating. Flash chromatography (FC): silica gel *Merck 60* (0.04–0.063 mm). Optical rotations: 1-dm cell at 25° and 589 nm. FT-IR: 1–2% soln. in the indicated solvent. ^1H-, ^{13}C-, and ^{31}P-NMR: at 200, 300, or 400 MHz, 50 or 75 MHz, and

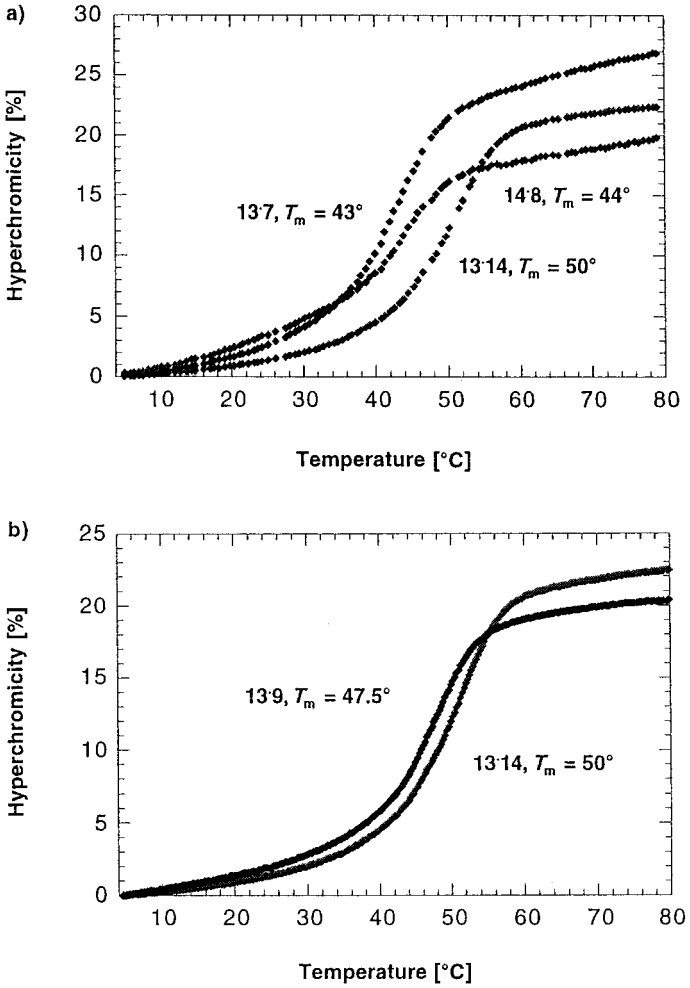


Fig. 5. a) *Temperature-dependent UV spectra* ('melting curves') *of the non-modified and modified DNA · DNA duplexes* **13 · 14**, **13 · 7**, *and* **14 · 8**, *and* b) **13 · 14** *and* **13 · 9**

121 MHz, respectively. MS: fast atom bombardment (FAB), matrix-assisted laser-desorption ionisation (MALDI), high resolution (HR). NOBA: 3-nitrobenzyl alcohol.

N^6-*Benzoyl-2′-deoxy-3′,5′-bis*-O-*(triethylsilyl)adenosine* (**16**). At 23°, a soln. of **15** [19] (11.6 g, 32.7 mmol) and imidazole (22.3 g, 328 mmol) in DMF (200 ml, distilled from CaH_2) was treated dropwise with Et_3SiCl (28 ml, 165 mmol), stirred for 16 h, treated with MeOH (6 ml), and evaporated. A soln. of the residue in CH_2Cl_2 was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 2:1) gave **16** (16.9 g, 88%). Colourless oil. R_f (AcOEt/hexane 6:1) 0.67. $[\alpha]_D^{25} = -4.8$ ($c = 1.3$, $CHCl_3$). IR ($CHCl_3$): 3407*w*, 2958*s*, 2912*m*, 2877*m*, 1707*m*, 1612*s*, 1584*m*, 1503*w*, 1455*s*, 1413*m*, 1352*w*, 1329*m*, 1111*m*, 1091*m*, 1071*m*, 1006*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): see *Table 5*; additionally, 9.10 (br. *s*, NH); 8.38 (*s*, H–C(8)); 8.10–8.00 (*m*, 2 arom. H); 7.66–7.45 (*m*, 3 arom. H); 1.10–0.85 (*m*, 2 ($MeCH_2$)$_3$Si); 0.75–0.45 (*m*, 2 ($MeCH_2$)$_3$Si). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 6*; additionally, 165.1 (*s*, C=O); 134.1 (*s*); 133.1 (*d*); 129.1 (*d*, 2 C); 128.1 (*d*, 2 C); 7.1, 6.8 (2*q*, 2 ($MeCH_2$)$_3$Si); 5.2, 5.0 (2*t*, 2 ($MeCH_2$)$_3$Si). FAB-MS (NOBA): 584 (64, $[M+H]^+$). HR-MALDI-MS: 606.291 ($[M+Na]^+$; calc. 606.291).

Table 5. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of* **3**, **5**, *and* **16**–**24** *in $CDCl_3$ Solution*

	16	**17**	**18**	**19**	**20**	**21**	**3**	**22**	**23**	**24**	**5**
H–C(1′)	6.56	6.47	6.39	6.38	6.29	6.40	6.37, 6.35	6.53	6.56	6.50	6.50, 6.48
$H_{pro\text{-}S}$–C(2′)	2.69	3.24	3.53	2.98	3.40	3.65	3.60, 3.50	3.53	3.08	3.50	3.42, 3.32
$H_{pro\text{-}R}$–C(2′)	2.50	2.34	2.20	2.26	2.34	2.38	2.50, 2.48	2.34	2.32	2.42	2.60–2.50
											2.50–2.42
H–C(3′)	4.63	4.71	4.73	4.76	5.05	5.62	5.17, 5.08	4.76	4.85–4.75	4.90	4.98, 4.90
H–C(4′)	4.06	4.00	3.92	4.14	4.11	4.30	4.30	4.00	4.22	4.13	4.27
H–C(5′)	3.84	3.88	3.92	3.97	4.65	4.70	4.76, 4.70	3.89	3.96	3.40	3.90–3.50
H–C(5′)	3.72	3.70	3.74	3.85–3.75	4.60	4.50	4.55, 4.52	3.72	3.77	3.40	3.90–3.50
CH–C(8)	–	5.00	4.55	4.58	4.55	4.55	4.48	5.69	5.65	5.65	5.72
CH′–C(8)	–	4.92	4.36	4.41	4.47	4.37	4.43	5.69	5.55	5.55	5.65
H–C(2)	8.80	8.75	8.77	8.76	8.60	8.46	8.70	8.80	8.73	8.60	8.63, 8.60
$J(1',2'_{pro\text{-}S})$	6.4	6.6	6.8	9.5	5.6	8.1		6.6	9.5	6.6	
$J(1',2'_{pro\text{-}R})$	6.4	6.6	6.8	5.4	7.5	6.2		6.6	5.4	6.6	
$J(2'_{pro\text{-}S}2'_{pro\text{-}R})$	12.9	13.4	13.1	13.3	13.1	14.0		13.3	13.3	13.4	
$J(2'_{pro\text{-}S}, 3')$	6.4	6.6	6.0	5.4	6.5	6.5		6.5	5.3	6.5	
$J(2'_{pro\text{-}R}, 3')$	3.7	3.4	3.1	< 1.5	5.6	2.6		3.7	< 1.5	4.1	
J(3′,4′)	3.8	3.7	2.8	< 1.5	5.0	3.0		3.3	< 1.5	4.1	
J(4′,5′)	3.8	3.7	5.0	< 1.5	4.4	5.0		4.5	< 1.5	5.0	
J(4′,5′)	3.8	4.4	6.9	< 1.5	5.0	5.3		5.6	< 1.5	5.0	
J(5′,5′)	11.2	11.2	13.4	12.4	12.1	11.8		10.8	12.8	< 1.5	
J(CH,CH′)	–	14.4	11.8	12.0	12.1	11.8		< 1.5	13.7	13.7	

Table 6. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of* **3**, **5**, *and* **16**–**24** *in $CDCl_3$ Solution*

	C(1′), C(4′)	C(2′)	C(3′)	C(5′)	CH_2–C(8)	C(2)	C(4)	C(5)	C(6)[a])	C(8)[a])
16	85.1, 88.6	41.6	72.1	62.6	–	153.1	149.6	123.6	151.6	141.6
17	85.1, 88.1	39.1	72.1	62.6	58.1	152.6	149.1	121.6	153.1	155.6
18	85.6, 88.4	37.5	73.0	63.1	59.7	152.6	149.6	122.6	152.9	152.4
19	87.3, 89.6	40.8	73.5	63.5	59.5	151.8	150.0	123.0	152.2	152.7
20	84.3, 84.3	38.1	71.6	64.0	59.6	152.4	149.4	122.3	152.6	152.6
21	82.3, 85.0	35.0	74.7	64.0	59.8	152.2	150.7	128.8	154.6	154.5
3	83.8, 83.7, 84.7, 84.6	37.3	73.3, 72.5	63.8	59.7, 59.5	152.6	149.4	122.7	152.6	152.3
22	85.3, 88.1	38.6	72.7	62.6	59.6	153.1	149.1	122.1	152.9	150.2
23	87.0, 90.0	40.8	73.4	63.6	59.2	153.0	149.2	123.3	152.2	150.7
24	85.0, 86.4	38.0	72.9	63.9	59.3	153.1	149.8	122.4	152.6	150.0
5	85.3, 85.1, 85.9, 86.0	37.3	74.5, 73.7	63.8, 63.5	59.6, 59.5	152.7	149.8, 149.6	122.3	152.7, 152.4	150.0, 149.9

[a]) Assignments for **17**–**24**, **3**, and **5** may be interchanged.

N^6-*Benzoyl-2′-deoxy-8-(hydroxymethyl)-3′,5′-bis*-O-*(triethylsilyl)adenosine* (**17**). At $-70°$, a soln. of (i-Pr)$_2$NH (10.3 ml, 72.9 mmol, distilled form CaH_2) in THF (40 ml) was treated dropwise with 1.6M BuLi in hexane (45 ml, 72 mmol), stirred at $-70°$ for 15 min and at 0° for 15 min, and transferred *via* a syringe to a cooled ($-70°$) soln. of **16** (8.45 g, 14.4 mmol) in THF (60 ml) within 10 min. The soln. was stirred at $-70°$ for 1 h, treated dropwise with DMF (28 ml, 363 mmol, distilled, from CaH_2) at $-70°$, stirred for 2.5 h at $-70°$, treated with AcOH (10 ml), allowed to warm to 23°, diluted with EtOH (100 ml), treated with $NaBH_4$ (1.76 g, 46 mmol), and stirred for 25 min. After evaporation, the residue was dissolved in CH_2Cl_2, washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:1) gave **17** (6.2 g, 70%). Colourless foam. R_f (AcOEt/hexane 2:1) 0.31. $[\alpha]_D^{25} = -8.9$ ($c = 0.65$, $CHCl_3$). IR ($CHCl_3$): 3405*m* (br.), 2958*s*, 2913*s*, 2878*s*, 1707*s*, 1655*w*, 1615*s*, 1587*m*, 1526*w*, 1459*s*, 1434*s*, 1345*m*, 1301*w*, 1096*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 5*;

additionally, 9.23 (br. *s*, NH); 8.06–7.96 (*m*, 2 arom. H); 7.64–7.45 (*m*, 3 arom. H); 3.80 (br. *s*, OH); 1.20–0.75 (*m*, 2 (MeCH$_2$)$_3$Si); 0.70–0.42 (*m*, 2 (MeCH_2)$_3$Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 6*; additionally, 165.6 (*s*, C=O); 134.1 (*s*); 133.1 (*d*); 128.9 (*d*, 2 C); 128.4 (*d*, 2 C); 6.6 (*q*, 2 (MeCH$_2$)$_3$Si); 4.9, 4.4 (2*t*, 2 (MeCH$_2$)$_3$Si). FAB-MS (NOBA): 614 (23, $[M+H]^+$). HR-FAB-MS: 614.3187 ($[M+H]^+$; calc. 614.3194).

N^6-*Benzoyl-2′-deoxy-8-[(4,4′-dimethoxytrityloxy)methyl]-3′,5′-bis*-O-*(triethylsilyl)adenosine* (**18**). A soln. of **17** (0.5 g, 0.815 mmol), EtN(i-Pr)$_2$ (0.55 ml, 3.2 mmol, distilled from CaH_2), and DMAP (50 mg, 0.4 mmol) in CH_2Cl_2 (5 ml) was treated with 4,4′-dimethoxytrityl chloride (1.1 g, 3.25 mmol) at 0°, stirred at 23° for 16 h, and evaporated. A soln. of the residue in CH_2Cl_2 was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:3) gave **18** (670 mg, 89%). Colourless foam. R_f (AcOEt/hexane 1:2) 0.46. $[\alpha]_D^{25} = -6.0$ ($c = 0.8$, $CHCl_3$). IR ($CHCl_3$): 3407*w* (br.), 2958*m*, 2912*m*, 2877*m*, 2839*w*, 1706*m*, 1614*s*, 1585*m*, 1509*s*, 1463*m*, 1429*m*, 1414*m*, 1344*m*, 1300*m*, 1116*m*, 1073*m*, 1033*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 5*; additionally, 9.02 (br. *s*, NH); 8.06–7.96 (*m*, 2 arom. H); 7.69–7.09 (*m*, 12 arom. H); 6.92–6.75 (*m*, 4 arom. H); 3.77 (*s*, 2 MeO); 1.05–0.80 (*m*, 2 (MeCH$_2$)$_3$Si); 0.70–0.42 (*m*, 2 (MeCH_2)$_3$Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 6*; additionally, 162.4 (*s*, C=O); 159.2 (*s*, 2 C); 144.5 (*s*); 135.6 (*s*); 135.3 (*s*); 134.4 (*s*); 132.8 (*d*); 130.4–127.2 (several *d*); 113.8, 113.7 (2*d*, 4 C); 87.9 (*s*, Ph$_3C$); 55.2 (*q*, 2 MeO); 6.7, 6.6 (2*q*, 2 (MeCH$_2$)$_3$Si); 4.6, 4.3 (2*t*, 2 (MeCH$_2$)$_3$Si). FAB-MS (NOBA): 916 (61, $[M+H]^+$), 303 (100, DMTr$^+$). HR-MALDI-MS: 938.432 ($[M+Na]^+$; calc. 938.432).

N^6-*Benzoyl-2′-deoxy-8-[(4,4′-dimethoxytrityloxy)methyl]adenosine* (**19**). A soln. of **18** (660 mg, 0.721 mmol) in THF (6 ml) was treated dropwise at 23° with 1M $Bu_4NF \cdot 3H_2O$ in THF (2.4 ml) for 1.5 h. Evaporation and FC (AcOEt/MeOH 100:1) gave **19** (470 mg, 95%). Colourless foam. R_f (AcOEt/MeOH 100:1) 0.39. $[\alpha]_D^{25} = -1.4$ ($c = 2.75$, $CHCl_3$). IR ($CHCl_3$): 3365*m* (br.), 3286*m* (br.), 2937*m*, 2839*w*, 1709*m*, 1614*s*, 1589*m*, 1509*s*, 1464*m*, 1432*m*, 1347*m*, 1301*m*, 1104*m*, 1062*m*, 1036*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): see *Table 5*; additionally, 9.20 (br. *s*, NH); 8.10–8.00 (*m*, 2 arom. H); 7.68–7.16 (*m*, 12 arom. H); 6.90–6.78 (*m*, 4 arom. H); 6.23 (br. *d*, $J = 11.2$, HO–C(5′)); 3.76 (*s*, 2 MeO); 2.30 (br. *s*, HO–C(3′)). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 6*; additionally, 165.0 (*s*, C=O); 159.1 (*s*, 2 C); 144.5 (*s*); 135.2 (*s*); 135.0 (*s*); 134.0 (*s*); 133.0 (*d*); 130.2–127.3 (several *d*); 113.7 (*d*, 4 C); 88.2 (*s*, Ph$_3C$); 55.5 (*q*, 2 MeO). FAB-MS (NOBA): 688 (100, $[M+H]^+$), 303 (75, DMTr$^+$). HR-FAB-MS: 688.2769 ($[M+H]^+$; calc. 688.2771).

N^6,5′-O-*Dibenzoyl-2′-deoxy-8-[(4,4′-dimethoxytrityloxy)methyl]adenosine* (**20**). At −10° (ice/NaCl), a soln. of **19** (454 mg, 0.66 mmol) in CH_2Cl_2 (10 ml) and pyridine (2 ml, 24.9 mmol, distilled from CaH_2) was treated dropwise with a soln. of BzCl (76 µl, 0.66 mmol) in CH_2Cl_2 (2 ml), stirred for 3 h, washed with H_2O (10 ml), dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 4:1) gave **20** (316 mg, 61%). Colourless foam. R_f (AcOEt/hexane 6:1) 0.55. $[\alpha]_D^{25} = -2.2$ ($c = 0.6$, $CHCl_3$). IR ($CHCl_3$): 3408*m* (br.), 2961*m*, 1711*s*, 1614*s*, 1586*m*, 1509*s*, 1463*m*, 1430*w*, 1352*w*, 1093*m*, 1070*m*, 1035*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 5*, additionally, 9.00 (br. *s*, NH); 8.10–7.90 (*m*, 4 arom. H); 7.71–7.15 (*m*, 15 arom. H); 6.90–6.75 (*m*, 4 arom. H); 3.75 (*s*, 2 MeO); 2.75–2.65 (*m*, HO–C(3′)). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 6*; additionally, 166.8 (*s*, O–C=O); 165.0 (*s*, N–C=O); 159.1 (*s*, 2 C); 144.3 (*s*); 135.3 (*s*); 135.2 (*s*); 134.2 (*s*); 133.4 (*d*); 132.9 (*d*); 129.9 (*s*); 130.3–127.4 (several *d*); 113.8 (*d*, 4 C); 88.0 (*s*, Ph$_3C$); 55.5 (*q*, 2 MeO). FAB-MS (NOBA): 792 (89, $[M+H]^+$), 303 (100, DMTr$^+$). HR-FAB-MS (NOBA): 792.3037 ($[M+H]^+$; calc. 792.3034).

N^6,3′-O-*Diacetyl*-N^6,5′-O-*dibenzoyl-2′-deoxy-8-[(4,4′-dimethoxytrityloxy)methyl]adenosine* (**21**). A soln. of **20** (30 mg, 0.044 mmol) in pyridine (1 ml, distilled from CaH_2) was treated with Ac_2O (0.5 ml) and stirred for 16 h at 23°. Evaporation and FC (AcOEt/hexane 1:1) gave **21** (20 mg, 53%). Colourless foam. R_f (AcOEt/hexane 1:1) 0.33. IR ($CHCl_3$): 2958*w*, 1719*s*, 1607*s*, 1577*m*, 1509*s*, 1449*m*, 1418*w*, 1367*m*, 1347*m*, 1087*m*, 1070*m*, 1035*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 5*; additionally, 8.10–8.00 (*m*, 2 arom. H); 7.75–7.65 (*m*, 2 arom. H); 7.60–7.20 (*m*, 15 arom. H); 6.90–6.80 (*m*, 4 arom. H); 3.80 (*s*, 2 MeO); 2.56 (*s*, AcN); 2.0 (*s*, AcO). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 6*; additionally, 173.1, 172.3 (2*s*, 2 O–C=O); 170.5, 166.8 (2*s*, 2 N–C=O); 159.3 (*s*, 2 C); 144.2 (*s*); 135.2 (*s*); 135.0 (*s*); 134.6 (*s*); 133.4 (*d*); 133.0 (*d*); 130.3 (*s*); 130.0–127.5 (several *d*); 113.8, 113.7 (2*d*, 4 C); 88.0 (*s*, Ph$_3C$); 55.2 (*q*, 2 MeO); 25.6 (*q*, Me); 22.2 (*q*, Me). FAB-MS (NOBA): 876 (14, $[M+H]^+$), 303 (100, DMTr$^+$).

N^6,5′-O-*Dibenzoyl-2′-deoxy-8-[(4,4′-dimethoxytrityloxy)methyl]adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**3**). A soln. of **20** (80 mg, 0.1 mmol) and EtN(i-Pr)$_2$ (46 µl, 0.26 mmol, distilled from CaH_2) in CH_2Cl_2 (4 ml) was treated with 2-cyanoethyl diisopropylphosphoramidochloridite (31 mg, 0.13 mmol) and stirred for 3 h at 23°. Evaporation and FC (AcOEt/hexane 2:3) gave **3** (81 mg, 82%, 3:2 ratio of 2 diastereoisomers). Colourless foam. R_f (AcOEt/hexane 2:1) 0.25, 0.34 (2 diastereoisomers). $[\alpha]_D^{25} = -7.1$ ($c = 1.63$, $CHCl_3$). IR ($CHCl_3$): 3407*w* (br.), 2970*m*, 2190*w*, 1712*s*, 1614*s*, 1586*m*, 1509*s*, 1463*m*, 1430*w*, 1365*w*, 1352*w*, 1070*m*. ^{1}H-NMR (300 MHz, $CDCl_3$, 3:2 mixture of 2 diastereoisomers): 8.95 (br. *s*, NH); 8.70 (*s*, H–C(2)); 8.05–7.90 (*m*, 4 arom. H); 7.66–7.20 (*m*, 15 arom. H); 6.95–6.75 (*m*, 4 arom. H); 6.37 (*dd*, $J = 7.4$, 5.6, 0.4 H),

6.35 (t, J = 6.0, 0.6 H) (H–C(1′)); 5.17 (ddt, J = 12.0, 7.0, 5.3, 0.6 H), 5.08 (ddt, J = 10.5, 7.0, 4.5, 0.4 H) (H–C(3′)); 4.75 (dd, J = 12.0, 4.5, 0.6 H), 4.70 (dd, J = 12.0, 4.5, 0.4 H), 4.55 (dd, J = 12.0, 4.5, 0.6 H), 4.52 (dd, J = 12.0, 4.5, 0.4 H) (2 H–C(5′)); 4.48, 4.43 (2d, J = 12.1) (CH_2–C(8)); 4.30 (dt, J = 5.3, 4.5, H–C(4′)); 3.90–3.65 (m, OCH_2CH_2CN); 3.65–3.55 (m, 0.4 $H_{pro\text{-}S}$–C(2′), $(Me_2CH)_2N$); 3.76 (s, 2 MeO); 3.50 (ddd, J = 13.0, 7.0, 6.0, 0.6 $H_{pro\text{-}S}$–C(2′)); 2.60, 2.53 (2t, J = 6.2, CH_2CN); 2.50 (ddd, J = 13.0, 7.0, 5.6, 0.4 H); 2.48 (ddd, J = 13.0, 7.0, 6.0, 0.6 H) ($H_{pro\text{-}R}$–C(2′)); 1.40–1.00 (m, $(Me_2CH)_2N$). ^{13}C-NMR (100 MHz, $CDCl_3$, 3:2 mixture of 2 diastereoisomers): see *Table 6*; additionally, 166.4, 166.3 (2s, O–C=O); 164.5 (s, N–C=O); 158.9 (s, 2 C); 144.3, 144.2 (2s, 1 C), 135.3, 135.2, 135.1, 134.2, 134.1 (several s, 3 C); 133.2 (d); 132.8 (d); 130.1 (s); 130.1–127.4 (several d); 117.5, 117.4 (2s, CN); 113.6, 113.5, 113.4 (3d, 4 C); 87.9, 87.8 (2s, Ph_3C); 84.7, 84.6 (2dd, ^3J(C,P) = 3.7, C(4′)); 73.3, 72.5 (2dd, ^2J(C,P) = 17, C(3′)); 58.9, 58.7 (2dt, ^2J(C,P) = 20, OCH_2CH_2CN); 55.4 (q, 2 MeO); 43.4, 43.3 (2dd, ^2J(C,P) = 7.5, $(Me_2CH)_2N$); 24.8–24.6 (several q, $(Me_2CH)_2N$); 20.5, 20.4 (2dt, ^3J(C,P) = 4.7, OCH_2CH_2CN). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 149.4, 149.3. FAB-MS (NOBA): 992 (29, $[M+H]^+$), 303 (100, $DMTr^+$); HR-MALDI-MS: 1014.393 ($[M+Na]^+$; calc. 1014.393).

N^6-*Benzoyl-8-[(benzoyloxy)methyl]-2′-deoxy-3′,5′-bis*-O-*(triethylsilyl)adenosine* (**22**). At −10° (ice/NaCl), a soln. of **17** (1.68 g, 2.74 mmol) and pyridine (9 ml, 110 mmol, distilled from CaH_2) in CH_2Cl_2 (20 ml) was treated dropwise with BzCl (0.32 ml, 2.74 mmol), stirred for 3 h, washed with H_2O (20 ml), dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 2:3) gave **22** (1.43 g, 73%). Colourless foam. R_f (AcOEt/hexane 1:2) 0.35. $[\alpha]_D^{25} = 4.3$ (c = 1.3, $CHCl_3$). IR ($CHCl_3$): 3325w (br.), 2958s, 2913m, 2877m, 1724s, 1700s, 1615s, 1586m, 1532m, 1461m, 1428m, 1354w, 1109s, 1094s, 1071s. ^{1}H-NMR (200 MHz, $CDCl_3$): see *Table 5*; additionally, 9.15 (br. s, NH); 8.14–7.95 (m, 4 arom. H); 7.65–7.40 (m, 6 arom. H); 1.05–0.80 (m, 2 $(MeCH_2)_3Si$); 0.75–0.44 (m, 2 $(MeCH_2)_3Si$). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 6*; additionally, 165.9 (s, O–C=O); 164.9 (s, N–C=O); 134.2 (s); 133.9 (d); 132.9 (d); 130.2 (d, 2 C); 129.4 (s); 129.1 (d, 2 C); 128.8 (d, 2 C); 128.1 (d, 2 C); 6.8, 6.7 (2q, 2 $(MeCH_2)_3Si$); 4.8, 4.3 (2t, 2 $(MeCH_2)_3Si$). FAB-MS (NOBA): 718 (27, $[M+H]^+$), 374 (100). HR-MALDI-MS: 740.330 ($[M+Na]^+$; calc. 740.328).

N^6-*Benzoyl-8-[(benzoyloxy)methyl]-2′-deoxyadenosine* (**23**). At 23°, a soln. of **22** (1.27 g, 1.77 mmol) in THF (40 ml) was treated dropwise with 1M $Bu_4NF \cdot 3\,H_2O$ in THF (4.3 ml, 4.3 mmol) and stirred for 1.5 h. Evaporation and FC (AcOEt/MeOH 100:1) gave **23** (720 mg, 83%). Colourless foam. R_f (AcOEt/MeOH 100:1) 0.42. $[\alpha]_D^{25} = 27$ (c = 1.13, $CHCl_3$). IR ($CHCl_3$): 3402m (br.), 3289 (br.), 2946w, 1726s (br.); 1615s, 1590m, 1532w, 1479s, 1464s, 1452s, 1430s, 1356s, 1106s, 1071s. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 5*; additionally, 9.26 (br. s, NH); 8.10–7.80 (m, 4 arom. H); 7.61–7.30 (m, 6 arom. H); 6.06 (dd, J = 11.0, 1.2, HO–C(5′)); 3.40 (d, J = 2.5, HO–C(3′)). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 6*; additionally, 166.2 (s, O–C=O); 165.2 (s, N–C=O); 134.2 (d); 134.0 (s); 133.4 (d); 130.3 (d, 2 C); 129.4 (s); 129.4 (d, 2 C); 129.0 (d, 2 C); 128.4 (d, 2 C). FAB-MS (NOBA): 490 (29, $[M+H]^+$), 374 (100).

N^6-*Benzoyl-8-[(benzoyloxy)methyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenosine* (**24**). At 0°, a soln. of **23** (678 mg, 1.385 mmol), $EtN(i\text{-}Pr)_2$ (0.55 ml, 3.2 mmol, distilled from CaH_2) and DMAP (50 mg, 0.4 mmol) in pyridine (7 ml, distilled from CaH_2) was treated with 4,4′-dimethoxytrityl chloride (0.564 g, 1.67 mmol), stirred for 16 h at 23°, and evaporated. A soln. of the residue in CH_2Cl_2 was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 4:1) gave **24** (790 mg, 72%). Colourless foam. R_f (AcOEt/hexane 4:1) 0.29. $[\alpha]_D^{25} = -2.4$ (c = 1.3, $CHCl_3$). IR ($CHCl_3$): 3405w (br.), 3064w, 2959s, 1725s, 1700s, 1614s, 1586s, 1509s, 1464s, 1452s, 1429s, 1353m, 1094s, 1071s, 1036s. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 5*; additionally, 9.34 (br. s, NH); 8.05–7.90 (m, 4 arom. H); 7.60–7.00 (m, 15 arom. H); 6.80–6.65 (m, 4 arom. H); 3.70 (s, 2 MeO). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 6*; additionally, 165.9 (s, O–C=O); 164.9 (s, N–C=O); 158.9 (s, 2 C); 144.9 (s); 136.1 (s); 136.0 (s); 133.8 (s); 133.7 (d); 133.1 (d); 129.2 (s); 130.3–127.1 (several d); 113.3 (d, 4 C); 86.4 (s, Ph_3C); 55.5 (q, 2 MeO). FAB-MS (NOBA): 792 (4, $[M+H]^+$), 303 (50, $DMTr^+$), 374 (100). HR-MALDI-MS: 814.284 ($[M+Na]^+$; calc. 814.285).

N^6-*Benzoyl-8-[(benzoyloxy)methyl]-2′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5**). At 23°, a soln. of **24** (188 mg, 0.237 mmol) and $EtN(i\text{-}Pr)_2$ (0.11 ml, 0.62 mmol, distilled from CaH_2) in CH_2Cl_2 (12 ml) was treated with 2-cyanoethyl diisopropylphosphoramidochloridite (53 µl, 0.238 mmol) and stirred for 3 h. Evaporation and FC (AcOEt/hexane 2:3) gave **5** (169 mg, 72%, 1:1 mixture of 2 diastereoisomers). Colourless foam. R_f (AcOEt/hexane 1:2) 0.26, 0.33 (2 diastereoisomers). $[\alpha]_D^{25} = -10.3$ (c = 1.15, $CHCl_3$). IR ($CHCl_3$): 3405w (br.), 2969m, 2935m, 2200w, 1725s, 1700s, 1614s, 1586m, 1509s, 1463s, 1428m, 1365m, 1353m, 1094s, 1071s, 1036s. ^{1}H-NMR (300 MHz, $CDCl_3$, 1:1 mixture of 2 diastereoisomers): 9.00 (br. s, NH); 8.63, 8.60 (2s, H–C(2)); 8.08 (d, 2 arom. H); 8.00 (d, 2 arom. H); 7.60–7.10 (m, 15 arom. H); 6.80–6.65 (m, 4 arom. H); 6.50, 6.48 (2t, J = 6.5, H–C(1′)); 5.72, 5.65 (2d, J = 13.6), CH_2–C(8)); 4.98 (ddt, J = 14.7, 5.6, 4.5, 0.5 H), 4.90 (ddt, J = 13.2, 5.6, 2.8, 0.5 H) (H–C(3′)); 4.27 (dt, J = 5.6, 4.9, H–C(4′)); 3.90–3.50 (m, 2H–C(5′), $(Me_2CH)_2N$, OCH_2CH_2CN); 3.68 (s, 2 MeO); 3.42 (ddd, J =

12.0, 6.5, 4.5, 0.5 H), 3.32 (*ddd*, $J = 10.5$, 6.5, 2.8, 0.5 H) ($H_{pro\text{-}S}$–C(2′)); 2.60–2.40 (*m*, $H_{pro\text{-}R}$–C(2′)); 2.57 (*td*, $J = 6.3$, 2.1); 2.45 (*t*, $J = 6.4$, CH_2CN); 1.25–1.00 (*m*, $(Me_2CH)_2N$). ^{13}C-NMR (100 MHz, $CDCl_3$, 1:1 mixture of 2 diastereoisomers): see *Table 6*; additionally, 165.7 (*s*, O–C=O); 164.5 (*s*, N–C=O); 158.55, 158.5, 158.45 (3*s*, 2 C); 144.8, 144.7 (2*s*, 1 C); 136.2, 136.1, 135.9, 134.0 (several *s*, 3 C); 133.7 (*d*); 132.8 (*d*); 130.2–126.8 (*s*, several *d*); 117.6, 117.56 (2*s*, CN); 113.1 (*d*, 4 C); 86.35, 86.3 (2*s*, Ph_3C); 74.3 (*dd*, ^2J(C,P) = 16.8), 73.5 (*dd*, ^2J(C,P) = 17.9) (C(3′)); 58.6, 58.5 (2*dt*, ^2J(C,P) = 16.2, OCH_2CH_2CN); 55.3, 55.2 (2*q*, 2 MeO); 43.35 (2*dd*, ^2J(C,P) = 12.1, $(Me_2CH)_2N$); 24.75–24.55 (several *q*, $(Me_2CH)_2N$); 20.5, 20.3 (2*dt*, ^3J(C,P) = 7.1, OCH_2CH_2CN). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 149.5, 149.2. FAB-MS (NOBA): 992 (23, $[M+H]^+$), 303 (100, $DMTr^+$). HR-MALDI-MS: 1014.393 ($[M+Na]^+$; calc. 1014.393).

*2′-Deoxy-3′,5′-bis-*O*-(triethylsilyl)uridine* (**25**). A soln. of 2′-deoxyuridine (10 g, 43.9 mmol) and imidazole (29.7 g, 436 mmol) in DMF (150 ml) was treated dropwise with Et_3SiCl (37 ml, 218 mmol), stirred for 16 h at 23°, treated with MeOH (8 ml), and evaporated. A soln. of the residue in CH_2Cl_2 was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:1) gave **25** (18.9 g, 94%). Colourless oil. R_f (AcOEt/hexane 1:1) 0.67. $[\alpha]_D^{25} = 31.8$ ($c = 0.4$, $CHCl_3$). IR ($CHCl_3$): 3392*m* (br.), 2958*s*, 2913*m*, 2878*s*, 1690*s*, 1634*w*, 1460*s*, 1415*m*, 1388*m*, 1116*s*, 1084*m*, 1067*m*, 1006*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): see *Table 7*, additionally, 8.67 (br. *s*, NH); 8.00 (*d*, $J = 8.3$, H–C(6)); 0.98–0.84 (*m*, 2 ($MeCH_2$)$_3$Si); 0.70–0.42 (*m*, 2 ($MeCH_2$)$_3$Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 8*; additionally, 6.6, 6.4 (2*q*, 2 ($MeCH_2$)$_3$Si); 4.6, 4.2 (2*t*, 2 ($MeCH_2$)$_3$Si). FAB-MS (NOBA): 457 (88, $[M+H]^+$). HR-MALDI-MS: 479.236 ($[M+Na]^+$; calc. 479.237).

*2′-Deoxy-6-(hydroxymethyl)-3′,5′-bis-*O*-(triethylsilyl)uridine* (**26**). At −70°, a soln. of (i-Pr)$_2$NH (10.3 ml, 72.9 mmol, distilled from CaH_2) in THF (40 ml) was treated dropwise with 1.6M BuLi in hexane (45 ml, 72 mmol), stirred at −70° for 15 min and at 0° for 15 min, and transferred *via* syringe to a cooled (−70°) soln. of **25** (6.6 g, 14.4 mmol) in THF (60 ml) within 10 min. The soln. was stirred at −70° for 1 h, treated with DMF (28 ml, 363 mmol, distilled from CaH_2), stirred for 2.5 h, treated with AcOH (10 ml), allowed to warm to 23°, diluted with EtOH (100 ml), treated with $NaBH_4$ (1.76 g, 46 mmol), and stirred for 25 min. After evaporation, the residue was dissolved in CH_2Cl_2, washed with H_2O and brine, and dried (Na_2SO_4). Evaporation and FC (AcOEt/hexane 1:1) gave **26** (2.1 g, 30%) and **25** (3 g, 46%). Colourless foam. R_f (AcOEt/hexane 1:1) 0.27. $[\alpha]_D^{25} = 22.5$ ($c = 0.4$, $CHCl_3$). IR ($CHCl_3$): 3392*m* (br.), 2958*s*, 2913*m*, 2878*m*, 1697*s*, 1639*s*, 1458*m*, 1412*m*,

Table 7. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of* **4**, *and* **25**–**30** *in $CDCl_3$ Solution*

	25	**26**	**27**	**28**	**29**	**30**	**4**
H–C(1′)	6.33	6.20	5.75	5.75	5.74	5.80	5.78–5.65
$H_{pro\text{-}S}$–C(2′)	2.10	2.60	2.92	2.96	2.94	3.18	2.94
$H_{pro\text{-}R}$–C(2′)	2.33	2.20	2.02	2.07	2.18	2.13	2.30–2.15
H–C(3′)	4.45	4.50	4.38	4.72	4.78	5.47	4.90–4.72
H–C(4′)	3.95	3.83	3.8–3.6	3.80	3.92	4.15	4.12–4.00
H–C(5′)	3.90	3.90	3.8–3.6	3.82	4.61	4.58	4.70, 4.60
H–C(5′)	3.75	3.80	3.8–3.6	3.76	4.50	4.50	4.42, 4.38
CH–C(6)	–	4.55	3.97	4.00	4.00	3.95	4.00
CH′–C(6)	–	4.45	3.91	3.93	3.95	3.95	4.00
H–C(5)	5.70	5.78	5.73	5.66	5.70	5.68	5.78–5.65
J(1′,2′$_{pro\text{-}S}$)	6.2	7.2	6.2	7.5	4.1	5.6	
J(1′,2′$_{pro\text{-}R}$)	6.2	7.2	8.1	7.8	8.7	8.1	
J(2′$_{pro\text{-}S}$, 2′$_{pro\text{-}R}$)	13.3	13.2	13.1	13.7	13.7	13.8	
J(2′$_{pro\text{-}S}$, 3′)	6.2	7.5	7.5	7.4	8.1	8.0	
J(2′$_{pro\text{-}R}$, 3′)	4.3	3.7	4.7	4.4	6.5	4.7	
J(3′,4′)	3.7	4.0	4.7	4.4	6.5	5.0	
J(4′,5′)	2.2	2.8	[a])	2.8	4.1	4.8	
J(4′,5′)	2.5	3.8	[a])	4.1	6.2	6.8	
J(5′,5′)	11.2	10.6	[a])	12.8	11.8	11.8	
J(CH,CH′)	–	14.0	12.9	14.3	13.4	< 1.5	

[a]) Not determined.

Table 8. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of* **4**, *and* **25**–**30** *in $CDCl_3$ Solution*

	C(1′), C(4′)	C(2′)	C(3′)	C(5′), CH_2–C(6)	C(2)	C(4)	C(5)	C(6)
25	85.3, 88.0	42.0	71.2	62.0, –	150.6	163.9	102.0	140.5
26	85.8, 87.9	39.2	71.8	63.0, 61.0	150.8	164.4	101.9	156.6
27	86.6, 88.3	38.4	72.6	63.8, 62.7	150.3	163.6	102.6	153.6
28	87.6, 87.2	38.7	71.2	62.8, 62.3	151.6	163.3	103.7	153.1
29	84.9, 86.4	38.9	72.1	65.3, 62.6	150.9	163.4	103.3	153.2
30	82.4, 86.8	35.7	74.8	64.9, 62.9	150.3	163.0	103.5	152.9
4	84.2, 83.7	38.1	73.8	64.7, 62.6	150.3	163.6	103.0	153.1
	86.7, 86.3		73.0					153.0

1373*m*, 1338*m*, 1084*s*, 1042*m*, 1006 m. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 7*; additionally, 8.20 (br. *s*, NH); 3.40 (br. *s*, OH); 1.05–0.84 (*m*, 2 ($Me$$CH_2$)$_3$Si); 0.70–0.50 (*m*, 2 (MeCH_2)$_3$Si). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 8*; additionally, 6.7 (*q*, 2 ($Me$$CH_2$)$_3$Si); 4.7, 4.3 (2*t*, 2 (MeCH_2)$_3$Si). FAB-MS (NOBA): 487 (48, $[M+H]^+$).

2′-Deoxy-6-[(4,4′-dimethoxytrityloxy)methyl]-3′,5′-bis-O-*(triethylsilyl)uridine* (**27**). A soln. of **26** (3.7 g, 7.6 mmol), EtN(i-Pr)$_2$ (5.2 ml, 30.2 mmol, distilled from CaH_2), and DMAP (50 mg, 0.4 mmol) in CH_2Cl_2 (60 ml) was treated with 4,4′-dimethoxytrityl chloride (10.3 g, 30.4 mmol) at 0°, stirred at 23° for 16 h, and evaporated. A soln. of the residue in CH_2Cl_2 was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 1:3) gave **27** (3.8 g, 63%). Colourless foam. R_f (AcOEt/hexane 1:3) 0.28. $[\alpha]_D^{25} = 9.9$ (c = 1.45, $CHCl_3$). IR ($CHCl_3$): 3390*w* (br.), 2958*s*, 2938*m*, 2912*m*, 2877*s*, 1691*s*, 1608*m*, 1584*w*, 1510*s*, 1464*s*, 1445*m*, 1414*m*, 1379*m*, 1342*w*, 1071*s*, 1035*s*, 1006*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): see *Table 7*; additionally, 8.30 (br. *s*, NH); 7.50–7.20 (*m*, 9 arom. H); 6.95–6.80 (*m*, 4 arom. H); 3.76 (*s*, 2 MeO)); 1.05–0.84 (*m*, 2 ($Me$$CH_2$)$_3$Si); 0.75–0.50 (*m*, 2 (MeCH_2)$_3$Si). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 8*; additionally, 159.2 (*s*, 2 C); 144.2 (*s*); 135.3 (*s*); 135.0 (*s*); 130.1–127.5 (several *d*); 113.8, 113.7 (2*d*, 4 C); 88.0 (*s*, Ph_3C); 55.3 (*q*, 2 MeO); 6.7 (*q*, 2 ($Me$$CH_2$)$_3$Si); 4.6, 4.2 (2*t*, 2 (MeCH_2)$_3$Si). FAB-MS (NOBA): 789 (2, $[M+H]^+$), 303 (100, $DMTr^+$); HR-FAB-MS: 788.3883 (M^+; calc. 788.3888).

2′-Deoxy-6-[(4,4′-dimethoxytrityloxy)methyl]uridine (**28**). A soln. of **27** (3.55 g, 4.5 mmol) in THF (60 ml) was treated dropwise at 23° with 1M $Bu_4NF \cdot 3\ H_2O$ in THF (10.9 ml, 10.9 mmol) and stirred for 1.5 h. Evaporation and FC (AcOEt/MeOH 100:1) gave **28** (2.2 g, 87%). Colourless foam. R_f (AcOEt/MeOH 100:1) 0.56. $[\alpha]_D^{25} = 3.8$ (c = 0.42, $CHCl_3$). IR ($CHCl_3$): 3390*m* (br.), 2959*m*, 2840*m*, 1694*s*, 1608*m*, 1584*m*, 1509*s*, 1464*m*, 1445*m*, 1411*m*, 1382*m*, 1303*m*, 1064*m*, 1035*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 7*; additionally, 7.50–7.20 (*m*, 9 arom. H); 6.86 (*d*, 4 arom. H); 3.80 (*s*, 2 MeO). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 8*; additionally, 159.4 (*s*, 2 C); 144.1 (*s*); 135.2 (*s*); 135.0 (*s*); 130.2–127.7 (several *d*); 113.9, 113.8 (2*d*, 4 C); 88.3 (*s*, Ph_3C); 55.5 (*q*, 2 MeO). FAB-MS (NOBA): 560 (13, M^+), 303 (100, $DMTr^+$).

*5′-*O*-Benzoyl-2′-deoxy-6-[(4,4′-dimethoxytrityloxy)methyl]uridine* (**29**). At −10° (ice/NaCl), a soln. of **28** (2.2 g, 2.92 mmol) in CH_2Cl_2 (100 ml) and pyridine (23 ml, 284 mmol, distilled from CaH_2) was treated dropwise with a soln. of BzCl (0.56 ml, 4.86 mmol) in CH_2Cl_2 (10 ml), stirred at −10° for 3 h, washed with H_2O (60 ml), dried (Na_2SO_4), and evaporated. FC (AcOEt/hexane 4:1) gave **29** (1.7 g, 65%) and **28** (0.5 g, 23%). Colourless foam. R_f (AcOEt/hexane 6:1) 0.44. $[\alpha]_D^{25} = 8.9$ (c = 1.32, $CHCl_3$). IR ($CHCl_3$): 3391*m* (br.), 3186*w* (br.), 2959*m*, 2936*m*, 2840*w*, 1694*s*, 1608*m*, 1584*w*, 1509*s*, 1464*m*, 1452*m*, 1409*m*, 1381*m*, 1316*m*, 1095*s*, 1070*s*, 1036*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 7*; additionally, 8.80 (br. *s*, NH); 8.12–8.00 (*m*, 2 arom. H); 7.64–7.20 (*m*, 12 arom. H); 6.94–6.83 (*m*, 4 arom. H); 3.80 (*s*, 2 MeO)); 2.30 (br. *d*, J = 4.0, OH). ^{13}C-NMR (50 MHz, $CDCl_3$): see *Table 8*; additionally, 167.2 (*s*, O–C=O); 159.4 (*s*, 2 C); 144.1 (*s*); 135.2 (*s*); 135.0 (*s*); 133.4 (*d*); 130.3–127.7 (*s*, several *d*); 113.9, 113.8 (2*d*, 4 C); 88.1 (*s*, Ph_3C); 55.5 (*q*, 2 MeO). FAB-MS (NOBA): 664 (4, M^+), 303 (100, $DMTr^+$). HR-FAB-MS (NOBA): 664.2417 (M^+; calc. 664.2421).

*3′-*O*-Acetyl-5′-*O*-benzoyl-2′-deoxy-6-[(4,4′-dimethoxytrityloxy)methyl]uridine* (**30**). At 23°, a soln. of **29** (20 mg, 0.03 mmol) in pyridine (1 ml, distilled from CaH_2) was treated with Ac_2O (0.5 ml) and stirred for 16 h. Evaporation and FC (AcOEt/hexane 1:1) gave **30** (16 mg, 75%). Colourless foam. $[\alpha]_D^{25} = 2.3$ (c = 0.92, $CHCl_3$). IR ($CHCl_3$): 3390*w* (br.), 2936*w*, 1722*s*, 1692*s*, 1608*m*, 1510*m*, 1462*m*, 1452*m*, 1378*m*, 1095*m*, 1070*m*, 1036*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 7*; additionally, 9.00 (br. *s*, NH); 8.10–8.00 (*m*, 2 arom. H); 7.60–7.20 (*m*, 12 arom. H); 6.90–6.80 (*m*, 4 arom. H); 3.80 (*s*, 2 MeO); 1.95 (*s*, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 8*; additionally, 170.5, 166.7 (2*s*, 2 C=O); 159.4 (*s*, 2 C); 144.2 (*s*); 135.2 (*s*); 135.0 (*s*); 133.4 (*d*); 130.3 (*s*); 130.2–127.7 (several *d*); 113.8, 113.7 (2*d*, 4 C); 88.2 (*s*, Ph_3C); 55.5 (*q*, 2 MeO); 20.9 (*q*, Me).

5′-O-Benzoyl-2′-deoxy-6-[(4,4′-dimethoxytrityloxy)methyl]uridine 3′-(2-Cyanoethyl) Diisopropylphosphoramidite) (**4**). A soln. of **29** (100 mg, 0.15 mmol) and EtN(i-Pr)$_2$ (90 µl, 0.52 mmol, distilled from CaH_2) in CH_2Cl_2 (4 ml) was treated with 2-cyanoethyl diisopropylphosphoramidochloridite (34 µl, 0.152 mmol) and stirred at 23° for 3 h. Evaporation and FC (AcOEt/hexane 2:3) gave **4** (93 mg, 72%, 2:1 ratio of 2 diastereoisomers). Colourless foam. R_f (AcOEt/hexane 1:2) 0.24, 0.33 (2 diastereoisomers). IR ($CHCl_3$): 3390*w* (br.), 2969*w*, 2935*w*, 1718*s*, 1694*s*, 1608*w*, 1509*m*, 1463*m*, 1414*w*, 1379*m*, 1366*w*, 1094*m*, 1070*m*, 1035*m*. ^{1}H-NMR (300 MHz, $CDCl_3$, 2:1 ratio of 2 diastereoisomers): 8.10–8.00 (*m*, 2 arom. H); 7.60–7.20 (*m*, 12 arom. H); 6.90–6.80 (*m*, 4 arom. H); 5.78–5.65 (*m*, H–C(5), H–C(1′)); 4.90–4.72 (*m*, H–C(3′)); 4.70 (*dd*, *J* = 11.8, 3.1, 0.67 H), 4.60 (*dd*, *J* = 12.0, 3.7, 0.33 H), 4.42 (*dd*, *J* = 11.8, 3.4, 0.67 H), 4.38 (*dd*, *J* = 12.0, 3.4, 0.33 H) (2 H–C(5′)); 4.12–4.00 (*m*, H–C(4′)); 4.00 (br. *s*, CH_2–C(6)); 3.90–3.40 (*m*, $(Me_2CH)_2N$, OCH_2CH_2CN); 3.76 (*s*, 2 MeO); 2.94 (*ddd*, *J* = 13.0, 8.4, 4.7, $H_{pro\text{-}S}$–C(2′)); 2.58, 2.57 (2*t*, *J* = 6.3, CH_2CN); 2.30–2.15 (*m*, $H_{pro\text{-}R}$–C(2′)); 1.10–1.00 (*m*, $(Me_2CH)_2N$). ^{13}C-NMR (75 MHz, $CDCl_3$, 2:1 ratio of 2 diastereoisomers): see *Table 8*; additionally, 166.7 (*s*, O–C=O); 159.3 (*s*, 2 C), 144.1 (*s*); 135.25, 135.2, 135.0 (3*s*, 2 C); 133.3 (*d*); 130.3 (*s*); 130.2–127.6 (several *d*); 117.8 (*s*, CN); 113.9 (*d*, 4 C); 88.1 (*s*, Ph_3C); 84.2, 83.7 (2*dd*, ^3J(C,P) = 4.5, C(4′)); 73.8 (*dd*, ^2J(C,P) = 19), 73.0 (*dd*, ^2J(C,P) = 17) (C(3′)); 59.0, 58.6 (2*dt*, ^2J(C,P) = 20, OCH_2CH_2CN); 55.4 (*q*, 2 MeO); 43.5, 43.2 (2*dd*, ^2J(C,P) = 12, $(Me_2CH)_2N$); 24.9–24.4 (several *q*, $(Me_2CH)_2N$); 20.6–20.1 (2*dt*, ^3J(C,P) = 4.6, OCH_2CH_2CN). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 149.9, 149.7. FAB-MS (NOBA): 865 (1, $[M+H]^+$), 303 (100, $DMTr^+$). HR-MALDI-MS: 887.340 ($[M+Na]^+$; calc. 887.340).

Oligonucleotide Synthesis. Oligonucleotide syntheses were performed on a *Pharmacia Gene Assembler* on a 1.3-µmol scale. The commercial phosphoramidites and the CPG solid supports were obtained from *Glen Research.* Solvents and reagents were prepared essentially according to the protocol for the synthesis of pRNA [10]. Detritylation was accomplished within 2 min by 3% $Cl_2CHCOOH$ in $(CH_2Cl)_2$. Couplings (0.16 ml of 0.1M phosphoramidite soln. + 0.36 ml of 0.35M 1*H*-tetrazole/0.15M 5-(4-nitrophenyl)-1*H*-tetrazole soln. in MeCN, or 0.36 ml 0.25M 1-(benzylthio)-1*H*-tetrazole soln. in MeCN, resp.) were performed within 6–10 min (DNA-phosphoramidites coupled on unmodified nucleotides), or 30 min (modified phosphoramidites, and DNA-phosphoramidites coupled on modified nucleotides, resp.). Capping and oxidation was accomplished under standard conditions [35].

Deprotection and Purification of Oligonucleotides. Removal of the protecting groups and detachment from the solid support was effected in conc. aq. NH_3 soln./MeOH 1:1 (2.5 ml) at 50° in 20 h (oligomers **6**, **7**, **9**, and **11**–**14**) or at 35° in 5 h (oligomer **8** containing dU*; see *Results and Discussion*). After filtration and evaporation, the crude oligomers were dissolved in aq. buffer soln. (0.1M HOAc, 0.1M Et_3N), purified by RP-HPLC, and desalted by RP-HPLC. Their composition was confirmed by MALDI-TOF-MS.

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Received January 3, 2001