From Phosphate to Bis(methylene) Sulfone: Non-Ionic Backbone Linkers in DNA

by **Daniel Hutter**[a]), **Monika O. Blaettler**[b]), and **Steven A. Benner***[a])

[a]) Department of Chemistry, University of Florida, Gainesville FL 32611-7200
(tel: (352)3927773; fax: (352)3927918; email: benner@chem.ufl.edu)
[b]) Departement Chemie, ETH Hönggerberg, CH-8093 Zürich

Chimeric DNA molecules containing four different linking groups, the natural phosphate, 5′-methylenephosphonate, bis(methylene)phosphinate, and bis(methylene) sulfone (see *Fig. 1*), were directly compared for their ability to form duplexes with complementary DNA and DNA chimeras. From melting temperatures for analogous complementary sequences, general conclusions about the impact of geometric distortion of the internucleotide linkage around the two P–O–C bridges were drawn, as were conclusions about the impact on duplex stability that arises from the removal of the negative charge in the linking group. Each structural perturbation diminished the melting temperature, by *ca.* −2.5° per modification for the 5′-methylenephosphonate, −3.5° per modification for the bis(methylene)phosphinate, and −4.5° per modification for the bis(methylene) sulfone linker. These results have implications for DNA chemistry including the design of 'antisense' candidates and the proposal of alternative genetic materials in the search for non-terrean life.

1. Introduction. – The repeating negative charge of DNA has emerged as a key structural-feature important for rule-based molecular recognition. Much of the evidence for this came from analogs of DNA intended to implement the 'antisense' strategy for blocking the expression of mRNA, where replacement of the charged phosphate group by an uncharged linking group was presumed to allow easier passage of a DNA analog across cell membranes, and to counteract the 'unfavorable' interstrand phosphate repulsion [1]. In most of these cases, it turned out that removing the charge did not strengthen duplex stability, as expected, but rather diminished it.

These results have generated a larger discussion about alternative genetic material, a discussion whose relevance has moved beyond biomedical research. In particular, the discussion has become relevant to the search for signatures of life in *NASA* missions to other bodies in the solar system. We and others have come to ask: What features of the structure of DNA will be universal in the genetic material of all life forms, regardless of their genesis? In particular, is the repeating negative charge a 'universal' in genetic material able to function in H_2O?

The impact of removing the charge has been investigated by substituting the phosphate group with the isoelectronic, non-ionic and non-stereogenic bis(methylene) sulfone linker [2]. The crystal structure of a duplex between sulfone-linked dimers [3] showed only minor differences in geometry compared to its natural analog [4] and clearly demonstrated that bis(methylene) sulfone-linked nucleic acid analogs are capable of *Watson-Crick* base pairing. This suggested that a negative charge is not essential for *Watson-Crick* duplex formation.

Upon expanding the size of the oligosulfones beyond the dinucleoside level, however, major differences in the physicochemical behavior compared with natural DNA emerged. Particularly remarkable was the oligosulfone r($A_{SO_2}U_{SO_2}G_{SO_2}G_{SO_2}U_{SO_2}C_{SO_2}A_{SO_2}U$) [5] ($_{SO_2}$ corresponds to $CH_2SO_2CH_2$ instead of $OP(=O)(O^-)O$). The molecule displayed an extraordinary thermal denaturation curve, melting at *ca.* 80°. Upon melting, a large hyperchromicity was observed (> 200%; 25% is typical for the melting of a DNA duplex). The sequence was not, in the *Watson-Crick* sense, self-complementary. Thus, it was concluded that this oligosulfone folded to a rather stable conformation, indeed one of the most stable single-stranded 'RNA' structures known. *Richert et al.* have subsequently examined the conformation of sulfone-linked oligonucleotide analogs, establishing details of the folding [5].

The results of these and other experiments suggested that each oligosulfone has its own unique properties and reactivity. Different oligosulfones differing (in some cases) by only one nucleobase displayed different levels of solubility, aggregation behavior, folding, chemical reactivity, and *Watson-Crick* base-pairing ability. Their properties were often influenced dramatically by adding a single charge to one end of the molecule [6].

These results suggested that removing *all* the phosphate groups of an oligonucleotide introduces such a big change in the molecule's physicochemical behavior that the effect cannot easily be interpreted. We, therefore, set out to synthesize chimeric sequences were the phosphate groups were replaced one at a time. We hoped to gain a more quantitative understanding of the influence of charge neutralization on duplex stability and conformation, which then would enable us by extrapolation to better understand the unexpected properties of the oligosulfones.

The results with these chimeras were again surprising. The incorporation of one bis(methylene) sulfone linker into an otherwise unchanged DNA duplex lowered the T_m by several degrees [7]. In addition, the incorporation of a modified linker at the place of the recognition sequence of *Eco*RV endonuclease resulted in a potent inhibitor for the enzyme, while a similar experiment with *Eco*RI and a bis(methylene) sulfone modification in its recognition sequence did not produce any significant inhibition [8]. These experiments strongly suggested that the modified linker introduced a kink into the conformation of the chimera.

Replacing the natural phosphate linker by a bis(methylene) sulfone linker does more than just remove the charge, however. It also replaces the bridging O-atoms by CH_2 groups, which are sterically more demanding and less easily solvated. To assign the effects to specific structural features, we considered a stepwise replacement of the linking phosphate by first introducing a 5′-methylenephosphonate (replacing one bridging O-atom by a CH_2 group, while retaining the charge), then a bis(methylene)-phosphinate (replacing the other bridging O-atom by a CH_2 group, still retaining the charge), and only then losing the charge with a bis(methylene)sulfone (*Fig. 1*).

Collingwood and co-workers had already examined DNA analogs with single phosphates replaced by bis(methylene)phosphinate [9] linkers. However, the comparison of their data with our measurements is complicated by the fact that identical sequences were not examined, nor were the conditions for measuring T_m the same.

We report here a systematic study that examines, in parallel, phosphate, 5′-methylenephosphonate, bis(methylene)phosphinate, and bis(methylene) sulfone linkers.

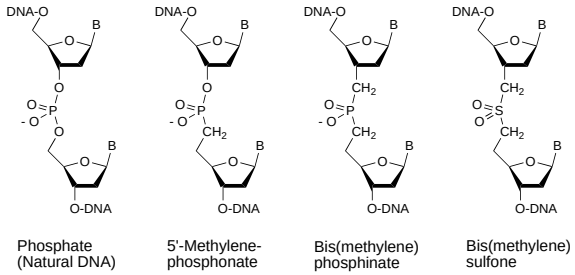


Fig. 1. *Backbone linkers investigated in this study*

We report the synthesis of all three modified linkers, incorporation of these at identical positions in various sequences, and the properties of the resulting chimeras determined in parallel measurements.

2. Synthesis of the Dinucleoside Analogs. – *Bis(methylene) sulfone Analogs.* The syntheses of the $T^{SO_2}T$[1]) and $A^{SO_2}T$[1]) bis(methylene) sulfone analogs **16** and **17** are outlined in *Scheme 1*. The synthesis of the starting nucleoside analogs **1** and **2**, carrying a 3′-carboxaldehyde, was already reported by *Sanghvi et al.* [10]. These aldehydes were reduced to the corresponding alcohols **3** and **4**. The alcohols were transformed by a *Mitsunobu* reaction with thioacetic acid to the thioacetates **5** and **6**. The adenine-bearing monomer was *N*-benzoylated, and the thioacetates were hydrolyzed to the corresponding thiols **7** and **8**. The thymine- and *N*-benzoyladenine-bearing monomers were obtained in 11 and 5% overall yield from commercially available thymidine and 2′-deoxyadenosine, respectively.

The 5′-homologated thymidine analog **9** was obtained in 32% overall yield from thymidine in a three-step synthesis described by *Baeschlin et al.* [11].

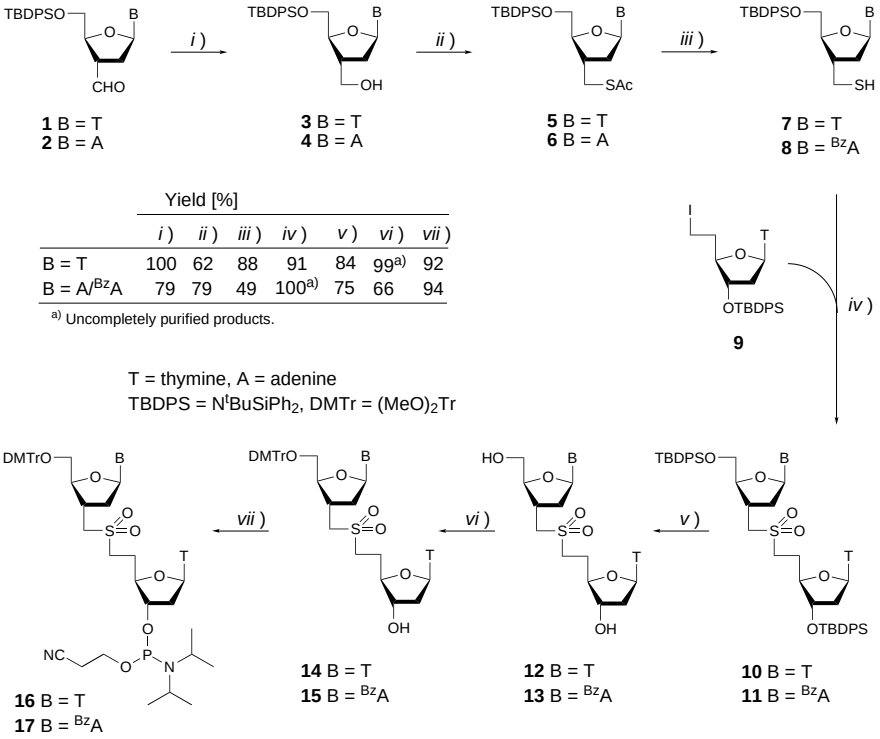
The coupling of iodide **9** with the thiols **7** and **8** was achieved almost quantitatively with Cs_2CO_3 in THF/DMF, following a method developed by *Richert et al.* in their work preparing an all-sulfone RNA octamer [5]. The resulting thioethers were quantitatively oxidized with Oxone® to the sulfones **10** and **11**. These dinucleoside analogs were deprotected to the diols **12** and **13**. Standard protocols were followed for the 5′-$(MeO)_2$Tr protection, furnishing the analogs **14** and **15**, and the subsequent 3′-activation to the phosphoramidites **16** and **17**. The overall yields of the dinucleoside steps were 70% for **16** and 42% for **17**.

Bis(methylene)phosphinate Analogs. The synthesis of the bis(methylene)phosphinate-modified $T^{POOEt}T$[1]) dimer **29** (POOEt corresponds to $CH_2P(=O)(OEt)CH_2$ instead of $OP(=O)(O^-)O$) was first reported by *Collingwood* and co-workers [9][12]. Their route was applied here for both the $T^{POOEt}T$ and $A^{POOEt}T$ analogs (*Scheme 2*). Compound **19** was synthesized in 62% yield following a procedure from *Horwitz et al.* [13]. The methylphosphinate reagent **18** was synthesized in two steps and 54% overall yield from hypophosphorous acid by protection with triethyl orthoacetate under acid catalysis as described by *Gallagher* and *Honegger* [14] and subsequent methylation of

---

[1]) If not indicated otherwise, one-letter nucleoside symbols designate 2′-deoxynucleosides.

Scheme 1



	Yield [%]						
	i)	*ii*)	*iii*)	*iv*)	*v*)	*vi*)	*vii*)
B = T	100	62	88	91	84	99[a)]	92
B = A/^{Bz}A	79	79	49	100[a)]	75	66	94

[a)] Uncompletely purified products.

T = thymine, A = adenine
TBDPS = N^{t}BuSiPh$_2$, DMTr = (MeO)$_2$Tr

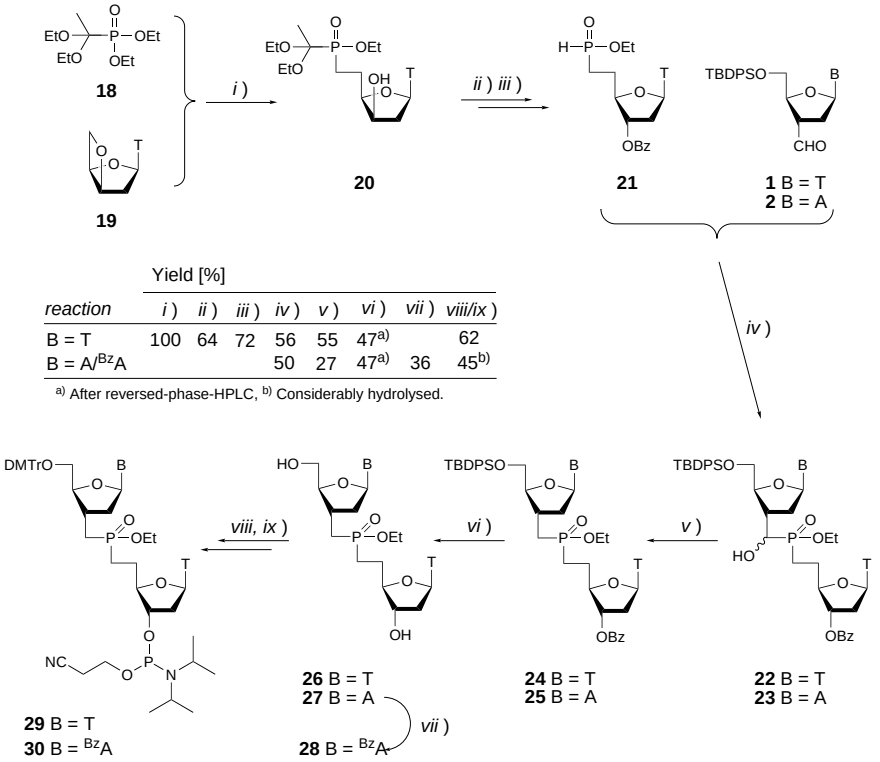
i) $NaBH_4$, EtOH/THF, r.t., 2 h. *ii*) PPh_3, diisopropyl azodicarboxylate (DIAD), AcSH, THF, 0° to r.t., 2 h. *iii*) for B = T: aq. NaOH soln., MeOH/THF, 0°, 1 h. For B = A: 1) BzCl, *N,N*-dimethylpyridin-4-amine (DMAP), py, r.t., 1 h; 2) H^+/H_2O; 3) aq. NaOH soln., EtOH/py, r.t., 10 min. *iv*) 1) Cs_2CO_3, THF, r.t., 3 h, DMF, r.t., 4–20 h, 2) *Oxone*®, aq. AcONa soln., THF/MeOH/H_2O, r.t., 2 h. *v*) HF, py, r.t., 20 h. *vi*) $(MeO)_2TrCl$, DMAP, Et_3N, py, r.t., 2 h. *vii*) $(^iPr_2N)_2P(OCH_2CH_2CN)$, diisopropylammonium tetrazolide (DIPAT), MeCN, r.t., 3 h.

the phosphinate. Following the strategy of *Collingwood* and co-workers, nucleophilic ring opening of the oxetane of **19** with **18** led quantitatively to 5′-methylenephosphinate analog **20**, which was transformed to phosphinic acid ester **21** in 45% overall yield.

Base-mediated addition of aldehyde **1** or **2** to this phosphinic acid ester **21** led to the TPOOEtT analog **22** or the APOOEtT analog **23** in *ca.* 50% yield. Still following the protocols of *Collingwood* and co-workers, the TPOOEtT phosphoramidite **29** was obtained in 16% overall yield from **22** by *Barton* deoxygenation (→**24**), deprotection to the diol (→**26**), 5′-$(MeO)_2$Tr protection and 3′-activation. The diol **26** was purified by reversed-phase HPLC. This synthesis of the TPOOEtT phosphoramidite **29** could be accomplished rather easily and in similar yields as those reported by *Collingwood* and *Baxter* [9].

The analogous synthesis of the APOOEtT phosphoramidite **30**, however, posed some major problems and was plagued by low-yielding steps (*Scheme 2*). The two primary

Scheme 2



	Yield [%]							
reaction	*i*)	*ii*)	*iii*)	*iv*)	*v*)	*vi*)	*vii*)	*viii/ix*)
B = T	100	64	72	56	55	47[a)]		62
B = A/^{Bz}A				50	27	47[a)]	36	45[b)]

[a)] After reversed-phase-HPLC, [b)] Considerably hydrolysed.

i) BuLi, $BF_3 \cdot OEt_2$, THF, −78°, 5 h. *ii*) PPh_3, benzoic acid, DIAD, THF, 0° to r.t., 20 h. *iii*) Me_3SiCl, EtOH/$CHCl_3$, 0° to r.t., 20 h. *iv*) 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), THF (for B = T) or DMF (for B = A), r.t., 20 h. *v*) 1) *p*-Tolyl chlorothionoformate (=4-methylphenyl carbonochloridothioate), DMAP, MeCN, r.t., 20 h; 2) Bu_3SnH, azobis[isobutyronitrile] (AIBN), toluene, 80°, 20 h. *vi*) 1) Bu_4NF (TBAF), AcOH, THF, r.t., 20 h; 2) EtONa, EtOH, r.t., 20 h. *vii*) 1) BzCl, DMAP, Et_3N, py, 0° to r.t., 20 h; 2) EtONa, THF/EtOH, r.t., 20 min. *viii*) $(MeO)_2TrCl$, DMAP, Et_3N, py, r.t., 20 h. *ix*) $^{i}Pr_2NPCl(OCH_2CH_2CN)$, $^{i}Pr_2EtN$ (DIPEA), DMAP, CH_2Cl_2, 0°, 75 min.

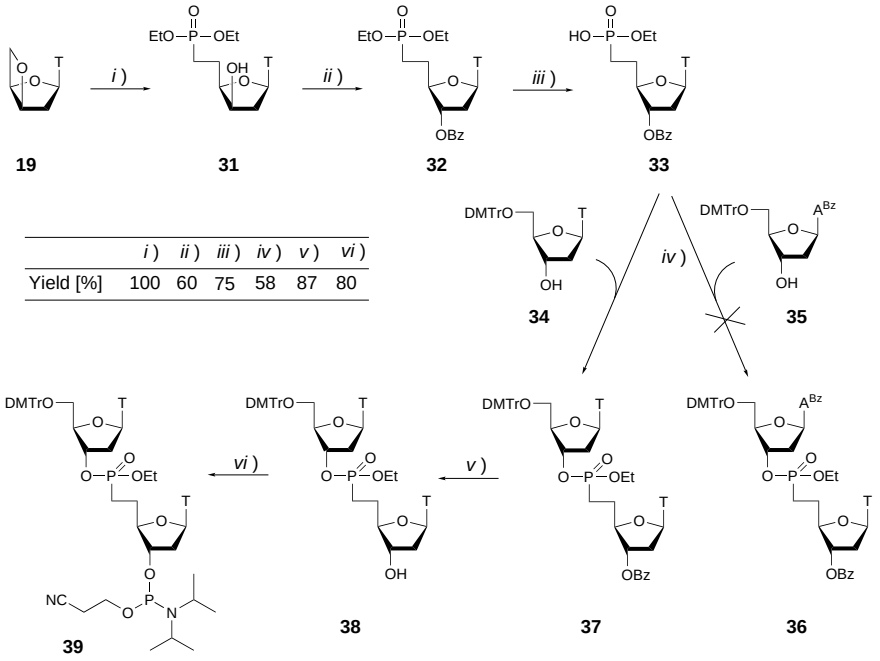
obstacles were the well known low reactivity of 2′-deoxyadenosine derivatives at the 3′-position, which significantly hampered the *Barton* deoxygenation of **23** to **25**, as well as the need for protecting the amino group of adenine, which could not be accomplished satisfactorily at any stage of the synthesis. All attempts to introduce the protective group on the monomer level failed, because the base could either not be protected at all or the protective group was cleaved again during later transformations. At last, the protective group was introduced on diol **27** (→ **28**), since this allowed for a purification of **27** by reversed-phase HPLC, while **28** could not be purified by reversed-phase HPLC. Phosphoramidite **30** was finally obtained in only 1% overall yield from dinucleoside analogue **23**. In addition, the final phosphoramidite could not be isolated as a pure compound, but contained significant amounts of hydzolyzed material, mainly due to the small scale of its preparation (30 mg). The yields of some of the reactions on

the dinucleoside level could certainly be improved, since they were done only once and were not optimized. The primary problems would persist, however, and they make this synthetic strategy rather unsuitable for the synthesis of the APOOEtT analog.

5′-Methylenephosphonate Analogs. Several publications have already addressed the synthesis of 5′-methylenephosphonate-modified nucleotides, dinucleotides, and oligomers [15][16]. An intriguing strategy for synthesizing 5′-methylenephosphonate-linked oligonucleotides was proposed by *Stawinski* and co-workers [17]. It was based on the synthesis of 3′-$(MeO)_2$Tr-protected 5′-methylenephosphonate analogs bearing any of the four nucleobases, where the phosphonate protective group is 4-methoxy-1-oxido-2-picolyl (=(4-methoxy-1-oxidopyridin-2-yl)methyl), an intramolecular nucleophilic catalyst. These monomers can, together with a suitable condensing agent, undergo fast and efficient formation of the 5′-methylenephosphonate internucleosidic bond. This offers the possibility of synthesizing all-phosphonate oligomers of any desirable sequence on solid support. So far, no oligonucleotide analogs synthesized in this way have been characterized, however.

We chose to prepare the 5′-methylenephosphonate-linked dinucleosides (*Scheme 3*) in a manner similar to that employed for the synthesis of the bis(methylene)phosphinates. In this strategy, the phosphonate group would be introduced by a route originally published by *Tanaka et al.* [18], which involves the nucleophilic ring

Scheme 3



	i)	*ii*)	*iii*)	*iv*)	*v*)	*vi*)
Yield [%]	100	60	75	58	87	80

i) Diethyl methylphosphonate, BuLi, $BF_3 \cdot OEt_2$, THF, −78°, 4 h. *ii*) PPh_3, benzoic acid, DIAD, THF, 0° to r.t., 20 h. *iii*) LiBr, py, reflux, 4 h. *iv*) 1-(2-Mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT), py, r.t., 7 d. *v*) EtONa, THF/EtOH, r.t., 20 h. *vi*) $^i Pr_2NPCl(OCH_2CH_2CN)$, $^i Pr_2EtN$, DMAP, CH_2Cl_2, 0°, 75 min.

opening of an oxetane with a dialkyl methylphosphonate. The principal disadvantage of this strategy compared to the *Moffatt-Jones* procedure [15] lies in the fact that it leads to an alkyl-protected phosphonate, which is more difficult to deprotect and is generally less reactive towards nucleophilic attack than its aryl-protected counterpart. The diethyl methylphosphonate **31** was prepared in an analogous fashion to the phosphinate analog **20**, but with diethyl methylphosphonate substituting for the phosphinate **18**. Again, the 3′-center was inverted and the alcohol function benzoyl-protected by *Mitsunobu* reaction to give **32**. The mono-deprotection of the phosphonate was accomplished by treatment with 5 equiv. of LiBr in pyridine under reflux [19]. The resulting monoester **33** was easily obtained in 45% yield overall after precipitation from 1M aq. HCl, which also removed the excess LiBr.

The dinucleotide analog was to be synthesized by condensing the phosphonate ester **33** with the 3′-OH group of a suitably protected nucleoside. Thymidine and 2′-deoxyadenosine were, therefore, protected at the 5′-OH group as the $(MeO)_2Tr$ ethers, and the amino group of adenine was benzoylated. This gave the thymidine analog **34** and the adenosine analog **35**. The coupling to the TOPOOEtT[1]) analog **37** (OPOOEt corresponds to $OP(=O)(OEt)CH_2$ instead of $OP(=O)(O^-)O$) was attempted first. The activation of **33** with 2 equiv. (to prevent dimerization of the nucleotide analog) of 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) went smoothly and was complete after 1 h. To enhance the coupling rate, 3 equiv. of **34** were added, and the amount of solvent was kept to a minimum. It nonetheless took 7 d at room temperature to generate a signficant amount of dimer. After that time, the reaction did not appear to go further, and some of the activated phosphonate had been hydrolyzed to **33**. The pure dimer **37** was obtained in 58% yield.

For the AOPOOEtT analog **36**, the same protocol was applied. But even after 10 d at room temperature and with 5 equiv. of the protected 2′-deoxyadenosine **35**, no product was detectable. As expected, the 3′-OH group of the 2′-deoxyadenosine monomer was much less reactive than that of the thymidine counterpart. Heating the solution to 80° resulted in the loss of the $(MeO)_2Tr$ protection of **35** without formation of any desired dinucleoside. The synthesis of the AOPOOEtT analog was therefore abandoned. The deprotection of the TOPOOEtT analog **37** to the 3′-alcohol **38** and the subsequent activation to the phosphoramidite **39**, by standard protocols, went on smoothly and in good overall yield of 70%.

The P-atom in a protected phosphodiester is, of course, a stereogenic center. This is also the case for the P-atoms in the protected bis(methylene)phosphinate and 5′-methylenephosphonate analogs described above. After incorporation of these dinucleotides into an oligonucleotide, however, the Et protective group is removed, and the diastereoisomerism is lost. For this reason, no attempts were made to separate or characterize the individual diastereoisomers.

3. Synthesis of the Chimeras. – The DNA chimeras **S-1** to **S-17**, **O-3** to **O-5**, **O-9**, **O-10**, and **O-14** to **O-17**, as well as **P-1** to **P-5**, **P-9**, **P-10**, and **P-14** to **P-17** (*Tables 1* and *2*) were synthesized on solid phase by the standard 2-cyanoethyl phosphoramidite method (**S** = bis(methylene) sulfone, **O** = 5′-methylenephosphonate, and **P** = bis(methylene)-phosphinate chimeras). The coupling time for the modified dinucleoside phosphoramidites was extended to 20 min. The coupling yields for the modified dinucleosides

Table 1. *Bis(methylene) Sulfone* (**S**) *Chimeras and Their Analysis by MALDI-TOF-MS.* s = Bis(methylene) sulfone linker.

	Sequence (5′ → 3′)d	Molecular mass	
		calc.	exper.
S-1	GCGTsTTTGCT	3020.1	3019.7
S-2	GCGTsTTsTGCT	3016.3	3015.8
S-3	CTCGTACCTsTTCCGGTCC	5372.6	5371.9
S-4	CTCGTACTsTTsTCCGGTCC	5382.8	5382.4
S-5	GACGATsTCAGCTCTCTsTCTAC	6332.2	6337.5
S-6	GACGTCATAsTTCTGCACG	5474.6	5467.3
S-7	CGTGCAGAAsTATGACGTC	5523.6	5515.5
S-8	GACGTCAsTATTCTGCACG	5474.6	5481.9
S-9	GACGTCATATsTCTGCACG	5474.6	5476.6
S-10	CGATCCATGACTsTCGTACCTCTAG	7263.8	7260.6
S-11	CTTCACGAsTCCTATAG	4816.2	4812.7
S-12	CTAsTAGGAACGTGAAG	4954.3	4953.0
S-13	CTTCACGTTCCTAsTAG	4807.2	4802.2
S-14	CTsTCACGTTCCTATAG	4807.2	4815.0
S-15	TsTCACGTTCCTATAG	4518.0	4512.3
S-16	CTsTCACGTsTCCTATAG	4807.2	4796.3
S-17	CTTCACGTsTCCTATAG	4807.2	4808.4

Table 2. *5′-Methylenephosphonate* (**O**) *and Bis(methylene)phosphinate* (**P**) *Chimeras and Their Analysis by Enzymatic Digestion.* o = 5′-methylenephosphonate, p = bis(methylene)phosphinate linker.

	Sequence (5′ → 3′)d	Calc.					Found				
		dC	dG	T	dA	X[a])	dC	dG	T	dA	X[a])
O-3	CTCGTACCToTTCCGGTCC	8	3	5	1	1	8.0	3.3	5.0	0.9	0.9
O-4	CTCGTACToTToTCCGGTCC	7	3	5	1	2	7.0	3.3	5.0	0.9	1.9
O-5	GACGAToTCAGCTCTCToTCTAC	7	3	5	4	2	7.0	3.3	5.0	3.7	1.9
O-9	GACGTCATAToTCTGCACG	5	4	4	4	1	5.0	4.4	3.9	3.8	0.9
O-10	CGATCCATGACToTCGTACCTCTAG	8	4	6	5	1	8.0	4.3	5.9	4.7	0.9
O-14	CToTCACGTTCCTATAG	5	2	5	3	1	5.1	2.2	5.0	2.9	0.9
O-15	ToTCACGTTCCTATAG	4	2	5	3	1	4.0	2.2	5.0	2.8	0.9
O-16	CToTCACGToTCCTATAG	5	2	4	3	2	5.0	2.2	4.0	2.8	1.9
O-17	CTTCACGToTCCTATAG	5	2	5	3	1	5.1	2.2	5.0	2.9	0.9
P-1	GCGTpTTTGCT	2	3	3	0	1	2.0	3.1	3.0	0.0	1.0
P-2	GCGTpTTpTGCT	2	3	1	0	2	1.9	3.0	0.9	0.0	1.8
P-3	CTCGTACCTpTTCCGGTCC	8	3	4	1	1	8.0	3.2	3.9	1.0	0.9
P-4	CTCGTACTpTTpTCCGGTCC	7	3	3	1	2	7.0	3.2	3.0	1.0	1.7
P-5	GACGATpTCAGCTCTCTpTCTAC	7	3	3	4	2	7.0	3.1	2.9	3.9	1.8
P-9	GACGTCATATpTCTGCACG	5	4	3	4	1	5.0	3.9	3.0	3.9	1.0
P-10	CGATCCATGACTpTCGTACCTCTAG	8	4	5	5	1	8.0	3.9	5.0	4.8	1.0
P-14	CTpTCACGTTCCTATAG	5	2	4	3	1	5.0	2.1	3.9	2.8	0.9
P-15	TpTCACGTTCCTATAG	4	2	4	3	1	4.1	2.2	4.0	2.8	1.0
P-16	CTpTCACGTpTCCTATAG	5	2	2	3	2	5.0	2.1	2.0	2.6	1.9
P-17	CTTCACGTpTCCTATAG	5	2	4	3	1	5.0	2.1	3.9	2.8	0.9

[a]) X = PO_3^{2-}T or TPO_2^-T.

(measured by trityl detection) depended very much on the particular batch of dinucleoside phosphoramidite. High-quality phosphoramidite batches were incorporated like the standard monomers, while batches of poorer quality (partially hydrolyzed) led to significant reductions in incorporation yield. The APOOEtT phosphoramidite **30** led to an almost complete stop of elongation.

The chimeras were cleaved (trityl-off) from the support and deprotected by the standard treatment (30% NH_4OH solution, 55°, overnight). This procedure cleaved all protective groups except the ethyl group of the bis(methylene)phosphinate and 5′-methylenephosphonate linkers. The bis(methylene)phosphinates were therefore fully deprotected with 2N NaOH at room temperature overnight. The same hydroxide treatment applied to the 5′-methylenephosphonate chimeras led to almost complete scission of the strand by cleaving the C(3′)–O bond by an elimination reaction. A more nucleophilic but less basic agent was therefore needed to selectively cleave the Et group. Since a common procedure for the deprotection of methyl phosphonates involves thiolate [20], 4-methoxybenzenethiolate (1M) in dioxane/H_2O was tried. Applying this combination at 55° for 40 h turned out to be highly selective for the deprotection of the 5′-methylenephosphonate chimeras. The thiolate was subsequently removed by extraction with AcOEt.

The fully deprotected chimeras (as well as all purchased natural oligonucleotides (**N**)) were purified by preparative ion-exchange HPLC. None of the chimeras containing the APOOEtT dinucleotide could be successfully purified. They were, therefore, discarded. Anal. HPLC plots of the purified chimeras showed pure compounds throughout (> 95% pure), with a few exceptions (**S-6** to **S-9**), which were used without further purification because the impurities were relatively minor and the amount of chimeras at hand was small. MALDI-TOF-MS analysis of the bis(methylene) sulfone chimeras (*Table 1*) and HPLC analysis of enzymatic digests of the other chimeras (*Table 2*) confirmed their correct constitution.

4. UV Thermal-Melting Experiments. – *Determination of the Melting Temperatures.* The chimeras were analyzed by UV thermal melting. The melting temperature (T_m) of a duplex depends, among other factors, on salt concentration. High salt concentrations lead to increased T_m values, although the amount of this effect strongly depends on the type of cation. This is usually explained as a consequence of the cations partially neutralizing the negative charge on the phosphate groups, thus reducing the interstrand backbone repulsion and facilitating duplex formation [21]. It would then be expected that the salt concentration would have a less significant influence on the T_m of neutral backbones.

Two standard ways are used to determine the T_m from the UV melting curve, the 'derivative' method and the '*van't Hoff*' method. The 'derivative' method uses the fact that the T_m is the temperature of the midpoint of a sigmoidal curve. Hence, the first derivative of the melting curve reaches a maximum at this temperature. In the '*van't Hoff*' method [22], the original melting curve is converted into a 1-α *vs.* temperature curve, where α equals the fraction of strands in the duplex state. This conversion needs, of course, the drawing of two base lines that indicate the theoretical absorbance of pure duplex or pure single-strands at any given temperature. Once the conversion is complete, T_m is obtained as the temperature at which $\alpha = 0.5$.

By comparing the melting temperatures of modified and natural duplexes, it is possible to make qualitative and semi-quantitative statements about the effect of the modification(s) on the duplex stability. Considerable effort has been directed towards elaborating methods for extracting more fundamental data from these UV melting curves, including the thermodynamic parameters ΔH, ΔS and ΔG. In particular, the '*van't Hoff*' method can be used to extract these parameters [22]. Its accuracy is very limited, however, partly because its underlying assumptions are known to be poor approximations of reality. For example, the transition enthalpy and entropy are not independent of temperature [22][23], even though they are assumed to be so in the model. Also, many linear asymptotes must be drawn manually during the calculation process; error in their drawing significantly influences the outcome of the calculation. Only calorimetric methods would allow for an accurate (largely model-independent) measurement of these thermodynamic parameters [24].

We, therefore, decided to base all our interpretations on melting temperatures, which can be calculated rather accurately from UV melting curves. In principle, comparing melting temperatures compares events occurring at different temperatures, problematic given the temperature dependence of the various parameters. This approximation does not appear to be more severe than the approximations (mentioned above) used to calculate thermodynamic values from the melting curves themselves, however, especially since we compared the chimeras with their natural counterparts of exactly the same sequence, under exactly the same conditions, and the differences in T_m were small enough to assume quasi-independence of temperature for the enthalpy and entropy.

The values obtained from the two methods ('derivative' and '*van't Hoff*') agreed with each other to within a few tenths of a degree. The errors of the T_m values as well as ΔT_m values depend not only on the statistical error of the measurements, but at least as much on the systematic error introduced by the calculation methods. The data are therefore not given with error bars, but the errors for T_m values as well as ΔT_m values are estimated to be *ca.* $\pm 0.5°$.

Thermal Melting Experiments with the Single Strands. The T_m is the temperature at which the equilibrium constant between the duplex and the single-strands is unity. This means that T_m is affected not only by the stability of the duplex, but also by the stability of the single strands [25]. Since these single strands may form secondary structures such as hairpins or stacked areas by themselves, or partial duplexes with another strand of the same sequence, T_m cannot be reliably interpreted without having at least an estimate of this possibility for competing single strand structure.

In our case, we were not primarily interested in the details of a melting transition of a particular duplex but only in the comparison between a particular duplex with some backbone modification and its natural counterpart. This suggested that single-strand structure might be neglected, as an approximation, if the modified and the natural strand showed the same thermal transition behavior. To assess this approximation, melting experiments were performed with the single strands alone in high-salt-concentation buffer (1M NaCl). If the modified and the natural strands behaved the same, the two melting curves would be superimposable, if there was no difference in single-strand structure at high salt concentration. The same might then be assumed for low salt concentration as well because of the very limited stability of any single-strand structure in general.

None of the sequences exhibited a strongly cooperative transition, and none of the curves obtained with chimeras deviated significantly from the ones obtained with the corresponding natural oligonucleotides (data not shown). Single strand structure was therefore neglected in the following discussion of the ΔT_m values.

Thermal-Melting Experiments with Blunt-End Duplexes. Duplexes were examined at concentrations of 2 μM for each single strand, at two different salt concentrations: 100 mM NaCl ('low salt') or 1M NaCl ('high salt'), 10 mM Na_2HPO_4/NaH_2PO_4, 0.1 mM EDTA, pH 7. The duplexes with the different backbone modifications were measured in the same experiment as the natural duplex in a multicell block to ensure identical conditions.

Table 3 collects most of the results of the oligonucleotide/chimera combinations investigated by melting experiments. The first goal was to make a general statement about impact of backbone modification on duplex stability. The sequences were also designed, however, to show in some detail the impact of factors such as chimera length,

Table 3. *UV Thermal-Melting Experiments with Natural and Chimeric Duplexes: Melting Temperatures of the Natural Duplexes* (**N** T_m) *and* ΔT_m *values of the Corresponding Modified Duplexes.* **N** = Natural oligonucleotides, **O** = 5′-methylenephosphonate, **P** = bis(methylene)phosphinate, and **S** = bis(methylene) sulfone chimeras; x = modified linker, _ = phosphate opposite modified linker.

Entry	Duplex	Sequence (5′ → 3′)d sequence (3′ → 5′)d	**N** T_m [°][a])	**O** ΔT_m [°][a])	**P** ΔT_m [°][a])	**S** ΔT_m [°][a])
1	**N-** or **P-1**	GCGTxTTTGCT	45.2		– 7.0	
	N-26	CGCA_AAACGA	53.3		– 6.8	
2	**N-**, **P-**, or **S-2**	GCGTxTTxTGCT	45.1		– 16.9	– 23.0
	N-26	CGCA_AA_ACGA	53.3		– 15.0	– 24.0
3	**N-**, **O-**, **P-**, or **S-3**	CTCGTACCTxTTCCGGTCC	63.0	– 2.4	– 3.8	– 4.3
	N-28	GAGCATGGA_AAGGCCAGG	72.5	– 2.4	– 3.9	– 5.1
4	**N-**, **O-**, **P-**, or **S-4**	CTCGTACTxTTxTCCGGTCC	62.0	– 4.6	– 7.1	– 8.5
	N-29	GAGCATGA_AA_AGGCCAGG	71.7	– 4.6	– 7.1	– 10.0
5	**N-**, **P-**, or **O-5**	GACGATxTCAGCTCTCTxTCTAC	62.3	– 4.5	– 5.5	
	N-30	CTGCTA_AGTCGAGAGA_AGATG	73.5	– 4.2	– 5.5	
6	**N-** or **S-11**	CTTCACGAxTCCTATAG	52.8			– 5.4
	N-35	GAAGTGCT_AGGATATC				
7	**N-** or **S-11**	CTTCACGAxTCCTATAG	43.0			– 3.9
	N-12	GAAGTGCA_AGGATATC				
8	**N-** or **S-13**	CTTCACGTTCCTAxTAG	51.7			– 4.7
	N-12	GAAGTGCAAGGAT_ATC				
9	**N-13**	CTTCACGTTCCTA_TAG	52.2			– 4.8
	N- or **S-12**	GAAGTGCAAGGATxATC				
10	**N-**, **O-**, **P-**, or **S-14**	CTxTCACGTTCCTATAG	51.7	– 1.6	– 3.1	– 4.1
	N-12	GA_AGTGCAAGGATATC	61.5	– 1.9	– 2.7	– 3.6
11	**N-**, **O-**, **P-**, or **S-17**	CTTCACGTxTCCTATAG	51.7	– 2.5	– 3.3	– 4.8
	N-12	GAAGTGCA_AGGATATC	61.5	– 2.6	– 3.5	– 5.4
12	**N-**, **O-**, **P-**, or **S-16**	CTxTCACGTxTCCTATAG	52.1	– 4.6	– 7.3	– 9.5
	N-12	GA_AGTGCA_AGGATATC	61.6	– 4.9	– 6.8	– 9.9
13	**N-**, **P-**, or **S-14**	CTxTCACGTTCCTATAG	42.8		– 3.5	– 4.5
	N-35	GA_AGTGCTAGGATATC	52.8		– 3.5	– 4.5
14	**N-**, **P-**, or **S-17**	CTTCACGTxTCCTATAG	42.8		– 2.5	– 2.9
	N-35	GAAGTGCT_AGGATATC	52.8		– 2.5	– 3.5
15	**N-** or **P-16**	CTxTCACGTxTCCTATAG	42.8		– 6.3	
	N-35	GA_AGTGCT_AGGATATC	52.8		– 6.3	

Table 3 (cont.)

Entry	Duplex	Sequence (5′→3′)d sequence (3′→5′)d	**N** T_m [°][a])	**O** ΔT_m [°][a])	**P** ΔT_m [°][a])	**S** ΔT_m [°][a])
16	**N-**, **P-**, or **S-15**	TxTCACGTTCCTATAG	49.4		– 1.4	– 1.0
	N-34	A_AGTGCAAGGATATC	58.8		– 1.0	– 1.0
17	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	66.5		– 3.0	– 3.5
	N-36	GCTAGGTACTGA_AGCATGGAGATC	76.1	– 1.5	– 2.8	– 4.1
18	**N-**, **O-**, **P-**, or **S-9**	GACGTCATATxTCTGCACG	60.8	– 2.7	– 2.5	– 3.5
	N-7	CTGCAGTATA_AGACGTGC	70.5	– 2.6	– 3.0	– 4.4
19	**N-** or **S-6**	GACGTCATAxTTCTGCACG	60.6			– 4.2
	N-7	CTGCAGTAT_AAGACGTGC				
20	**N-** or **S-8**	GACGTCAxTATTCTGCACG	60.8			– 4.5
	N-7	CTGCAGT_ATAAGACGTGC	70.4			– 5.5
21	**N-6**	GACGTCATA_TTCTGCACG	60.3			– 4.4
	N- or **S-7**	CTGCAGTATxAAGACGTGC				
22	**S-17**	CTTCACGTxTCCTA_TAG	52.2			– 10.6
	S-12	GAAGTGCA_AGGATxATC				
23	**S-6**	GACGTCATAxTTCTGCACG	60.3			– 7.5
	S-7	CTGCAGTATxAAGACGTGC	70.2			– 9.7
24	**S-8**	GACGTCAxTA_TTCTGCACG	60.3			– 8.3
	S-7	CTGCAGT_ATxAAGACGTGC	70.2			– 10.3
25	**S-9**	GACGTCATA_TxTCTGCACG	60.3			– 7.7
	S-7	CTGCAGTATxA_AGACGTGC	70.2			– 9.5

[a]) The top numbers are for 'low salt' buffer (10 mM Na_2HPO_4/NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7), the bottom numbers for 'high salt' buffer (10 mM Na_2HPO_4/NaH_2PO_4, 1M NaCl, 0.1 mM EDTA, pH 7). All standard deviations are estimated to be $\pm 0.5°$.

number of modifications, place of the modifications within the chimera, and mismatches on stability. Some of the sequences also formed duplexes with modifications in both strands, to see whether the impact could be mitigated by compensation.

The most striking result evident from these data was that introducing a backbone modification never influenced the impact of salt concentration on T_m, even with the uncharged bis(methylene) sulfone modification (**S** series). Perhaps naively, chimeras containing the neutral bis(methylene) sulfones were expected to show less of a salt effect than their natural counterparts (or the other modifications).

In general, one 5′-methylenephosphonate unit lowered the T_m by 2–3° (**O** series), one bis(methylene)phosphinate lowered the T_m by 3–4° (**P** series) and one bis(methylene) sulfone lowered the T_m by 4–5° (**S** series). These values were remarkably independent of duplex length, except for the shortest duplexes (*Entries 1* and *2*). The amount of destabilization was very much dependent on the placement of the modification within the sequence, however. If the modification was at the very end of the duplex (*Entry 16*), then the ΔT_m was significantly smaller and virtually independent of the nature of the modification. This was probably due to the fact that a change in conformation can more easily be accommodated at the end of the duplex than in the middle, where it will influence the conformation on both sides of the modification.

Surprisingly, if the dinucleotide modification was introduced even just one G·C base pair from the end of the duplex (*Entry 10*), then it generated nearly the same destabilization as if it was in the middle of the duplex (*Entries 8*, *9*, and *11*). The G·C

base pair obviously restricts the duplex structure very strongly. Additional information about this effect was obtained through examination of duplexes with an overhang (see below). The nature of the base pair (G·C *vs.* A·T) flanking the modified dinucleotide seemed not to significantly influence the destabilization, however, as long as the modification was placed several base pairs from the end of the duplex (*Entries 8* and *9 vs. 10* and *Entry 3 vs. 11*). As expected, there was also no significant difference in ΔT_m for duplexes with AxT modifications compared to TxT modifications (*Entry 6 vs. 11*).

Remarkably, the destabilization by two modifications was approximately additive, no matter whether the two modifications were in the same strand (*Entry 1 vs. 2, Entry 3 vs. 4* and *5*, and *Entries 10* and *11 vs. 12*) or in opposite strands (*Entries 22–25*). Additivity was also observed when two modifications in opposite strands were directly opposite to each other (*Entries 23–25*) or half a turn apart (*Entry 22*). No compensation was observed.

A mismatch directly opposite to the modification (*Entries 7*, *14*, and *15*) resulted in a smaller ΔT_m than a mismatch opposite to natural DNA, or half a turn apart from the modification (*Entries 13* and *15*). The chimera, thus, base-paired with less sequence-specificity than natural DNA. The actual T_m was still *ca.* 10° lower than that of the fully matched duplex, of course.

Thermal-Melting Experiments with Duplexes with Overhang. The possibility was considered that a modification introduced a geometric distortion that made it difficult to accommodate structural features, such as stacking, that stabilize the DNA duplex. This distortion might be relieved by breaking, or gapping, the backbone. To this end, sequences **N-**, **O-**, **P-**, and **S-10** were synthesized to investigate how a gap in the complementary strand would influence the melting temperature of the modified oligonucleotides compared to the natural one.

Since T_m strongly depends on the length of the duplex, substituting two short strands for one longer one should decrease T_m, irrespective of a modification in the complementary strand. Comparison with the gapped natural duplex would provide the appropriate reference. The additional questions emerged, therefore: Do the two short strands stabilize each other through base stacking and result in a T_m that is higher than for each of the two short strands by itself? Would this stabilization be influenced by the modification in the long complement? Could a gap be treated as an uncharged 'pseudo-linker', and how might this pseudo-linker influence T_m of a duplex with a modification such as a neutral bis(methylene) sulfone in the opposite strand?

To answer these questions, melting curves were measured for the duplex between oligonucleotide or chimera **N-**, **O-**, **P-**, and **S-10** and a series of short strands that should pairwise bind to the complementary strand of sequences **10**. The curves were not interpretable, however, because each short strand led to a separate melting transition, and the close overlap of the two transitions made the determination of either melting temperature impossible.

This strategy was therefore abandoned. Instead, the duplexes were measured with only one of the short complements, which was gradually increased in length, moving its 5′- or 3′-end step by step along oligonucleotide or chimera **N-**, **O-**, **P-**, and **S-10**. The melting temperatures extracted from these curves are given in *Table 4*. The data from the 'high-salt' measurements with the bis(methylene)phosphinate and bis(methylene) sulfone chimeras are also plotted in *Fig. 2*. The data for the 5′-methylenephosphonate

Table 4. *UV Thermal-Melting Experiments with Oligonucleotide/Chimera* **N-**, **O-**, **P-**, or **S-10** *and Its Short Complements* (duplexes with overhang): *Melting Temperatures of the Natural Duplexes and* ΔT_m *values of the Corresponding Modified Duplexes.* **N** = natural oligonucleotides; **O** = 5′-methylenephosphonate; **P** = bis(methylene)phosphinate; **S** = bis(methylene) sulfone chimeras; x = modified linker, _ = phosphate opposite modified linker.

Entry	Duplex	Sequence (5′ → 3′)d sequence (3′ → 5′d	**N** T_m [°][a])	**O** ΔT_m [°][a])	**P** ΔT_m [°][a])	**S** ΔT_m [°][a])
17	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	66.5		− 3.0	− 3.5
	N-36	GCTAGGTACTGA_AGCATGGAGATC	76.1	− 1.5	− 2.8	− 4.1
26	**N-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	28.0		− 1.4	+ 2.3
	N-37	ATGGAGATC	38.3		− 1.4	+ 1.0
27	**N-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	33.1		+ 0.3	+ 0.6
	N-38	CATGGAGATC	41.8		+ 0.5	+ 0.5
28	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	40.2		+ 1.0	+ 1.3
	N-39	GCATGGAGATC	49.3	+ 0.5	+ 0.1	+ 0.4
29	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	40.0		+ 0.4	+ 0.2
	N-40	AGCATGGAGATC	52.3	+ 0.3	− 0.1	− 0.2
30	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	45.2		− 2.0	− 2.3
	N-41	A_AGCATGGAGATC	55.6	− 1.6	− 2.2	− 2.9
31	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	49.1		− 4.7	− 6.4
	N-42	GA_AGCATGGAGATC	59.6	− 2.6	− 4.5	− 6.0
32	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	49.7		− 4.9	− 6.8
	N-43	TGA_AGCATGGAGATC	61.2	− 3.0	− 4.6	− 6.8
33	**N-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	52.7		− 4.4	− 5.7
	N-44	CTGA_AGCATGGAGATC				
34	**N-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	54.7		− 4.0	− 5.4
	N-45	ACTGA_AGCATGGAGATC				
35	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	55.8		− 3.8	− 5.1
	N-46	TACTGA_AGCATGGAGATC	67.4	− 2.5	− 3.8	− 5.7
36	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	59.3		− 3.2	− 4.2
	N-47	GCTAGGTACTGA_AGCATG	70.4	− 2.2	− 3.0	− 4.7
37	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	56.8		− 3.7	− 5.0
	N-48	GCTAGGTACTGA_AGCAT	67.8	− 2.8	− 3.5	− 5.4
38	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	57.0		− 3.7	− 5.2
	N-49	GCTAGGTACTGA_AGCA	67.2	− 2.6	− 3.6	− 5.4
39	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	56.0		− 3.9	− 5.0
	N-50	GCTAGGTACTGA_AGC	65.0	− 3.0	− 3.5	− 5.2
40	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	54.0		− 3.6	− 4.2
	N-51	GCTAGGTACTGA_AG	62.5	− 2.7	− 3.2	− 4.5
41	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	48.9		+ 0.5	+ 0.3
	N-52	GCTAGGTACTGA_A	58.7	− 0.5	+ 0.2	− 0.5
42	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	47.3		+ 0.2	+ 0.6
	N-53	GCTAGGTACTGA	57.7	− 0.2	− 0.9	− 1.2
43	**N-**, **O-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	45.7		+ 0.4	+ 1.2
	N-54	GCTAGGTACTG	55.9	− 0.3	− 0.8	− 0.1
44	**N-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	36.4		− 0.5	+ 0.1
	N-55	GCTAGGTACT	47.2		− 0.9	− 0.4
45	**N-**, **P-**, or **S-10**	CGATCCATGACTxTCGTACCTCTAG	34.7		± 0.0	− 0.1
	N-56	GCTAGGTAC	44.3		− 0.9	− 0.5

[a]) The top numbers are for 'low-salt' buffer (10 mM Na_2HPO_4/NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7), the bottom numbers for 'high-salt' buffer (10 mM Na_2HPO_4/NaH_2PO_4, 1M NaCl, 0.1 mM EDTA, pH 7). All standard deviations are estimated to be ± 0.5°.

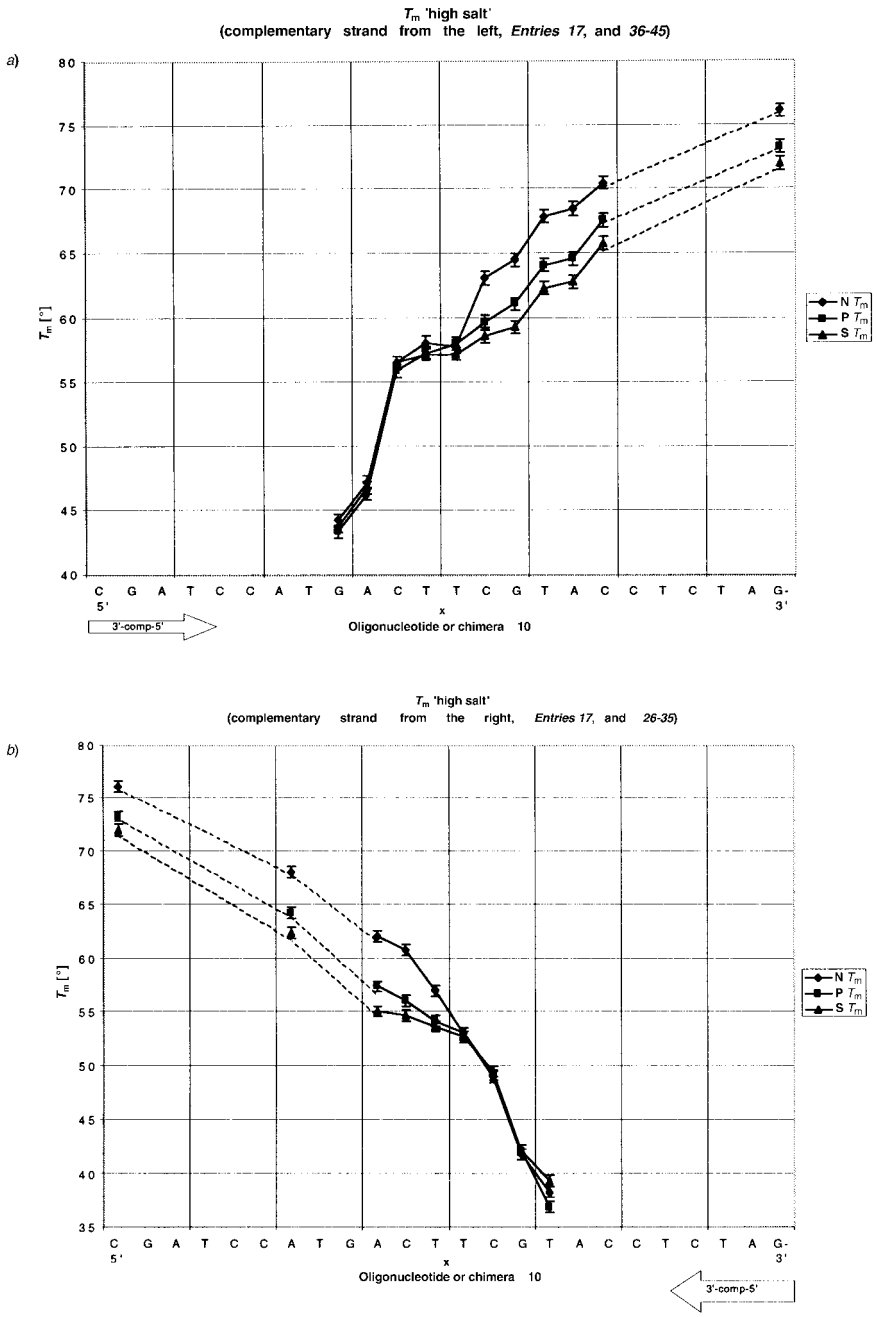


Fig. 2. *Melting temperatures of oligonucleotide or chimeras* **N-**, **P-**, *or* **S-10** (natural, bis(methylene)phosphinate, and bis(methylene) sulfone) *with the partially complementary strands of increasing length* a) *from the 5′-end of* **10** *and* b) *from the 3′-end of* **10**. Buffer: 10 mM Na_2HPO_4/NaH_2PO_4, 1M NaCl, 0.1 mM EDTA, pH 7. (The data are the same as in *Table 4, Entries 17* and *36–45* and *26–35,* resp.)

chimera are omitted from these plots for clarity; the trends were the same as for the other two modifications.

First, it is obvious from the data that the modifications had, as expected, no impact on the stability of the duplex if the complementary strand did not reach the modified site (*Entries 26–28* and *43–45*). The difference in *Entry 26* was probably artifactual, due to the very short length of the complement. The data for the duplexes with the end of the complement opposite to the modification (*Entries 29*, *30*, *41*, and *42*) seem ambiguous, showing a significant ΔT_m for some (*Entry 30*) and a drop in overall stability for others (*Entries 29* and *41* at 'high salt'). These differences may well have been artifacts as well due to the very flat transitions of the corresponding melting curves and the resulting difficulties extracting the T_m. The terminal AA·TT 'dimer' may have decreased the cooperativity of the melting transition somewhat because of fraying.

For the duplexes where the complement reached further than the modified dinucleotide (*Entries 17* and *31–40*), the destabilization first 'overshot' to $-5°$ to $-7°$ (*Entry 31* and *Entries 32*, *37–40*), due to the restrictions by the flanking C·G (in accordance with the interpretation of the data from *Table 3*), and after a few more base pairs gradually returned to the value for the full-length duplex (*Entry 17*). A closer inspection of *Fig. 2* showed that these big ΔT_m values right after the complementary strand had passed the modification (*Entries 31* and *32*, *Entries 37–40*) were primarily due to the fact that the modified duplexes were only little stabilized by the following base pairs (C·G *etc.*), while the T_m of the natural duplex rose much more with every additional base pair. It seems that the modified duplexes needed several base pairs to get back into a 'normal' helix, stabilized by base pairing.

5. Discussion. – The preparation of chimeric DNA molecules having identical sequences with the phosphate, bis(methylene) sulfone, bis(methylene)phosphinate, or 5′-methylenephosphonate linkers interchanged at various positions allowed us to dissect the impact of different charge from different linking-group geometry. A comparison of the melting temperatures obtained for the duplexes of these chimeras and their complementary DNA strands with all of the different linkers measured in parallel permitted us to draw general conclusions about the impact of geometric distortion of the internucleotide linkage around the two P–O–C bridges, and to compare this impact with the impact on duplex stability that arises from the removal of the negative charge.

The data can be roughly summarized as follows. All the modifications destabilized the duplex. The most dramatic change in thermal stability ($-2.5°$ in general) was introduced by substituting O(5′) with a CH_2 group. Additional substitution of O(3′) with a CH_2 group lowered T_m by another degree, and final removal of the charge lowered T_m by yet another degree. All these numbers were largely independent of salt concentration.

The change in duplex stability encountered after replacing O(5′) with a CH_2 group can be explained by steric effects. In the standard B-DNA conformation, this O-atom lies over the deoxyribose ring, *ca.* 4 Å from C(6) of the pyrimidine nucleobase. This is a tight contact, and is not compatible with a CH_2-group replacement. Indeed, in the crystal structure of *Roughton*'s r[GSO_2C]$_2$ duplex [3], the backbone adjusts to relieve

this steric clash. The C(6′)–C(5′)–C(4′) bond angle is increased to 115°, and torsion angle δ is increased to 90°, resulting in a change of sugar pucker from C(3′)-*endo* to C(4′)-*exo*. The H_R–C(6′) H-atom thereby occupies the approximate position assumed by O(5′) in the natural duplex. These structural rearrangements contribute to a slide of *ca.* 1.2 Å. Changing the P–O(5′)–C(5′) bond angle of 120° to 111° for the S–C(6′)–C(5′) angle results in an unwinding of the helix by *ca.* 9° per nucleoside. Since relief of the steric clash goes hand in hand with the increase of the S–C(6′)–C(5′) bond angle, it is difficult to assess which one of these two effects is more responsible for the change in conformation and, thus, destabilization of the 5′-methylenephosphonate chimeras.

The impact of replacing O(3′) by a CH_2 group could be interpreted in terms of sugar pucker. The sterically more demanding CH_2 group replacing the bridging O-atom prefers an equatorial position, which drives the sugar to accept the C(3′)-*endo*/C(2′)-*exo* conformation. This is more like the RNA conformation, and might be expected to decrease T_m with complementary DNA, while increasing it with complementary RNA.

The impact could also be interpreted in light of two crystal structures. *Heinemann et al.* determined the crystal structure of the self complementary chimeric octamer d(GCCCGpGGC), where 'p' denotes a 3′-methylenephosphonate linker [26]. They found that this modification resulted in only minor changes in conformation, most notably in the change of sugar pucker from C(3′)-*endo* to C(2′)-*exo*. This particular sequence, however, forms an A-type helix, not the standard B-helix, and the impact of a change in sugar pucker should be more dramatic in a B-DNA oligomer.

The C(3′)–CH_2–P bond angle should also influence hybridization, and an angle close to 109° might generate a kink in the overall helix conformation. *Heinemann et al.* do not report any bond angles, but examination of the structure deposited in the Protein Data Bank (1D26.pdb) with *RasMol* found a C(3′)–CH_2–P bond angle of 108°, in excellent agreement with sp^3-hybridization at CH_2. *Egli et al.* determined the crystal structure of another A-DNA chimera of the sequence d(GCGTAmpTACGC) where 'mpT' denotes a 2′-methoxy-3′-methylenephosphonate modified thymidine [27]. They again report that the modified backbone locks the conformation of the sugar in the RNA-like C(3′)-*endo* pucker. The C(3′)–CH_2–P bond angle was again determined with *RasMol* from the corresponding coordinates from the Protein Data Bank (1DPL.pdb) and found to be 115–118° (the value differed for the two strands). This number is not as low as expected from theory, but it is clearly lower than all the C(3′)–O(3′)–P bond angles (*ca.* 120°) within the same crystal structure. The exact number could also be affected by the modelling calculations used to fit electron density to the diffraction data.

A disruption of the solvation from replacing the ester O-atom by CH_2 groups is an alternative explanation for the decreased thermal stabilities of the 5′-methylenephosphonate and bis(methylene)phosphinate chimeras. *Schneider* and co-workers analyzed the hydration of DNA [28] and found that the ester O-atoms O(5′) and O(3′) are hydrated least in all three DNA helical forms (A, B, Z). O(5′) in the right handed form is sterically inaccessible; the reasons for poor hydration of O(3′) are not clear. A change in solvation, therefore, does not seem to be a likely explanation for the reduced thermal stability of duplexes incorporating the 5′-methylenephosphonate and bis(methylene)phosphinate chimeras.

The literature shows that most modifications that lead to an RNA-like sugar conformation (C(3′)-*endo*) improve binding to complementary RNA while diminishing binding to complementary DNA [29]. In the natural DNA · RNA hybrid, this unfavorable sugar-pucker effect is compensated by the improved solvation of the 2′-OH, rendering the hybrid duplex about as stable as the DNA · DNA duplex. A 2′-deoxy 3′-methylenephosphonate chimera is, therefore, expected to form a less stable duplex with complementary DNA than natural DNA does, while the opposite is reported for its duplexes with complementary RNA [30]. This suggests that the difference in T_m values between the 5′-methylenephosphonate and bis(methylene)phosphinate chimeras is largely due to the change in sugar pucker and not to the change in bond angle. This also suggests that, if the effect of the sp^3-hybridization of the 5′-CH_2 group can be compared to that of the 3′-CH_2 group, the destabilization due to the 5′-CH_2 group is mostly a result of the steric clash between the methylene H-atoms and the nucleobase, and only to a small degree because of the change in P−(CH_2)−C(5′) bond angle forced by a change in hybridization.

The most remarkable observation of our melting experiments is that the bis(methylene) sulfone chimeras form the least stable duplexes. This runs counter to the (perhaps naive) expectation that the loss of a negative charge would diminish interstrand coulombic repulsion and, thus, enhance duplex stability. The bis(methylene)sulfone linker differs from the bis(methylene)phosphinate linker by a single proton; the two linkers are isoelectronic. There is no significant difference in bond lengths either. The S−C(6′) bond length in *Roughton*'s bis(methylene) sulfone crystal structure is 1.78 Å [3], while the corresponding CH_2−P (=C(3″)−P) bond length in the aforementioned Protein Data Bank file of *Egli*'s 3′-methylenephosphonate chimera was measured to be 1.74 Å. Therefore, the effect must be attributed, directly or indirectly, to the change in charge.

More surprising was the fact, that the difference between the T_m values with bis(methylene)phosphinate and bis(methylene) sulfone chimeras, with identical bridging groups, was largely insensitive to a 10-fold increase in the concentration of NaCl. This suggested that the different duplex stability created by the two linkers is not a coulombic effect, meaning that it does not involve the interaction between two charged species.

The most plausible explanation for the decreased stability of the duplexes formed with the bis(methylene) sulfone chimeras *vs.* the bis(methylene)phosphinate chimeras would be a change in solvation. The phosphinate O-atoms are highly hydrated, as discussed above. The sulfone O-atoms, however, are significantly weaker H-acceptors because of their lack of charge.

Differential solvation of the sulfone is evident from the crystal structure of *Roughton*'s bis(methylene) sulfone dimer [3]. Instead of forming contact with a Na^+ ion and three H_2O molecules as seen with the phosphate in the analogous natural RNA dimer [4], the O-atoms of the bis(methylene) sulfone group formed contacts with one H_2O molecule, a cytosine NH_2 group from a neighboring duplex, and the Me group of a MeOH molecule. This would destabilize the duplex if, *e.g.*, the sulfone were not able to participate as effectively in a solvation network that stabilizes the duplex structure.

These results suggest that the impact of the loss of a single charge is not due to single-strand structure (presumably the case when all of the charges are lost [2]), but

rather a perturbation of the solvation necessary for duplex stability. Earlier crystal structures did not associate the H_2O molecules solvating the phosphate groups with any longer-range hydration network as they did for the spine of hydration interconnecting the nucleobases in the minor groove of B-DNA [28][31].

Only recently has the resolution of low-temperature crystal structures been improved to the extent of enabling the detection of significant parts of second- and third-shell hydration [27]. These structures show well-defined networks of H_2O molecules in both grooves, linking the first hydration shell of the phosphate groups with the deeper lying hydration shell of the nucleobases. The reduced hydration of the charge-neutral sulfone might, therefore, significantly disrupt the solvation of the resulting duplex. This is corroborated by the data reported by *Cohen* and co-workers about the different duplex stabilities of natural oligonucleotides (PO_2) and their all-phosphorothioate (POS) or all-phosphorodithioate (PS_2) analogs with complementary RNA or with themselves [32]. The major difference between $P-O^-$ and $P-S^-$ is obviously the change in delocalization of the negative charge, which leads to different solvation patterns. *Cohen* and co-workers found the duplex stability to decrease in the order $PO_2 > POS > PS_2$, in agreement with a disruption of duplex solvation. The effect of disrupting the hydration network would also explain the different physicochemical properties and structures obtained with [*P*(*R*)]- and [*P*(*S*)]-phosphorothioate substitutions [33], since the S-atoms interact with different parts of the solvation structure.

Many of the uncharged DNA backbones reported in the literature face problems with reduced solvation, of course. Solubility problems in particular plagued the syntheses of oligosulfones as well [2]. It is, therefore, not surprising that all the modified backbones that exhibit improved binding to complementary DNA or RNA have a backbone that allows for a good solvation (except PNA, see below).

In this light, it seems surprising that most of the chimeras with positively charged linkers, such as amines and guanidinium, form duplexes that exhibit stabilities as low as the ones obtained with bis(methylene) sulfone chimeras. *Lynn* and co-workers report an NMR structure of a DNA hexamer with a positively charged aminoethyl linker [34]. The analysis shows that the positively charged linker 'collapses' onto the hydrophobic core, inducing a bend in the helix due to the reorganization of the phosphates. A similar mechanism could be used to explain the different T_m values of the bis(methylene) sulfone and bis(methylene)phosphinate chimeras. This would, however, not explain the puzzling independence of the different stabilities on salt concentration. The collapse of the bis(methylene) sulfone unit onto the hydrophobic core would be independent of salt concentration, but the bending of the helix because of intrastrand phosphate repulsion would not. The only way out of this dilemma would be the possibility that the dependence of this mechanism on salt concentration is too small to be visible in our melting experiments. The average $\Delta\Delta T_m$ between bis(methylene)phosphinate and bis(methylene) sulfone chimeras is *ca.* $1 \pm 0.5°$. It is not clear whether this accuracy allows for a definitive distinction between a bend induced by intrastrand phosphate repulsion and an effect of different solvation only.

The suggestion that a backbone charge is necessary for successful *Watson-Crick* base-pairing behavior seems to be contradicted by peptide nucleic acid (PNA). PNAs display rule-based molecular recognition [35]. Many PNA·PNA duplexes and PNA·DNA(RNA) hybrid duplexes are more stable than DNA·DNA or DNA·RNA

duplexes, with ΔT_m values of *ca.* 1 – 1.5° per base pair [36]. The hybridization of PNAs to complementary sequences is characterized by good mismatch discrimination [37]. PNAs also possess high rates of association for duplex DNA [38] and a remarkable propensity for the invasion of double-stranded structure. PNAs are not hydrolyzed by nucleases or proteases [39]. These phenomena are so striking that PNAs are being examined closely for a variety of technological applications [35][40]. The range of its applications has encouraged *Corey* and co-workers to call PNA 'one of the most successful designed macromolecules' [41]. *Orgel* and co-workers have conducted experiments to explore PNA as possibly the primordial DNA [42]. PNA may be the example that disproves the hypothesis requiring a polyelectrolyte in the universal genetic molecule.

Other data suggest an alternative interpretation. Just as long bis(methylene) sulfone analogs do not support *Watson-Crick* base pairing well even though short ones do, long PNA backbones do not seem to support *Watson-Crick* molecular recognition as well as sequences of modest length. Molecular recognition in PNA does not disappear with increasing length as rapidly as it disappears in oligosulfones. PNA derivatives as long as 20 nucleotides have been reported that continue to bind to complementary DNA by *Watson-Crick* rules [37]. But their propensity to self-aggregate is recorded in the literature [43], as is single strand structure that appears to interfere with PNA·DNA duplex formation [44]. Longer PNA molecules suffer aggregation and other physical behaviors that interfere with their ability to recognize complementary DNA. PNAs also change their physical properties substantially (and largely unpredictably) with small changes in sequence [45], although adding charged appendages reduces this sequence dependence [46] (as it does with bis(methylene) sulfones). One may expect that different non-ionic backbones will sustain *Watson-Crick* rule-based molecular recognition up to different lengths, depending on the backbone, its interaction with the solvent, and its potential to interact with itself and the heterocycles that it carries. But it seems that all non-ionic analogs must arrive at a length where they prefer to fold, aggregate, and precipitate rather than template.

This work is suggestive of the hypothesis that charge-charge repulsion is necessary for a templating system to prevent non-*Watson-Crick* interactions in the duplex. To test this hypothesis, complementary oligomers with all linkers positively charged would have to be synthesized. These oligomers are predicted to form stable and sequence-selective duplexes with each other, but not with the negatively charged DNA or RNA. *Bruice* and co-workers have prepared some positively charged DNA analogs with guanidinium linkers [47]. They have reported melting studies only with triplexes formed by pentameric or hexameric all-guanidinium-linked oligoT or oligodA with complementary DNA. These studies reveal huge hysteresis, implying the presence of other processes different form simple *Watson-Crick* base pairing. This fits into the hypothesis above. It would be interesting to see whether the guanidinium-linked oligonucleotides can form stable and sequence-selective duplexes with each other. Another way to test the hypothesis would be to synthesize oligonucleosides with all-amine linkages, such as those in dinucleosides prepared by *Kruse* and co-workers [48]. These linkers would again replace O(5′) with a CH_2 group, however.

The most striking result of this work and most other work with backbone-modified oligonucleotide analogs is how often 'small steps' taken away from the natural DNA

structure seem to destabilize the duplex. This implies that the structure of DNA is somehow 'optimal'. This conclusion is remarkable, given the difficulty of conceiving a mechanism of continuous structural variation that would seem to be a requirement for *Darwinian* evolution. In the absence of such a mechanism, this behavior suggests that there was a coupling between prebiotic chemistry during the time when nucleic acids originated. While many in the 'origins' community accept this view, it will remain discomforting until some prebiotic experiment comes close to generating, without the involvement of life, something that resembles a phosphate-linked polymer of ribo- or deoxyribonucleotides. In this light, it remains remarkable that the removal of a 2′-OH group that converts RNA into DNA does not have a larger impact than it does. There is no other chemical system, in H_2O, where removal of a dozens (or more, depending on the length of the gene) of OH groups leaves an important physical property unchanged.

Last, for the purpose of identifying chemical markers that can be used experimentally to detect evidence of life on *NASA* planetary missions, a need for polyelectrolyte character may be coupled to other demands of universal genetic molecules in constructing a probe. *Dougherty*, *e.g.*, has recently pointed out that tacticity, stereoregularity in a polymer that contains multiple stereogenic centers, may be another universal structural feature of genetic molecules [49]. A polymer that is both stereoregular and a polyelectrolyte might be persuasively viewed as the product having biological origin.

6. Conclusions. – We describe here for the first time the impact of neutralizing the backbone charge of a nucleic acid analog by the simple addition of a proton to the phosphate nucleus. By comparing the bis(methylene) sulfone and the bis(methylene)-phosphinate chimeras with each other, the effect of charge neutralization on conformation and thermal stability of the duplex can be examined without having to take into account any other factor. In addition, the systematic modification of the phosphate group allowed us to also determine the impact on duplex stability of substituting the bridging O-atoms of the phosphodiester.

The data obtained from synchronous melting experiments of natural oligonucleotides as well as 5′-methylenephosphonate, bis(methylene)phosphinate, and bis(methylene) sulfone chimeras with their complementary DNA led to three simple rules (the only real exception to these rules is the still little understood case of PNA):

1) Replacing O(5′) results in a significant destabilization of the duplex, mainly due to a steric clash between the bulkier substituent and the *syn* face of the nucleobase.

2) Removing the charge leads to further destabilization, in addition to the often encountered problem of low solubility, due to reduced solvation and/or a collapse of the neutralized linker onto the hydrophobic core of the helix.

3) Replacing O(3′) decreases the stability of a duplex with complementary DNA, while usually improving the stability of a hybrid duplex with complementary RNA, mainly due to a change in sugar pucker to the RNA-like C(3′)-*endo* conformation.

The second rule can be rewritten as stating that any successful genetic molecule forcing a *Watson-Crick*-like reversible templating behavior must be a polyelectrolyte, regardless of its genesis. This may answer the question why Nature chose phosphates. As *Westheimer* noted [50], phosphates are an easy way to get a polyelectrolyte; there are few other linking units that are as easy to create that confer the same property.

Experimental Part

General. All reactions were carried out under Ar. All reagents were purchased from *Fluka AG* or *Aldrich Co.* at the highest quality available and used without further purification (AIBN was recrystallized from MeOH), technical-grade solvents for extraction and chromatography were purchased from *Fisher Co*. Reagents were dried over P_2O_5/high vacuum or by coevaporation with pyridine, solvents for reactions were dried over 4-Å molecular sieves. 'Evaporation' refers to removal of volatile solvents with a membrane pump at 40°. Flash chromatography (FC): silica gel (230–425 mesh, *Fisher*). TLC: silica-gel plates from *Aldrich*; visualization by staining with a Ce/Mo reagent (2.5% phosphormolybdic acid, 1% $Ce^{IV}(SO_4)_2 \cdot 4H_2O$, 6% H_2SO_4 in H_2O) and heating. NMR: *Varian Gemini-200*, *Varian XL-300*, *Bruker AMX-500*; δ in ppm; calibration to $SiMe_4$ (^1H), residual solvent peak (^{13}C), or H_3PO_4 (= 0 ppm) as external standard (^{31}P); *J* in Hz; ^{13}C multiplicities derived from DEPT spectra; annotation: for TxT dinucleosides; 5′-T = (T1), 3′-T = (T2), and for AxT dinucleosides, (A) or (T). MS: spectra recorded with a *VG-ZAB2-SEQ* spectrometer by the Spectroscopic Services of the Chemistry Departments at the Swiss Federal Institute of Technology (LR spectra) and at the University of Florida (HR spectra); NOBA (3-nitrobenzyl alcohol) matrix; *in m/z* with % rel. intensity compared to base peak.

*1-[(3′*R*,4′*S*)-5′*-O-*[(*tert*-Butyl)diphenylsilyl]-2′,3′-dideoxy-3′*-C-*(hydroxymethyl)-β-pentofuranosyl}thymine* (**3**). Aldehyde **1** [10] (375 mg, 760 μmol) was dissolved in EtOH (3 ml). $NaBH_4$ (9 mg, 238 μmol) was added, and the soln. stirred at r.t. for 2 h. AcOH (3M) was added until the foaming ceased. The soln. was diluted with AcOEt and washed with brine. The org. layer was dried ($MgSO_4$) and evaporated: **3** (375 mg, quant.). Colorless foam. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.24. ^1H-NMR ($CDCl_3$, 500 MHz): 1.10 (*s*, ^{t}Bu); 1.64 (*d*, *J* = 1.2, Me–C(5)); 2.12 (*m*, 1 H–C(2′)); 2.33 (*m*, 1 H–C(2′)); 2.60 (*m*, H–C(3′)); 3.64 (*dd*, *J* = 10.9, 6.2, 1 H, HOCH_2–C(3′)); 3.67 (*dd*, *J* = 10.9, 5.8, 1 H, HOCH_2–C(3′)); 3.82 (*dd*, *J* = 11.2, 3.2, 1 H–C(5′)); 3.95 (*m*, H–C(4′)); 4.01 (*dd*, *J* = 11.2, 3.5, 1 H–C(5′)); 6.13 (*dd*, *J* = 6.0, 6.0, H–C(1′)); 7.37–7.47 (*m*, H–C(6), 6 arom. H); 7.66–7.68 (*m*, 4 arom. H); 9.19 (br. *s*, H–N(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 12.2 (*q*, *Me*–C(5)); 19.4 (*s*, Me_3C; 27.0 (*q*, Me_3C); 35.6 (*t*, C(2′)); 41.6 (*d*, C(3′)); 63.3, 65.0 (2*t*, (HOCH_2–C(3′), C(5′)); 83.2 (*d*, C(4′)); 84.3 (*d*, C(1′)); 110.9 (*s*, C(5)); 127.9, 128.0, 130.0, 130.1 (4*d*, arom. C); 132.6, 133.0 (2*s*, arom. C); 135.4, 135.6 (2*d*, C(6), arom. C); 150.5 (*s*, C(2)); 164.0 (*s*, C(4)). FAB-MS: 517 (12, $[M + Na]^+$), 495 (33, $[M + H]^+$).

*9-[(3′*R*,4′*S*)-5′*-O-*[(*tert*-Butyl)diphenylsilyl]-2′,3′-dideoxy-3′*-C-*(hydroxymethyl)-β-pentofuranosyl}adenine* (**4**). As described for **3**, with **2** [10] (158 mg, 315 μmol), EtOH (1.5 ml), THF (1.5 ml), and $NaBH_4$ (6 mg, 157 μmol): **4** (125 mg, 79%). Colorless foam. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.21. ^1H-NMR ($CDCl_3$, 300 MHz): 1.07 (*s*, ^{t}Bu); 2.44 (*ddd*, *J* = 13.0, 9.0, 6.9, 1 H–C(2′)); 2.61 (*ddd*, *J* = 13.0, 8.0, 3.4, 1 H–C(2′)); 2.78 (*m*, H–C(3′)); 3.70 (*d*, *J* = 5.9, 1 H, HOCH_2–C(3′)); 3.83 (*dd*, *J* = 11.0 , 4.0, 1 H–C(5′)); 3.94 (*dd*, *J* = 11.0, 4.7, 1 H–C(5′)); 4.06 (*m*, H–C(4′)); 6.13 (br. *s*, H_2N–C(6)); 6.30 (*dd*, *J* = 6.9, 3.4, H–C(1′)); 7.32–7.45 (*m*, 6 arom. H); 7.61–7.68 (*m*, 4 arom. H); 8.11 (*s*, H–C(8)); 8.30 (*s*, H–C(2)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 19.2 (*s*, Me_3C); 26.9 (*q*, Me_3C); 36.2 (*t*, C(2′)); 42.0 (*d*, C(3′)); 62.9 (*t*, HOCH_2–(C(3′)); 65.0 (*t*, C(5′)); 84.3, 84.7 (2*d*, C(1′), C(4′)); 119.9 (*s*, C(5)); 127.9, 128.0, 130.0 (3*d*, arom. C); 132.6, 132.7 (2*s*, arom. C); 135.5, 135.6 (2*d*, arom. C); 138.6 (*d*, C(8)); 149.2 (*s*, C(4)); 152.9 (*d*, C(2)); 155.5 (*s*, C(6)). FAB-MS: 504 (100, $[M + H]^+$).

*1-[(3′*S*,4′*S*)-3′*-C-*[(Acetylthio)methyl]-5′*-O-*[(*tert*-butyl)diphenylsilyl]-2′,3′-dideoxy-β-pentofuranosyl}thymine* (**5**). PPh_3 (370 mg, 1.44 mmol) was dissolved in THF (6 ml). DIAD (275 μl, 1.44 mmol) was added at 0° and the soln. stirred for 15 min. (→ white suspension). A soln. of **3** (355 mg, 718 μmol) in THF (6 ml) was added to the PPh_3DIAD suspension at 0°, followed by thioacetic acid (103 μl, 1.44 mmol). The now clear soln. was stirred for 2 h while allowed to warm to r.t., then MeOH (1 ml) was added, and the soln. evaporated. The oily product was purified by FC (CH_2Cl_2/AcOEt 4:1 → 1:1): **5** (238 mg, 62%). Colorless foam. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.67. ^1H-NMR ($CDCl_3$, 300 MHz): 1.10 (*s*, ^{t}Bu); 1.61 (*d*, *J* = 1.1, Me–C(5)); 2.22 (*m*, H–C(2′)); 2.34 (*s*, MeCO); 2.69 (*m*, H–C(3′)); 2.95 (*m*, S–CH_2–C(3′)); 3.82 (*m*, 2 H–C(5′)); 4.07 (*m*, H–C(4′)); 6.14 (*dd*, *J* = 5.9, 5.9, H–C(1′)); 7.37–7.47 (*m*, H–C(6), 9 arom. H); 7.66–7.70 (*m*, 6 arom. H); 8.68 (br. *s*, H–N(3)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 11.9 (*q*, *Me*–C(5)); 19.2 (*s*, Me_3C); 21.7 (*t*, S–CH_2–C(3′)); 26.8 (*q*, Me_3C); 30.3 (*q*, *Me*CO); 37.6 (*t*, C(2′)); 38.0 (*d*, C(3′)); 63.6 (*t*, C(5′)); 84.2, 84.6 (2*d*, C(1′), C(4′)); 110.5 (*s*, C(5)); 127.7, 129.7, 129.8 (3*d*, arom. C); 132.3, 132.8 (2*s*, arom. C); 135.0, 135.1, 135.3 (3*d*, C(6), arom. C); 150.0 (*s*, C(2)); 163.5 (*s*, C(4)); 194.5 (*s*, C=O). FAB-MS: 553 (48, $[M + H]^+$).

*9-[(3′*S*,4′*S*)-3′*-C-*[(Acetylthio)methyl]-5′*-O-*[(*tert*-butyl)diphenylsilyl]-2′,3′-dideoxy-β-pentofuranosyl}adenine* (**6**). As described for **5**, with PPh_3 (47 mg, 179 μmol), THF (0.7 ml), DIAD (35 μl, 179 μmol), **4** (60 mg, 119 μmol), THF (0.7 ml), and thioacetic acid (13 μl, 179 μmol). FC (CH_2Cl_2/AcOEt 1:1 with 0–5% MeOH): **6** (53 mg, 79%). Colorless foam. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.13. ^1H-NMR ($CDCl_3$, 300 MHz): 1.08 (*s*, ^{t}Bu); 2.33 (*s*, MeCO); 2.35 (*m*, 1 H–C(2′)); 2.63 (*ddd*, *J* = 13.5, 7.0, 3.0, 1 H–C(2′)); 2.81 (*m*, H–C(3′)); 2.94 (*dd*, *J* = 13.6, 7.2, 1 H, S–CH_2–C(3′)); 3.03 (*dd*, *J* = 13.6, 5.8, 1 H, S–CH_2–C(3′)); 3.79 (*dd*, *J* = 11.4, 3.5, 1 H–C(5′));

3.89 (*m*, H–C(4′)); 4.01 (*dd*, *J* = 11.4 , 3.4, 1 H–C(5′)); 6.05 (br. *s*, H_2N–C(6)); 6.32 (*dd*, *J* = 6.8, 3.0, H–C(1′)); 7.34–7.46 (*m*, 6 arom. H); 7.64–7.71 (*m*, 4 arom. H); 8.17 (*s*, H–C(8)); 8.31 (*s*, H–C(2)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 18.9 (*s*, Me_3C); 26.7 (*q*, Me_3C); 29.7 (*q*, *Me*CO); 30.3 (*t*, S–CH_2–C(3′)); 37.8 (*t*, C(2′)); 38.2 (*d*, C(3′)); 63.6 (*t*, C(5′)); 84.1, 85.2 (2*d*, C(1′), C(4′)); 119.8 (*s*, C(5)); 127.6, 129.6, 129.7 (3*d*, arom. C); 132.4, 132.5 (2*s*, arom. C); 135.2, 135.4 (2*d*, arom. C); 138.4 (*d*, C(8)); 148.9 (*s*, C(4)); 152.6 (*d*, C(2)); 155.2 (*s*, C(6)); 194.5 (*s*, C=O). FAB-MS: 562 (64, $[M+H]^+$).

*1-[(3′*S*,4′*S*)-5′*-O-*[(*tert*-Butyl)diphenylsilyl]-2′,3′-dideoxy-3′*-C-*(mercaptomethyl)-β-pentofuranosyl]thymine* (**7**). A soln. of **5** (108 mg, 200 μmol) in THF (1.5 ml) and MeOH (1.5 ml) was degassed by repeated freeze-thaw cycles. Degassed 1N aq. NaOH (0.4 ml, 400 μmol) was added at 0°, the soln. stirred at 0° for 1 h and then neutralized with 3M aq. AcOH (1 ml). This soln. was extracted with AcOEt. The org. layer was dried ($MgSO_4$) and evaporated: **7** (90 mg, 88%). Colorless foam. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.55. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.11 (*s*, ^{t}Bu); 1.66 (*d*, *J* = 1.2, Me–C(5)); 2.26 (*m*, 2 H–C(2′)); 2.51–2.61 (*m*, H–C(3′), S–CH_2–C(3′)); 3.79 (*dd*, *J* = 11.5, 3.3, 1 H–C(5′)); 3.87 (*m*, H–C(4′)); 4.06 (*dd*, *J* = 11.5, 2.7, 1 H–C(5′)); 6.13 (*dd*, *J* = 6.5, 5.5, H–C(1′)); 7.39–7.48 (*m*, H–C(6), 6 arom. H); 7.52–7.69 (*m*, 4 arom. H); 8.45 (br. *s*, H–N(3)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.3 (*q*, *Me*–C(5)); 19.4 (*s*, Me_3C); 26.7 (*t*, S–CH_2–C(3′)); 27.1 (*q*, Me_3C); 38.1 (*t*, C(2′)); 41.4 (*d*, C(3′)); 64.2 (*t*, C(5′)); 84.5, 84.6 (2*d*, C(1′), C(4′)); 110.9 (*s*, C(5)); 128.0, 128.0, 130.1, 130.1 (4*d*, arom. C); 132.6, 133.0 (2*s*, arom. C); 135.4, 135.6 (2*d*, C(6), arom. C); 150.4 (*s*, C(2)); 164.0 (*s*, C(4)). FAB-MS: 511 (10, $[M+H]^+$).

N^6*-Benzoyl-9-[(3′*S*,4′*S*)-5′*-O-*[(*tert*-butyl)diphenylsilyl]-2′,3′-dideoxy-3′*-C-*(mercaptomethyl)-β-pentofuranosyl]adenine* (**8**). Thioacetate **6** (1.72 g, 3.07 mmol) and DMAP (345 mg, 3.07 mmol) were dissolved in pyridine (15 ml). BzCl (1.72 mL, 15.3 mmol) was added, and the mixture stirred at r.t. for 1 h. The reaction was quenched with MeOH (1 ml). The soln. was diluted with CH_2Cl_2 and extracted with sat. aq. $NaHCO_3$ soln. The org. layer was dried ($MgSO_4$) and evaporated to yield the intermediate amino-protected thioacetate as a brown oil, which was dissolved in pyridine (9 ml) and EtOH (18 ml). The soln. was degassed by repeated freeze-thaw cycles. Degassed 2N aq. NaOH (9 ml, 18 mmol) was added. The soln. was stirred at r.t. for 10 min and then neutralized with 3M aq. AcOH. The soln. was diluted with sat. aq. $NaHCO_3$ soln. and extracted with CH_2Cl_2, the org. layer dried ($MgSO_4$) and evaporated, and the residue purified by FC (CH_2Cl_2/AcOEt 1:1): **8** (937 mg, 49%). Yellowish foam. TLC (CH_2Cl_2/AcOEt 1:1): R_f 0.31. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.08 (*s*, ^{t}Bu); 1.35 (*t*, *J* = 8.2, SH); 2.41–2.67 (*m*, 2 H–C(2′), H–C(3′)); 2.78 (*m*, S–CH_2–C(3′)); 3.81 (*m*, 1 H–C(5′)); 3.96–4.04 (*m*, H–C(4′), 1 H–C(5′)); 6.39 (*dd*, *J* = 6.9, 2.5, H–C(1′)); 7.34–7.69 (*m*, 13 arom. H); 8.34 (*m*, 2 arom. H); 8.37 (*s*, H–C(8)); 8.78 (*s*, H–C(2)); 9.23 (br. *s*, HN–C(6)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 19.2 (*s*, Me_3C); 26.0 (*t*, S–CH_2–C(3′)); 27.0 (*q*, Me_3C); 35.4 (*t*, C(2′)); 41.4 (*d*, C(3′)); 62.2 (*t*, C(5′)); 84.9, 85.2 (2*d*, C(1′), C(4′)); 123.5 (*s*, C(5)); 127.9, 128.1, 128.7, 130.0, 130.1, 132.7, 132.8 (7*d*, arom. C); 133.8, 134.4 (2*s*, arom. C); 135.5, 135.6 (2*d*, arom. C); 141.4 (*d*, C(8)); 149.6 (*s*, C(4)); 151.1 (*s*, C(6)); 152.4 (*d*, C(2)); 165.0 (*s*, C=O). FAB-MS: 624 (5, $[M+H]^+$).

5′-O-*[(*tert*-Butyl)diphenylsilyl]-2′-deoxy-3′-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3′→5′)-3′*-O-*[(*tert*-Butyl)diphenylsilyl]-2′,5′-dideoxythymidine* (*TBDPSO-TsO_2T-OTBDPS*; **10**). A soln. of **7** (90 mg, 176 μmol) in THF (3 ml) was transferred into a flask containing dry iodide **9** [11] (97 mg, 160 μmol) and Cs_2CO_3 (156 mg, 480 μmol). The suspension was degassed by repeated freeze-thaw cycles. The degassed suspension was stirred at r.t. for 3 h. DMF (1 ml) was added, the suspension degassed again and then stirred at r.t. for 4 h. The reaction was quenched with aq. acetate buffer (2 ml; 3M AcOH, 1M AcONa). The soln. was diluted with H_2O and extracted with CH_2Cl_2. The org. layer was dried ($MgSO_4$) and evaporated to yield the intermediary sulfide, which was redissolved in THF (3.8 ml) and MeOH (25 ml). A soln. of *Oxone*® (420 mg, 680 μmol) and AcONa (188 mg, 2.2 mmol) in H_2O (6.3 ml) was added dropwise. The white suspension was stirred at r.t. for 90 min and then concentrated to 1/3 of its volume. The suspension was diluted with CH_2Cl_2 and extracted with sat. aq. $Na_2S_2O_3$ soln. The org. layer was dried ($MgSO_4$) and evaporated. The residue was purified by FC (CH_2Cl_2/AcOEt/H_2O 80:20:0.25 with 3–8% MeOH): **10** (148 mg, 91%). Colorless solid. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.20. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.08, 1.09 (2*s*, 2 ^{t}Bu); 1.67 (*d*, *J* = 1.1, Me–C(5)(T1)); 1.78 (*m*, 2 H–C(5′)(T2)); 1.88 (*d*, *J* = 1.0, Me–C(5)(T2)); 2.14–2.36 (*m*, 1 H–C(2′)(T1), 2 H–C(2′)(T2)); 2.43–2.52 (*m*, 1 H–C(2′)(T1)); 2.70–2.78 (*m*, H–C(3′)(T1)); 2.80–2.98 (*m*, CH_2–C(5′)(T2)); 3.02–3.13 (*m*, SO_2CH_2–C(3′)(T1)); 3.83 (*dd*, *J* = 11.2, 3.0, 1 H–C(5′)(T1)); 3.84–3.90 (*m*, 2 H–C(4′)); 4.00 (*dd*, *J* = 11.2, 2.7, 1 H–C(5′)(T1)); 4.17 (*m*, H–C(3′)(T2)); 6.11–6.18 (*m*, 2 H–C(1′)); 6.90 (*d*, *J* = 1.2, H–C(6)(T2)); 7.33–7.48 (*m*, H–C(6)(T1), 12 arom. H); 7.50–7.68 (*m*, 8 arom. H); 8.75, 8.90 (2br. *s*, 2 HN–C(6)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.3, 12.4 (2*q*, 2 *Me*–C(5)); 19.1, 19.4 (2*s*, 2 Me_3C); 25.4 (*t*, C(5′)(T2)); 26.9, 27.0 (2*q*, 2 Me_3C); 32.4 (*d*, C(3′)(T1)); 38.2, 38.5 (2*t*, 2 C(2′)); 50.7 (*t*, CH_2–C(5′)(T2)); 55.0 (*t*, SO_2CH_2–C(3′)(T1)); 63.8 (*t*, C(5′)(T1)); 75.9 (*d*, C(3′)(T2)); 84.2, 84.6, 87.3 (3*d*, 2 C(1′), 2 C(4′)); 111.4, 111.5 (2*s*, 2 C(5)); 128.0, 128.0, 130.1, 130.1, 130.3,

130.3 (6*d*, arom. C); 132.2, 132.8, 132.8, 132.9 (4*s*, arom. C); 135.4, 135.6, 135.7, 135.7 (4*d*, arom. C); 136.5 (*d*, 2 C(6)); 150.2, 150.5 (2*s*, 2 C(2)); 163.6 (*s*, 2 C(4)). FAB-MS: 1019 (47, $[M+H]^+$).

N^6-*Benzoyl-5′*-O-*[(*tert-*butyl)diphenylsilyl]-2′-deoxy-3′-de(phosphinicooxy)adenylylmethylenesulfonyl-methylene-(3′ → 5′)-3′*-O-*[(*tert-*butyl)diphenylsilyl]-2′,5′-dideoxythymidine* (*TBDPSO-^{Bz}AsO$_2$T-OTBDPS;* **11**). A soln. of **8** (576 mg, 924 μmol) in THF (15 ml) was degassed by repeated freeze-thaw cycles and then transferred into a flask containing dry iodide **9** [11] (500 mg, 828 μmol) and Cs_2CO_3 (803 mg, 2.46 mmol). The suspension was stirred at r.t. for 3 h. Degassed DMF (5 ml) was added and the suspension stirred at r.t. overnight. The reaction was quenched with 3M aq. AcOH. The soln. was diluted with CH_2Cl_2 and extracted with sat. aq. $NaHCO_3$ soln. The org. layer was dried ($MgSO_4$) and evaporated to yield the intermediary sulfide, which was redissolved in THF (20 ml) and MeOH (130 ml). A soln. of *Oxone*® (2.21 g, 3.6 mmol) and AcONa (985 mg, 11.5 mmol) in H_2O (33 ml) was added dropwise. The white suspension was stirred at r.t. for 2 h and then concentrated to 1/3 of its volume. The suspension was diluted with CH_2Cl_2 and extracted with sat. aq. $Na_2S_2O_3$ soln. The org. layer was dried ($MgSO_4$) and evaporated. Crude **11** (985 mg, quant.) was used for the subsequent deprotection without further purification. An anal. sample was purified by FC (CH_2Cl_2/AcOEt/H_2O 80:20:0.25). Colorless solid. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.65. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 0.90, 1.03 (2*s*, 2 *t*Bu); 1.71 (*s*, Me–C(5)(T)); 1.74–1.86 (*m*, 2 H–C(5′)(T)); 2.05–2.16 (*m*, 1 H–C(2′)(T)); 2.16–2.23 (*m*, 1 H–C(2′)(T)); 2.56 (*m*, 1 H–C(2′)(A)); 2.90–2.94 (*m*, 1 H–C(2′)(A)); 2.95–3.13 (*m*, 1 H of CH_2–C(5′)(T), H–C(3′)(A)); 3.20–3.42 (*m*, 1 H of CH_2–C(5′)(T), SO_2CH_2–C(3′)(A)); 3.80 (*m*, 1 H–C(5′)(A)); 3.93–4.06 (*m*, 1 H–C(5′)(A), 2 H–C(4′)); 4.26 (*m*, H–C(3′)(T)); 6.26 (*dd*, *J* = 7.0, 7.0, H–C(1′)); 6.47 (*dd*, J = 7.5, 2.6, H–C(1′)); 7.23–7.66 (*m*, 23 arom. H, H–C(6)(T)); 8.05 (*m*, 2 arom. H); 8.58, 8.64 (2*s*, H–C(2)(A), H–C(8)(A)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.3 (*q*, *Me*–C(5)(T)); 19.0, 19.1 (2*s*, 2 Me_3C); 25.3 (*t*, C(5′)(T)); 26.9 (*q*, 2 Me_3C); 33.9 (*d*, C(3′)(A)); 38.2, 38.8 (2*t*, 2 C(2′)); 49.7, 54.5 (2*t*, SO_2CH_2–C(3′)(A), CH_2–C(5′)(T)); 64.3 (*t*, C(5′)(A)); 76.0 (*d*, C(3′)(T)); 84.7, 84.8, 85.2, 88.2 (4*d*, 2 C(1′), 2 C(4′)); 111.4 (*s*, C(5)(T)); 123.3 (*s*, C(5)(A)); 127.9, 128.0, 128.1, 128.3, 128.6, 130.1, 130.1, 130.3, 130.4 (9*d*, arom. C); 132.4, 132.5, 132.6, 132.8, 132.8 (5*s*, arom. C); 133.7, 135.4, 135.6, 135.7 (4*d*, arom. C); 137.1 (*d*, C(6)(T)); 141.6 (*d*, C(8)(A)); 149.9, 150.3, 151.0 (3*s*, C(4)(A), C(2)(T), C(6)(A)); 152.7 (*d*, C(2)(A)); 164.0, 165.1 (2*s*, C(4)(T), Ph*C*=O). FAB-MS: 1154 (31, $[M+Na]^+$), 1132 (62, $[M+H]^+$).

2′-Deoxy-3′-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine (*HO-TsO$_2$T-OH;* **12**). A soln. of **10** (116 mg, 114 μmol) in pyridine (0.7 ml) was transferred into a plastic tube with septum. A soln. of 4.7M HF in pyridine (0.7 ml, 3.3 mmol) was added and the soln. stirred at r.t. overnight. The reaction was quenched with $MeOSiMe_3$ (4 ml, 35 mmol). The soln. was transferred into a glass flask and evaporated. The residue was purified by FC (CH_2Cl_2/EtOH/MeOH/H_2O 86:7:7:0.25 → 83:9:8:0.25): **12** (52 mg, 84%). Colorless solid. TLC (CH_2Cl_2/AcOEt/MeOH 4:6:1): R_f 0.05. ^{1}H-NMR ((D$_6$)DMSO/D$_2$O 4:1, 300 MHz): 1.74 (*d*, *J* = 1.1, Me–C(5)); 1.76 (*d*, *J* = 1.1, Me–C(5)); 1.93 (*m*, 1 H–C(5′)(T2)); 2.02–2.11 (*m*, 1 H–C(2′)(T2), 2 H–C(5′)(T2)); 2.18–2.30 (*m*, 1 H–C(2′)(T2), 2 H–C(2′)(T1)); 2.69 (*m*, H–C(3′)(T1)); 3.13–3.25 (*m*, CH_2–C(5′)(T2), 1 H, SO_2CH_2–C(3′)(T1)); 3.36 (*dd*, *J* = 13.8, 3.6, 1 H, SO_2CH_2–C(3′)(T1)); 3.58 (*dd*, *J* = 12.7, 3.4, 1 H–C(5′)(T1)); 3.69–3.76 (*m*, 2 H–C(5′)(T1), 2 H–C(4′)); 4.12 (*m*, H–C(3′)(T2)); 5.96 (*dd*, *J* = 6.3, 4.0, H–C(1′)(T1)); 6.09 (*dd*, *J* = 7.0, 7.0, H–C(1′)(T2)); 7.36 (*d*, *J* = 1.1, H–C(6)); 7.80 (*d*, *J* = 1.1, H–C(6)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 12.0, 12.2 (2*q*, 2 *Me*–C(5)); 24.9 (*t*, C(5′)(T2)); 31.0 (*d*, C(3′)(T1)); 37.6, 38.0 (2*t*, 2 C(2′)); 49.4 (*t*, CH_2–C(5′)(T2)); 53.4 (*t*, SO_2CH_2–C(3′)(T1)); 60.1 (*t*, C(5′)(T1)); 72.5 (*d*, C(3′)(T2)); 83.3, 83.7, 84.0, 84.6 (4*d*, 2 C(1′), 2 C(4′)); 109.7, 109.9 (2*s*, 2 C(5)); 136.1 (*d*, 2 C(6)); 150.2, 150.4 (2*s*, 2 C(2)); 163.6, 163.7 (2*s*, 2 C(4)). FAB-MS: 543 (100, $[M+H]^+$).

N^6*-Benzoyl-2′-deoxy-3′-de(phosphinicooxy)adenylylmethylenesulfonylmethylene-(3′ → 5′)-2′,5′-dideoxy-thymidine* (*HO-^{Bz}AsO$_2$T-OH;* **13**). Crude **11** (885 mg, *ca.* 740 μmol) was transferred into a plastic tube with septum, dried under high vacuum, and dissolved in pyridine (2 ml). A soln. of 4.7M HF in pyridine (3 ml, 14 mmol) was added, and the soln. stirred at r.t. overnight. The reaction was quenched with $MeOSiMe_3$ (4 ml, 35 mmol). The soln. was transferred into a glass flask and evaporated. The residue was purified by FC (CH_2Cl_2/EtOH/MeOH/H_2O 86:7:7:0.25): **13** (349 mg, *ca.* 75%). Colorless solid. TLC (CH_2Cl_2/MeOH/EtOH 10:1:1): R_f 0.25. ^{1}H-NMR ((D$_6$)DMSO, 500 MHz): 1.79 (*d*, *J* = 1.0, Me–C(5)(T)); 2.00 (*m*, 1 H–C(5′)(T)); 2.07 (*ddd*, *J* = 13.6, 6.7, 4.1, 1 H–C(2′)(T)); 2.13 (*m*, 1 H–C(5′)(T)); 2.26 (*m*, 1 H–C(2′)(T)); 2.57 (*ddd*, *J* = 13.7, 9.4, 7.3, 1 H–C(2′)(A)); 2.83 (*ddd*, *J* = 13.7, 7.8, 2.8, 1 H–C(2′)(A)); 3.05 (*m*, H–C(3′)(A)); 3.22–3.30 (*m*, CH_2–C(5′)(T)); 3.38 (*dd*, *J* = 14.0, 10.1, 1 H, SO_2CH_2–C(3′)(A)); 3.55 (*dd*, *J* = 14.0, 3.8, 1 H, SO_2CH_2–C(3′)-(A)); 3.63 (*m*, 1 H–C(5′)(A)); 3.73–3.77 (*m*, 1 H–C(5′)(A), H–C(4′)(T)); 3.92 (*m*, H–C(4′)(A)); 4.15 (*m*, H–C(3′)(T)); 5.09 (*t*, *J* = 5.2, OH(A)); 5.36 (*d*, *J* = 4.0, OH(T)); 6.17 (*dd*, *J* = 6.7, 6.7, H–C(1′)(T)); 6.45 (*dd*, *J* = 7.3, 2.8, H–C(1′)(A)); 7.44 (*d*, *J* = 1.0, H–C(6)(T)); 7.54 (*m*, 2 H (Bz)); 7.65 (*m*, 1 H (Bz)); 8.04 (*m*, 2 H (Bz)); 8.72, 8.73 (2*s*, H–C(2)(A), H–C(8)(A)); 11.20, 11.27 (2br. *s*, 2 NH). ^{13}C-NMR ((D$_6$)DMSO, 125 MHz):

12.0 (*q*, *Me*–C(5)(T)); 24.9 (*t*, C(5′)(T)); 31.9 (*d*, C(3′)(A)); 37.7, 38.0 (2*t*, 2 C(2′)); 49.3, 53.4 (2*t*, SO_2CH_2–C(3′)(A), CH_2–C(5′)(T)); 60.7 (*t*, C(5′)(A)); 72.8 (*d*, C(3′)(T)); 83.4, 83.7, 84.1, 85.4 (4*d*, 2 C(1′), 2 C(4′)); 109.9 (*s*, C(5)(T)); 125.8 (*s*, C(5)(A)); 128.4 (*d*, Bz); 132.3 (*d*, Bz); 133.4 (*s*, Bz); 136.1 (*d*, C(6)(T)); 142.7 (*d*, C(8)(A)); 150.2 (*s*, C(4)(A), C(2)(T)); 151.3 (*d*, C(2)(A)); 151.4 (*s*, C(6)(A)); 163.6 (*s*, C(4)(T)); 165.6 (*s*, Ph*C*=O). FAB-MS: 678 (6, $[M+Na]^+$), 656 (100, $[M+H]^+$).

*2′-Deoxy-3′-de(phosphinicooxy)-5′-*O*-(4,4′-dimethoxytrityl)thymidylylmethylenesulfonylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine* (*$(MeO)_2$TrO-TsO_2T-OH*; **14**). To a soln. of **14** (85 mg, 157 μmol), DMAP (3 mg, 25 μmol), Et_3N (55 μl, 390 μmol) in pyridine (2 ml), and $(MeO)_2$TrCl (110 mg, 314 μmol) were added. After stirring at r.t. for 2 h, the reaction was quenched with MeOH. The soln. was evaporated and the residue purified by FC (CH_2Cl_2/MeOH 50:1, 20:1, and 10:1, with 2% Et_3N): **14** (132 mg, 99%; still containing significant amounts of Et_3N). Colorless foam. TLC (CH_2Cl_2/MeOH/EtOH 10:1:1): R_f 0.55. ^{1}H-NMR ((D_6)DMSO, 400 MHz): 1.50 (*d*, *J* = 1.1, Me–C(5)(T1)); 1.76 (*d*, *J* = 1.1, Me–C(5)(T2)); 1.96 (*m*, 1 H–C(5′)(T2)); 2.03–2.14 (*m*, 1 H–C(2′)(T2), 1 H–C(5′)(T2)); 2.26 (*m*, 1 H–C(2′)(T2)); 2.32–2.40 (*m*, 1 H–C(2′)(T1)); 2.92 (*m*, H–C(3′)(T1)); 3.12–3.35 (*m*, CH_2–C(5′)(T2), SO_2CH_2–C(3′)(T1), H–C(5′)(T1)); 3.70 (*m*, H–C(4′)(T1)); 3.73, 3.73 (2*s*, $(MeO)_2$Tr); 3.89 (*m*, H–C(4′)(T2)); 4.13 (*m*, H–C(3′)(T2)); 5.34 (br. *s*, OH); 6.06 (*dd*, *J* = 7.0, 4.8, H–C(1′)(T1)); 6.14 (*dd*, *J* = 7.0, 7.0, H–C(1′)(T2)); 6.87 (*m*, 4 arom. H); 7.20–7.31 (*m*, 7 arom. H); 7.38–7.42 (*m*, 2 arom. H, H–C(6)); 7.53 (*d*, *J* = 1.1, H–C(6)). ^{13}C-NMR ((D_6)DMSO, 100 MHz): 11.9, 12.0 (2*q*, 2 *Me*–C(5)); 25.0 (*t*, C(5′)(T2)); 31.9 (*d*, C(3′)(T1)); 37.2, 38.1 (2*t*, 2 C(2′)); 49.5 (*t*, CH_2–C(5′)(T2)); 53.6 (*t*, SO_2CH_2–C(3′)(T1)); 55.0 (*q*, $(MeO)_2$Tr); 63.0 (*t*, C(5′)(T1)); 72.8 (*d*, C(3′)(T2)); 82.6, 83.4, 83.7, 84.2 (4*d*, 2 C(1′), 2 C(4′)); 85.8 (*s*, Ph$C(Ar)_2$); 109.2, 109.9 (2*s*, 2 C(5)); 113.2 (*d*, MeOC_6H_4); 126.7, 127.6, 127.8, 129.7 (4*d*, arom. C); 135.3, 135.3 (2*s*, *Me*OC_6H_4); 135.7, 136.1 (2*d*, 2 C(6)); 144, 7 (*s*, arom. C); 150.3, 150.4 (2*s*, 2 C(2)); 158.1 (*d*, MeOC_6H_4); 163.6, 163.7 (2*s*, 2 C(4)). FAB-MS: 867 (5, $[M+Na]^+$); 844 (14, $[M+H]^+$); 303 (100, $(MeO)_2Tr^+$).

N^6*-Benzoyl-2′-deoxy-3′-de(phosphinicooxy)-5′-*O*-(4,4′-dimethoxytrityl)adenylylmethylenesulfonylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine* (*$(MeO)_2$TrO-^{Bz}ASO_2T-OH*; **15**). As described for **14**, with **13** (298 mg, 485 μmol), DMAP (3 mg, 25 μmol), Et_3N (160 μl, 1.14 mmol), pyridine (6 ml), and $(MeO)_2$TrCl (241 mg, 711 μmol). FC (CH_2Cl_2/MeOH 50:1, 20:1, and 10:1) gave **15** (283 mg, 66%). Colorless foam. TLC (CH_2Cl_2/MeOH 10:1): R_f 0.35. ^{1}H-NMR ((D_6)DMSO, 400 MHz): 1.76 (*d*, *J* = 1.1, Me–C(5)(T)); 1.93–2.16 (*m*, 2 H–C(5′)(T)); 2.25 (*m*, 2 H–C(2′)(T)); 2.58 (*m*, 1 H–C(2′)(A)); 3.00 (*m*, 1 H–C(2′)(A)); 3.17–3.52 (*m*, CH_2–C(5′)(T), SO_2CH_2–C(3′)(A), 2 H–C(5′)(A), H–C(3′)(A)); 3.70, 3.70 (2*s*, $(MeO)_2$Tr); 3.73, 4.04 (2*m*, 2 H–C(4′)); 4.14 (*m*, H–C(3′)(T)); 5.35 (*d*, *J* = 4.5, OH); 6.15 (*dd*, *J* = 7.0, 7.0, H–C(1′)(T)); 6.46 (*dd*, *J* = 7.5, 2.9, H–C(1′)(A)); 6.79 (*m*, 4 arom. H); 7.14–7.23 (*m*, 6 arom. H); 7.30 (*m*, 2 arom. H); 7.42 (*d*, *J* = 1.1, H–C(6)(T)); 7.55 (*m*, 2 H (Bz)); 7.64 (*m*, 1 H (Bz)); 8.04 (*m*, 2 H (Bz)); 8.31 (*m*, 1 arom. H); 8.55, 8.69 (2*s*, H–C(2)(A), H–C(8)(A)). ^{13}C-NMR ((D_6)DMSO, 100 MHz): 12.0 (*q*, *Me*–C(5)(T)); 25.0 (*t*, C(5′)(T)); 33.0 (*d*, C(3′)(A)); 36.7, 38.1 (2*t*, 2 C(2′)); 49.3 (*t*, CH_2–C(5′)(T)); 53.6 (*t*, SO_2CH_2–C(3′)(A)); 54.9 (*q*, $(MeO)_2$Tr); 63.5 (*t*, C(5′)(A)); 72.8 (*d*, C(3′)(T)); 79.1, 83.4, 83.7, 84.2 (4*d*, 2 C(1′), 2 C(4′)); 85.6 (*s*, Ph$C(Ar)_2$); 109.9 (*s*, C(5)(T)); 113.0 (*d*, MeOC_6H_4); 125.8 (*s*, C(5)(A)); 126.5, 127.5, 127.6, 128.4, 128.4, 129.5, 132.3 (7*d*, arom. C); 133.3, 135.4, 135.4 (3*s*, arom. C); 136.1 (*d*, C(6)(T)); 143.0 (*d*, C(8)(A)); 151.4 (*d*, C(2)(A)); 151.4, 151.5 (2*s*, C(2)(T), C(4)(A), C(6)(A)); 157.9 (*d*, MeOC_6H_4); 163.6 (*s*, C(4)(T)); 165.5 (*s*, Ph*C*=O). FAB-MS: 980 (24, $[M+Na]^+$); 958 (100, $[M+H]^+$); 303 (58, $(MeO)_2Tr^+$).

*2′-Deoxy-3′-de(phosphinicooxy)-5′-*O*-(4,4′-dimethoxytrityl)thymidylylmethylenesulfonylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (*$(MeO)_2$TrO-TsO_2T-OP(OCH_2CH_2CN)(N^iPr_2)*; **16**). A mixture of **14** (100 mg, 118 μmol) and DIPAT (10 mg, 58 μmol) was dried at 40° under high vacuum and dissolved in MeCN (0.5 ml). Then 2-cyanoethyl tetraisopropylphosphorodiamidite (51 μl, 157 μmol) was added and the soln. stirred at r.t. for 3 h. The soln. was diluted with CH_2Cl_2 and extracted with sat. aq. $NaHCO_3$ soln. The org. layer was washed with brine, dried ($MgSO_4$) and evaporated. The residue was redissolved in CH_2Cl_2 and precipitated from hexane (20 ml) at –20° for 1 h. The soln. was decanted, evaporated and once again precipitated from CH_2Cl_2/hexane. The combined solids were dried under high vacuum: **16** (113 mg, 92%). Colorless foam. TLC (CH_2Cl_2/MeOH 10:1): R_f 0.50. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.18 (*m*, 2 $(Me_2CH)_2N$); 1.56 (*s*, Me–C(5)(T1)); 1.91, 1.92 (2*s*, Me–C(5)(T2)); 2.12–2.60 (*m*, 2 H–C(2′)(T1), H–C(3′)(T1), 2 H–C(5′)(T2), 2 H–C(2′)(T2)); 2.65 (*m*, CH_2CN); 3.01–3.20 (*m*, CH_2–C(3′)(T1), CH_2–C(5′)(T2)); 3.33 (*dd*, *J* = 11.0, 3.0, 1 H–C(5′)(T1)); 3.53 (*m*, 1 H–C(5′)(T1)); 3.58–3.74 (*m*, $POCH_2$, 2 Me_2CH); 3.78 (*s*, $(MeO)_2$Tr); 3.83–4.03 (*m*, 2 H–C(4′)); 4.37 (*m*, H–C(3′)(T2)); 6.04 (*dd*, *J* = 6.8, 6.8, 0.5 H–C(1′)); 6.09 (*dd*, *J* = 6.5, 6.5, 0.5 H–C(1′)); 6.17 (*dd*, *J* = 4.3, 4.3, 0.5 H–C(1′)); 6.18 (*dd*, *J* = 4.7, 4.7, 0.5 H–C(1′)); 6.83 (*m*, 4 arom. H); 7.03 (*d*, *J* = 1.0, 0.5 H–C(6)(T2)); 7.04 (*d*, *J* = 1.0, 0.5 H–C(6)(T2)); 7.22–7.32 (*m*, 7 arom. H); 7.40–7.43 (*m*, 2 arom. H); 7.53 (*d*, *J* = 1.0, 0.5 H–C(6)(T1)); 7.54 (*d*, *J* = 1.0,

0.5 H–C(6)(T1)). ^{31}P–NMR ($CDCl_3$, 120 MHz): 148.8, 149.4. FAB-MS: 1045 (62, $[M+H]^+$), 303 (100, $(MeO)_2Tr^+$).

N^6-*Benzoyl-2′-deoxy-3′-de(phosphinicooxy)-5′*-O-*(4,4′-dimethoxytrityl)adenylylmethylenesulfonylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (*$(MeO)_2TrO$-^{Bz}ASO_2T-OP(OCH_2CH_2CN)(N^iPr_2)*; **17**). As described for **16**, with **15** (100 mg, 106 μmol), DIPAT (9 mg, 52 μmol), MeCN (0.5 ml) and 2-cyanoethyl tetraisopropylphosphorodiamidite (46 μl, 142 μmol). **17** (113 mg, 94%). Colorless foam. TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.35. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.18 (*m*, 2 ($Me_2CH)_2$N); 1.87 (*m*, Me–C(5)(T)); 2.10 (*m*, 1 H–C(5′)(T); 2.23–2.42 (*m*, 2 H–C(2′)(T), 1 H–C(5′)(T)); 2.47–2.78 (*m*, 2 H–C(2′)(A), CH_2CN); 2.95 (*m*, H–C(3′)(A)); 3.05–3.27 (*m*, CH_2–C(3′)(A), CH_2–C(5′)(T)); 3.32 (*m*, 1 H–C(5′)(A)); 3.38–3.51 (*m*, 1 H–C(5′)(A), 1 H of $POCH_2$); 3.52–3.89 (*m*, 1 H of $POCH_2$, 2 Me_2CH); 3.76 (*s*, ($MeO)_2$Tr); 3.91–4.09 (*m*, 2 H–C(4′)); 4.32 (*m*, H–C(3′)(T)); 5.99 (*dd*, *J* = 7.1, 7.1, 0.5 H–C(1′)(T)); 6.07 (*dd*, *J* = 6.9, 6.9, 0.5 H–C(1′)(T)); 6.43 (*d*, *J* = 6.5, H–C(1′)(A)); 6.79 (*m*, 4 arom. H); 7.03 (*d*, *J* = 1.3, 0.5 H–C(6)(T)); 7.04 (*d*, *J* = 1.3, 0.5 H–C(6)(T)); 7.16–7.63 (*m*, 12 arom. H); 8.07–8.12 (*m*, 2 arom. H); 8.32 (*s*, 0.5 H–C(8)(A)); 8.34 (*s*, 0.5 H–C(8)(A)); 8.80 (*s*, H–C(2)(A)). ^{31}P-NMR ($CDCl_3$, 120 MHz): 148.9, 149.5. FAB-MS: 1180 (6, $[M+Na]^+$), 1158 (100, $[M+H]^+$), 303 (96, $(MeO)_2Tr^+$).

*1-[(3′*R*,4′*R*)-2′,5′,6′-trideoxy-6′-(diethoxyphosphinyl)-β-hexofuranosyl]thymine* (**31**). Diethyl methylphosphonate (3.0 g, 20 mmol) was dissolved in THF (40 ml) and cooled to −78°. BuLi (2.5M in hexane; 8.0 ml, 20 mmol) was added dropwise over 20 min. The resulting bright orange soln. was stirred at −78° for 90 min. $BF_3 \cdot Et_2O$ (2.5 ml, 20 mmol) was added dropwise over 5 min. The soln. was stirred for another 5 min, during which time the color faded almost completely. A soln. of **19** [13] (900 mg, 4 mmol) in THF (40 ml) was slowly added over 2 h. After another 2 h at −78°, the reaction was quenched with sat. aq. $NaHCO_3$ soln. (2.5 ml) and solid $NaHCO_3$ (0.8 g). The now white suspension was allowed to warm to r.t. overnight. The solvent was evaporated, the resulting slimy residue resuspended in CH_2Cl_2 (150 ml), and the mixture filtered through *Celite*. The white residue was twice removed from the top of the *Celite*, washed with additional CH_2Cl_2 (100 ml), and refiltered. The combined org. layers were concentrated to *ca.* 30 ml and purified by FC (CH_2Cl_2/MeOH 30 : 1 and 20 : 1): **31** (1.7 g; quant.; still containing some diethyl methylphosphonate). Colorless foam. An anal. sample was further purified by prep. HPLC (silica gel; $CHCl_3$/EtOH 20 : 1, 7 ml/min). TLC (CH_2Cl_2/MeOH 10 : 1): R_f 0.35. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.34, 1.35 (2*t*, *J* = 7.0, 2 $POCH_2Me$); 1.64–1.80 (*m*, 1 H–C(6′)); 1.92 (*d*, *J* = 1.0, Me–C(5)); 1.90–2.04 (*m*, 1 H–C(6′)); 2.05–2.18 (*m*, 2 H–C(5′), 1 H–C(2′)); 2.58–2.70 (*m*, 1 H–C(2′)); 3.75–3.81 (*m*, H–C(4′)); 4.04–4.24 (*m*, 2 $POCH_2$Me); 4.31–4.35 (*m*, H–C(3′)); 6.12 (*dd*, *J* = 2.2, 8.5, H–C(1′)); 7.72 (*d*, *J* = 1, H–C(6)); 8.57 (br. *s*, H–N(3)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.8 (*q*, *Me*–C(5)); 16.6, 16.6 (2*q*, *J*(C,P) = 5, 2 *Me*CH_2O); 21.4 (*t*, *J*(C,P) = 4, C(5′)); 21.7 (*t*, *J*(C,P) = 143, C(6′)); 41.2 (*t*, C(2′)); 62.1, 62.3 (2*t*, *J*(C,P) = 7, 2 Me*C*H_2O); 69.5 (*d*, C(3′)); 84.9 (*d*, C(1′)); 85.0 (*d*, *J*(C,P) = 14, C(4′)); 110.4 (*s*, C(5)); 138.0 (*d*, C(6)); 151.0 (*s*, C(2)); 164.5 (*s*, C(4)). ^{31}P-NMR ($CDCl_3$, 120 MHz): 28.8. HR-FAB-MS: 377.1515 (11, $[M+H]^+$).

*1-[(3′*S*,4′*R*)-3′*-O-*Benzoyl-2′,5′,6′-trideoxy-6′-(diethoxyphosphinyl)-β-hexofuranosyl]thymine* (**32**). A soln. of **31** (1.3 g, *ca.* 3 mmol; still containing some diethyl methylphosphonate), benzoic acid (610 mg, 5 mmol) and Ph_3P (1.3 g, 5 mmol) in THF (60 ml) was cooled to 0°. DIAD (965 μl; 5 mmol) was added dropwise. The soln. was allowed to warm to r.t. overnight. The reaction was quenched with Et_3N (0.5 ml) and MeOH (1 ml). The soln. was evaporated, and the residue purified by FC (CH_2Cl_2/MeOH 30 : 1, 20 : 1, and 10 : 1): **32** (850 mg, *ca.* 60%). Colorless foam. TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.20. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.34, 1.34 (2*t*, *J* = 7.0, 2 *Me*CH_2O); 1.75–1.88 (*m*, 1 H–C(6′)); 1.99 (*s*, Me–C(5)); 1.89–2.08 (*m*, 1 H–C(5′); 1 H–C(6′)); 2.08–2.22 (*m*, 1 H–C(5′)); 2.25–2.35 (*m*, 1 H–C(2′)); 2.58 (*ddd*, *J* = 14.5, 5.6, 2.2, 1 H–C(2′)); 4.06–4.20 (*m*, H–C(4′), 2 MeCH_2O); 5.27–5.32 (*m*, H–C(3′)); 6.39 (*dd*, *J* = 6.0, 8.3, H–C(1′)); 7.20 (*s*, H–C(6)); 7.44–7.51 (*m*, 2 H (Bz)); 7.58–7.64 (*m*, 1 H (Bz)); 8.00–8.10 (*m*, 2 H (Bz)); 8.91 (br. *s*, H–N(3)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 12.7 (*q*, *Me*–C(5)); 16.6 (*q*, *J*(C,P) = 6, 2 *Me*CH_2O); 22.0 (*t*, *J*(C,P) = 144, C(6′)); 27.1 (*t*, *J*(C,P) = 4, C(5′)); 36.9 (*t*, C(2′)); 61.9 (*t*, 2 Me*C*H_2O); 76.7 (*d*, C(3′)); 83.9 (*d*, *J*(C,P) = 18, C(4′)); 84.6 (*d*, C(1′)); 111.9 (*s*, C(5)); 128.6 (*d*, Bz); 129.2 (*s*, Bz); 129.8, 133.6 (2*d*, Bz); 134.7 (*d*, C(6)); 150.7 (*s*, C(2)); 164.0 (*s*, C(4)); 166.0 (*s*, Ph*C*=O). ^{31}P-NMR ($CDCl_3$, 120 MHz): 31.6. HR-FAB-MS: 481.1701 (36, $[M+H]^+$).

*1-[(3′*S*,4′*R*)-3′-Benzoyl-2′,5′,6′-trideoxy-6′-(ethoxyhydroxyphosphinyl)-β-hexofuranosyl]thymine* (**33**). A soln. of **32** (1.17 g, 2.4 mmol) and LiBr (1.0 g, 11.5 mmol) in pyridine (10 ml) was heated under reflux for 4 h. The soln. was evaporated and the crude product redissolved in H_2O (20 ml) and precipitated from 1M aq. HCl (250 ml). The suspension was centrifuged (6000 rpm, 30 min), the supernatant was decanted, and the residue resuspended in 1M aq. HCl (100 ml). The suspension was again centrifuged, and the supernatant decanted. The residue was redissolved in MeOH and dried: **33** (830 mg, 75%). Colorless solid. TLC (AcOEt/MeOH/H_2O 80 : 17 : 3): R_f 0.1. ^{1}H-NMR ((D_6)DMSO, 300 MHz): 1.20 (*t*, *J* = 7.0, *Me*CH_2O); 1.66–1.80 (*m*, H–C(6′)); 1.82 (*s*, Me–C(5)); 1.84–2.05 (*m*, 2 H–C(5′)); 2.38 (*ddd*, *J* = 14.2, 6.0, 2.0, 1 H–C(2′)); 2.58–2.69 (*m*, 1 H–C(2′));

3.86–3.98 (*m*, MeCH_2O); 4.05–4.13 (*m*, H–C(4′)); 5.31–5.37 (*m*, H–C(3′)); 6.24 (*dd*, *J* = 6.4, 8.0, H–C(1′)); 7.53–7.60 (*m*, H–C(6), 2 H (Bz)); 7.67–7.73 (*m*, 1 H (Bz)); 7.99–8.04 (*m*, 2 H (Bz)); 11.4 (br. *s*, H–N(3)). ^{13}C-NMR ((D$_6$)DMSO, 75 MHz): 12.1 (*q*, *Me*–C(5)); 16.4 (*q*, *J*(C,P) = 6, *Me*CH_2O); 22.2 (*t*, *J*(C,P) = 139, C(6′)); 26.5 (*t*, *J*(C,P) = 4, C(5′)); 35.2 (*t*, C(2′)); 60.0 (*t*, *J*(C,P) = 6, MeCH_2O); 76.8 (*d*, C(3′)); 83.1 (*d*, *J*(C,P) = 18, C(4′)); 83.7 (*d*, C(1′)); 110.1 (*s*, C(5)); 128.8 (*d*, Bz); 129.3 (*s*, Bz); 129.4, 133.7 (2*d*, Bz); 136.2 (*d*, C(6)); 150.5 (*s*, C(2)); 163.7 (*s*, C(4)); 165.2 (*s*, Ph*C*=O). ^{31}P-NMR ((D$_6$)DMSO, 120 MHz): 29.2. HR-FAB-MS: 453.1432 (40, $[M+H]^+$).

*2′-Deoxy-*O^P*-ethyl-5′-*O*-(4,4′-dimethoxytrityl)thymidylylmethylene-(3′ → 5′)-3′-*O*-benzoyl-2′,5′-dideoxythymidine* (*(MeO)$_2$TrO-TOPOOEtT-OBz*; **37**). A soln. of **33** (452 mg, 1.0 mmol) in pyridine (5 ml) was transferred into a flask containing MSNT (590 mg, 2.0 mmol). The resulting soln. was stirred in the dark for 1 h and then transferred into a flask containing **34** (1.65 g, 3.0 mmol). The resulting soln. was stirred at r.t. in the dark for 7 d. The reaction was quenched with H_2O (300 µl) and the soln. evaporated. The residue was subjected to a preliminary FC (CH_2Cl_2/MeOH 30:1, 20:1, and 10:1) to separate the (MeO)$_2$Tr-protected compounds from the rest. The pure product was obtained by a second FC (pure AcOEt, AcOEt/EtOH 3:1): **37** (570 mg, 58%). Coarse, brownish foam. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.20. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.33 (*t*, *J* = 7.0, *Me*CH_2O); 1.38–1.40 (*m*, Me–C(5)(T1)); 1.95 (*s*, Me–C(5)(T2)); 1.84–2.22 (*m*, 2 H–C(5′)(T2), CH_2–C(5′)(T2)); 2.35–2.47 (*m*, 1 H–C(2′)(T1)); 2.50–2.64 (*m*, 1 H–C(2′)(T1), 2 H–C(2′)(T2)); 3.33–3.54 (*m*, 2 H–C(5′)(T1)); 3.77–3.79 (*m*, (*Me*O)$_2$Tr); 3.85–4.16 (*m*, H–C(4′)(T2), MeCH_2O); 4.20–4.30 (*m*, H–C(4′)(T1)); 5.18–5.26 (*m*, H–C(3′)(T1)); 5.28–5.34 (*m*, H–C(3′)(T2)); 6.24–6.32 (*m*, H–C(1′)(T1)); 6.46 (*dd*, *J* = 5.3, 8.8, H–C(1′)(T2)); 6.82–6.86 (*m*, 4 H, Tr); 7.13–7.17 (*m*, H–C(6)(T2)); 7.22–7.32 (*m*, 7 H, Tr); 7.35–7.39 (*m*, 2 H, Tr); 7.42–7.49 (*m*, 2 H (Bz)); 7.54–7.62 (*m*, 1 H (Bz), H–C(6)(T1)); 8.00–8.05 (*m*, 2 H (Bz)). ^{13}C-NMR ($CDCl_3$, 75 MHz): 11.8, 12.8 (2*q*, 2 *Me*–C(5)); 16.5, 16.6 (2*q*, *Me*CH_2O); 21.6, 23.1, 23.5, 23.9 (4*t*, *C*H$_2$–C(5′)); 26.9 (*t*, C(5′)(T2)); 36.7 (*t*, C(2′)(T2)); 39.6 (*t*, C(2′)(T1)); 55.4 (*q*, (*Me*O)$_2$Tr); 62.3, 62.4, 62.5, 62.6 (4*t*, MeCH$_2$O); 63.3, 63.4 (2*t*, C(5′)(T1)); 76.3, 76.4, 76.8 (3*d*, 2 C(3′)); 83.6, 83.9, 84.4, 85.0, 85.3 (5*d*, 2 C(1′), 2 C(4′)); 87.3, 87.3 (2*s*, Ph*C*(Ar)$_2$); 111.8, 111.9, 111.9 (3*s*, 2 C(5)); 113.4 (*d*, MeO*C*$_6$H$_4$); 127.3, 128.1, 128.2, 128.6 (4*d*, arom. C); 129.2 (*s*, Bz); 129.8, 130.2, 133.7 (3*d*, arom. C); 135.2 (*s*, MeO*C*$_6$H$_4$), 135.3, 135.5 (2*d*, 2 C(6)); 144.1, 144.2 (2*s*, arom. C); 150.6, 150.8 (2*s*, 2 C(2)); 158.8 (*d*, MeO*C*$_6$H$_4$); 164.0, 164.1, 164.2 (3*s*, 2 C(4)); 166.0 (*s*, Ph*C*=O). ^{31}P-NMR ($CDCl_3$, 120 MHz): 31.9, 32.1. HR-FAB-MS: 979.3525 (1, $[M+H]^+$), 303 (229, (MeO)$_2$Tr$^+$).

*2′-Deoxy-*O^P*-ethyl-5′-*O*-(4,4′-dimethoxytrityl)thymidylylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine* (*(MeO)$_2$TrO-TOPOOEtT-OH*; **38**). To a soln. of **37** (294 mg, 0.30 mmol) in THF (1 ml) and EtOH (8 ml), 0.71M EtONa in EtOH (1.5 ml, 1.06 mmol) was added. The soln. was stirred at r.t. overnight (→white precipitate). The reaction was quenched with AcOH (60 µl, 1.05 mmol) in EtOH (1 ml) and Et_3N (30 µl), which resulted in the dissolution of all the precipitate. The soln. was evaporated and the residue purified by FC (CH_2Cl_2/MeOH 20:1 and 10:1; partial separation of the two diastereoisomers at the P-atom): **38** (230 mg, 87%). Colorless foam. TLC (CH_2Cl_2/MeOH 10:1): R_f 0.35. ^{1}H-NMR ($CDCl_3$, 300 MHz; diastereoisomerically pure sample): 1.32 (*t*, *J* = 7.0, *Me*CH_2O); 1.41 (*s*, Me–C(5)(T1)); 1.90 (*s*, Me–C(5)(T2)); 1.80–2.04 (*m*, 2 H–C(5′)(T2), CH_2–C(5′)(T2)); 2.11–2.22 (*m*, 1 H–C(2′)(T1)); 2.30–2.46 (*m*, 1 H–C(2′)(T1), 1 H–C(2′)(T2)); 2.50–2.55 (*m*, 1 H–C(2′)(T2)); 3.40 (*dd*, *J* = 8.5, 2.0, 1 H–C(5′)(T1)); 3.52 (*dd*, *J* = 8.5, 2.0, 1 H–C(5′)(T1)); 3.78 (*s*, (*Me*O)$_2$Tr); 3.78–3.86 (*m*, H–C(4′)(T2)); 4.02–4.24 (*m*, H–C(3′)(T2), MeCH_2O); 4.28–4.32 (*m*, H–C(4′)(T1)); 5.13–5.19 (*m*, H–C(3′)(T1)); 6.17 (*dd*, *J* = 6.6, 6.6, H–C(1′)(T1)); 6.44 (*dd*, *J* = 5.5, 8.6, H–C(1′)(T2)); 6.82–6.86 (*m*, 4 H (Tr)); 7.08 (*s*, H–C(6)(T2)); 7.22–7.32 (*m*, 7 H (Tr)); 7.35–7.39 (*m*, 2 H (Tr)); 7.58 (*s*, H–C(6)(T1)). ^{13}C-NMR ($CDCl_3$, 75 MHz; diastereoisomerically pure sample): 11.9, 12.8 (2*q*, 2 Me–C(5)); 16.6 (*q*, *J*(C,P) = 6, *Me*CH$_2$O); 22.5 (*t*, *J*(C,P) = 142, *C*H$_2$–C(5′)); 26.6 (*t*, C(5′)(T2)); 39.4, 39.7 (2*t*, 2 C(2′)); 55.4 (*q*, (*Me*O)$_2$Tr); 62.7 (*t*, *J*(C,P) = 6, MeCH$_2$O); 63.5 (*t*, C(5′)(T1)); 73.9 (*d*, C(3′)(T2)); 76.8 (*d*, C(3′)(T1)); 84.5, 84.9, 85.0, 85.0, 85.6, 85.8 (6*d*, 2 C(1′), 2 C(4′)); 87.3, (*s*, Ph*C*(Ar)$_2$); 111.4, 111.9 (2*s*, 2 C(5)); 113.4 (*d*, MeO*C*$_6$H$_4$); 127.4, 128.2, 130.2 (3*d*, arom. C); 135.2 (*s*, MeO*C*$_6$H$_4$), 135.4, 135.6 (2*d*, 2 C(6)); 144.2 (*s*, arom. C); 150.8, 151.1 (2*s*, 2 C(2)); 158.8 (*d*, MeO*C*$_6$H$_4$); 164.3, 164.4 (2*s*, 2 C(4)). ^{31}P-NMR ($CDCl_3$, 120 MHz; mixture of diastereoisomers): 32.3, 32.4. HR-FAB-MS: 875.3273 (2, $[M+H]^+$), 874 (2, M^+), 303 (100, (MeO)$_2$Tr$^+$).

*2′-Deoxy-*O^P*-ethyl-5′-*O*-(4,4′-dimethoxytrityl)thymidylylmethylene-(3′ → 5′)-2′,5′-dideoxythymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (*(MeO)$_2$TrO-TOPOOETT-OP(OCH$_2$CH$_2$CN)(N^{i}Pr$_2$)*; **39**). A soln. of **38** (320 mg, 360 µmol) and DMAP (16 mg, 130 µmol) in CH_2Cl_2 (6 ml) was cooled to 0°. ^{i}Pr$_2$EtN (250 µl, 1.44 mmol) was added, followed by 2-cyanoethyl diisopropylphosphoramidochloridite (161 µl, 720 µmol). The soln. was stirred for 75 min at 0°, diluted with CH_2Cl_2 (40 ml), and quickly washed with sat. aq. $NaHCO_3$ soln. (45 ml). The org. layer was dried (Na_2SO_4) and evaporated. The residue was redissolved in dry CH_2Cl_2 (2.5 ml)

and precipitated from hexane (40 ml) as described for **16**: **39** (310 mg, 80%). Colorless powder. TLC (AcOEt/MeOH 10:1): R_f 0.55. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.15–1.20 (*m*, 2 *Me*$_2$CH); 1.25–1.30 (*m*, *Me*CH$_2$O); 1.36–1.40 (*m*, Me–C(5)(T1)); 1.91 (*s*, Me–C(5)(T2)); 1.77–2.08 (*m*, 2 H–C(5′)(T2), CH$_2$–C(5′)(T2)); 2.16–2.48 (*m*, 2 H–C(2′)(T1), 1 H–C(2′)(T2)); 2.52–2.78 (*m*, 1 H–C(2′)(T2), CH$_2$CN); 3.33–3.67 (*m*, 2 H–C(5′)(T1), POCH$_2$, 2 Me$_2$C*H*); 3.79 (*s*, (*Me*O)$_2$Tr); 3.82–4.32 (*m*, H–C(4′)(T1), H–C(4′)(T2), H–C(3′)(T2), MeC*H*$_2$O); 5.17–5.26 (*m*, H–C(3′)(T1)); 6.12–6.21 (*m*, H–C(1′)(T1)); 6.46 (*dd*, *J* = 5.5, 8.2, H–C(1′)(T2)); 6.82–6.86 (*m*, 4 H (Tr)); 7.05–7.11 (*m*, H–C(6)(T2)); 7.22–7.40 (*m*, 9 H (Tr)); 7.55–7.60 (*m*, H–C(6)(T1)); 9.1 (br. *s*, 2 H–N(3)). ^{31}P-NMR ($CDCl_3$, 120 MHz mixture of diastereoisomers): 32.1, 32.3 (2 P), 32.5, 149.1, 149.2, 149.3, 149.4. HR-FAB-MS: 1075.437 (0.6, $[M+H]^+$), 303 (68, $(MeO)_2Tr^+$).

Synthesis, Purification, and Characterization of the Chimeras. The chimeras (*Tables 1* and *2*) were prepared from the modified dinucleotide phosphoramidites **16**, **17**, *2′-deoxy-3′-de(phosphinicooxy)-5′-*O*-(4,4′-dimethoxytrityl)thymidylylmethylene(ethoxyphosphinylidene)methylene-(3′ → 5′)-2′,5′-dideoxythymidine 3′-(2-cyanoethyl diisopropylphosphoramidite)* (**29**), N^6*-benzoyl-2′-deoxy-3′-de(phosphinicooxy)-5′-*O*-(4,4′-dimethoxytrityl)-adenylylmethylene(ethoxyphosphinylidene)methylene-(3′ → 5′)-2′,5′-dideoxythymidine 3′-(2-cyanoethyl diisopropylphosporamidite)* (**30**), and **39** and commercially available dA, dC, dG, and T phosphoramidites (*Glen Research*) and deoxynucleoside-CPG (1.3 μmol, *Glen Research*) with a *PerSeptive-Biosystems-Expedite™-8909* nucleic acid synthesis system by the standard 2-cyanoethyl diisopropylphosphoramidite method, with the coupling time for the modified dinucleosides extended to 20 min. The efficiency of the individual incorporations was monitored by a trityl detector. The chimeras ('trityl-off') were cleaved from the resin and deprotected by treatment with 1 ml of conc. aq. NH_3 at 55° overnight. The ethyl protective groups of the bis(methylene)phosphinates were removed by additional treatment of the chimeras with 1 ml of 2M aq. NaOH at r.t. overnight. The ethyl protective groups of the 5′-methylenephosphonates were removed by treatment of the chimeras with 1M 4-methoxybenzenethiole and ^{i}Pr$_2$EtN in dioxane/H_2O (10:1) at 55° for 40 h and subsequent extraction of the fully deprotected chimeras (AcOEt/H_2O). All crude chimeras as well as the natural oligonucleotides ('standard desalting' quality, *Integrated DNA Technologies*) were purified by prep. ion-exchange HPLC at pH 12 (*Dionex DNAPac™-PA-100* column (9 × 250 mm), *Waters PrepLC-4000* pump, *Waters 486-Tunable-Absorbance* detector, flow rate 2 ml/min; solvent *A* = 20 mM NaOH; solvent *B* = 20 mM NaOH, 1M NaCl; gradient *I:* 10 → 30% *B* in 2 min, then → 60% *B* in 30 min; gradient *II:* 10–38% *B* in 2 min, then → 60% *B* in 30 min.). The isolated oligonucleotides were desalted over *Sep-Pak®-Plus-C18* cartridges (*Waters*) following the manufacturer's protocol. The purity of the chimeras was confirmed by anal. ion-exchange HPLC (*Dionex-DNAPac™-PA-100* column (4 × 250 mm), *Waters Alliance* system, *Waters 996-PDA* detector, controlled by *Waters Millennium^{32}*™ software); same gradients as used for the purification, but flow rate 0.5 ml/min.

The integrity of the bis(methylene) sulfone chimeras was confirmed by MALDI-TOF-MS (*Voyager-DE-PRO*, pos. mode, 2,5-dihydroxybenzoic acid matrix), that of the other chimeras by enzymatic digestion. For the digestions, an aliquot of the chimera (3–5 μl, 0.2–1 nmol) was mixed with the same volume of enzyme mix (60 μl of buffer (100 mM *Tris* · HCl, 20 mM $MgCl_2$, pH 8.3), 4 μl of phosphodiesterase (*Crotalus durissus*, 2 mg/ml, *Boehringer Mannheim*); 1 μl of alkaline phosphatase (calf intestine, 1 U/μl, *Boehringer Mannheim*) and then incubated at 37° overnight. The reaction was quenched by the addition of aq. (Et_3NH)OAc buffer (50 mM; pH 7; 100 μl) and the whole mixture was analyzed by anal. reversed-phase HPLC (*Waters Nova-Pak®-C18* column (3.9 × 150 mm), *Waters Alliance* system, *Waters 996-PDA* detector, controlled by *Waters Millennium^{32}*™ software; flow rate 0.5 ml/min; solvent *A* = (Et_3NH)OAc (25 mM; pH 7), solvent *B* = 20% MeCN in solvent *A*; 5 min *A*, then → 11% *B* in 15 min (curve = 10), then → 17% *B* in 3 min, another 3 min at constant 17% *B*, then → 35% *B* in 8 min, and finally → 100% *B* in 11 min (all curves linear except the one mentioned)): t_R of dC 8, of dG 26, of T 27, of dA 33, of pO_3^{2-}T 24, of TpO_2^-T 41, and of TpooEtT (doublet) 50 min. The peaks were defined manually and integrated with the *Millennium* software. The peak areas were then divided by the respective extinction coefficients (ε [$M^{-1}cm^{-1}$]: dC 7300, dG 11700, T, and pO_3^{2-}T 8800, TpO_2^-T 17600, and dA 15400 [51]) and normalized to the most abundant monomer.

UV Melting Experiments. The UV melting curves were measured on a *Varian-Cary-Bio*-UV/VIS spectrophotometer equipped with a 6 × 6 multicell holder and a *Cary* temperature controller. The curves of the single strands were measured one sample at a time, at 260 nm with 1-ml samples at a strand concentration of 2 μM. The temp. was taken with an internal temp. probe. The oligonucleotides or chimeras were heated fast to 80° and then cooled once to 15° at a rate of 1°/min. The measurements of the duplexes were performed at 260 nm with 1 ml samples at a concentration of 2 μM per single strand. The salt concentrations were 100 mM ('low salt') or 1M ('high salt') NaCl, 10 mM Na_2HPO_4/NaH_2PO_4, 0.1 mM EDTA, pH 7.0. Each sample was heated fast to 85° and then four times cooled to 15° and reheated to 85°, at a rate of 0.5°/min. The temp. was taken as the temp. of the heating block (with no internal temp. probes in the cuvettes). The chimeras were measured in the

same experiment as their natural counterparts to allow for an exact comparison of the melting temps. These melting temps. were extracted from the melting curves by the *Cary OS/2* software provided with the instrument. For the analysis by the 'derivative' method, each heating or cooling ramp was individually smoothed by the *Savitzky-Golay* procedure [52] with filter 29 and interval 0.5 and the first derivative calculated on this new curve. The reported T_m is the average of these eight values. For the 'hyperchromicity' or '*van't Hoff*' method, the eight ramps were first, without smoothing, averaged to a single 'mean' from which the T_m was determined by the '*van't Hoff*' plot as described in the text (*Sect. 4*).

REFERENCES

[1] E. Uhlmann, A. Peyman, *Chem. Rev.* **1990**, *90*, 543; A. De Mesmaeker, R. Haener, P. Martin, H. E. Moser, *Acc. Chem. Res.* **1995**, *28*, 366; S. M. Freier, K.-H. Altmann, *Nucleic Acids Res.* **1997**, *25*, 4429.

[2] S. A. Benner, D. Hutter, *Bioorg. Chem.* **2002**, *30*, 62.

[3] A. L. Roughton, S. Portmann, S. A. Benner, M. Egli, *J. Am. Chem. Soc.* **1995**, *117*, 7249.

[4] R. O. Day, N. C. Seeman, J. M. Rosenberg, A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 849; J. M. Rosenberg, N. C. Seeman, R. O. Day, A. Rich, *J. Mol. Biol.* **1976**, *104*, 145.

[5] C. Richert, A. L. Roughton, S. A. Benner, *J. Am. Chem. Soc.* **1996**, *118*, 4518.

[6] Z. Huang, Ph.D. Thesis No. 10429, Swiss Federal Institute of Technology (ETH), Zürich, 1993; B. Eschgfaeller, Ph.D. Thesis No. 12582, Swiss Federal Institute of Technology (ETH), Zürich, 1998.

[7] M. O. Blaettler, Ph.D. Thesis No. 12109, Swiss Federal Institute of Technology (ETH), Zürich, 1997.

[8] M. O. Blaettler, C. Wenz, A. Pingoud, S. A. Benner, *J. Am. Chem. Soc.* **1998**, *120*, 2674.

[9] S. P. Collingwood, A. D. Baxter, *Synlett* **1995**, 703.

[10] Y. S. Sanghvi, R. Bharadwaj, F. Debart, A. De Mesmaeker, *Synthesis* **1994**, 1163; Y. S. Sanghvi, B. Ross, R. Bharadwaj, J.-J. Vasseur, *Tetrahedron Lett.* **1994**, *35*, 4697.

[11] D. K. Baeschlin, M. Daube, M. O. Blaettler, S. A. Benner, C. Richert, *Tetrahedron Lett.* **1996**, *37*, 1591.

[12] A. D. Baxter, E. K. Baylis, S. P. Collingwood, R. J. Taylor, A. De Mesmaeker, C. Schmit, Eur. Pat. 0 614 906 A1, 1994; A. D. Baxter, E. K. Baylis, S. P. Collingwood, R. J. Taylor, A. De Mesmaeker, C. Schmit, Eur. Pat. 0 614 907 A1, 1994.

[13] J. P. Horwitz, J. Chua, M. A. Da Rooge, M. Noel, I. L. Klundt, *J. Org. Chem.* **1966**, *31*, 205.

[14] M. J. Gallagher, H. Honegger, *Aust. J. Chem.* **1980**, *33*, 287.

[15] G. H. Jones, J. G. Moffatt, *J. Am. Chem. Soc.* **1968**, *90*, 5337; G. H. Jones, H. P. Albrecht, N. P. Damodaran, J. G. Moffatt, *J. Am. Chem. Soc.* **1970**, *92*, 5510.

[16] S. N. Mikhailov, N. S. Padyukova, M. Y. Karpeiskii, L. I. Kolobushkina, L. N. Beigelman, *Collect. Czech. Chem. Commun.* **1989,** *54*, 1055; M. Oivanen, S. N. Mikhailov, N. S. Padyukova, H. Loennberg, *J. Org. Chem.* **1993**, *58*, 1617; M. P. Boehringer, D. Graff, M. H. Caruthers, *Tetrahedron Lett.* **1993**, *34*, 2723.

[17] T. Szabo, A. Kers, J. Stawinski, *Nucleic Acids Res.* **1995**, *23*, 893; T. Szabo, J. Stawinksi, *Tetrahedron* **1995**, *51*, 4145; A. Kers, T. Szabo, J. Stawinski, *J. Chem. Soc., Perkin Trans. 1* **1999**, 2585.

[18] H. Tanaka, M. Fukui, K. Haraguchi, M. Masaki, T. Miyasaka, *Tetrahedron Lett.* **1989**, *30*, 2567.

[19] H. Krawczyk, *Synth. Commun.* **1997**, *27*, 3151.

[20] A. Andrus, S. L. Beaucage, *Tetrahedron Lett.* **1988**, *29*, 5479.

[21] G. S. Manning, *Q. Rev. Biophys.* **1978**, *11*, 179; M. T. Record Jr., C. F. Anderson, T. M. Lohman, *Q. Rev. Biophys.* **1978**, *11*, 103; J. P. Bond, C. F. Anderson, M. T. Record Jr., *Biophys. J.* **1994**, *67*, 825; C. F. Anderson, M. T. Record Jr., *Ann. Rev. Phys. Chem.* **1995**, *46*, 657.

[22] L. A. Marky, K. J. Breslauer, *Biopolymers* **1987**, *26*, 1601.

[23] I. Rouzina, V. A. Bloomfield, *Biophys. J.* **1999**, *77*, 3242; J. A. Holbrook, M. W. Capp, R. M. Saecker, M. T. Record Jr., *Biochemistry* **1999**, *38*, 8409; T. V. Chalikian, J. Voelker, G. E. Plum, K. J. Breslauer, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7853.

[24] G. E. Plum, K. J. Breslauer, *Curr. Opin. Struct. Biol.* **1995**, *5*, 682.

[25] G. Vesnaver, K. J. Breslauer, *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3569.

[26] U. Heinemann, L.-N. Rudolph, C. Alings, M. Morr, W. Heikens, R. Frank, H. Bloecker, *Nucleic Acids Res.* **1991**, *19*, 427.

[27] M. Egli, V. Tereshko, M. Teplova, G. Minasov, A. Joachimiak, R. Sanishvili, C. M. Weeks, R. Miller, M. A. Maier, H. An, P. D. Cook, M. Manoharan, *Biopolymers* **1998**, *48*, 234.

[28] B. Schneider, D. Cohen, H. M. Berman, *Biopolymers* **1992**, *32*, 725; B. Schneider, K. Patel, H. M. Berman, *Biophys. J.* **1998**, *75*, 2422.

[29] V. K. Rajwanshi, A. E. Hakansson, M. D. Sorensen, S. Pitsch, S. K. Singh, R. Kumar, P. Nielsen, J. Wengel, *Angew. Chem., Int. Ed.* **2000**, *39*, 1656; M. J. Damha, B. Meng, D. Wang, C. G. Yannopoulos, G. Just, *Nucleic Acids Res.* **1995**, *23*, 3967; K.-Y. Lin, J. S. Pudlo, R. J. Jones, N. Bischofberger, M. D. Matteucci, B. C. Froehler, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1061; J. S. Rice, X. Gao, *Biochemistry* **1997**, *36*, 399.
[30] S. P. Collingwood, M. E. Douglas, F. Natt, U. Pieles, *Phosphorus, Sulfur Silicon Relat. Elem.* **1999**, *144–146*, 645.
[31] H. R. Drew, R. E. Dickerson, *J. Mol. Biol.* **1981**, *151*, 535; M. L. Kopka, A. V. Fratini, H. R. Drew, R. E. Dickerson, *J. Mol. Biol.* **1983**, *163*, 129.
[32] M. K. Ghosh, K. Ghosh, O. Dahl, J. S. Cohen, *Nucleic Acids Res.* **1993**, *21*, 5761; J. W. Jaroszewski, V. Clausen, J. S. Cohen, O. Dahl, *Nucleic Acids Res.* **1996**, *24*, 829.
[33] S. V. Patil, R. B. Mane, M. M. Salunkhe, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2663; Y. Tamura, H. Miyoshi, T. Yokota, K. Makino, A. Murakami, *Nucleosides Nucleotides* **1998**, *17*, 269; M. Boczkowska, P. Guga, B. Karwowki, A. Maciaszek, *Biochemistry* **2000**, *39*, 11057.
[34] P. Luo, J. C. Leitzel, Z.-Y. J. Zhan, D. G. Lynn, *J. Am. Chem. Soc*. **1998**, *120*, 3019.
[35] D. R. Corey, *Trends Biotechnol.* **1997**, *15*, 224; A. Ray, B. Norden, *FASEB J.* **2000**, *14*, 1041.
[36] M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, P. E. Nielsen, *Nature (London)* **1993**, *365*, 566.
[37] T. Ratilainen, A. Holmen, E. Tuite, P. E. Nielsen, B. Norden, *Biochemistry* **2000**, *39*, 7781.
[38] S. V. Smulevitch, C. G. Simmons, J. C. Norton, T. W. Wise, D. R. Corey, *Nat. Biotechnol.* **1996**, *14*, 1700.
[39] V. V. Demidov, V. N. Potaman, M. D. Frank-Kamenetskii, M. Egholm, O. Buchardt, S. H. Sonnichsen, P. E. Nielsen, *Biochem. Pharmacol.* **1994**, *48*, 1310.
[40] H. J. Larson, T. Bentin, P. E. Nielsen, *Biochim. Biophys. Acta* **1999**, *1489*, 159.
[41] D. F. Doyle, D. A. Braasch, C. G. Simmons, B. A. Janowski, D. R. Corey, *Biochemistry* **2001**, *40*, 53.
[42] J. G. Schmidt, L. Christensen, P. E. Nielsen, L. E. Orgel, *Nucleic Acids Res.* **1997**, *25*, 4792.
[43] M. Egholm, O. Buchardt, P. E. Nielsen, R. H. Berg, *J. Am. Chem. Soc.* **1992**, *114*, 1895.
[44] K. L. Dueholm, K. H. Peterson, D. K. Jensen, P. E. Nielsen, M. Egholm, O. Buchardt, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1077.
[45] F. Bergmann, W. Bannwarth, S. Tam, *Tetrahedron Lett.* **1995**, *36*, 6823; B. D. Gildea, S. Casey, J. MacNeill, H. Perry-O'Keefe, D. Sorensen, J. M. Coull, *Tetrahedron Lett.* **1998,** *39*, 7255.
[46] B. P. Gangamani, V. A. Kumar, K. N. Ganesh, *Biochem. Biophys. Res. Commun.* **1997**, *240*, 778.
[47] A. Blasko, R. O. Dempcy, E. E. Minyat, T. C. Bruice, *J. Am. Chem. Soc.* **1996**, *118*, 7892; D. P. Arya, T. C. Bruice, *J. Am. Chem. Soc*. **1998**, *120*, 12419; B. A. Linkletter, I. E. Szabo, T. C. Bruice, *Nucleic Acids Res.* **2001**, *29*, 2370.
[48] T. J. Caulfield, C. V. C. Prasad, C. P. Prouty, A. K. Saha, M. P. Sardaro, W. C. Schairer, A. Yawman, D. A. Upson, L. I. Kruse, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2771; C. V. C. Prasad, T. J. Caulfield, C. P. Prouty, A. K. Saha, W. C. Schairer, A. Yawman, D. A. Upson, L. I. Kruse, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 411.
[49] D. A. Dougherty, *Enantiomer* **2001**, 101.
[50] F. H. Westheimer, *Science (Washington, D.C.)* **1987**, *235*, 1173.
[51] F. Seela, F. Lampe, *Helv. Chim. Acta* **1991**, *74*, 1790.
[52] A. Savitzky, M. J. E. Golay, *Anal. Chem.* **1964**, *36*, 1627.

Received May 6, 2002