

Synthesis and Properties of Oligodeoxynucleotide Analogs with Bis(methylene) Sulfone Bridges

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A convergent, solution-phase synthesis was developed for the bis(methylene) sulfone-bridged oligodeoxynucleotide analogs (SNA) 5'-d(HOCH₂-Tso₂Tso₂Tso₂Cso₂Tso₂Tso₂Tso₂T-CH₂SO₃⁻)-3' (**35b**) and 5'-d(HOCH₂-Tso₂Tso₂Tso₂Tso₂Tso₂Tso₂Tso₂T-CH₂SO₃⁻)-3' (**34c**) (SO₂ corresponds to CH₂SO₂CH₂ instead of OP(=O)(O⁻)(O)). In these, the phosphodiester linkages are replaced by non-ionic bis(methylene) sulfone linkers. The general strategy involved convergent coupling of 3',5'-bishomo-β-D-deoxyribonucleotide analogs functionalized at the 6'-end (=CH₂-C(5')) as bromides or mesylates and at the CH₂-C(3') position as thiols, with the resulting thioether being oxidized to the corresponding sulfone. A single charge was introduced at the terminal CH₂-C(3') position of the octamers to increase their solubility in water. During the synthesis, it became apparent that the key intermediates generated secondary structures through either folding or aggregation in a variety of solvents. This generated unusual reactivity and was unique for very similar structures. For example, although the dimeric thiol d(BzOCH₂-Tso₂C-CH₂SH) (**14b**) was a well-behaved synthetic intermediate, the tetrameric thiol d(TrOCH₂-Tso₂Tso₂Tso₂C-CH₂SH) derived from the corresponding thioacetate was rapidly converted to a disulfide by very small amounts of oxidant (**28** → **29**, Scheme 6), while the analogous tetrameric thiol d(BzOCH₂-Tso₂TsTso₂T-CH₂SH) (**26**), differing only by a single heterocycle, was oxidized much more slowly (Bz = PhCO, Tr = Ph₃C, to = 2-MeC₆H₄CO (at N⁴ of dc)). The sequence-dependent reactivity, well known in many classes of natural products (including polypeptides), is not prominent in natural oligonucleotides. These results are discussed in light of the proposal that the repeating negative charge in nucleic acids is key to their ability to serve as genetic molecules, in particular, their capability to support Darwinian evolution. The ability of 5'-d(HOCH₂-Tso₂Tso₂Tso₂Cso₂Tso₂Tso₂T-CH₂SO₃⁻)-3' (**35b**) to bind as a third strand to duplex DNA was also examined. No triple-helix-forming propensity was detected in this molecule.

Introduction. – A considerable amount of work has been devoted over the past few years to analyze the 'chemical etiology' of nucleic acids [1]. To this end, effort has been devoted towards chemical synthesis of alternative forms of nucleic acids. This work has inquired what structures might support the rule-based molecular recognition required for genetics, and how genetic molecules might appear if they emerged elsewhere in the cosmos, independent of life on Earth [2][3]. Considerable progress has been made in understanding the degree to which the heterocyclic nucleobases can tolerate modification [4–8], and an entirely new genetic alphabet has been created, shown to expand the number of amino acids that can be encoded in proteins [9], and incorporated into commercial diagnostics and drug-discovery products [10]. Likewise, the sugar units of DNA have been modified in DNA analogs [11–14], as have the phosphate linkages [15][16]. Some strikingly attractive analogs of DNA have emerged that appear to support rule-based molecular recognition quite well [17][18].

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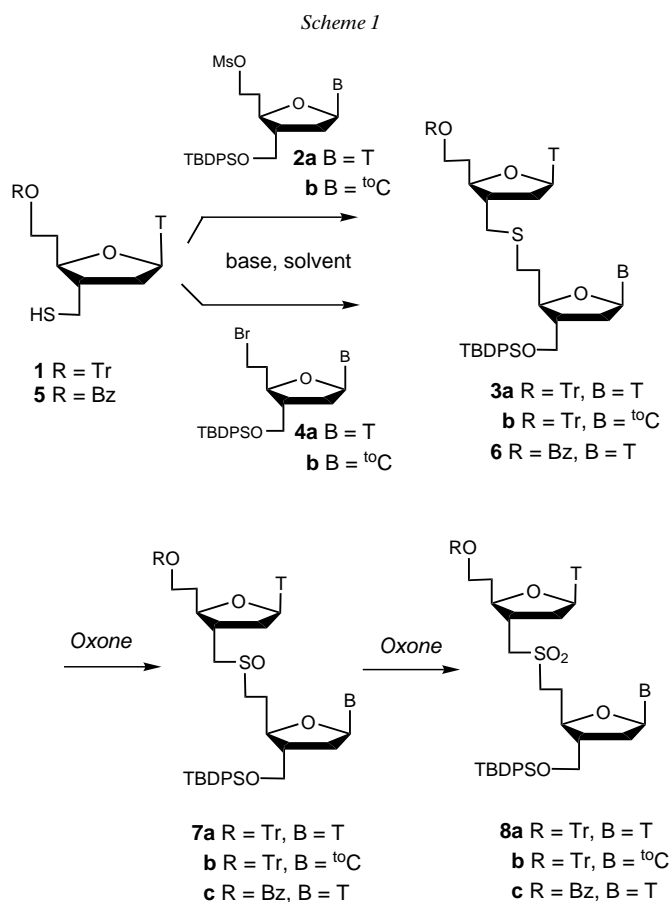
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The interaction between the phosphate, sugar, and nucleobase pieces in DNA is, of course, a key to the etiology of DNA, as it is with other classes of molecules. The emerging view holds that the backbone plays a more-important role in the process of molecular recognition than was explicitly incorporated into the *Watson-Crick* model [19][20]. In part, this view was based on oligonucleotide analogs in which the bridging phosphodiester groups were replaced by non-ionic groups, including phosphonates [21], amide backbones [21], and bis(methylene) sulfones [22][23]. The last are sulfone-linked oligonucleotide analogs (SNAs) that are isoelectronic, largely isosteric, chemically stable, and contain no stereocenters. A radiolabeled tetrameric rSNA showed remarkably good bioavailability in a mouse study [24], and a short sulfone-linked RNA dinucleotide analog formed a *Watson-Crick* duplex in a crystal [25]. Longer sulfone-linked RNA analogs displayed rich conformational properties, however, far broader than those allowed by simple *Watson-Crick* rules [26].

While the conformation of non-ionic analogs of nucleic acids has proven to be remarkably complex, certain areas remain where non-ionic oligonucleotide analogs might be useful. For example, triple helices have a high charge density; they may be more easily formed by non-ionic nucleic acid analogs. Further, the degree to which physical and chemical properties of large molecules are changed by small changes in sequences, first suggested in an SNA matrix, needs to be further explored. Given the availability of substantial amounts of building blocks (see the preceding paper [27]), we have explored in detail the synthesis of octameric dSNAs and tested their molecular-recognition properties. The details of the synthesis provide insights into the change in reactivity as one proceeds from small molecules to large molecules.

Results. – *Synthesis of SNAs.* The general strategy for the synthesis of SNAs in solution involves the formation of a thioether *via* the reaction of a thiolate of one unit with a C-atom carrying a leaving group at the other unit. The resulting thioether is then oxidized to yield the sulfone. The synthesis is convergent, with monomers yielding dimers, dimers yielding tetramers, and tetramers yielding octamers [28].

Two leaving groups at the 6'-position ($=\text{CH}_2-\text{C}(5')$), mesylate and bromide, were examined in the formation of dinucleotide analogs. In trial runs, 6'-*O*-trityl(Tr)-protected thiol **1** and **2b** carrying a 6'-mexyloxy group were coupled in degassed DMF in the presence of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) at room temperature to yield the dinucleotide analog **3b** in 80% yield after chromatography (*Scheme 1*). The analogous reaction in THF as solvent gave **3b** in 72% yield. With 6'-bromo derivative **4b** as the electrophile in the presence of Cs_2CO_3 as the base in degassed DMF at room temperature, **3b** was obtained in 83% yield after chromatography. Similar results were achieved in coupling reactions of mesylate **2a** and thiol **1** in the presence of Cs_2CO_3 in DMF at 45° (82% yield of **3a**), as well as of bromide **4a** and thiol **1** in the presence of Cs_2CO_3 in THF at 45° (84% yield of **3a**). Modestly higher yields were obtained when Cs_2CO_3 was used as base, even though Cs_2CO_3 did not dissolve completely in the organic solvents [29]. Coupling was slower in THF than in DMF. This became especially noticeable for the coupling of dimeric and tetrameric SNAs to give tetramers and octamers, respectively. *Richert* performed analogous reactions in MeCN/H₂O mixtures, which enabled the complete solubilization of Cs_2CO_3 but led to up to six times longer reaction times [30]. Following optimization, conditions for coupling 6'-*O*-



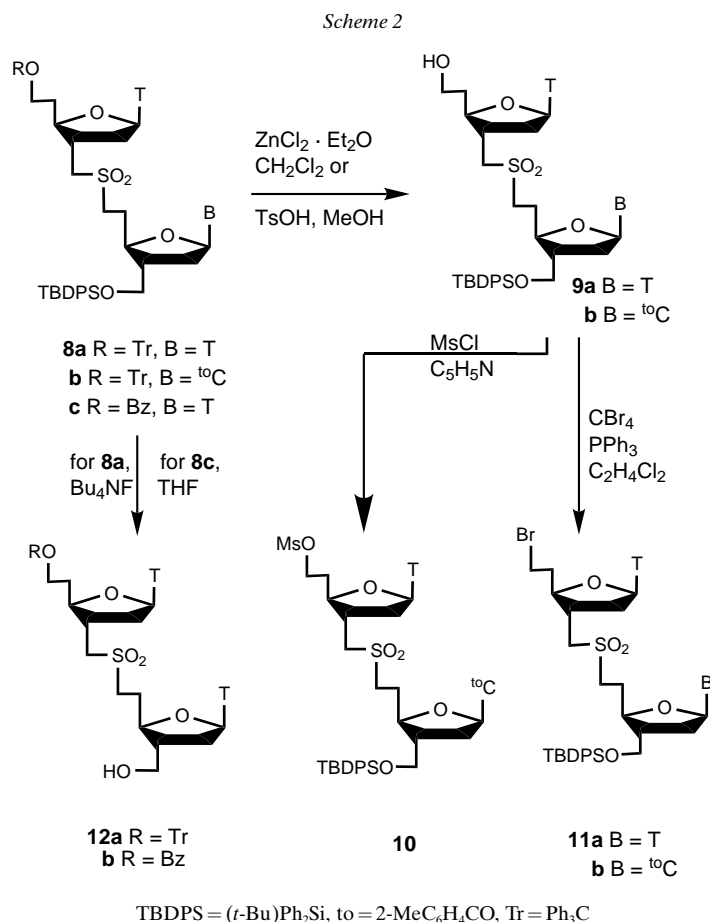
TBDPS = (*t*-Bu)₂Ph₂Si, ^{to} = 2-MeC₆H₄CO, Tr = Ph₃C

benzoyl(Bz)-protected thiol **5** and bromide **4a** with Cs₂CO₃ in degassed DMF at 50° over 4 h generated **6** in 99% yield.

Oxone, a 2 KHSO₄·K₂SO₄·KHSO₄ triple salt, converts sulfides to sulfones selectively [31]. To avoid detritylation and depurination, oxidations of sulfides **3a,b** and **6** were performed in THF/MeOH/H₂O mixtures buffered with NaOAc. Under these conditions, the sulfides were oxidized within few minutes to the mixtures **7a–c** of diastereoisomeric sulfoxides, which were not separated (*Scheme 1*). Subsequent oxidation converted the sulfoxides **7a–c** to the corresponding sulfones **8a–c**. Under optimized oxidation conditions, **8b** and **8c** were obtained in essentially quantitative yields, and **8a** in 96% yield.

The Tr group was removed from dimers **8a** and **8b** in view of their transformation to become electrophiles for the next cycle of coupling. Initially, TsOH in MeOH was used to deprotect the dimer, *e.g.*, **8b**; the reaction mixture was neutralized with NaHCO₃ before direct chromatography of the crude product to yield **9b** in 91% yield

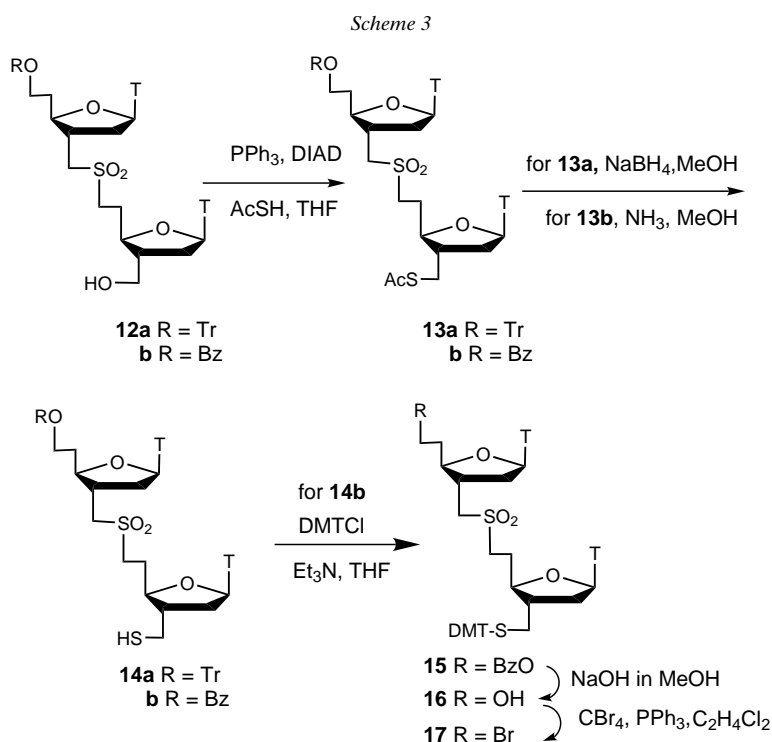
(Scheme 2). This method generated a small amount of by-product after prolonged reaction times. Cleavage with mild *Lewis* acids was, therefore, examined. For example, **8b** was dissolved in CH_2Cl_2 and treated with 5 equiv. of $\text{ZnCl}_2 \cdot \text{Et}_2\text{O}$ solution (Scheme 2). The resulting turbid, yellow suspension was filtered through a layer of silica gel after 10 min, and **9b** was obtained in quantitative yield after chromatography. Likewise, **8a** was deprotected with 10 equiv. of $\text{ZnCl}_2 \cdot \text{Et}_2\text{O}$ solution to give **9a** in 99% yield.



For the next step of a convergent synthesis of SNAs, a leaving group was required at the 6'-end of the dimers. Preliminary work suggested that better coupling yields might be obtained if the electrophile were a mesylate rather than a bromide. But mesylation of **9b** in pyridine generated **10** in only 47% yield (Scheme 2). However, bromination of **9b** in 1,2-dichloroethane with CBr_4 and PPh_3 first at 0° and then at room temperature (75 min) gave, after workup and chromatography, **11b** in 86% yield. Compound **9a** was similarly converted to bromide **11a** in 97% yield. Removal of the (*t*-Bu) Ph_2Si group at $\text{OCH}_2\text{-C}(3')$ was also necessary to support convergent synthesis of SNAs. This proved

to be readily done with Bu_4NF in THF. Under these conditions, compounds **8a** and **8c** gave **12a** (93%) and **12c** (91%), respectively, after chromatography. Loss of the benzoyl group in the deprotection of **8c** was not detected.

The *Mitsunobu* reaction was used to introduce the S-atom into the dimers to generate the nucleophile for higher-order couplings. Thioacetates **13a,b** were prepared from **12a,b** with PPh_3 , diisopropyl azodicarboxylate (DIAD), and thioacetic acid in anhydrous THF in 91 and 99% yield, respectively, after chromatography (Scheme 3). Thioacetate **13a** was then converted to thiol **14a**, either by treatment with NaBH_4 in degassed MeOH or by ammonolysis in degassed MeOH in quantitative yield. Treatment of **13b** with NaBH_4 led to the loss of the 6'-O-benzoyl group; therefore, ammonolysis was used to generate **14b**, in quantitative yield. Disulfide was observed neither during the reaction steps nor during the purification, a result that was to become significant as the convergent synthesis proceeded.



Tr = Ph_3C , DIAD = diisopropyl azodicarboxylate, DMT = $(\text{MeO})_2\text{Tr} = (4\text{-MeOC}_6\text{H}_4)_2\text{PhC}$

These optimized conditions for preparing dimeric SNAs became less satisfactory in the case of longer-SNA synthesis. In previous work, *Huang* synthesized an all-sulfide-linked octamer and attempted to oxidize the oligomer in a single step at the end of the synthesis [28]. The oxidation was very slow and gave only poor yields; this was attributed to the poor solubility of the intermediate, but the observation could not then be examined in greater detail due to lack of sufficient starting material [28].

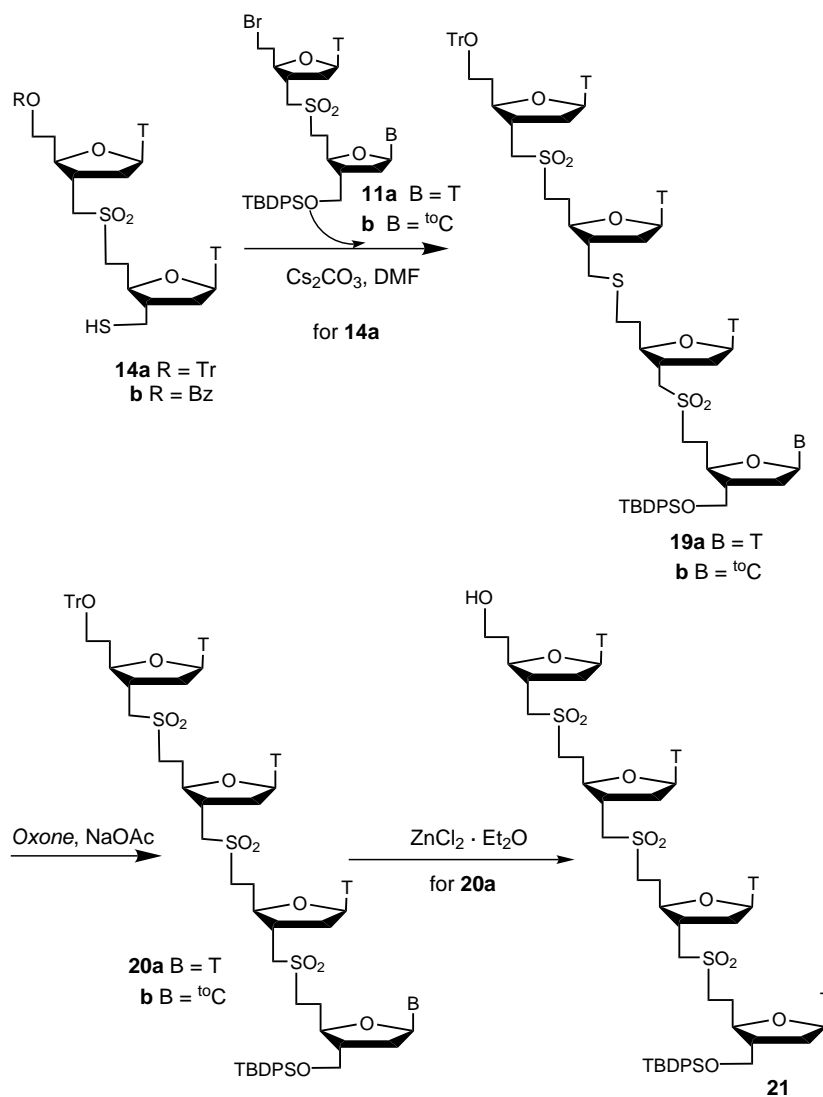
Aggregation also appeared to be more severe in oligomers with sulfide-containing linkers than with sulfoxide- or sulfone-containing linkers. For this reason, *Richert* oxidized thioethers immediately after every coupling step with *Oxone* [30]. *Blättler* and *König* also chose this strategy for the synthesis of their dSNAs and rSNAs, respectively [32][33]. One way to manage solubility in longer oligonucleotide analogs is to introduce an anionic group at the end of the oligomer. *Huang*, e.g., treated a $(\text{MeO})_2\text{TrSCH}_2\text{-C}(3')$ thioether with *Oxone* to generate a terminal $\text{KOSO}_2\text{CH}_2\text{-C}(3')$ group, and found its solubility to be enhanced. *König* introduced such a sulfonate group at the 6'-position by analogous oxidation of a $\text{TrSCH}_2\text{-C}(5')$ thioether [33]. Thus, a $(\text{MeO})_2\text{Tr}$ thioether group was introduced strategically at an early stage in the present synthesis, with the intent of oxidizing it to a sulfonate with *Oxone* at the end of the synthesis. Accordingly, the mercapto group CH_2SH at C(3') of **14b** was protected with $(\text{MeO})_2\text{TrCl}$ and Et_3N in anh. THF to give **15** in 99% yield after chromatography (*Scheme 3*). The overall yield for the three steps from **12b** to **15** was 98%. The benzoyl protection of **15** was removed with 2M NaOH in MeOH to give **16** (98%), which was treated with PPh_3 and CBr_4 in 1,2-dichloroethane to rapidly yield 6'-bromo derivative **17** (97%). A trace of $(\text{MeO})_2\text{Tr}$ -cleavage product was observed under these conditions.

To couple dimers to give tetramers, Cs_2CO_3 and DMF were used under a set of optimized conditions. Coupling **14a** and **11a** at room temperature gave tetramer **19a** (85%), and coupling **14a** and **11b** at 45° gave tetramer **19b** (81%) (*Scheme 4*). Coupling **14b** and **17** at 45° gave tetramer **18** (92%) (*Scheme 5*). In general, the coupling at 40–50° seemed preferable. The tetrameric monosulfide derivatives **19a** and **19b** were oxidized with *Oxone* in MeOH/THF 2:1 in the presence of NaOAc to give the tetrameric trisulfone derivatives **20a** (92%) and **20b** (90%) (*Scheme 4*). The thioether linkage in **18** could not be selectively oxidized to the corresponding sulfone without also oxidatively cleaving the $(\text{MeO})_2\text{Tr}$ thioether at the 3'-end. Compound **18**, therefore, entered subsequent coupling reactions as the mixed sulfoxide-sulfide derivative. The 6'-*O*-trityl group of **20a** was cleaved with $\text{ZnCl}_2 \cdot \text{Et}_2\text{O}$ solution in CH_2Cl_2 within 10 min to yield **21** in quantitative yield after chromatography (*Scheme 4*). Removal of the 6'-*O*-benzoyl group of **18** was achieved by hydrolysis with 2M NaOH in MeOH/THF 3:1 for 30 min to yield **22** in 96% yield after chromatography (*Scheme 5*).

As the SNA intermediates became longer, issues relating to purification were revisited. For monomers and oligomers, flash chromatography (FC) on silica gel with a stepwise gradient 0% → 20% MeOH/ CH_2Cl_2 sufficed; for dimers and tetramers, addition of 0.25% H_2O gave improved separations (*cf. Sect. 9* in the *Exper. Part*). For 3',5'-deprotected tetramers, MeOH (2.5–5%) and H_2O (0.25%) in the starting solvent were essential to obtain good separation. With pure CH_2Cl_2 , the SNAs eluted from the silica gel only in a broad band.

The 6'-OH compounds **21** and **22** were activated by introduction of a bromo substituent as leaving group (*Scheme 5*). The small scale of the bromination reaction was problematic, given that traces of H_2O would consume the reagents, while excess reagent generated side reactions. This problem was circumvented by addition of a secondary educt by *Richert*, who added greater than stoichiometric amounts of a 6'-OH uridine analog to his tetramer to enhance the absolute alcohol concentration in the bromination reaction (turnover rate 80%, yielding 60% brominated monomer and

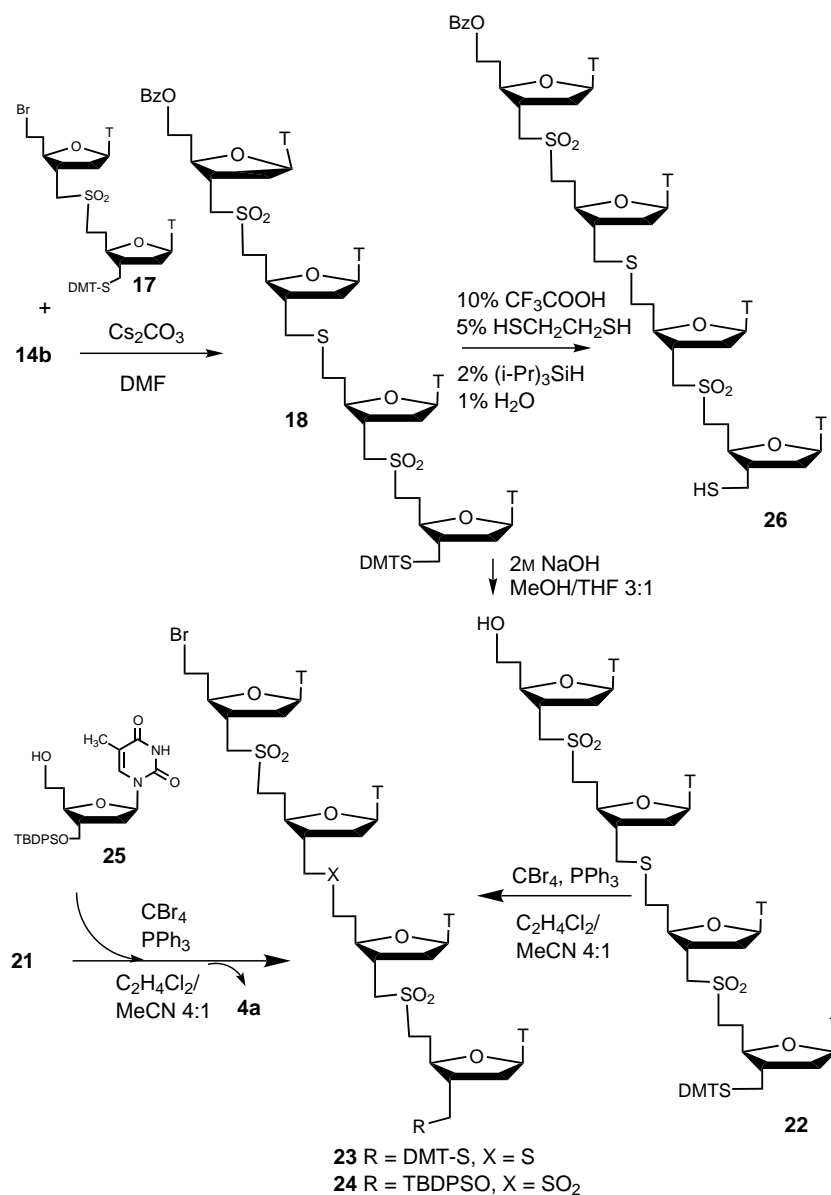
Scheme 4



Tr = Ph₃C, ^{to} = 2-MeC₆H₄ CO, TBDPS = (*t*-Bu)Ph₂Si

40% brominated tetramer [30]). Following this strategy, tetramer **21** and monomer **25** (compound **11** of the preceding paper [27]) were brominated with PPh₃ and CBr₄ in 1,2-dichloroethane/MeCN 4:1 (Scheme 5). TLC Monitoring showed the complete turnover of starting material after 90 min. By-products were not detected. The crude products were purified by FC (silica gel) to give tetramer **24** in 91% yield and monomeric bromide **4a** in 96% yield. The 6'-OH compound **22** was treated with PPh₃ and CBr₄ in 1,2-dichloroethane/MeCN 4:1 to yield the corresponding bromo derivative

Scheme 5



DMT = (MeO)₂Tr = (4-MeOC₆H₄)₂PhC, TBDPS = (*t*-Bu)Ph₂Si

23 in 74% yield (95% based on recovered starting material); as with dimer **16**, small amounts of detritylation product were observed (Scheme 5).

Huang described the cleavage of (MeO)₂Tr thioethers with silver nitrate in H₂O/MeOH/THF and treatment of the remaining silver thiolate with dithioerythrol (DTE)

to obtain the deprotected thiol [28]. This reaction was generally low yielding (< 50%). *Huang* was not able to use acidic cleavage due to the presence of a $(\text{MeO})_2\text{Tr}$ ether at the 6'-end of his building blocks [28]. The use of the 6'-*O*-benzoyl protecting group in this work (see **18**) makes acidic cleavage strategically acceptable. Thus, the $(\text{MeO})_2\text{Tr-S}$ bond was cleaved in tetrameric **18** to yield the corresponding thiol **26** with 10% CF_3COOH , 5% ethane-1,2-dithiol, 2% triisopropylsilane [34], and 1% H_2O as scavengers in CH_2Cl_2 for 5 min (*Scheme 5*). Starting material **18** (6% yield) and thiol **26** (80% yield; 85% based on recovered starting material) were obtained after chromatography. The formation of the disulfide of **26** was not problematic.

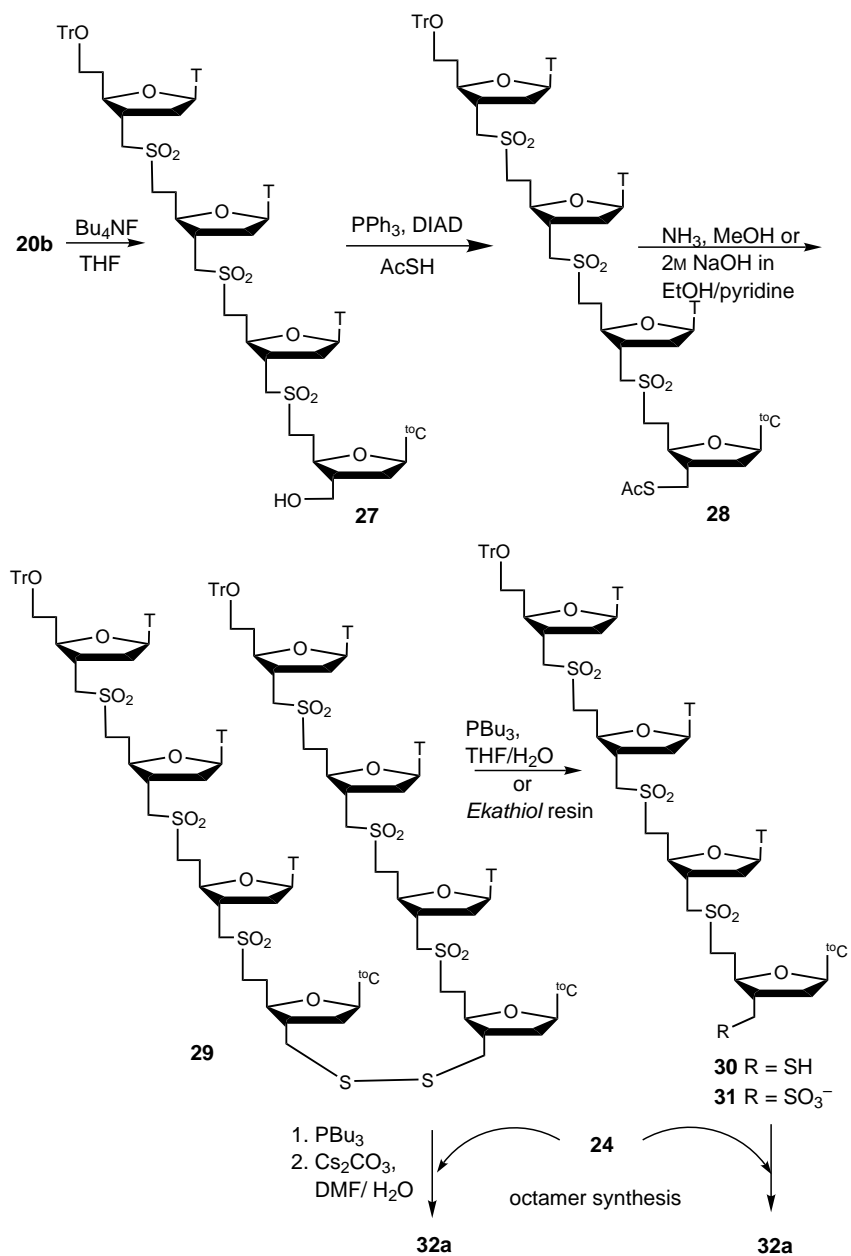
A second nucleophilic tetramer was created by the removal of the silyl protecting group from **20b** (*Scheme 6*). Buffering the Bu_4NF solution with AcOH to pH 5–6 prevented the loss of the N^4 -(*o*-toluoyl) protecting group [35]. The (*t*-Bu) Ph_2Si group of **20b** was, thus, cleaved in THF within 2 h to give **27** in 93% yield. Compound **27** with a CH_2OH group at C(3') was converted to the corresponding thioacetate on a small scale with a secondary educt to increase the overall concentration, in analogy to *Richert's* mixed bromination procedure [30]. Reaction with PPh_3 , diisopropyl azodicarboxylate (DIAD), and thioacetic acid gave **28** (94% yield) as a colorless foam after chromatography.

Treatment of **28** with either ammonia or 2M NaOH in pyridine/EtOH generated disulfide **29** (*Scheme 6*) as the sole product, even though all solutions were degassed, first with Ar (1 h), and then by repeated freeze-thaw cycles. No thiol was detectable by TLC at any stage during the reaction. Even though thiols in basic media are known to be sensitive to oxidation, the result was surprising, as the expected thiol was structurally quite similar to the tetrameric thiol **26** that was prepared in over 80% yield by closely analogous procedures without yielding disulfide. To further investigate the sequence-dependence of disulfide formation, tetramer thiol **26** was dissolved in degassed MeOH, and treated either with gaseous ammonia or 2M NaOH. In both cases, the corresponding disulfide could not be detected. The only difference between **26** and **30** was that the latter had an N^4 -(*o*-toluoyl)cytosine replacing a thymine moiety at the analogous position in the sequence, and the 6'-*O*-protecting groups were benzoyl and trityl respectively. In natural DNA, such differences would not change reactivity dramatically; here they did.

Disulfide **29** was reduced to the corresponding thiol **30** with PBU_3 in degassed THF/ H_2O [33][36–38] (*Scheme 6*). Attempts to purify **30** by chromatography on silica gel yielded disulfide **29** as the single product, the oxidation presumably occurring during the purification step. Efforts to obtain the stable thiol from **29** were pursued further. *Ekathiol*, a resin carrying immobilized dithiothreitol, reduces disulfides in aqueous or organic solvents under neutral, mildly acidic, or mildly basic conditions when present in stoichiometric excess. Purification is achieved by simply separating the solution from the resin. Disulfide **29** and 10 equiv. of *Ekathiol* resin were suspended in degassed THF and shaken for 4 h at room temperature. After filtration and evaporation at 0° , **30** was isolated in essentially quantitative yield.

The instability of **30** with respect to the formation of disulfide proved an obstacle to the synthesis of octamers from tetramers. The first attempts to couple the tetramers **24** and **30** in Cs_2CO_3 in DMF at room temperature followed by oxidation with *Oxone* generated only tetrameric sulfonate **31** resulting from oxidation of disulfide **29**

Scheme 6



Tr = Ph₃C, to = 2-MeC₆H₄CO, DIAD = diisopropyl azodicarboxylate

(Scheme 6). A similar result was achieved when disulfide **29** was reduced with *Ekathiol* resin in degassed DMF, even when the solution was passed with filtration directly under Ar into a mixture of **24** and Cs_2CO_3 , further degassed with multiple freeze-pump cycles, and stirred at 45° overnight. *Huang*, for $\text{d}(\text{UsUsUsUsUsUsUsU})$ [28], and *Richert*, for $\text{r}(\text{AsO}_2\text{UsO}_2\text{GsO}_2\text{GsUsO}_2\text{CsO}_2\text{AsO}_2\text{U})$ [30], had also observed that the coupling of the functionalized tetrameric SNA to yield the corresponding octamers proved to be more difficult than the coupling of smaller fragments. *Huang* performed his coupling reaction with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in THF/MeOH 10:1, in 20–30% yield. The addition of MeOH was necessary in this case because the precursor tetrameric thiolate precipitated in THF. *Richert* reported very long reaction times with considerable formation of by-products in the coupling reaction when MeCN/H₂O or THF was used as solvent. Disulfide was detected as well, and the overall coupling yield was only 50–70%.

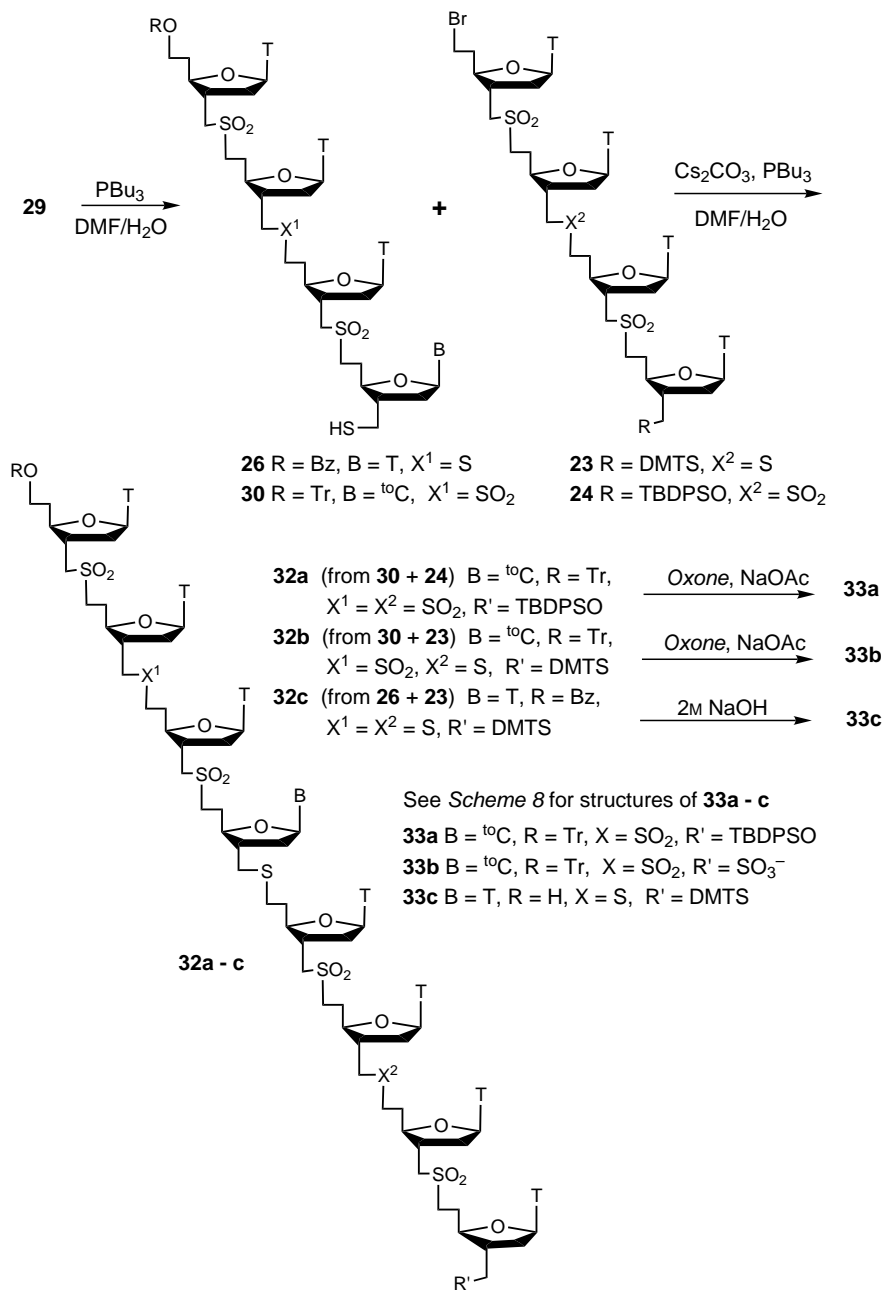
In the next coupling attempt (Scheme 7), disulfide **29** was dissolved in degassed DMF/H₂O 5:1, PBU_3 was added, and the mixture was stirred for 2 h. The solution containing thiol **30** was transferred *via* capillary under Ar pressure to a solution of bromide **24** and Cs_2CO_3 in DMF/H₂O. The reaction mixture was again degassed with multiple freeze-pump cycles and stirred for 6 h at 45° . Crude **32a** was directly oxidized with *Oxone* and NaOAc in MeOH/THF/H₂O 4:2:1 for 12 h to the corresponding sulfone **33a**. **33a** was analyzed by HPLC and the molecular mass of the octamer was confirmed by MALDI-TOF MS. As previously reported for other octameric SNAs by *Huang* and *Richert*, the HPLC trace for **33a** changed depending on the amount of injected SNA, resulting in either a single peak for low concentrations or a twin peak for higher concentrations.

To prepare the analogous octamer carrying a CH_2SO_3^- group at the 3'-end, disulfide **29** was again reduced with PBU_3 in DMF/H₂O 5:1 prior to the coupling and the resulting **30** directly added *via* capillary transfer to bromide **23** and Cs_2CO_3 (Scheme 7). After multiple freeze-pump cycles, the mixture was stirred at 45° overnight. The crude product **32b** was immediately oxidized with *Oxone* and NaOAc. TLC Monitoring showed the complete oxidation of the terminal $(\text{MeO})_2\text{Tr}$ thioether to sulfonate after 1 h. HPLC Analysis and MALDI-TOF MS showed the formation of the singly charged octamer **33b**.

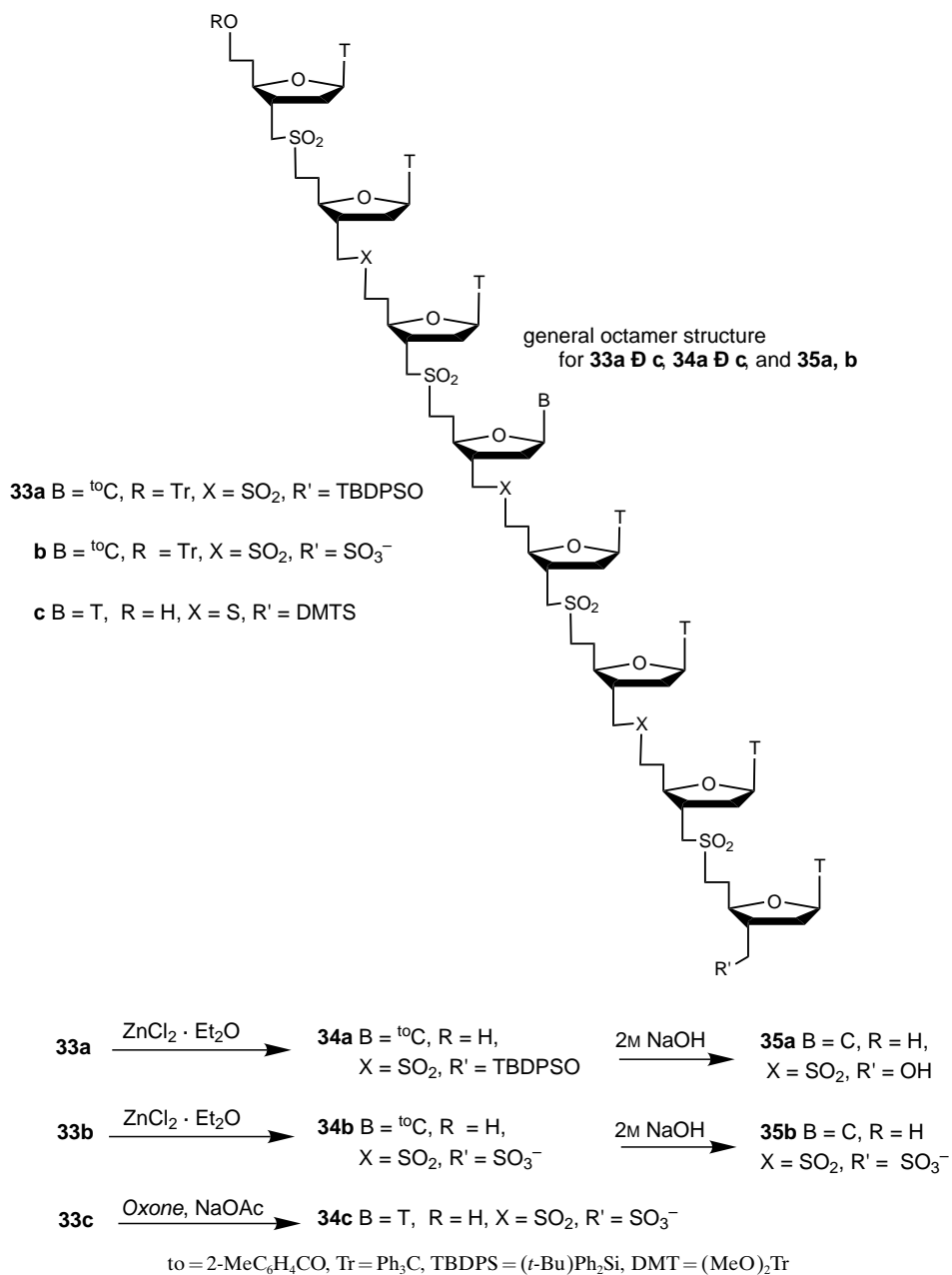
In contrast to the synthesis of **32a** and **32b**, octamers containing only thymine as the nucleobase could be prepared without *in situ* reduction of the disulfide. Thiol **26**, bromide **23**, and Cs_2CO_3 were mixed in degassed DMF at 45° for 7 h and worked up as the octamers above. No disulfide formation was observed. Crude **32c** was first debenzoylated at the 6'-end with 2M NaOH in MeOH/THF/H₂O to give **33c** (Scheme 7). The starting material was surprisingly difficult to dissolve in aqueous base, even though the thymidine moieties should be deprotonated. The product mixture was neutralized, and desalted with *SepPak-C₁₈* cartridges. Oxidation of **33c** with *Oxone* also proved to be difficult; after 12 h at room temperature, MALDI-TOF MS showed that the octamer was not completely oxidized, requiring the oxidation procedure to be extended by treatment with additional reagent for 24 h. The fully oxidized, singly charged, and deprotected all-dT octamer **34c** was then purified by HPLC (Scheme 8).

Octamer **33a** was detritylated with 2.2M $\text{ZnCl}_2 \cdot \text{Et}_2\text{O}$ solution in CH_2Cl_2 to give **34a** (Scheme 8). HPLC Analysis and MALDI-TOF MS showed that *ca.* 80% was

Scheme 7



Scheme 8



detritylated and 20% remained protected, even after prolonged and repeated treatment under the acidic conditions. Crude **34a** was then treated with 2M NaOH in MeOH/THF/H₂O at 45° for 18 h to give **35a**. The deprotection was monitored by

HPLC; no considerable by-products were detected. The mixture was buffered with acetate and desalted thrice with *SepPak-C₁₈* cartridges followed by purification by HPLC.

Octamer **33b** was deprotected via **34b** as described for **33a**, or treated first with 2M NaOH in MeOH/THF/H₂O at 40° for 20 h, followed by detritylation with TsOH · H₂O in MeOH to yield **35b**. The trityl cleavage with TsOH · H₂O was monitored by HPLC. After *ca.* 3 h, the formation of a less polar by-product was detected, and after 5.5 h, *ca.* 90% octamer was deprotected, 5% remained protected, and 5% by-product was formed. The reaction mixture was neutralized and desalted five times with a *SepPak-C₁₈* cartridge. In test reactions, elongated reaction times resulted in up to 50% by-product formation. The crude deprotected, singly charged octamer **35b** was purified by HPLC.

Fig. 1 shows an HPLC profile of purified, deprotected all-dT octamer sulfonate **34c**. The molecules **34c** and **35a,b** were further characterized by mass spectrometry. For molecules of this size at the boundary between classical organic and biomolecules, standard analytical methods fail to provide data having the same precision as obtained for precursors to the octamer (see *Sect. 9* in the *Exper. Part*). Nevertheless, the samples were sufficiently well characterized to permit them to serve as the starting point for biophysics-type experiments.

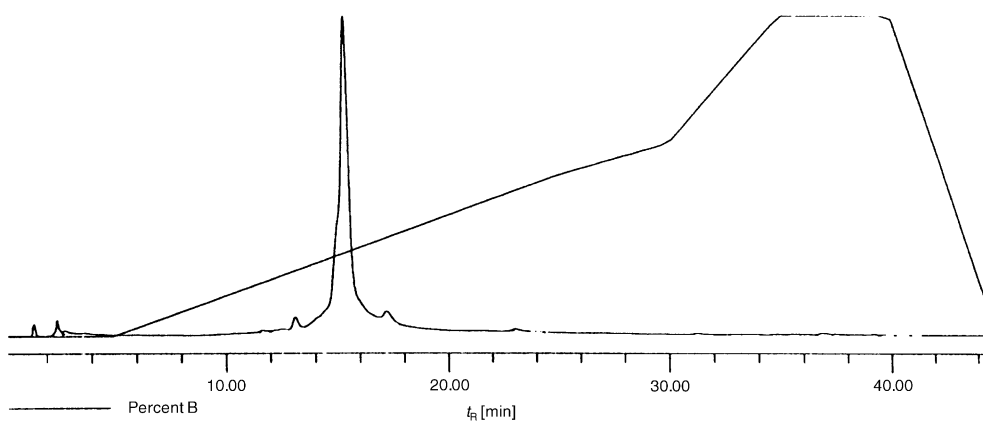


Fig. 1. HPLC Profile of purified, deprotected all-d octamer sulfonate **34c** (absorbance at λ 260 nm vs. time)

Melting Studies with SNAs. We first asked whether SNAs could form duplexes similar to those formed by standard DNA. Several different aqueous buffers were used for the pertinent analyses (*Table 1*). The structures of the studied SNAs and DNAs are given in *Table 2*.

Table 1. Buffers for Melting-Curve Experiments

	NaCl	MgCl ₂	Tris · HCl	pH
Buffer 1	1M	50 mM	10 mM	7.0
Buffer 2	1M	50 mM	10 mM	5.0
Buffer 3	–	100 mM	10 mM	5.0
Buffer 4	0.15M	–	10 mM	7.0

Table 2. Sequences of the SNAs and DNAs for the Melting Curves

	Sequence ^{a)}	Backbone
35a	5'-d(HOCH ₂ -Tso ₂ Tso ₂ Tso ₂ Cso ₂ Tso ₂ Tso ₂ Tso ₂ T-CH ₂ OH)-3'	SNA
35b	5'-d(HOCH ₂ -Tso ₂ Tso ₂ Tso ₂ Cso ₂ Tso ₂ Tso ₂ Tso ₂ T-CH ₂ SO ₃ ⁻)-3'	SNA
34c	5'-d(HOCH ₂ -Tso ₂ Tso ₂ Tso ₂ Tso ₂ Tso ₂ Tso ₂ Tso ₂ T-CH ₂ SO ₃ ⁻)-3'	SNA
DNA1	5'-d(HO-GCGAAAAGAAAACGC-OH)-3'	DNA
DNA2	5'-d(HO-GCGTTTTCTTTTCGC-OH)-3'	DNA
DNA3	5'-d(HO-GCGAAAAGAAAACGC-OH)-3'	DNA
DNA4	5'-d(HO-GCGTTTTTTTTTCGC-OH)-3'	DNA
DNA5	5'-d(HO-GCGAAAAAAAACGC-OH)-3'	DNA
DNA6	5'-d(HO-TTTCTTTT-OH)-3'	DNA
DNA7	5'-d(HO-AAAAGAAA-OH)-3'	DNA
DNA8	5'-d(HO-TTTTTTTT-OH)-3'	DNA
DNA9	5'-d(HO-AAAAAAA-OH)-3'	DNA

^{a)} so₂ in **35a,b** and **34c** corresponds to the linker CH₂SO₂CH₂ instead of the implied standard linker OP(=O)(O⁻)O in **DNA1–DNA9**.

First, as a control, evidence for self-folding of the DNA single strands used in this work was sought by examining the UV absorbance (λ 260 nm) of **DNA2** and **DNA3** as a function of temperature (Fig. 2). These ‘melting curves’ for **DNA2** showed no evidence of self-folding, while the curve for **DNA3** did, slightly. The hyperchromicity normally associated with unfolding was small, only 12–14% between 10 and 80°C, and occurred slowly over the entire temperature interval.

To determine whether an SNA could bind to a complementary DNA, the octameric SNA **35a** was mixed with its complementary oligomeric DNA **DNA1** (1 μ M/oligomer, 1°/min). No melting transition above those assigned to the single-strand melting was observed (Fig. 3, a) in all buffers between 0 and 90°. In contrast, the DNA·DNA pair from **DNA6** and **DNA7** showed a classical melting transition (Fig. 3, b). These results suggest that no duplex forms between this SNA sequence and its complementary DNA under conditions where DNA·DNA duplexes are formed.

Next, evidence for triple-helix formation was sought. DNA Octamers having the sequences d(TTTTTTTT) and d(TTTCTTTT) are well known to bind to duplex DNA in the major groove, with thymine binding by *Hoogsteen* base pairing to adenine in the triplex, and protonated cytosine binding by *Hoogsteen* base pairing to guanine [39]. Reasoning that the absence of charge on the SNA might make it an especially good binder as a third helix, the SNAs **35a**, **35b**, and **34c** were mixed with the DNA duplex formed between sequences **DNA2** and **DNA3**, and melting transitions were sought. The DNA sequences had a GCG moiety at the 5'-end and a CGC at the 3'-end to enhance the DNA duplex stability and ensure tight hybridization at the ends of the DNA strands. The study concentrations of 3 μ M/oligomer and a lower heating (0.5°/min) rate were chosen in consideration of the observation that association-dissociation rates of triple helices are more than 100 times lower than those of double helices [40]. Here, the 8mer duplex **DNA6**·**DNA7** melted at 18° (buffer 4, see Table 1) (Fig. 3, b), while the 14mer duplex **DNA2**·**DNA3** melted at 50° (buffer 3, see Table 1) and 62° (buffers 1 and 2, see Table 1), respectively. As is frequently observed, the melting temperature of the natural duplex increases at the higher salt concentrations of

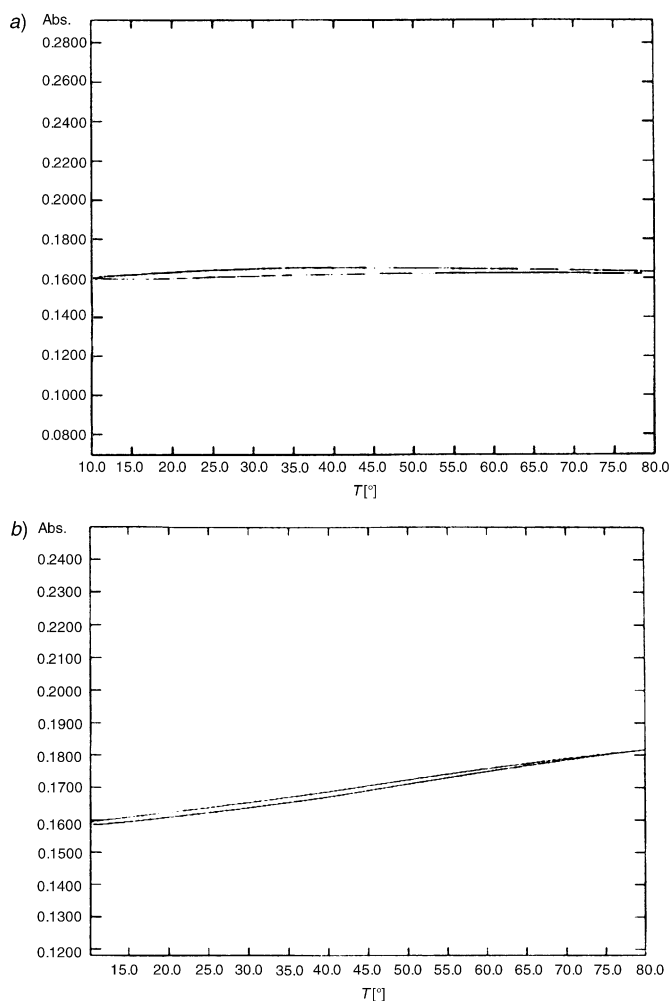


Fig. 2. Melting curves of the single-stranded DNA oligomers (absorbance at λ 260 nm vs. T in $^{\circ}$, in buffer 4), seeking self-folding: a) **DNA2**, showing no significant hyperchromicity, implying no self-folding; b) **DNA3**, showing only modest hyperchromicity, implying little self-folding

buffers 1 and 2, whereas the pH difference between buffer 1 and 2 does not seem to have an effect on duplex melting.

Triple-helix formation could be detected from melting curves in mixtures of three DNA strands. For example, melting of the triplex formed by **DNA2** + **DNA3** + **DNA6** (in buffer 1) occurred at *ca.* 4° , the duplex melted at 62° (Fig. 4, a). Accurate determination of the triplex T_m was difficult because the melting process was not complete at -10° , the temperature at which the aqueous buffers started to freeze. No UV transition at all was observed that could be assigned to the melting of a triple joining SNA **35a,b** or **34c** with **DNA2**·**DNA3** (Fig. 4, b–d) or **DNA4**·**DNA5**,

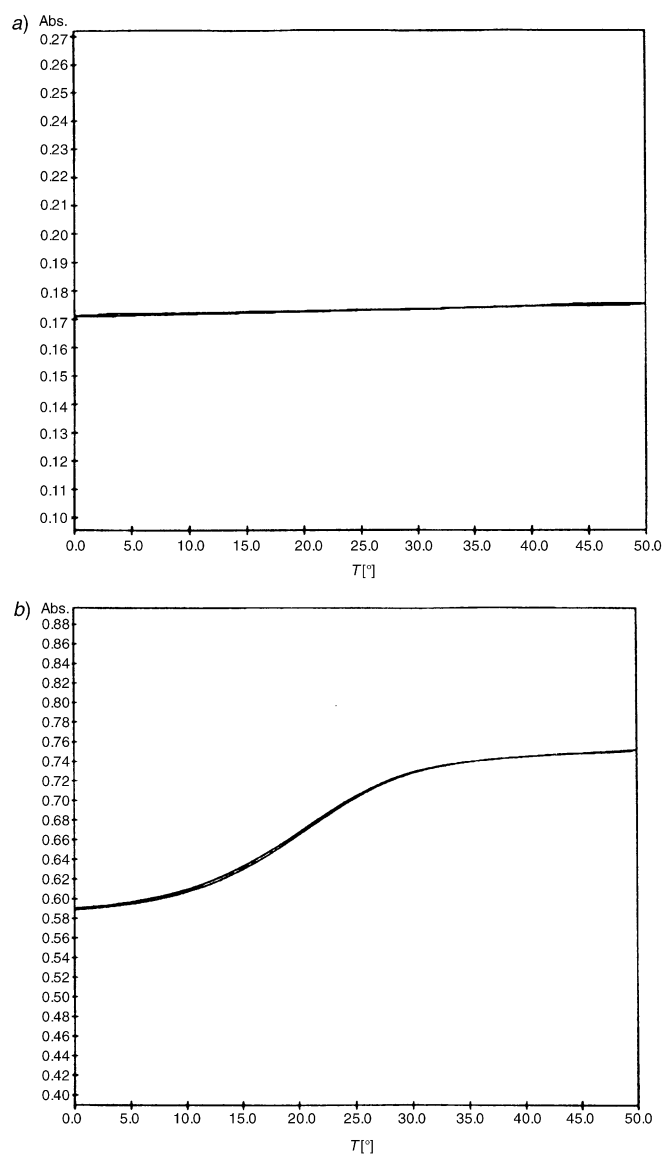


Fig. 3. Seeking SNA · DNA duplex structure by Means of melting curves (absorbance at λ 260 nm vs. T in $^{\circ}$, in buffer 4): a) melting curves of SNA 35a and DNA1, showing slight hyperchromicity, i.e., no evidence for duplex formation, b) melting of DNA6 + DNA7, showing the classical melting curve, i.e., evidence of duplex formation

however. The only hyperchromicity observed was that for the melting of the DNA duplex and melting of the single-stranded SNA. Therefore, it must be concluded that the SNAs examined here do not form duplexes or triplexes with natural DNA oligonucleotides that are more stable than those formed by DNA itself.

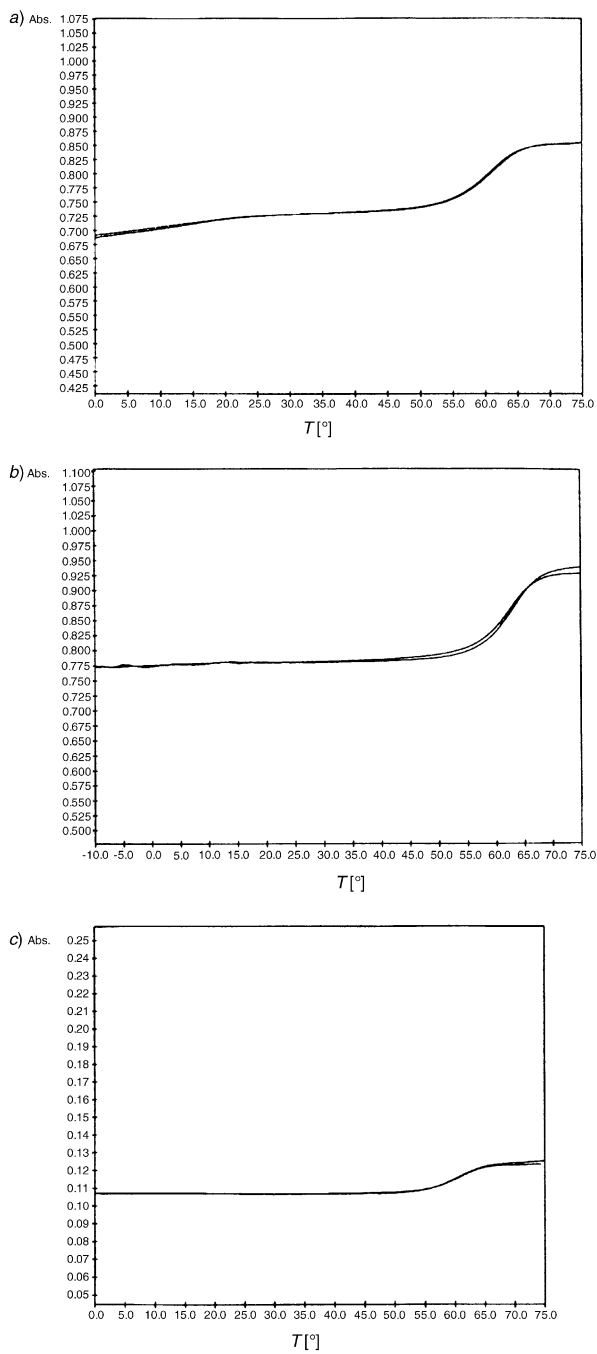


Fig. 4. Seeking the ability of SNA to bind as a third strand to duplex DNA by means of melting curves (absorbance at λ 260 nm vs. T in $^{\circ}$): a) control containing the duplex DNA2·DNA3 with DNA6 as a third strand; b) duplex DNA2·DNA3 in the presence of SNA 35a; c) duplex DNA2·DNA3 in the presence of SNA 35b; d) duplex DNA2·DNA3 in the presence of SNA 34c. Each panel has two curves, representing experiments in buffers 1 and 2.

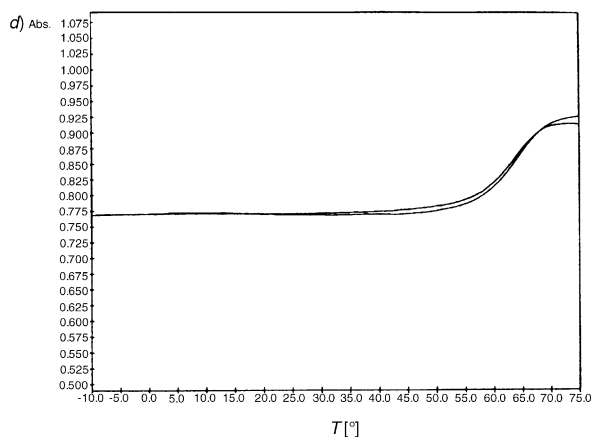


Fig. 4. (cont.)

Discussion. – One of the most-characteristic features of nucleic acids is that their reactivity does *not* change with small (or even large!) changes in chemical composition. Changing a DNA sequence from d(TTTTTTTT) to d(TTTCCTTTT), for example, has essentially no impact on physical behavior, chemical reactivity, or molecular recognition, which continues to follow the *Watson-Crick* rules (A pairs with T, G pairs with C). Indeed, the physical, reactivity, and molecular-recognition properties of DNA remain rule-based for virtually any DNA sequence, of which there are 65536 for an octamer, and far more for longer oligonucleotides.

The insensitivity of the physical behavior of a DNA molecule with respect to changes in nucleobase sequence is, of course, central to its functioning as a genetic molecule. Life as we know it is no more (and no less) than a special type of chemistry, one that has joined a property common in organic molecules (the ability to undergo spontaneous chemical transformation) with an uncommon property (the ability to direct the synthesis of copies of itself) in a way that allows changes in molecular structure arising from spontaneous transformation to themselves be copied. Any chemical system having this combination is expected to undergo natural selection, evolving in structure to replicate faster through more-efficient use of available molecular resources and energy. This will generate life, which may be defined as a self-sustaining chemical system capable of undergoing Darwinian evolution [41].

To support Darwinian evolution, a biopolymer must be able to change its structure without changing its overall physical properties, at least not to the level that such changes will disrupt whatever processes that are essential to its replication. This is arguably the most-fundamental etiological principle behind the DNA structure.

As these and other results suggest, the ability of DNA to retain its overall physical properties, even as it changes its sequence, arises from its repeating anion backbone. This polyelectrolyte character has been discussed elsewhere from this perspective [20]. The work reported here contains one striking example of how changes in molecular structure that would be considered insignificant in the context of a DNA create quite

significant changes in the behavior of the SNA: the susceptibility of thiolated tetramers to disulfide formation (see *Scheme 6*, **28** → **29** (via **30**) vs. stable **26**). Were SNAs incorporated into living systems, the idiosyncratic behavioral changes that are a consequence of small structural changes would create as many problems in their biosynthesis as they created for us in their abiological synthesis.

From this, we can conclude that SNAs are not likely to be alternative genetic substances. This conclusion is made despite the observation that short SNAs form canonical *Watson-Crick* base pairs [25]. Similar behavior is observed with PNA, another non-ionic DNA analog, whose practical application is hampered by limited solubility in aqueous systems and pronounced self-organization [42]. In PNA, addition of a negative charge to one end of the molecule diminishes aggregation and improves solubility. This was also observed in the SNA analogs, where adding a CH_2SO_3^- group at the 3'-end diminished aggregation and improved solubility in these species. Nevertheless, it remains to be explained why the *Watson-Crick*-type molecular interactions survive in PNA molecules past the dinucleotide level of oligomerization.

Experimental Part

1. *General*. See [27].

2. *Monomers*. 1-[3'-{[(*tert*-Butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-6'-O-(methylsulfonyl)- β -D-erythro-hexofuranosyl]thymine (**2a**). The 1-[3'-{[(*tert*-Butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy- β -D-erythro-hexofuranosyl]thymine (compound **11** in the preceding paper [27]; 278 mg, 0.546 mmol) was co-evaporated 3 × with pyridine and dissolved in pyridine/ CH_2Cl_2 4:1 (5 ml). The soln. was cooled to 0° and methanesulfonyl chloride (65 μl , 0.84 mmol) was added dropwise. The mixture was allowed to warm to r.t., stirred for 3 h, and hydrolyzed with 1.6% H_2SO_4 soln. (2 ml). CH_2Cl_2 (50 ml) was added, the soln. washed with 1.6% H_2SO_4 soln. (3 × 10 ml) and brine (3 × 10 ml), dried (Na_2SO_4), and evaporated, and the crude product chromatographed (silica gel, AcOEt/petroleum ether 1:1, 3:1): **2a** (299 mg, 93%). Colorless foam. UV (MeCN): 203 (26700), 266 (8900). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 1.07 (*s*, *t*-Bu); 1.96 (*s*, Me-C(5)); 1.99–2.08 (*m*, 2 H-C(5')); 2.17–2.36 (*m*, H-C(3'), 2 H-C(2')); 2.99 (*s*, MeSO_2); 3.63–3.73 (*m*, CH_2 -C(3')); 3.98 (*dt*, $J=2.6, 8.8$, H-C(4')); 4.27–4.37 (*m*, 1 H-C(6')); 4.38–4.47 (*m*, 1 H-C(6')); 6.05 (*dd*, $J=4.1, 7.1$, H-C(1')); 7.14 (2*s*, H-C(6)); 7.39–7.47 (*m*, 6 H, Ph_2Si); 7.62–7.66 (*m*, 4 H, Ph_2Si); 9.26 (br., NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.62 (*q*, Me-C(5)); 19.61 (*s*, Me_3C); 26.84 (*q*, Me_3C); 34.34 (*t*, C(5')); 34.69 (*t*, C(2')); 37.31 (*q*, MeSO_2); 45.05 (*d*, C(3')); 63.43 (*t*, CH_2 -C(3')); 66.92 (*t*, C(6')); 78.79 (*d*, C(4')); 84.94 (*d*, C(1')); 111.21 (*s*, C(5)); 127.90, 130.01 (2*d*, Ph_2Si); 132.80 (*s*, Ph_2Si); 135.20 (*d*, C(6)); 135.55 (*d*, Ph_2Si); 150.20 (*d*, C(2)); 163.74 (*s*, C(4)). ESI-MS (pos.): 1194.8 ($[2M + \text{Na}]^+$), 1172.7 ($[2M + \text{H}]^+$), 609.2 ($[M + \text{Na}]^+$), 586.9 ($[M + \text{H}]^+$).

1-[6'-Bromo-3'-{[(*tert*-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy- β -D-erythro-hexofuranosyl]thymine (**4a**). The 1-[3'-{[(*tert*-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy- β -D-erythro-hexofuranosyl]thymine (compound **11** in the preceding paper [27]; 50 mg, 98 μmol) and PPh_3 (51 mg, 196 μmol) were co-evaporated 3 × with toluene, dried overnight under high vacuum at r.t., and then dissolved in 1,2-dichloroethane/MeCN 4:1 (10 ml). A soln. of CBr_2 (65 mg, 196 μmol) in 1,2-dichloroethane (2 ml) was added, and the mixture was stirred for 1.5 h at r.t. MeOH (1 ml) was added to quench the reaction. The solvents were evaporated and the residue submitted to FC (silica gel, $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 1:1): **4a** (54 mg, 97%). Colorless foam. UV (MeCN): 215 (17000), 265 (10400). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 1.08 (*s*, *t*-Bu); 1.95 (*s*, Me-C(5)); 1.98–2.09 (*m*, 1 H-C(5')); 2.10–2.23 (*m*, 1 H-C(5'), H-C(3')); 2.25–2.38 (*m*, 2 H-C(2')); 3.41–3.59 (*m*, 2 H-C(6')); 3.67 (*d*, $J=4.0$, CH_2 -C(3')); 4.03 (*dt*, $J=2.5, 9.0$, H-C(4')); 6.06 (*dd*, $J=4.4, 7.1$, H-C(1')); 7.11, 7.12 (2*s*, H-C(6)); 7.26–7.44 (*m*, 6 H, Ph_2Si); 7.64–7.66 (*m*, 4 H, Ph_2Si); 9.17 (br., NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.70 (*q*, Me-C(5)); 19.15 (*s*, Me_3C); 26.83 (*q*, Me_3C); 29.48 (*t*, C(6')); 34.87 (*t*, C(5')); 38.07 (*t*, C(2')); 44.87 (*d*, C(3')); 63.40 (*t*, CH_2 -C(3')); 80.63 (*d*, C(4')); 87.89 (*d*, C(1')); 110.89 (*s*, C(5)); 127.82, 129.92 (2*d*, Ph_2Si); 132.85 (*s*, Ph_2Si); 135.20 (*d*, C(6)); 135.55 (*d*, Ph_2Si); 150.22 (*d*, C(2)); 163.76 (*s*, C(4)). FAB-MS (NOBA; pos.): 573 (24, $[M_1 + \text{H}]^+$), 572 (10, $[M_2 + 2\text{H}]^+$), 571 (25, $[M_2 + \text{H}]^+$), 559 (14), 515 (23), 513 (21), 492 (37, $[M - \text{Br} + \text{H}]^+$), 491 (100, $[M - \text{Br}]^+$), 433 (17), 289 (20), 287 (22), 269 (32), 263 (20), 261 (29), 251 (22),

247 (31), 244 (21), 243 (74), 239 (44), 237 (18), 235 (60), 233 (19), 229 (18), 227 (35), 225 (34), 223 (24), 213 (28), 211 (19), 209 (21), 207 (22), 203 (24), 201 (31), 200 (33), 190, 189, 136, 135.

1-[3'-{[(tert-Butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-6'-O-(methylsulfonyl)-β-D-erythro-hexofuranosyl]-N⁴-(*o*-toluoyl)cytosine (**2b**). As described for **2a**, with 1-[3'-{[(tert-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-β-D-erythro-hexofuranosyl]-N⁴-(*o*-toluoyl)cytosine (compound **14a** in the preceding paper [27]; 380 mg, 0.62 mmol), pyridine (3 ×), pyridine/CH₂Cl₂ 1:2 (6 ml), and methanesulfonyl chloride (67 μl, 0.86 mmol). Chromatography (silica gel, AcOEt) yielded **2b** (321 mg, 95%). Colorless foam. UV (MeCN): 253 (19700), 309 (8600). ¹H-NMR (CDCl₃, 300 MHz): 1.08 (*s*, *t*-Bu); 1.98–2.15 (*m*, 2 H–C(5')); 2.16–2.23 (*m*, H–C(3')); 2.28–2.40 (*m*, 1 H–C(2')); 2.46–2.58 (*m*, 1 H–C(2')); 2.52 (*s*, Me–C(5)); 3.02 (*s*, MeSO₂); 3.65–3.74 (*m*, CH₂–C(3')); 4.13 (*dt*, *J* = 2.0, 9.0, H–C(4')); 4.35–4.50 (*m*, 2 H–C(6')); 6.06 (*dd*, *J* = 3.0, 7.0, H–C(1')); 7.26–7.32 (*m*, 2 H, *to*); 7.37–7.46 (*m*, 7 H, Ph₂Si, H–C(5)); 7.48–7.53 (*m*, 1 H, *to*); 7.55–7.60 (*m*, 1 H, *to*); 7.60–7.67 (*m*, 4 H, Ph₂Si); 7.95 (*d*, *J* = 7.0, H–C(6)); 8.34 (*br.*, NH). ¹³C-NMR (CDCl₃, 75 MHz): 19.23 (*s*, Me₃C); 20.12 (*q*, Me (*to*)); 26.04 (*q*, Me₃C); 36.16 (*t*, C(5')); 37.18 (*t*, C(2')); 37.53 (*q*, MeSO₂); 44.61 (*d*, C(3')); 62.67 (*t*, C(6')); 62.92 (*t*, CH₂–C(3')); 83.12 (*d*, C(4')); 87.65 (*d*, C(1')); 95.95 (*d*, C(5)); 126.17, 126.98 (*2d*, *to*); 127.87, 129.94 (*2d*, Ph₂Si); 131.57, 131.80 (*2d*, *to*); 132.83 (*s*, Ph₂Si); 134.15 (*s*, C_{ipso} (*to*)); 135.55 (*d*, Ph₂Si); 137.45 (*s*, C_o (*to*)); 144.04 (*d*, C(6)); 162.10 (*s*, C=O). FAB-MS (NOBA; pos.): 1380 (27, 2M⁺), 692 (18, [M + 2H]⁺), 691 (42, [M + H]⁺), 690 (100, M⁺), 632 (28), 594 (13), 365 (15), 277, 230 ([MeC₆H₄CONHC₄H₉N₂O + H]⁺).

1-[6'-Bromo-3'-{[(tert-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-β-D-erythro-hexofuranosyl]-N⁴-(*o*-toluoyl)cytosine (**4b**). As described for **4a**, with 1-[3'-{[(tert-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-β-D-erythro-hexofuranosyl]-N⁴-(*o*-toluoyl)cytosine (compound **14a** in the preceding paper [27]; 50 mg, 82 μmol), PPh₃ (43 mg, 163 μmol), toluene (3 ×), 1,2-dichloroethane (10 ml), and CBr₄ (49 mg, 147 μmol) in 1,2-dichloroethane (2 ml) (added at 0°). The mixture was allowed to warm to r.t. and stirred for 2 h. The soln. was poured into sat. NaHCO₃ soln. (15 ml) containing ice (10 g). CH₂Cl₂ (60 ml) was added, the aq. phase extracted with CH₂Cl₂ (4 × 20 ml), the combined org. phase dried (MgSO₄) and evaporated (30° water-bath temp.), and the residue submitted to FC (silica gel, CH₂Cl₂ (100 ml), CH₂Cl₂/AcOEt 3:1 (200 ml): **4b** (41 mg, 74%). Colorless foam. UV (MeCN): 254 (15400), 308 (7500). ¹H-NMR (CDCl₃, 300 MHz): 1.06 (*s*, *t*-Bu); 1.98–2.28 (*m*, 2 H–C(5'), H–C(3')); 2.28–2.42 (*m*, 1 H–C(2')); 2.43–2.61 (*m*, 1 H–C(2')); 2.52 (*s*, Me (*to*)); 3.48–3.67 (*m*, 2 H–C(6')); 3.68–3.74 (*d*, CH₂–C(3')); 4.19 (*dt*, H–C(4')); 6.04 (*dd*, H–C(1')); 7.25–7.31 (*m*, 2 H, *to*); 7.36–7.52 (*m*, 7 H, Ph₂Si, *to*); 7.52–7.61 (*m*, 3 H, *to*, H–C(5)); 7.61–7.70 (*m*, 4 H, Ph₂Si, *to*); 7.94 (*d*, *J* = 7.0, H–C(6)); 8.38 (*br.*, NH). ¹³C-NMR (CDCl₃, 75 MHz): 19.18 (*s*, Me₃C); 20.14 (*q*, Me (*to*)); 26.84 (*q*, Me₃C); 29.41 (*t*, C(6')); 36.18 (*t*, C(5')); 38.09 (*t*, C(2')); 44.16 (*d*, C(3')); 62.90 (*t*, CH₂–C(3')); 82.04 (*d*, C(4')); 87.46 (*d*, C(1')); 95.77 (*d*, C(5)); 126.19, 126.91 (*2d*, *to*); 127.86, 129.95 (*2d*, Ph₂Si); 131.60, 131.83 (*2d*, *to*); 132.82 (*s*, Ph₂Si); 134.16 (*s*, C_{ipso} (*to*)); 135.55 (*d*, Ph₂Si); 137.48 (*s*, C_o (*to*)); 143.72 (*d*, C(6)); 162.03 (*s*, C=O). FAB-MS (NOBA; pos.): 677 (38, [M₁ + 2H]⁺), 676 (50, [M₁ + H]⁺), 675 (77, [M₂ + H]⁺), 674 (95, [M₂ + H]⁺), 663 (25), 618 (23, M₁ – (*t*-Bu) + H]⁺), 616 (23, [M₂ – (*t*-Bu) + H]⁺), 573 (35), 572 (30), 571 (50), 561 (20), 515 (70), 513 (63), 448 (21), 447 (62), 446 (23), 445 (66), 389 (55), 387 (47), 383 (25), 369 (25), 365 (29), 341 (25), 339 (32), 337 (24), 335 (21), 329 (23), 327 (32), 326 (20), 325 (50), 323 (20), 319 (29), 317 (35), 316 (29), 315 (34), 313 (22), 230 ([MeC₆H₄CONHC₄H₉N₂O + H]⁺), 190, 189, 136, 135.

3. *Dimers: Coupling and Oxidation.* 5'-Deoxy-3'-de(phosphinicooxy)-5'-[trityloxy]methyl]thymidyl-methylenethiomethylene-(3' → 5')-3'-{[(tert-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-N⁴-(*o*-toluoyl)-cytidine (*d*(TrOCH₂-T₃^oC-CH₂OTBDPS); **3b**). *Method 1:* Thiol **1** (159 mg, 0.30 mmol) and mesylate **2b** (173 mg, 0.25 mmol) were dried overnight under high vacuum and dissolved in degassed DMF (5 ml, 1 h Ar). The soln. was degassed a second time by performing three freeze-pump cycles. The soln. was cooled to 0°, and DBU (49 μl, 0.33 mmol) was added. The soln. was allowed to warm to r.t., stirred overnight, and cooled again to 0°. AcOH was added until pH 6 was reached. The solvent was evaporated at max. 40° and the remaining crude product chromatographed (silica gel, AcOEt/CH₂Cl₂ 3:1): **3b** (224 mg, 80%). Colorless foam.

Method 2: Thiol **1** (178 mg, 337 μmol), bromide **4b** (220 mg, 319 μmol), and Cs₂CO₃ (189 mg, 933 μmol) were dissolved in degassed DMF (5 ml, 1 h Ar). The soln. was degassed a second time by performing three freeze-pump cycles. The mixture was stirred overnight at r.t. Acetate buffer (3M AcOH/1M AcONa; 470 μl) was added and the mixture evaporated at max. 40°. The crude product was chromatographed (silica gel, 0–5% MeOH/CH₂Cl₂): **3b** (298 mg, 83%). Colorless foam. UV (MeCN): 256 (21400), 308 (7500). ¹H-NMR (CDCl₃, 500 MHz): 1.06 (*s*, *t*-Bu); 1.88 (*2s*, Me–C(5)(T)); 1.85–1.92 (*m*, 1 H–C(5)(T), 1 H–C(5)(C)); 1.97–2.10 (*m*, 1 H–C(5)(T), H–C(3)(C), 1 H–C(5)(C)); 2.13–2.19 (*m*, 1 H–C(2)(C), 1 H–C(2)(T), H–C(3)(T)); 2.21–2.27 (*m*, 1 H–C(2)(T)); 2.46–2.52 (*m*, 1 H–C(2)(C), 1 H–C(6)(C)); 2.52 (*s*, Me (*to*)); 2.62–2.68 (*m*, 1 H–C(6)(C), 1 H of CH₂–C(3)(T)); 2.73–2.78 (*m*, 1 H of CH₂–C(3)(T)); 3.30 (*t*, *J* = 6.5,

2 H–C(6')(T)); 3.64–3.71 (*m*, CH₂–C(3')(C)); 3.87–3.90 (*m*, H–C(4')(T)); 4.07–4.11 (*dd*, *J* = 2.9, 8.0, H–C(4')(C)); 5.98 (*dd*, *J* = 3.9, 6.8, H–C(1')(T)); 6.04 (*dd*, *J* = 2.9, 6.8, H–C(1')(C)); 7.10 (2*s*, H–C(6)(T)); 7.20–7.24 (*m*, 3 H, Tr); 7.27–7.30 (*m*, 8 H, Tr, to); 7.37–7.45 (*m*, 13 arom. H); 7.51 (*d*, *J* = 7.4, H–C(5)(C)); 7.55–7.56 (*m*, 1 H, to); 7.61–7.64 (*m*, 4 H, Ph₂Si); 7.94 (*d*, *J* = 7.4, H–C(6)(C)); 8.30 (br., 1 NH); 8.43 (br., 1 NH). ¹³C-NMR (CDCl₃, 125 MHz): 12.72 (*q*, Me–C(5)(T)); 19.24 (*s*, Me₃C); 20.18 (*q*, Me (to)); 26.90 (*q*, Me₃C); 29.70 (*t*, C(5')(T)); 30.18 (*t*, C(5')(C)); 34.75 (*t*, CH₂–C(3')(T)); 35.06 (*t*, C(6')(C)); 36.37 (*t*, C(2')(C)); 38.49 (*t*, C(2')(T)); 43.14 (*d*, C(3')(T)); 44.42 (*d*, C(3')(C)); 60.65 (*t*, C(6')(T)); 63.31 (*t*, CH₂–C(3')(C)); 82.16 (*d*, C(4')(T)); 82.84 (*d*, C(4')(C)); 84.90 (*d*, C(1')(T)); 86.90 (*s*, Tr); 87.43 (*d*, C(1')(C)); 95.84 (*d*, C(5)(C)); 110.56 (*s*, C(5)(T)); 126.20, 126.99 (2*d*, to); 127.07 (*d*, Tr); 127.83, 127.89 (2*d*, Ph₂Si); 128.63, 128.85 (2*d*, Tr); 129.97, 129.99 (*d*, Ph₂Si); 131.64, 131.86 (2*d*, to); 132.94 (*s*, Ph₂Si); 134.06 (*s*, C_{ipso}(to)); 135.13 (*d*, C(6)(T)); 135.56 (*d*, Ph₂Si); 137.55 (*s*, C₂(to)); 143.79 (*s*, Tr); 144.06 (*s*, C(6)(C)); 149.96 (*s*, C(2)(T)); 155.02 (*d*, C(2)(C)); 162.09 (*s*, C=O); 163.40 (*s*, C(4)(T)); 168.60 (*s*, C(4)(C)). MALDI-TOF (*A* = dimer in CH₂Cl₂, *B* = 0.1M 2,5-DHB (2,5-dihydroxybenzoic acid) in MeCN/EtOH/H₂O 50 : 45 : 5, *C* = *A/B* 1 : 1): 1163.0 (*[M + K]⁺*), 1145.7 (*[M + Na]⁺*).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylylmethylenethiomethylene-(3' → 5')-3'-[[[(tert-butyl)diphenylsilyloxy)methyl]-3',5'-dideoxythymidine (*d*(TrOCH₂-TsT-CH₂OTBDPS); **3a**). *Method 1*: Thiol **1** (264 mg, 0.50 mmol), mesylate **2a** (270 mg, 0.46 mmol), and Cs₂CO₃ (450 mg, 1.38 mmol) were dissolved in degassed DMF (15 ml, 1 h Ar). The soln. was degassed a second time by performing three freeze-pump cycles. The mixture was warmed to 45° and stirred for 4 h. Acetate buffer (3M AcOH/1M AcONa, 690 μl) was added, and the solvent was evaporated at max. 40°. The crude product was purified by FC (silica gel, CH₂Cl₂/AcOEt 3 : 1, 1 : 1): **3a** (383 mg, 82%). Colorless foam.

Method 2: Thiol **1** (50 mg, 95 μmol), bromide **4a** (50 mg, 87 μmol), and Cs₂CO₃ (114 mg, 350 μmol) were dissolved in degassed THF (10 ml). The soln. was degassed a second time by performing three freeze-pump cycles. The mixture was warmed to 45° and stirred for 4 h. Acetate buffer (3M AcOH/1M AcONa; 175 μl) was added, and the solvent was evaporated at max. 40°. The crude product was chromatographed (silica gel, CH₂Cl₂/AcOEt 3 : 1, 1 : 1): **3a** (75 mg, 84%). Colorless foam. UV (MeCN): 266 (21200). ¹H-NMR (CDCl₃, 300 MHz): 1.06 (*s*, *t*-Bu); 1.91, 1.93 (2*s*, 6 H, Me–C(5)(T)); 1.81–2.06 (*m*, 2 H–C(5')(T1), 2 H–C(5')(T2), H–C(3')(T2)); 2.07–2.38 (*m*, 1 H–C(2')(T1), 1 H–C(2')(T2), H–C(3')(T1)); 2.44–2.75 (*m*, 2 H–C(6')(T2), CH₂–C(3')(T1)); 3.29 (*t*, *J* = 6.0, 2 H–C(6')(T1)); 3.68 (*t*, *J* = 4.8, CH₂–C(3')(T2)); 3.83–3.98 (*m*, H–C(4')(T1), H–C(4')(T2)); 5.96–6.08 (*m*, H–C(1')(T1), H–C(1')(T2)); 7.10, 7.13 (2*s*, 2 H, H–C(6)(T)); 7.21–7.33 (*m*, 9 H, Tr); 7.36–7.49 (*m*, 12 H, 6 H of Ph₂Si, 6 H of Tr); 7.60–7.69 (*m*, 4 H, Ph₂Si); 8.79 (br., 1 NH); 8.81 (br., 1 NH). ¹³C-NMR (CDCl₃, 125 MHz)³: 12.73, 12.77 (2*q*, Me–C(5)(T)); 19.24 (*s*, Me₃C); 26.91 (*q*, Me₃C); 30.00 (*t*, C(5')(T1), C(5')(T2)); 34.69 (*t*, CH₂–C(3')(T1)); 35.06, 35.08 (2*t*, C(6')(T2), C(2')(T2)); 38.49 (*t*, C(2')(T1)); 43.09 (*d*, C(3')(T1)); 45.13 (*d*, C(3')(T2)); 60.66 (*t*, C(6')(T1)); 63.77 (*t*, CH₂–C(3')(T2)); 81.38 (*d*, C(4')(T1)); 82.14 (*d*, C(4')(T2)); 84.83, 84.84 (2*d*, C(1')(T2)); 86.90 (*s*, Tr); 110.62, 110.90 (2*s*, C(5)(T)); 127.06 (*d*, Tr); 127.82, 127.88, 127.89, 128.63 (4*d*, Ph₂Si, Tr); 129.98, 130.00 (2*d*, Ph₂Si); 132.95, 132.99 (2*s*, Ph₂Si); 135.08, 135.24 (2*d*, C(6)(T)); 135.55, 135.58 (2*d*, Ph₂Si); 144.07 (*s*, Tr); 150.15, 150.19 (*s*, C(2)(T)); 163.60, 163.63 (2*s*, C(4)(T)). ESI-MS (pos.): 1041.3 (*[M + Na]⁺*).

5'-[(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenethiomethylene-(3' → 5')-3'-[[[(tert-butyl)diphenylsilyloxy)methyl]-3',5'-dideoxythymidine (*d*(BzOCH₂-TsT-CH₂OTBDPS); **6**). Thiol **5** (123 mg, 314 μmol), bromide **4a** (175 mg, 305 μmol), and Cs₂CO₃ (399 mg, 1.22 mmol) were dissolved in degassed DMF (10 ml, 1 h Ar). The soln. was degassed a second time by performing three freeze-pump cycles. The mixture was warmed to 50° and stirred for 4 h. Acetate buffer (3M AcOH/1M AcONa, 610 μl) was added, and the solvent was evaporated at max. 40°. The crude product was purified by FC (silica gel, CH₂Cl₂/MeOH 100 : 0, 40 : 1, 20 : 1, 10 : 1): **6** (267 mg, 99%). Colorless foam. ¹H-NMR (CDCl₃, 300 MHz): 1.06 (*s*, 9 *t*-Bu); 1.92, 1.93 (2*s*, 6 H, Me–C(5)(T)); 1.84–2.34 (*m*, 2 H–C(5')(T1), 2 H–C(5')(T2), H–C(3')(T2), 2 H–C(2')(T1), 2 H–C(2')(T2), H–C(3')(T1)); 2.54–2.78 (*m*, 2 H–C(6')(T2), CH₂–C(3')(T1)); 3.68 (*d*, *J* = 4.7, CH₂–C(3')(T2)); 3.90–3.98 (*m*, H–C(4')(T1), H–C(4')(T2)); 4.39–4.47 (*m*, 1 H–C(6')(T1)); 4.54–4.62 (*m*, 1 H–C(6')(T1)); 6.04–6.09 (*m*, H–C(1')(T1), H–C(1')(T2)); 7.17, 7.24 (2*s*, 2 H, H–C(6)(T)); 7.36–7.47 (*m*, 2 H of Bz, 6 H of Ph₂Si); 7.53–7.58 (*t*, *J* = 7.4, 1 H, Bz); 7.63–7.65 (*m*, 4 H, Ph₂Si); 8.02–8.04 (*m*, 2 H, Bz); 10.22 (br., 2 NH).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[[[(tert-butyl)diphenylsilyloxy)methyl]-2',3',5'-trideoxy-N⁴-(*o*-toluoyl)cytidine (*d*(TrOCH₂-Tso₂¹⁰C-

³) Spectrum kindly provided by Daniel Hutter [43].

CH₂OTBDPS); **8b**). To a soln. of **3b** (39 mg, 35 μ mol) in MeOH/THF 4 : 1 (10 ml), *Oxone* (107 mg, 175 μ mol) and NaOAc (49 mg, 600 μ mol) in deionized H₂O (0.6 ml) were added with vigorous stirring (\rightarrow opaque soln.). After 5 min, TLC showed the complete conversion to two diastereoisomeric sulfoxides, which were further oxidized to **8b** after 2 h. Excess *Oxone* was reduced with sat. Na₂S₂O₃ soln. (5 ml). The soln. was concentrated to 50% of the original volume. CH₂Cl₂ (40 ml) was added, the aq. phase extracted with CH₂Cl₂ (2 \times 20 ml), the combined org. phase washed with brine (2 \times 20 ml), which was reextracted with CH₂Cl₂ (2 \times 20 ml), and the combined org. phase dried (MgSO₄) and evaporated. Filtration through a layer of silica gel (CH₂Cl₂/MeOH 10 : 1) yielded **8b** (40 mg, quant.). Colorless foam. UV (MeCN): 205 (70200), 255 (19200), 309 (5400). ¹H-NMR (CDCl₃, 500 MHz): 1.07 (*s*, *t*-Bu); 1.90 (*s*, Me-C(5)); 1.86–2.04 (*m*, 2 H-C(5')(T)); 2.10–2.24 (*m*, H-C(3')(T), 2 H-C(5')(C)); 2.33–2.61 (*m*, 2 H-C(2')(C), 2 H-C(2')(T), H-C(3')(T)); 2.52 (*s*, Me (to)); 2.87–3.05 (*m*, 1 H-C(6')(C), 1 H of CH₂-C(3')(T)); 3.13–3.25 (*m*, 1 H-C(6')(C), 1 H of CH₂-C(3')(T)); 3.29–3.33 (*m*, 2 H-C(6')(T)); 3.65–3.76 (*m*, CH₂-C(3')); 3.82–3.89 (*m*, H-C(4')(T)); 3.95–4.01 (*dt*, H-C(4')(C)); 6.03–6.05 (*m*, H-C(1')(T), H-C(1')(C)); 7.04 (*s*, H-C(6)(T)); 7.20–7.31 (*m*, 9 H of Tr, 2 H of to); 7.38–7.47 (*m*, 6 H of Tr, 6 H of Ph₂Si, H-C(5)(C)); 7.49–7.51 (*m*, 1 H, to); 7.57–7.64 (*m*, 1 H of to, 4 H of Ph₂Si); 7.86 (*d*, *J* = 7.4, H-C(6)(C)); 8.20 (br., 1 NH); 8.42 (br., 1 NH). ¹³C-NMR (CDCl₃, 125 MHz): 12.87 (*q*, Me-C(5)(T)); 19.21 (*s*, Me₃C); 20.13 (*q*, Me(to)); 26.89 (*q*, Me₃C); 33.12 (*t*, C(5')(T)); 35.17 (*t*, C(5')(C)); 35.71 (*d*, C(3')(T)); 36.37 (*t*, C(2')(C)); 38.09 (*t*, C(2')(T)); 44.83 (*d*, C(3')(C)); 50.98 (*t*, CH₂-C(3')(T)); 54.51 (*t*, C(6')(C)); 60.40 (*t*, C(6')(T)); 63.31 (*t*, CH₂-C(3')(C)); 82.16 (*d*, C(4')(T)); 82.84 (*d*, C(4')(C)); 84.72 (*d*, C(1')(T)); 86.90 (*s*, Tr); 87.56 (*d*, C(1')(C)); 95.84 (*d*, C(5)(C)); 110.56 (*s*, C(5)(T)); 126.17, 126.99 (*2d*, to); 127.13 (*d*, Tr); 127.88, 127.89 (*2d*, Ph₂Si); 128.55, 128.85 (*2d*, Tr); 130.01; 130.05 (*2d*, Ph₂Si); 131.66, 131.83 (*2d*, to); 132.77 (*s*, Ph₂Si); 134.08 (*s*, C_{ipso}(to)); 135.14 (*d*, C(6)(T)); 135.52 (*d*, Ph₂Si); 137.56 (*s*, C₂(to)); 143.86 (*s*, Tr); 144.10 (*d*, C(6)(C)); 149.99 (*s*, C(2)(T)); 155.07 (*d*, C(2)(C)); 162.08 (*s*, C=O); 163.43 (*s*, C(4)(T)); 168.62 (*s*, C(4)(C)). FAB-MS (NOBA; pos.): 1176 ([*M* + Na]⁺), 1154 (*M*⁺), 289 (3), 259 (2), 245 (2), 244 (22), 243 (100), 231 (3), 230 (17). MALDI-TOF MS (*A* = dimer in CH₂Cl₂, *B* = 0.1M CCA (α -cyano-4-hydroxycinnamic acid) in MeCN/EtOH/H₂O 50 : 45 : 5, *C* = *A/B* 1 : 1): 1177.4 ([*M* + Na]⁺).

Corresponding sulfoxide. FAB-MS (NOBA; pos.): 1162 ([*M* + Na]⁺), 1139 ([*M* + H]⁺), 1138 (*M*⁺), 290 (3), 289 (7), 273 (2), 259 (3), 245 (3), 244 (21), 243 (100), 242 (2), 230 (8).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylmethylenesulfonylmethylene-(3' \rightarrow 5')-3'-[[[(tert-butyl)diphenylsilyloxy)methyl]-3',5'-dideoxythymidine (*d*(TrOCH₂-Tso₂T-CH₂OTBDPS); **8a**). As described for **8b**, with **3a** (354 mg, 347 μ mol), MeOH/THF 6 : 1 (70 ml), *Oxone* (850 mg, 1.39 mmol), NaOAc (370 mg, 4.50 mmol), and deionized H₂O (15 ml). Workup with sat. Na₂S₂O₃ soln. (15 ml) and CH₂Cl₂ (60 ml): **8a** (350 mg, 96%). Colorless foam. UV (MeCN): 265 (23700). ¹H-NMR (CDCl₃, 300 MHz): 1.07 (*s*, *t*-Bu); 1.89, 1.93 (2*s*, 6 H, Me-C(5)(T)); 1.86–2.04 (*m*, 2 H-C(5')(T₂)); 2.05–2.18 (*m*, 1 H-C(2')(T₂), H-C(3')(T₂)); 2.21–2.39 (*m*, 1 H-C(2')(T₁), 1 H-C(2')(T₂), 2 H-C(5')(T₁)); 2.40–2.62 (*m*, H-C(2')(T₁), H-C(3')(T₁)); 2.83–2.91 (*m*, 1 H-C(6')(T₂)); 2.92–3.02 (*m*, 1 H of CH₂-C(3)(T₂)); 3.07–3.12 (*m*, 1 H-C(6')(T₂)); 3.12–3.21 (*m*, 1 H of CH₂-C(3')(T₂)); 3.32 (*t*, *J* = 6.0, 2 H-C(6')(T₁)); 3.75–3.83 (*m*, CH₂-C(3')(T₂)); 3.80–3.91 (*m*, H-C(4')(T₁), H-C(4')(T₂)); 5.95–6.03 (*m*, H-C(1')(T₁), H-C(1')(T₂)); 7.03, 7.05 (2*s*, 2 H, H-C(6)(T)); 7.21–7.34 (*m*, 9 H, Tr); 7.38–7.50 (*m*, 6 H of Ph₂Si, 6 H of Tr); 7.61–7.68 (*m*, 4 H, Ph₂Si); 8.42 (br., 1 NH); 8.56 (br., 1 NH). ¹³C-NMR (CDCl₃, 125 MHz): 12.63, 12.70 (2*q*, Me-C(5)(T)); 19.23 (*s*, Me₃C); 26.61 (*t*, C(5')(T₁)); 26.93 (*q*, Me₃C); 34.17, 34.25 (2*t*, C(5')(T₂), C(2')(T₂)); 36.61 (*d*, C(3')(T₁)); 38.41 (*t*, C(2')(T₁)); 45.34 (*d*, C(3')(T₂)); 51.05 (*t*, CH₂-C(3')(T₁)); 55.00 (*t*, C(6')(T₂)); 60.44 (*t*, C(6')(T₁)); 63.58 (*t*, CH₂-C(3')(T₂)); 80.64 (*d*, C(4')(T₁)); 81.59 (*d*, C(4')(T₂)); 84.87 (*d*, C(1')(T₁)); 85.69 (*d*, C(1')(T₂)); 87.05 (*s*, Tr); 111.14, 111.36 (2*s*, C(5)(T₅)); 127.14 (*d*, Tr); 127.90, 127.94, 127.96, 128.59 (4*d*, Ph₂Si, Tr); 130.06, 130.08 (2*d*, Ph₂Si); 132.86, 132.88 (2*s*, Ph₂Si); 135.10 (*d*, C(6)(T)); 135.57 (*d*, Ph₂Si); 135.69 (*d*, C(6)(T)); 143.92 (*s*, Tr); 150.25, 150.27 (2*s*, C(2)(T)); 163.57, 163.65 (2*s*, C(4)(T)). MALDI-TOF MS (*A* = dimer in CH₂Cl₂, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50 : 45 : 5, *C* = *A/B* 1 : 1): 1097 ([*M* + 2Na - H]⁺), 1090 ([*M* + K - H]⁺), 1074 ([*M* + Na - H]⁺).

5'-[(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylmethylenesulfonylmethylene-(3' \rightarrow 5')-3'-[[[(tert-butyl)diphenylsilyloxy)methyl]-3',5'-dideoxythymidine (*d*(BzOCH₂-Tso₂T-CH₂OTBDPS); **8c**). *Method 1*: As described for **8b**, with **6** (270 mg, 306 μ mol), MeOH/THF 6 : 1 (60 ml), *Oxone* (752 mg, 1.22 mmol), NaOAc (550 mg, 4.04 mmol), and deionized H₂O (10 ml) (conversion to sulfoxides within 2 min). Workup with sat. Na₂S₂O₃ soln. (20 ml) and CH₂Cl₂ (50 ml), then extraction with CH₂Cl₂ (3 \times 20 ml) (no washing with brine): **8c** (279 mg, quant.). Colorless foam.

Method 2: Dimer **9a** (95 mg, 117 μ mol) was co-evaporated 3 \times with pyridine and dissolved in pyridine (15 ml). *N,N*-Dimethylpyridin-4-amine (DMAP; 0.5 mg) was added, the soln. cooled to 0^o, and benzoyl chloride

(27.2 μl , 234 μmol) slowly added dropwise over 5 min. The soln. was allowed to warm to r.t. and stirred for 5 h. After cooling to 0°, the reaction was terminated by the slow addition of sat. NaHCO_3 soln. (5 ml). The mixture was concentrated to ca. 10 ml, CH_2Cl_2 (20 ml) and deionized H_2O (5 ml) were added, and the aq. phase was extracted with CH_2Cl_2 (2×25 ml, 2×15 ml). The org. phases were washed with 5% HCl soln. (2×10 ml), sat. NaHCO_3 soln. (1×15 ml), and brine (1×10 ml) and the aq. phases reextracted with CH_2Cl_2 (1×20 ml). The combined org. phase was evaporated. FC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100 : 0, 20 : 1, 10 : 1) yielded **8c** (76 mg, 71%). Colorless foam. The products of the two methods were identical according to TLC and $^1\text{H-NMR}$. UV (MeCN): 219 (27000), 265 (17200). $^1\text{H-NMR}$ ((D_6) DMSO, 300 MHz): 1.00 (s, *t*-Bu); 1.78, 1.80 (2s, 6 H, Me-C(5)(T)); 2.00–2.17 (m, 2 H-C(5')(T1), 2 H-C(5')(T2)); 2.20–2.34 (m, H-C(3')(T2), 1 H-C(2')(T1), 2 H-C(2')(T2)); 2.39–2.50 (m, 1 H-C(2')(T1)); 2.62–2.73 (m, H-C(3')(T1)); 2.18–3.36 (m, 2 H-C(6')(T2), 1 H of $\text{CH}_2\text{-C}(3')(\text{T1})$); 3.45–3.53 (m, 1 H of $\text{CH}_2\text{-C}(3')(\text{T1})$, partly under DMSO); 3.65–3.72 (m, $\text{CH}_2\text{-C}(3')(\text{T2})$); 3.80–3.91 (m, H-C(4')(T1), H-C(4')(T2)); 4.28–4.37 (m, 1 H-C(6')(T1)); 4.39–4.49 (m, 1 H-C(6')(T1)); 6.03–6.08 (m, 2 H-C(1')(T1), H-C(1')(T2)); 7.40–7.55 (m, 2 H-C(6)(T), 2 H of Bz, 6 H of Ph_2Si); 7.60–7.68 (m, 1 H of Bz, 4 H of Ph_2Si); 7.96 (d, $J=7.4$, 2 H, Bz); 11.28 (br., 2 NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.50 (2q, Me-C(5)(T)); 19.11 (s, Me_3C); 26.80 (q, Me_3C); 32.69 (t, C(5')(T1)); 34.04 (t, C(5')(T2)); 36.44 (d, C(3')(T2)); 37.89 (2t, C(2')(T)); 45.19 (d, C(3')(T1)); 50.99 (t, $\text{CH}_2\text{-C}(3')(\text{T1})$); 54.63 (t, C(6')(T2)); 61.64 (t, C(6')(T1)); 63.51 (t, $\text{CH}_2\text{-C}(3')(\text{T2})$); 80.61 (d, C(4')(T2)); 80.98 (d, C(4')(T1)); 84.95 (d, C(1')(T2)); 85.83 (d, C(1')(T1)); 111.15, 111.30 (2s, C(5)(T)); 127.82 (d, Ph_2Si); 128.34, 129.46 (2d, Bz); 129.93 (d, Ph_2Si); 129.94 (s, Bz); 132.76 (s, Ph_2Si); 133.06 (d, Bz); 135.17 (d, C(6)(T)); 135.43 (d, Ph_2Si); 135.88 (d, C(6)(T)); 150.42 (2s, C(2)(T)); 163.91 (2s, C(4)(T)); 166.36 (s, C=O). ESI-MS (pos.): 957.2 ($[M-\text{H}+2\text{Na}]^+$), 935.3 ($[M+\text{Na}]^+$), 809.3 ($[M-\text{T}\cdot\text{H}+\text{Na}]^+$); 683.3 ($M-2\text{T}\cdot\text{H}+\text{Na}^+$).

4. *Functionalization of the Dimers. 5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylmethylensulfonylmethylene-(3' \rightarrow 5')-3'-{[(tert-butyl)diphenylsilyloxy)methyl]-2,3',5'-trideoxy-N⁴-(o-toluoyl)cytidine (d(HOCH₂-Tso₂-C-CH₂OTBDPS); **9b**). Method 1:* TsOH (20 mg, 0.1 mmol) was dissolved in MeOH (10 ml) and a soln. of **8b** (200 mg, 173 μmol) in THF (4 ml) was added. The mixture was stirred overnight at r.t. Sat. NaHCO_3 soln. was added until pH 8 was reached. The mixture was filtered and evaporated. FC of the crude product (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20 : 1) yielded **9b** (144 mg, 91%). Colorless foam.

Method 2: To a soln. of **8b** (177 mg, 153 μmol) in CH_2Cl_2 (3 ml), 2.2M $\text{ZnCl}_2\cdot\text{Et}_2\text{O}$ in CH_2Cl_2 (348 μl , 765 μmol) was added (\rightarrow yellow soln., and after ca. 250 μl , yellow precipitate). The deprotection was complete after 10 min. The suspension was filtered through a layer of silica gel and the filtrate evaporated. FC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100 : 0, 40 : 1, 20 : 1, 10 : 1) yielded **9b** (140 mg, quant.). Colorless foam. UV (MeCN): 256 (20000), 309 (6500). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 1.07 (s, *t*-Bu); 1.92, 1.93 (2s, Me-C(5)(T)); 1.89–1.97 (m, 1 H-C(5')(T)); 1.99–2.05 (m, 1 H-C(5)(T)); 2.13–2.30 (m, H-C(3')(C), 2 H-C(5')(C)); 2.32–2.54 (m, 2 H-C(2')(C), 2 H-C(2')(T)); 2.51 (s, Me(to)); 2.70–2.79 (m, H-C(3')(T)); 2.97 (dd, $J=9.8$, 13.7, 1 H-C(6)(C)); 3.10 (ddd, $J=5.3$, 10.5, 13.7, 1 H-C(6)(C)); 3.29–3.37 (m, $\text{CH}_2\text{-C}(3')(\text{T})$); 3.67–3.76 (m, 2 H-C(6')(T)); 3.77–3.92 (m, $\text{CH}_2\text{-C}(3')(\text{C})$, H-C(4')(T)); 4.00 (dd, $J=2.8$, 9.0, H-C(4')(C)); 5.97 (dd, $J=3.6$, 7.1, H-C(1')(T)); 6.05 (dd, $J=4.2$, 7.7, H-C(1')(C)); 7.12, 7.13 (2s, H-C(6)(T)); 7.27–7.30 (m, 2 H, to); 7.36–7.43 (m, 6 H, Ph_2Si); 7.45 (d, $J=7.3$, H-C(5)(C)); 7.50–7.52 (d, $J=7.9$, 1 H, to); 7.58–7.59 (m, 1 H, to); 7.62–7.65 (m, 4 H, Ph_2Si); 7.84 (d, $J=7.4$, H-C(6)(C)); 8.49 (br., NH); 8.62 (br., NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.33 (q, Me-C(5)(T)); 18.98 (s, Me_3C); 19.77 (q, Me(to)); 26.67 (q, Me_3C); 35.47 (t, C(5')(C)); 35.54 (d, C(3')(C)); 35.58 (t, C(5)(T)); 36.16 (t, C(2')(C)); 37.89 (t, C(2')(T)); 44.53 (d, C(3')(T)); 50.86 (t, $\text{CH}_2\text{-C}(3')(\text{T})$); 54.29 (t, C(6)(C)); 58.66 (t, C(6)(T)); 63.01 (t, $\text{CH}_2\text{-C}(3')(\text{C})$); 81.49 (d, C(4')(T)); 82.05 (d, C(4)(C)); 84.60 (d, C(1')(C)); 87.50 (d, C(1')(T)); 96.95 (d, C(5)(C)); 111.07 (s, C(5)(T)); 125.69, 127.19 (2d, to); 127.74 (d, Ph_2Si); 129.85 (d, Ph_2Si); 131.14, 131.24 (2d, to); 132.61 (s, Ph_2Si); 134.09 (s, C_{ipso} (to)); 135.47 (d, C(6)(T)); 135.56 (d, Ph_2Si); 137.04 (s, C_o (to)); 143.99 (d, C(6)(C)); 150.44 (s, C(2)(T)); 155.63 (d, C(2)(C)); 163.73 (s, C=O); 164.11 (s, C(4)(T)); 169.53 (s, C(4)(C)). FAB-MS (NOBA; pos.): 945 (1, $[M+\text{Na}]^+$), 914 (2, $[M+\text{H}]^+$), 913 (7, M^+), 301 (13), 243 (11), 231 (17), 230 (100), 199 (15), 197 (14), 183 (5), 165 (7), 163 (5), 155 (6), 154 (14), 149 (6), 139 (10), 138 (9), 137 (19), 136 (19), 135 (37), 128 (5), 127 (32), 125 (6), 123 (11), 121 (10), 119 (47), 111 (7), 109 (33), 107 (14), 105 (11).

*5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylmethylensulfonylmethylene-(3' \rightarrow 5')-3'-{[(tert-butyl)diphenylsilyloxy)methyl]-3',5'-dideoxythymidine (d(HOCH₂-Tso₂-T-CH₂OTBDPS); **9a**). As described for **9b** (Method 2), with **8a** (75 mg, 72 μmol), CH_2Cl_2 (1.5 ml), and 2.2M $\text{ZnCl}_2\cdot\text{Et}_2\text{O}$ in CH_2Cl_2 (326 μl , 717 μmol): **9a** (58 mg, 99%). Colorless foam. $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 1.08 (s, *t*-Bu); 1.93 (2s, 6 H, Me-C(5)(T)); 1.84–2.05 (m, 2 H-C(5')(T1)); 2.08–2.21 (m, H-C(5')(T2)); 2.22–2.40 (m, 1 H-C(5')(T2), H-C(3')(T2), 1 H-C(2')(T1), 2 H-C(2')(T2)); 2.43–2.54 (m, 1 H-C(2')(T1)); 2.71–2.80 (m, H-C(3')(T1)); 2.94–3.12 (m, 1 H of $\text{CH}_2\text{-C}(3')(\text{T1})$, 1 H-C(6')(T2)); 3.22–3.32 (m, 1 H of*

CH₂-C(3')(T1), 1 H-C(6')(T2)); 3.68–3.72 (*m*, 2 H-C(6')(T1)); 3.74–3.91 (*m*, CH₂-C(3')(T2), H-C(4')(T1), H-C(4')(T2)); 5.85–5.88 (*m*, H-C(1')(T2)); 6.03 (*dd*, *J*=4.1, 7.4, H-C(1')(T1)); 7.04, 7.12 (2*s*, 2 H, H-C(6)(T)); 7.39–7.48 (*m*, 6 H, Ph₂Si); 7.62–7.65 (*m*, 4 H, Ph₂Si); 9.15 (br., 1 NH); 9.36 (br., 1 NH). ¹³C-NMR (CDCl₃, 75 MHz): 12.47, 12.56 (2*q*, Me-C(5)(T)); 19.16 (*s*, Me₃C); 26.83 (*q*, Me₃C); 33.73 (*t*, C(5')(T1)); 35.57 (*t*, C(5')(T2)); 36.36 (*d*, C(3')(T2)); 38.00 (2*t*, C(2')(T)); 45.25 (*d*, C(3')(T1)); 50.75 (*t*, CH₂-C(3')(T1)); 54.66 (*t*, C(6')(T2)); 59.03 (*t*, C(6')(T1)); 63.52 (*t*, CH₂-C(3')(T2)); 80.63 (*d*, C(4')(T2)); 81.83 (*d*, C(4')(T1)); 85.08 (*d*, C(1')(T2)); 86.62 (*d*, C(1')(T1)); 111.24, 111.34 (2*s*, C(5)(T)); 127.87, 129.99 (2*d*, Ph₂Si); 132.80 (*s*, Ph₂Si); 135.50 (*d*, Ph₂Si); 136.47 (2*d*, C(6)(T)); 150.36, 150.44 (2*s*, C(2)(T)); 163.83, 164.04 (2*s*, C(4)(T)). FAB-MS (NOBA; pos.): 810 (12, [M+H]⁺), 809 (25, M⁺), 327 (10), 289 (13), 281 (18), 221 (14), 207 (22), 156 (10), 155 (32), 154 (88), 153 (11), 152 (14), 148 (12), 147 (52), 139 (24), 138 (42), 137 (73), 136 (100), 133 (14) (only *m/z* > 130 were considered).

5'-(Bromomethyl)-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[[[(tert-butyl)diphenylsilyloxy]methyl]-2',3',5'-trideoxy-N⁴-(o-toluoyl)cytidine (d(BrCH₂-Tso₂¹⁰C-CH₂OTBDPS); **11b**). Compound **9b** (76 mg, 83 μmol) and PPh₃ (59 mg, 225 μmol) were dried overnight under high vacuum at r.t. and dissolved in 1,2-dichloroethane (6 ml). The soln. was cooled to 0°, and CBr₄ (75 mg, 225 μmol) in 1,2-dichloroethane (1.5 ml) was added. The mixture was allowed to warm to r.t. and stirred for 75 min. The soln. was poured into sat. NaHCO₃ soln. (15 ml) containing ice (10 g). CH₂Cl₂ (60 ml) was added, the aq. phase extracted with CH₂Cl₂ (3 × 20 ml), and combined org. phase dried (MgSO₄) and evaporated (30° water-bath temp.). FC (silica gel, CH₂Cl₂/MeOH 100:0, 40:1, 20:1) yielded **11b** (70 mg, 86%). Colorless foam. UV (MeCN): 256 (17600), 309 (5700). ¹H-NMR (CDCl₃, 300 MHz): 1.05 (*s*, *t*-Bu); 1.92 (*s*, Me-C(5)(T)); 2.06–2.28 (*m*, 2 H-C(5')(T), H-C(3')(C), 2 H-C(5')(C)); 2.30–2.55 (*m*, 2 H-C(2')(C), 2 H-C(2')(T)); 2.50 (*s*, Me(to)); 2.65–2.73 (*m*, H-C(3')(T)); 2.98 (*dd*, *J*=9.3, 13.5, 1 H-C(6')(C)); 3.06–3.16 (*m*, 1 H-C(6')(C), 1 H of CH₂-C(3')(T)); 3.24–3.35 (*m*, 1 H of CH₂-C(3')(T)); 3.44–3.61 (*m*, 2 H-C(6')(T)); 3.64–3.76 (*m*, CH₂-C(3')(C)); 3.82–3.87 (*dt*, H-C(4')(T)); 3.96–4.03 (*m*, H-C(4')(C)); 6.00 (*dd*, *J*=3.5, 7.0, H-C(1')(T)); 6.05 (*dd*, *J*=4.2, 7.5, H-C(1')(C)); 7.03, 7.04 (2*s*, H-C(6)(T)); 7.25–7.28 (*m*, 2 H, to); 7.37–7.47 (*m*, 7 H, Ph₂Si, H-C(5)(C)); 7.53 (*dd*, *J*=1.4, 7.4, 1 H, to); 7.59–7.68 (*m*, 5 H, to, Ph₂Si); 7.85 (*d*, *J*=7.4, H-C(6)(C)); 8.36 (br., 1 NH); 8.57 (br., 1 NH). ¹³C-NMR (CDCl₃, 75 MHz): 12.33 (*q*, Me-C(5)(T)); 18.98 (*s*, Me₃C); 19.77 (*q*, Me (to)); 26.67 (*q*, Me₃C); 35.47 (*t*, C(5')(C)); 35.54 (*d*, C(3')(C)); 35.58 (*t*, C(5')(T)); 36.16 (*t*, C(2')(C)); 37.89 (*t*, C(2')(T)); 44.53 (*d*, C(3')(T)); 50.86 (*t*, CH₂-C(3')(T)); 54.29 (*t*, C(6')(C)); 58.66 (*t*, C(6')(T)); 63.01 (*t*, CH₂-C(3')(C)); 81.49 (*d*, C(4')(T)); 82.05 (*d*, C(4')(C)); 84.60 (*d*, C(1')(C)); 87.50 (*d*, C(1')(T)); 96.95 (*d*, C(5)(C)); 111.07 (*s*, C(5)(5)); 125.69, 127.19 (2*d*, to); 127.74 (*d*, Ph₂Si); 129.85 (*d*, Ph₂Si); 131.14, 131.24 (*d*, to); 132.61 (*s*, Ph₂Si); 134.09 (*s*, C_{ipso}(to)); 135.47 (*d*, C(6)(T)); 135.56 (*d*, Ph₂Si); 137.04 (*s*, C_{ipso}(to)); 143.99 (*d*, C(6)(C)); 150.44 (*s*, C(2)(T)); 155.63 (*d*, C(2)(C)); 163.73 (*s*, C=O); 164.11 (*s*, C(4)(T)); 169.53 (*s*, C(4)(C)). FAB-MS (NOBA; pos.): 977 (26, [M+2H]⁺), 976 (37, [M₁+H]⁺), 974 (51, [M₂+H]⁺), 613 (16), 292 (12), 291 (15), 290 (20), 289 (44), 273 (10), 230 (26), 167 (14), 166 (16), 165 (16), 154.

5'-(Bromomethyl)-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[[[(tert-butyl)diphenylsilyloxy]methyl]-3',5'-dideoxythymidine (d(BrCH₂-Tso₂T-CH₂OTBDPS); **11a**). Compound **9a** (60 mg, 74 μmol) and PPh₃ (39 mg, 148 μmol) were dried overnight under high vacuum at r.t. and dissolved in 1,2-dichloroethane (4 ml). CBr₄ (49 mg, 148 μmol) in 1,2-dichloroethane (1 ml) was added, and the mixture was stirred for 60 min. The soln. was poured into sat. NaHCO₃ soln. (15 ml) containing ice (10 g). CH₂Cl₂ (60 ml) was added, the aq. phase extracted with CH₂Cl₂ (4 × 20 ml), and the combined org. phase dried (MgSO₄) and evaporated (30° water-bath temp.). FC (silica gel, CH₂Cl₂/MeOH 100:0, 40:1, 20:1, 10:1) yielded **11a** (63 mg, 97%). Colorless foam. UV (MeCN): 265 (14400). ¹H-NMR (CDCl₃, 300 MHz): 1.09 (*s*, *t*-Bu); 1.94 (2*s*, 6 H, Me-C(5)(T)); 2.05–2.41 (*m*, 4 H-C(5'), H-C(3')(T2), 1 H-C(2')(T1), 2 H-C(2')(T2)); 2.46–2.55 (*m*, H-C(2')(T1)); 2.68–2.75 (*m*, H-C(3')(T1)); 2.97–3.15 (*m*, 1 H of CH₂-C(3')(T1), 2 H-C(6')(T2)); 3.22–3.31 (*m*, 1 H of CH₂-C(3')(T1)); 3.48–3.62 (*m*, 2 H-C(6')(T1)); 3.82–3.95 (*m*, H-C(4')(T1), H-C(4')(T2)); 5.92–5.96 (*m*, H-C(1')(T2)); 6.03–6.09 (*dd*, H-C(1')(T1)); 7.06 (2*s*, 2 H, H-C(6)(T)); 7.39–7.50 (*m*, 6 H, Ph₂Si); 7.61–7.66 (*m*, 4 H, Ph₂Si); 9.08 (br., 1 NH); 9.17 (br., 1 NH). ¹³C-NMR (CDCl₃, 75 MHz): 12.61, 13.24 (2*q*, Me-C(5)(T)); 19.34 (*s*, Me₃C); 26.94 (*q*, Me₃C); 29.72 (*t*, C(6)(T1)); 30.98 (*t*, C(2')(T1)); 33.31 (*t*, C(5')(T1)); 34.84 (*t*, C(5')(T2)); 38.02 (*t*, C(2')(T2)); 38.44 (*d*, C(3')(T2)); 46.28 (*d*, C(3')(T1)); 48.94 (*t*, CH₂-C(3')(T1)); 52.88 (*t*, C(6)(T2)); 64.80 (*t*, CH₂-C(3')(T2)); 80.83 (*d*, C(4)(T2)); 83.95 (*d*, C(4)(T1)); 84.78 (*d*, C(1')(T2)); 92.81 (*d*, C(1')(T1)); 110.23, 111.84 (2*s*, C(5)(T)); 127.94, 130.13 (2*d*, Ph₂Si); 132.80 (*s*, Ph₂Si); 135.62 (*d*, Ph₂Si); 137.12 (2*d*, C(6)(T)); 150.11, 151.23 (2*s*, C(2)(T)); 163.82 (2*s*, C(4)(T)). FAB-MS (NOBA; pos.): 873 (14, [M₁+H]⁺), 871 (14, [M₂+H]⁺), 669 (12), 667 (11), 491 (15), 489 (13), 366 (14), 365 (72), 364 (14), 363 (68), 289 (17), 287 (16), 279 (21), 269 (15), 265 (11), 262 (11), 257

(11), 251 (10), 247 (20), 243 (13), 239 (23), 237 (11), 235 (13), 233 (19), 229 (11), 227 (21), 225 (15), 223 (10), 217 (10), 199 (93), 189 (100), 183 (36), 165 (45), 154, 135.

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(hydroxymethyl)thymidine (*d*(TrOCH₂-Tso₂T-CH₂OH); **12a**). Compound **8a** (70 mg, 67 μmol) was dissolved in THF (10 ml) at r.t., and 1M Bu₄NF in THF (0.25 ml, 0.25 mmol) was added (→ immediately yellow). The mixture was stirred for 2 h at r.t. Me₃SiOMe (0.23 ml, 165 μmol) was added, stirring continued for 10 min, and the mixture filtered through a layer of silica gel (CH₂Cl₂/MeOH 20 : 1). The filtrate was evaporated and the residue chromatographed (silica gel, CH₂Cl₂/MeOH 20 : 0, 10 : 1): **12a** (50 mg, 93%). Colorless foam. UV (MeCN): 203 (43100), 265 (13200). ¹H-NMR (CDCl₃, 300 MHz): 1.88, 1.91 (2s, Me-C(5)(T)); 1.83–2.04 (m, 2 H-C(5')(T1)); 2.09–2.27 (m, 2 H-C(5')(T2)); 2.30–2.50 (m, H-C(3')(T2), 1 H-C(2')(T1), 1 H-C(2')(T2)); 2.51–2.64 (m, 1 H-C(2')(T1)); 2.80–2.89 (m, H-C(3')(T1)); 3.00 (dd, *J* = 9.7, 14.2, 1 H of CH₂-C(3')(T1)); 3.09–3.23 (m, 1 H of CH₂-C(3')(T1), 2 H-C(6')(T2)); 3.30 (*t*, *J* = 6.0, 2 H-C(6')(T1)); 3.60–3.68 (m, 1 H of CH₂-C(3')(T2)); 3.70–3.77 (m, 1 H of CH₂-C(3')(T2)); 3.83–3.96 (m, H-C(4')(T1), H-C(4')(T2)); 5.93–6.00 (m, H-C(1')(T1), H-C(1')(T2)); 7.08, 7.14 (2s, 2 H, H-C(6)(T)); 7.20–7.32 (m, 9 H, Tr); 7.40–7.45 (m, 6 H, Tr); 9.4 (br., 2 NH). ¹³C-NMR (CDCl₃, 75 MHz): 12.61, 12.70 (2*q*, Me-C(5)(T)); 34.17 (*t*, C(5')(T1)); 34.41 (*t*, C(5')(T1)); 34.41 (*t*, C(5')(T2)); 36.79 (*d*, C(3')(T2)); 38.39 (2*t*, C(2')(T1)); 44.99 (*d*, C(3')(T1)); 50.63 (*t*, CH₂-C(3')(T1)); 54.97 (*t*, C(6')(T2)); 60.46 (*t*, C(6')(T1)); 62.63 (*t*, CH₂-C(3')(T2)); 80.93 (*d*, C(4')(T2)); 81.78 (*d*, C(4')(T1)); 85.56 (*m*, C(1')(T2)); 85.76 (*d*, C(1')(T1)); 87.03 (*s*, Tr); 110.99, 111.34 (2*s*, C(5)(T)); 127.15, 127.92, 128.58 (3*d*, Tr); 135.49, 135.94 (2*d*, C(6)(T)); 143.91 (*s*, Tr); 150.40 (*s*, C(2)(T)); 163.78 (*s*, C(4)(T)). FAB-MS (NOBA; pos.): 814 (19, [M + H]⁺), 766 (20), 664 (39), 663 (23), 648 (24), 622 (20), 614 (38), 613 (100), 597 (21), 596 (31), 595 (22), 576 (29), 566 (21), 552 (23), 535 (31), 530 (31), 289, 243, 207, 155, 153 (only *m/z* > 150 were considered). MALDI-TOF MS (0.1M 2,5-DHB in MeCN/EtOH/H₂O 50 : 45 : 5): 835.5 ([M + Na]⁺).

5'-[(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(hydroxymethyl)thymidine (*d*(BzOCH₂-Tso₂T-CH₂OH); **12b**). As described for **12a**, with **8c** (275 mg, 301 μmol), THF (10 ml), and 1M Bu₄NF in THF (0.92 ml, 0.25 mmol) for 1.5 h, then with Me₃SiOMe (104 μl, 753 μmol). Chromatography (silica gel, CH₂Cl₂/MeOH 20 : 0, 10 : 1, 5 : 1) yielded **12b** (185 mg, 91%). Colorless foam. ¹H-NMR (CDCl₃/MeOH 4 : 1, 300 MHz): 1.89, 1.90 (2s, 6 H, Me-C(5)(T)); 2.03–2.51 (m, 2 H-C(5')(T1), 2 H-C(5')(T2), 2 H-C(2')(T1), 2 H-C(2')(T2), H-C(3')(T2)); 2.64–2.69 (m, H-C(3')(T1)); 3.09–3.35 (m, CH₂-C(3')(T1), 2 H-C(6')(T2)); 3.56–3.62 (m, 1 H of CH₂-C(3')(T2)); 3.62–3.70 (m, 1 H of CH₂-C(3')(T2)); 3.83–3.96 (m, H-C(4')(T1), H-C(4')(T2)); 4.38–4.49 (m, 1 H-C(6')(T1)); 4.51–4.61 (m, 1 H-C(6')(T1)); 5.96–6.02 (m, H-C(1')(T2)); 6.04–6.09 (m, H-C(1')(T1)); 7.22, 7.23 (2s, 2 H, H-C(6)(T)); 7.39–7.46 (m, 2 H, Bz); 7.53–7.60 (m, 1 H, Bz); 7.98–8.02 (m, 2 H, Bz). ¹³C-NMR (CDCl₃/MeOH 4 : 1, 75 MHz): 11.99 (2*q*, Me-C(5)(T)); 26.59; 32.26 (*t*, C(5')(T1)); 34.16 (*t*, C(5')(T2)); 36.17 (*d*, C(3')(T2)); 37.57 (2*t*, C(2')(T1)); 44.89 (*d*, C(3')(T1)); 50.66 (*t*, CH₂-C(3')(T1)); 54.01 (*t*, C(6')(T2)); 61.61 (2*t*, C(6')(T1), CH₂-C(3')(T2)); 80.59, 80.80 (2*d*, C(4')(T1)); 84.84 (2*d*, C(1')(T1)); 110.90 (2*s*, C(5)(T)); 128.16, 129.22 (2*d*, Bz); 129.46 (*s*, Bz); 133.98 (*d*, Bz); 135.39, 135.64 (2*d*, C(6)(T)); 150.39, 150.44 (2*s*, C(2)(T)); 164.31 (2*s*, C(4)(T)); 166.53 (*s*, C=O).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[(acetylthio)methyl]-3',5'-dideoxythymidine (*d*(TrOCH₂-Tso₂T-CH₂SAc); **13a**). PPh₃ (77 mg, 295 μmol) was dried under high vacuum at 45° for 3 h and was dissolved in THF (1 ml). The soln. was cooled to 0°, and DIAD (42 μl, 216 μmol) was added dropwise. The soln. was stirred for 30 min at 0°. A white precipitate was formed after 10 min. Thioacetic acid (15.4 μl, 216 μmol) and **12a** (80 mg, 98 μmol; dried overnight under high vacuum at r.t.) were dissolved separately in THF (each 1 ml) and alternately added dropwise, beginning with thioacetic acid. The mixture was allowed to warm to r.t., stirred for 2 h, and quenched with Et₃N/MeOH 2 : 1 (1 ml). The soln. was evaporated and the crude residue chromatographed (silica gel, CH₂Cl₂/MeOH 100 : 0, 40 : 1, 20 : 1): **13a** (78 mg, 91%). Colorless foam. UV (MeCN): 203 (48300), 265 (13400). ¹H-NMR (CDCl₃, 300 MHz): 1.90, 1.92 (2s, 6 H, Me-C(5)(T)); 1.85–2.04 (m, 1 H-C(5')(T1)); 2.36 (*s*, Ac); 2.06–2.53 (m, 1 H-C(5')(T1), 2 H-C(5')(T2), H-C(3')(T2), 2 H-C(2')(T1), 2 H-C(2')(T2)); 2.58–2.67 (m, H-C(3')(T1)); 2.92–3.11 (m, 1 H of CH₂-C(3')(T1), CH₂-C(3')(T2), 1 H-C(6')(T2)); 3.16–3.27 (m, 1 H of CH₂-C(3')(T1), 1 H-C(6')(T2)); 3.32 (*t*, *J* = 6.0, 2 H-C(6')(T1)); 3.68–3.74 (m, H-C(4')(T2)); 3.83–3.96 (m, H-C(4')(T1)); 5.96–6.00 (dd, H-C(1')(T2)); 6.00–6.05 (dd, H-C(1')(T1)); 7.05, 7.06 (2s, 2 H-C(6)(T)); 7.22–7.34 (m, 9 H, Tr); 7.40–7.45 (m, 6 H, Tr); 8.92 (br., 1 NH); 9.05 (br., 1 NH). ¹³C-NMR (CDCl₃, 75 MHz): 12.69 (2*q*, Me-C(5)(T)); 25.95 (*t*, CH₂-C(3')(T2)); 29.97 (*t*, C(5')(T2)); 30.65 (*q*, MeCO); 34.13 (*t*, C(5')(T1)); 36.69 (*d*, C(3')(T2)); 37.10, 38.38 (2*t*, C(2')(T1)); 42.85 (*d*, C(3')(T1)); 50.69 (*t*, CH₂-C(3')(T1)); 55.01 (*t*, C(6')(T2)); 60.41 (*t*, C(6')(T1)); 81.61 (*d*, C(4')(T2)); 81.84 (*d*, C(4')(T1)); 85.07 (*d*, C(1')(T2)); 85.62 (*d*, C(1')(T1)); 87.00

(s, Tr); 111.07, 111.39 (2s, C(5)(T)); 127.11, 127.87, 128.55 (3d, Tr); 135.18, 135.75 (2d, C(6)(T)); 143.88 (s, Tr); 150.24 (s, C(2)(T)); 163.62 (s, C(4)(T)); 195.17 (s, MeCO). MALDI-TOF MS ($A = \text{dimer in } \text{CH}_2\text{Cl}_2, B = 0.1\text{M } 2,5\text{-DHB in MeCN/EtOH/H}_2\text{O } 50:45:5, C = A/B 1:1$): 895 ($[M + H + \text{Na}]^+$).

5'-[(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[(acetylthio)methyl]-3',5'-dideoxythymidine ($d(\text{BzOCH}_2\text{-Tso}_2\text{T-CH}_2\text{SAC})$; **13b**). As described for **13a**, with PPh_3 (212 mg, 809 μmol), THF (5 ml), DIAD (115 μl , 593 μmol), thioacetic acid (42.3 μl , 593 μmol), **12b** (182 mg, 270 μmol); dried overnight under high vacuum at r.t., and THF (each 1 ml); reaction for 1.5 h and quenching with $\text{Et}_3\text{N/MeOH } 2:1$ (2 ml). Chromatography (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH } 100:0, 40:1, 20:1, 10:1$) yielded **13b** (195 mg, 99%). Colorless foam. UV (MeCN): 221 (17300), 265 (16400). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$, 300 MHz): 1.78 (s, 6 H, Me-C(5)(T)); 2.03–2.43 (m, 2 H-C(5')(T1), 2 H-C(5')(T2), 2 H-C(2')(T1), 2 H-C(2')(T2), H-C(3')(T2)); 2.35 (s, Ac); 2.62–2.67 (m, H-C(3')(T1)); 2.93 (dd, $J = 5.4, 13.6$, 1 H of $\text{CH}_2\text{-C(3')(T2)}$); 3.06 (dd, $J = 7.5, 13.6$, 1 H of $\text{CH}_2\text{-C(3')(T2)}$); 3.21–3.32 (m, 1 H of $\text{CH}_2\text{-C(3')(T1)}$, 2 H-C(6')(T2)); 3.21–3.32 (m, 1 H of $\text{CH}_2\text{-C(3')(T1)}$); 3.56–3.62 (m, 1 H of $\text{CH}_2\text{-C(3')(T2)}$); 3.62–3.70 (dt, H-C(4')(T2)); 3.80–3.87 (dt, H-C(4')(T1)); 4.28–4.37 (m, 1 H-C(6')(T1)); 4.40–4.48 (m, 1 H-C(6')(T1)); 6.00–6.07 (m, H-C(1')(T2), H-C(1')(T1)); 7.44, 7.47 (2s, 2 H, H-C(6)(T)); 7.50–7.53 (m, 2 H, Bz); 7.62–7.67 (m, 1 H, Bz); 7.95–7.97 (m, 2 H, Bz); 11.30 (br., 1 NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.45 (2q, Me-C(5)(T)); 26.00 (t, $\text{CH}_2\text{-C(3')(T2)}$); 29.87 (t, C(5')(T2)); 30.50 (q, MeCO); 32.60 (t, C(5')(T1)); 36.49 (t, C(2')(T1)); 36.88 (d, C(3')(T2)); 37.78 (t, C(2')(T2)); 42.73 (d, C(3')(T1)); 50.58 (t, $\text{CH}_2\text{-C(3')(T1)}$); 54.55 (t, C(6')(T2)); 61.71 (t, C(6')(T1)); 81.00, 81.77 (2d, C(4')(T1)); 85.12, 85.37 (2d, C(1')(T1)); 111.12, 111.14 (2s, C(5)(T)); 128.31, 129.41 (2d, Bz); 129.75 (s, Bz); 133.03 (d, Bz); 135.34, 135.89 (2d, C(6)(T)); 150.45, 150.52 (2s, C(2)(T)); 163.96 (2s, C(4)(T)); 166.32 (s, C=O); 195.22 (s, MeCO). ESI-MS (neg.): 767.1 ($[M + \text{Cl}]^-$), 737.1 ($[M - \text{H}]^-$), 688.9 ($[M - \text{Ac}]^-$), 611.2 ($[M - \text{Bz}]^-$).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(mercaptomethyl)thymidine ($d(\text{TrOCH}_2\text{-Tso}_2\text{T-CH}_2\text{SH})$; **14a**). Method 1: NaBH_4 (6.2 mg, 164 μmol) was dissolved in degassed MeOH (2 ml, 1 h Ar), a soln. of NaOMe (0.5 mg) in degassed MeOH (0.5 ml, 1 h Ar) was added, and the soln. was cooled to 0°. Then **13a** (57 mg, 65 μmol) in degassed MeOH/THF 1:1 (4 ml, 1 h Ar) was slowly added dropwise. The mixture was allowed to warm to r.t., stirred for 4 h, and cooled again to 0°. AcOH was added until pH 5 was reached. The soln. was concentrated to 25% of the original volume, CH_2Cl_2 (20 ml) was added, and the soln. was filtered through alox B and silica gel. The filtrate was evaporated and the residue chromatographed (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH } 100:0, 40:1, 20:1, 10:1$): **14a** (53 mg, 98%). Colorless foam.

Method 2: A soln. of **13a** (70 mg, 566 μmol) in degassed MeOH (2 ml, 1 h Ar) was cooled to 0°. Ammonia was bubbled through the soln. for 15 min, and stirring was continued for 2 h. The mixture was carefully evaporated at a water-bath temp. of 0°. The acetamide was removed under high vacuum at r.t. overnight: **14a** (300 mg, quant.). Colorless foam. UV (MeCN): 208 (37500), 265 (16800). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 1.47 (t, $J = 16.6$, SH); 1.88, 1.92 (2s, Me-C(5)(T)); 1.80–2.02 (m, 2 H-C(5')(T1)); 2.08–2.19 (m, 1 H-C(5')(T2)); 2.22–2.49 (m, 1 H-C(5')(T2), H-C(3')(T2), 2 H-C(2')(T1), 2 H-C(2')(T2)); 2.53–2.72 (m, H-C(3')(T1), 1 H of $\text{CH}_2\text{-C(3')(T2)}$); 2.90–3.13 (m, 1 H of $\text{CH}_2\text{-C(3')(T1)}$, 1 H-C(6')(T2)); 3.13–3.26 (m, 1 H of $\text{CH}_2\text{-C(3')(T1)}$, 1 H-C(6')(T2)); 3.31 (t, $J = 6.0$, 2 H-C(6')(T1)); 3.77–3.85 (m, H-C(4')(T2)); 3.86–3.92 (m, H-C(4')(T1)); 5.97–6.04 (m, H-C(1')(T2), H-C(1')(T1)); 7.06, 7.08 (2s, 2 H, H-C(6)(T)); 7.21–7.32 (m, 9 H, Tr); 7.40–7.45 (m, 6 H, Tr); 9.21 (br., 1 NH); 9.31 (br., 1 NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.60, 12.68 (2q, Me-C(5)(T)); 26.01 (t, $\text{CH}_2\text{-C(3')(T2)}$); 34.13 (t, C(5')(T1)); 36.67 (d, C(3')(T2)); 36.90 (t, C(5')(T2)); 38.39 (t, C(2')(T1)); 45.74 (d, C(3')(T1)); 50.54 (t, $\text{CH}_2\text{-C(3')(T1)}$); 50.67 (t, C(6')(T2)); 55.17 (t, C(2')(T2)); 60.40 (t, C(6')(T1)); 81.61 (d, C(4')(T2)); 81.66 (d, C(4')(T1)); 85.14 (d, C(1')(T2)); 85.76 (d, C(1')(T1)); 87.00 (s, Tr); 1101.04, 111.44 (2s, C(5)(T)); 127.11, 127.88, 128.53 (3d, Tr); 135.22, 135.78 (2d, C(6)(T)); 143.85 (s, Tr); 150.29 (s, C(2)(T)); 163.65 (s, C(4)(T)); MALDI-TOF MS ($A = \text{dimer in } \text{CH}_2\text{Cl}_2, B = 0.1\text{M } 2,5\text{-DHB in MeCN/EtOH/H}_2\text{O } 50:45:5, C = A/B 1:1$): 1681.3 ($[2M + \text{Na}]^+$), 852.5 ($[M + \text{Na}]^+$).

5'-[(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(mercaptomethyl)thymidine ($d(\text{BzOCH}_2\text{-Tso}_2\text{T-CH}_2\text{SH})$; **14b**). As described for **14a** (Method 2), with **13b** (25 mg, 34 μmol), MeOH (3 ml, 1 h Ar), and ammonia (95 min): **14b**. (300 mg, quant.). Colorless foam. UV (MeCN): 265 (16100). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): 1.92 (s, 6 H, Me-C(5)(T)); 2.01–2.45 (m, 2 H-C(5')(T1), 2 H-C(5')(T2), 1 H-C(2')(T1), 2 H-C(2')(T2), H-C(3')(T2)); 2.46–2.82 (m, 1 H-C(2')(T1), H-C(3')(T1), $\text{CH}_2\text{-C(3')(T2)}$); 3.03–3.37 (m, $\text{CH}_2\text{-C(3')(T1)}$, 2 H-C(6')(T2)); 3.78–3.86 (m, H-C(4')(T2)); 3.87–3.96 (m, H-C(4')(T1)); 4.40–4.49 (m, H-C(6')(T1)); 4.54–4.63 (m, H-C(6')(T1)); 5.92–5.99 (m, H-C(1')(T2)); 6.02–6.09 (m, H-C(1')(T1)); 7.07/7.18 (2s, 2 H, H-C(6)(T)); 7.41–7.48 (m, 2 H, Bz); 7.56–7.61 (m, 1 H, Bz); 8.01–8.05 (m, 2 H, Bz); 9.58 (br., 1 NH).

^{13}C -NMR (CDCl_3 , 75 MHz): 12.56 (2q, Me–C(5)(T)); 26.16 (t, CH_2 –C(3')(T2)); 32.76 (2t, C(5')); 36.75 (d, C(3')(T2)); 37.52, 37.97 (2t, C(2')); 45.72 (d, C(3')(T1)); 50.61 (t, CH_2 –C(3')(T1)); 55.00 (t, C(6')(T2)); 61.70 (t, C(6')(T1)); 81.14 (d, C(4')(T2)); 81.69 (d, C(4')(T1)); 85.50 (d, C(1')(T2)); 86.11 (d, C(1')(T1)); 111.36 (2s, C(5)(T)); 128.45 (d, $\text{C}_m(\text{Bz})$); 129.54 (d, $\text{C}_o(\text{Bz})$); 129.84 (s, $\text{C}_{ipso}(\text{Bz})$); 133.17 (d, $\text{C}_o(\text{Bz})$); 135.40, 136.14 (2d, C(6)(T)); 150.39 (2s, C(2)(T)); 163.76 (2s, C(4)(T)); 166.46 (s, PhCO).

5'-[(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-[[4,4'-dimethoxytrityl]thio]methyl]thymidine (*d*(BzOCH₂-Tso₂T-CH₂S₂Tr(OMe)); **15**). Compound **14b** (110 mg, 159 μmol) and (MeO)₂TrCl (108 mg, 318 μmol) were dried overnight under high vacuum at r.t. and then dissolved in THF (10 ml). Et₃N (0.5 ml) was added (clear red → turbid yellow soln.). The mixture was stirred for 20 min at r.t. and then quenched with MeOH (1 ml) and evaporated. The residue was chromatographed (silica gel (column loaded with 1% Et₃N), CH₂Cl₂/MeOH 100:0, 40:1, 20:1): **15** (157 mg, 99%). Colorless foam. UV (MeCN): 209 (52600), 228 (34400), 265 (17100). ^1H -NMR (CDCl_3 , 300 MHz): 1.89 (s, 6 H, Me–C(5)(T)); 1.75–1.99 (m, 2 H–C(5')(T2)); 2.00–2.19 (m, 2 H–C(5')(T1), H–C(3')(T2), 1 H–C(2')(T2)); 2.22–2.41 (m, 1 H–C(2')(T1), CH₂–C(3')(T2), 1 H–C(2')(T2)); 2.44–2.55 (m, 1 H–C(2')(T1)); 2.66–2.78 (m, H–C(3')(T1)); 2.96–3.10 (m, 1 H of CH₂–C(3')(T1), 1 H–C(6')(T2)); 3.14–3.27 (m, 1 H of CH₂–C(3')(T1), 1 H–C(6')(T2)); 3.46–3.54 (dt, H–C(4')(T2)); 3.78 (s, 2 MeO); 3.84–3.93 (m, H–C(4')(T1)); 4.38–4.47 (m, 1 H–C(6')(T1)); 4.52–4.61 (m, 1 H–C(6')(T1)); 5.86 (dd, *J* = 4.2, 6.8, H–C(1')(T2)); 6.06 (dd, *J* = 4.4, 6.6, bH–C(1')(T1)); 6.80–6.83 (m, *J* = 8.8, 4 H, H_m of Ar₂PhC); 6.92, 7.15 (2s, 2 H, H–C(6)(T)); 7.18–7.24 (m, 1 H, (MeO)₂Tr); 7.26–7.32 (m, 6 H, (MeO)₂Tr); 7.36–7.44 (m, 4 H, H_o of Ar₂PhC, 2 H of Bz); 7.51–7.56 (m, 1 H, Bz); 8.01 (d, *J* = 7.1, 2 H, Bz); 9.83 (br., 1 NH). ^{13}C -NMR (CDCl_3 , 75 MHz): 12.50, 12.53 (2q, Me–C(5)(T)); 25.88 (t, CH₂–C(3')(T2)); 32.67, 33.43 (2t, C(5')(T)); 36.51 (d, C(3')(T2)); 37.43, 37.89 (2t, C(2')(T)); 42.61 (d, C(3')(T1)); 50.61 (t, CH₂–C(3')(T1)); 54.81 (t, C(6')(T2)); 55.17 (q, MeO); 61.65 (t, C(6')(T1)); 66.27 (s, Ar₂PhC); 81.03 (d, C(4')(T2)); 82.03 (d, C(4')(T1)); 85.15 (2d, C(1')(T)); 111.20, 111.25 (2s, C(5)(T)); 113.78 (d, $\text{C}_m(\text{Ar}_2\text{PhC})$); 126.70 (d, $\text{C}_p(\text{Ar}_2\text{PhC})$); 127.93 (d, $\text{C}_o(\text{Ar}_2\text{PhC})$); 128.35 (d, $\text{C}_m(\text{Bz})$); 129.19 (d, $\text{C}_m(\text{Ar}_2\text{PhC})$); 129.46 (d, $\text{C}_o(\text{Bz})$); 129.78 (s, $\text{C}_{ipso}(\text{Bz})$); 130.52 (d, $\text{C}_o(\text{Ar}_2\text{PhC})$); 133.07 (d, $\text{C}_p(\text{Bz})$); 135.23, 135.64 (2d, C(6)(T)); 136.52 (s, $\text{C}_{ipso}(\text{Ar}_2\text{PhC})$); 144.85 (s, $\text{C}_{ipso}(\text{Ar}_2\text{PhC})$); 150.40, 150.46 (2s, C(2)(T)); 158.08 (s, $\text{C}_p(\text{Ar}_2\text{PhC})$); 163.85 (2s, C(4)(T)); 166.35 (s, PhCO). ESI-MS (neg.): 1027.4 ([*M* + C][−]), 991.6 ([*M* − H][−]), 948.0

5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-[[4,4'-dimethoxytrityl]thio]methyl]thymidine (*d*(HOCH₂-Tso₂T-CH₂S₂Tr(OMe)₂); **16**). To a suspension of **15** (157 mg, 158 μmol) in MeOH (10 ml), 2M NaOH (1.0 ml) was added slowly (→ soln.). After 30 min, the mixture was neutralized with acetate buffer (3M AcOH/1M AcONa 3:1; 1.0 ml) and immediately poured into sat. NaHCO₃ soln. (15 ml) containing ice (10 g). CH₂Cl₂ (60 ml) was added and the org. phase washed with brine (2 × 15 ml). The aq. phases were reextracted with CH₂Cl₂ (3 × 20 ml). The combined org. phase was dried (MgSO₄) and evaporated. FC (silica gel (column loaded with 1% Et₃N), CH₂Cl₂/MeOH 100:0, 20:1, 10:1, 5:1) yielded **16** (138 mg, 98%). Colorless foam. UV (MeCN): 235 (211000), 264 (19000). ^1H -NMR (CDCl_3 , 500 MHz): 1.82–1.91 (m, 2 H–C(5')(T2)); 1.91, 1.92 (2s, 6 H, Me–C(5)(T)); 1.97–2.12 (m, 2 H–C(5')(T1), 1 H–C(2')(T2)); 2.19–2.28 (m, 1 H–C(2')(T2), 1 H of CH₂–C(3')(T2)); 2.29–2.37 (m, H–C(3')(T2), 1 H–C(2)(T2)); 2.22–2.41 (m, 1 H–C(2')(T1), CH₂–C(3')(T2), 1 H–C(2')(T2)); 2.44–2.55 (m, 1 H of CH₂–C(3')(T1), 1 H–C(2')(T1)); 2.46–2.52 (m, 1 H–C(2')(T1)); 2.70–2.78 (m, H–C(3')(T1)); 2.97 (dd, *J* = 10.2, 13.8, 1 H of CH₂–C(3')(T1)); 3.01–3.06 (m, 1 H–C(6')(T2)); 3.15–3.21 (m, 1 H–C(6')(T2)); 3.27 (dd, *J* = 3.5, 13.7, 1 H of CH₂–C(3')(T1)); 3.51–3.56 (dt, H–C(4')(T2)); 3.74–3.79 (m, 1 H–C(6')(T1)); 3.79 (s, 2 MeO); 3.82–3.88 (m, 1 H–C(6')(T1), H–C(4')(T1)); 5.80 (dd, *J* = 4.2, 7.6, H–C(1')(T2)); 6.00 (dd, *J* = 4.1, 7.6, H–C(1')(T1)); 6.80–6.84 (m, *J* = 9.0, 4 H, H_m of Ar₂PhC); 6.91, 6.92 (2s, 1 H, H–C(6)(T)); 7.12 (2s, 1 H, H–C(6)(T)); 7.19–7.23 (m, 1 H, (MeO)₂Tr); 7.26–7.31 (m, 6 H, (MeO)₂Tr); 7.37–7.39 (m, 2 H, H_o of Ar₂PhC). ^{13}C -NMR (CDCl_3 , 75 MHz): 12.55 (2q, Me–C(5)(T)); 26.03 (t, CH₂–C(3')(T2)); 33.45, 35.59 (2t, C(5')(T)); 36.39 (d, C(3')(T2)); 37.32, 37.97 (2t, C(2')(T)); 42.66 (d, C(3')(T1)); 50.40 (t, CH₂–C(3')(T1)); 54.65 (t, C(6')(T2)); 55.18 (q, MeO); 58.93 (t, C(6')(T1)); 66.26 (s, Ar₂PhC); 81.82, 82.13 (2d, C(4')(T)); 84.99, 85.29 (2d, C(1')(T)); 111.20 (2s, C(5)(T)); 113.17 (d, $\text{C}_m(\text{Ar}_2\text{PhC})$); 126.71 (s, $\text{C}_p(\text{Ar}_2\text{PhC})$); 127.92 (d, $\text{C}_o(\text{Ar}_2\text{PhC})$); 129.19 (d, $\text{C}_m(\text{Ar}_2\text{PhC})$); 130.53 (d, $\text{C}_o(\text{Ar}_2\text{PhC})$); 135.53, 135.80 (2d, C(6)(T)); 136.54 (s, $\text{C}_{ipso}(\text{Ar}_2\text{PhC})$); 144.86 (s, $\text{C}_{ipso}(\text{Ar}_2\text{PhC})$); 150.55, 150.64 (2s, C(2)(T)); 158.07 (s, $\text{C}_p(\text{Ar}_2\text{PhC})$); 164.02 (2s, C(4)(T)). ESI-MS (pos.): 911.0 ([*M* + Na]⁺), 865.3, 785.9 ([*M* − T + Na]⁺), 303 (Tr⁺).

5'-(Bromomethyl)-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-[[4,4'-dimethoxytrityl]thio]methyl]thymidine (*d*(BrCH₂-Tso₂T-CH₂S₂Tr(OMe)₂); **17**). Compound **16** (138 mg, 155 μmol) and PPh₃ (79 mg, 300 μmol) were dried overnight under high vacuum at 45° and then dissolved in 1,2-dichloroethane (10 ml). Then CBr₄ (80 mg, 240 μmol) in 1,2-dichloroethane (1 ml) was added

slowly. After the addition of *ca.* 1.2 equiv. of CBr_4 the soln. turned slightly orange because of negligible detritylation. The mixture was stirred for 60 min at r.t., quenched with $\text{Et}_3\text{N}/\text{MeOH}$ 2 : 1 (3 ml), and evaporated (30° water-bath temperature). FC (silica gel (column loaded with 1% Et_3N), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100 : 0, 20 : 1, 10 : 1, 5 : 1) yielded **17** (143 mg, 97%). Colorless foam. UV (MeCN): 207 (49700), 265 (17300). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): 1.81–1.91 (*m*, 2 H–C(5')(T2)); 1.94 (2s, 6 H, Me–C(5)(T)); 1.98–2.15 (*m*, 2 H–C(2')(T2)); 2.18–2.30 (*m*, 2 H–C(5')(T1), 1 H of $\text{CH}_2\text{–C}(3')(T2)$, H–C(3')(T2)); 2.33–2.39 (*m*, 1 H of $\text{CH}_2\text{–C}(3')(T2)$, 1 H–C(2')(T1)); 2.50–2.55 (*m*, 1 H–C(2')(T1)); 2.70–2.73 (*m*, H–C(3')(T1)); 2.98 (*dd*, $J=9.8, 13.7$, 1 H, $\text{CH}_2\text{–C}(3')(T1)$); 2.99–3.05 (*m*, 1 H–C(6')(T2)); 3.10 (*dd*, $J=3.5, 13.7$, 1 H, $\text{CH}_2\text{–C}(3')(T1)$); 3.13–3.19 (*m*, 1 H–C(6')(T2)); 3.49–3.61 (*m*, 2 H–C(6')(T1), H–C(4')(T2)); 3.80 (*s*, 2 MeO); 3.87 (*dd*, $J=3.4, 8.3$, H–C(4')(T1)); 5.83 (*dd*, $J=4.4, 7.8$, H–C(1')(T2)); 6.00 (*dd*, $J=3.9, 7.8$, H–C(1')(T1)); 6.81–6.84 (*m*, $J=9.8, 4$ H, H_m of Ar_2PhC); 6.92 (2s, 1 H, H–C(6)(T)); 7.04 (2s, 1 H, H–C(6)(T)); 7.21–7.24 (*m*, 1 H, $(\text{MeO})_2\text{Tr}$); 7.28–7.31 (*m*, 6 H, $(\text{MeO})_2\text{Tr}$); 7.39 (*d*, $J=7.3$, 2 H, H_o of Ar_2PhC); 8.33 (br., 1 NH); 8.42 (br., 1 NH). $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): 12.53 (2*q*, Me–C(5)(T)); 25.90 (*t*, $\text{CH}_2\text{–C}(3')(T2)$); 29.38 (*t*, C(6')(T1)); 33.39, 36.01 (2*t*, C(5')(T)); 36.45 (*d*, C(3')(T2)); 37.43, 37.83 (2*t*, C(2')(T)); 42.54 (*d*, C(3')(T1)); 50.53 (*t*, $\text{CH}_2\text{–C}(3')(T1)$); 54.61 (*t*, C(6')(T2)); 55.14 (*q*, MeO); 66.22 (*s*, Ar_2PhC); 81.45 (*d*, C(4')(T2)); 82.05 (*d*, C(4')(T1)); 85.17 (2*d*, C(1')(T)); 111.14, 111.25 (2*s*, C(5)(T)); 113.14 (*d*, $\text{C}_m(\text{Ar}_2\text{PhC})$); 126.66 (*s*, $\text{C}_o(\text{Ar}_2\text{PhO})$); 127.79 (*d*, $\text{C}_o(\text{Ar}_2\text{PhC})$); 129.15 (*d*, $\text{C}_m(\text{Ar}_2\text{PhC})$); 130.48 (*d*, $\text{C}_o(\text{Ar}_2\text{PhC})$); 135.41 (2*d*, C(6)(T)); 136.48 (*s*, $\text{C}_{ipso}(\text{Ar}_2\text{PhC})$); 144.80 (*s*, $\text{C}_{ipso}(\text{Ar}_2\text{PhC})$); 150.37, 150.39 (2*s*, C(2)(T)); 158.04 (*s*, $\text{C}_o(\text{Ar}_2\text{PhC})$); 163.75, 163.83 (2*s*, C(4)(T)). ESI-MS (neg.): 1031.7 ($[\text{M} + \text{Br}]^-$), 987.0 ($[\text{M} + \text{Cl}]^-$), 950.9, 948.9 (M^-), 905.1 ($[\text{M} - \text{Br} + \text{Cl}]^-$).

5. Tetramers: Coupling and Oxidation. 5'-Deoxy-3'-de(phosphinicooxy)-5'-I(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-{[(tert-butyl)diphenylsilyloxy]methyl}-2',3',5'-trideoxy-N⁴-(*o*-toluoyl)cytidine (*d*($\text{TrOCH}_2\text{-Tso}_2\text{TsTso}_2\text{-C-CH}_2\text{OTBDPS}$); **19b**). Thiol **14a** (40 mg, 48 μmol), bromide **11b** (44 mg, 45 μmol), and Cs_2CO_3 (64 mg, 180 μmol) were dissolved in degassed DMF (3 ml, 1 h Ar). The soln. was degassed a second time by performing three freeze-pump cycles. The mixture was warmed to 45° and stirred for 5 h. Acetate buffer (3M AcOH/1M AcONa; 90 μl) was added, and the solvent was evaporated at max. 40° . The crude product was chromatographed (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100 : 0, 40 : 1, 20 : 1, 10 : 1): **19b** (63 mg, 81%). Colorless foam. $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$, 500 MHz): 1.00 (*s*, *t*-Bu); 1.75, 1.77, 1.78 (6*s*, 9 H, Me–C(5)(T)); 1.83–1.94 (*m*, 2 H, H–C(5')); 1.96–2.41 (*m*, 17 H, 6 H–C(5'), 8 H–C(2'), 3 H–C(3')); 2.36 (*s*, Me(to)); 2.50–2.54 (*m*, partly under DMSO, 1 H, H–C(3')); 2.55–2.62 (*m*, 2 H, CH_2SCH_2); 2.67–2.74 (*m*, 2 H, CH_2SCH_2); 3.02–3.08 (*m*, 2 H, $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.18–3.37 (*m*, partly under H $_2\text{O}$, 6 H, $\text{CH}_2\text{SO}_2\text{CH}_2$, 2 H–C(6')(T1)); 3.40–3.48 (*m*, 2 H, $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.65–3.73 (*m*, 4 H, 2 H–C(4'), $\text{CH}_2\text{–C}(3')(C)$); 3.73–3.79 (*dt*, 1 H, H–C(4')); 3.99–4.04 (*dt*, 1 H, H–C(4')); 5.97–6.00 (*dd*, 1 H, H–C(1')); 6.00–6.04 (*m*, 3 H, H–C(1')); 7.20, 7.21, 7.22 (3*s*, 3 H–C(6)(T)); 7.24–7.31 (*m*, 8 arom. H); 7.33–7.40 (*m*, 9 arom. H); 7.41–7.48 (*m*, 9 H); 7.60–7.64 (*m*, 4 H, Ph_2Si); 8.15 (*d*, $J=7.4$, H–C(6)(C)); 11.18, 11.25 (2 br., 4 H, NH). $^{13}\text{C-NMR}$ ($(\text{D}_6)\text{DMSO}$, 125 MHz): 12.05, 12.09 (2*q*, Me–C(5)(T)); 18.73 (*s*, Me_3C); 19.46 (*q*, Me(to)); 25.44, 26.24 (2*t*, C(5')); 26.62 (*q*, Me_3C); 28.75 (*t*, CH_2SCH_2); 32.68, 33.02, 33.44 (3*t*, C(5'), CH_2SCH_2); 35.09 (*t*, C(5')); 35.75, 36.24 (2*d*, C(3')); 36.63, 36.81, 36.89 (3*t*, C(2')); 42.02 (*d*, C(3')(T1)); 43.91 (*d*, C(3')(C)); 49.52, 49.80 (2*t*, C(6')(T2), C(6')(T4)); 53.69 (*t*, $\text{CH}_2\text{–C}(3')(T1)$, $\text{CH}_2\text{–C}(3')(T3)$); 60.65 (*t*, C(6')(T1)); 63.72 (*t*, $\text{CH}_2\text{–C}(3')(T4)$); 80.35, 81.74, 81.83, 81.89 (4*d*, C(4')); 83.31, 83.62, 83.68 (3*d*, C(1')); 86.04 (*s*, Tr); 86.11 (*d*, C(1')(C)); 96.06 (*d*, C(5)(C)); 109.62, 109.76, 109.88 (2*s*, C(5)(T)); 125.53 (*d*, to); 126.93 (*d*, Tr); 127.81 (*d*, Tr); 127.95 (*d*, Ph_2Si); 128.16 (*d*, Tr); 129.91 (*d*, Ph_2Si); 130.52, 130.63 (2*d*, to); 132.60, 132.68 (2*s*, Ph_2Si); 135.07, 135.08 (2*d*, Ph_2Si); 135.17, 135.86 (2*s*, $\text{C}_o(\text{to})$); 135.98, 136.12, 136.19 (3*d*, C(6)(T)); 143.87 (*s*, Tr); 145.14 (*d*, C(6)(C)); 150.28, 150.31, 150.37 (3*s*, C(2)(T)); 154.36 (*d*, C(2)(C)); 162.64 (*s*, C=O); 163.67 (*s*, C(4)(T)); 169.50 (*s*, C(4)(C)). MALDI-TOF MS ($A = \text{dimer in } \text{CH}_2\text{Cl}_2/\text{MeOH } 10 : 1, B = 0.1\text{M CCA in MeCN/EtOH/H}_2\text{O } 50 : 45 : 5, C = A/B } 1 : 1$): 1744.6 ($[\text{M} + \text{Na}]^+$).

5'-Deoxy-3'-de(phosphinicooxy)-5'-I(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-{[(tert-butyl)diphenylsilyloxy]methyl}-3',5'-dideoxythymidine (*d*($\text{TrOCH}_2\text{-Tso}_2\text{TsTso}_2\text{-T-CH}_2\text{OTBDPS}$); **19a**). As described for **19b**, with **14a** (32 mg, 38.6 μmol), **11a** (31 mg, 35.6 μmol), Cs_2CO_3 (35 mg, 107 μmol), DMF (2 ml, 1 h Ar) (stirring of r.t. overnight), and acetate buffer (3M AcOH/1M AcONa; 54 μl): **19a** (49 mg, 85%). Colorless foam. UV ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20 : 1): 229, 266. $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): 1.06 (*s*, *t*-Bu); 1.84–2.05 (*m*, 5 H); 1.89, 1.89, 1.90, 1.90 (4*s*, 12 H, Me–C(5)(T)); 2.06–2.17 (*m*, 2 H); 2.18–2.40 (*m*, 10 H); 2.42–2.51 (*m*, 2 H); 2.54–2.70 (*m*, 4 H, CH_2SCH_2); 2.72–2.80 (*m*, 1 H, H–C(3')(T)); 2.99–3.26 (*m*, 8 H, $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.27–3.31 (*m*, 2 H, H–C(6')(T1)); 3.65–3.91

(*m*, 6 H, H–C(4'), CH₂–C(3')(T4')); 5.87–5.91 (*dd*, 1 H, H–C(1')); 5.92–5.96 (*m*, 2 H, H–C(1')); 6.04 (*dd*, *J* = 4.5, 7.3, 1 H, H–C(1')); 7.03, 7.07, 7.08, 7.11 (4*s*, 4 H, H–C(6)(T)); 7.19–7.29 (*m*, 9 H, Tr); 7.37–7.47 (*m*, 12 H, 6 H of Ph₂Si, 6 H of Tr); 7.60–7.64 (*m*, 4 H, Ph₂Si); 9.53, 9.61, 9.68, 9.82 (4 *br.*, 4 NH). ¹³C-NMR (CDCl₃, 125 MHz): 12.56, 12.59, 12.69 (3*q*, Me–C(5)(T)); 19.24 (*s*, Me₃C); 26.03 (*t*); 26.94 (*q*, Me₃C); 29.54, 33.47, 33.72, 34.19, 34.54 (5*t*, C(5'), CH₂–C(3')(T2), C(6')(T3)); 36.30, 36.74 (2*d*, C(3')(T2), C(3')(T3)); 37.33, 37.47, 37.76, 38.43 (4*t*, C(2')); 42.82 (*d*, C(3')(T1)); 45.38 (*d*, C(3')(T4)); 50.38, 51.02 (2*t*, C(6')(T2), C(6')(T4)); 54.73, 55.12 (2*t*, CH₂–C(3')(T1), CH₂–C(3')(T3)); 60.52 (*t*, C(6')(T1)); 63.65 (*t*, CH₂–C(3')(T4)); 80.79, 81.85, 82.06, 82.46 (4*d*, C(4')); 84.82, 85.58, 85.89, 86.83 (4*d*, C(1')); 87.00 (*s*, Tr); 110.83, 111.20, 111.35, 111.53 (*s*, C(5)(T)); 127.13 (*d*, Tr); 127.90, 127.93, 127.95, 127.99 (*d*, Ph₂Si); 128.59 (*d*, Tr); 130.03, 130.05 (2*d*, Ph₂Si); 132.82, 132.95 (2*s*, Ph₂Si); 135.44, 135.53 (2*d*, C(6)(T)); 135.57 (*d*, Ph₂Si); 136.13, 136.77 (2*d*, C(6)(T)); 143.95 (*s*, Tr); 150.50, 150.58, 150.61 (3*s*, C(2)(T)); 163.78, 163.81, 163.95, 164.03 (4*s*, C(4)(T)). MALDI-TOF-MS (*A* = dimer in CH₂Cl₂, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50 : 45 : 5, *C* = *A/B* 1 : 1): 1642.5 ([*M* + Na]⁺).

5'-[*(Benzoyloxy)methyl*]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenethiomethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-[[*(4,4'*-dimethoxytrityl)thio]methyl]thymidine (*d*(*Bz*OCH₂-Tso₂Tso₂T-CH₂STr(OMe)₂); **18**). As described for **19b**, with **14b** (78 mg, 113 μmol), **17** (114 mg, 120 μmol), Cs₂CO₃ (147 mg, 452 μmol), DMF (10 ml, 1 h Ar), (stirring at 45° for 4.5 h), and acetate buffer (3M AcOH/1M AcONa; 226 μl). Chromatography (silica gel (column loaded with 1% Et₃N), CH₂Cl₂/MeOH 100 : 0, 40 : 1, 20 : 1, 15 : 1, 5 : 1) yielded **18** (162 mg, 92%). Colorless foam. UV (CH₂Cl₂/CH₃OH 20 : 1): 228 (56600), 267 (51400). ¹H-NMR (CDCl₃, 500 MHz): 1.84–1.93 (*m*, 2 H); 1.89, 1.89, 1.90, 1.90 (4*s*, 12 H, Me–C(5)(T)); 1.98–2.06 (*m*, 3 H); 2.08–2.17 (*m*, 3 H); 2.17–2.41 (*m*, 11 H); 2.43–2.48 (*m*, 1 H); 2.49–2.68 (*m*, 4 H); 2.72–2.79 (*m*, 2 H); 3.03–3.32 (*m*, 8 H, CH₂SO₂CH₂); 3.52–3.57 (*m*, 1 H, H–C(4')); 3.74–3.85 (*m*, 2 H, H–C(4')); 3.79 (*s*, 2 MeO); 3.88–3.92 (*dt*, 1 H, H–C(4')); 4.40–4.46 (*m*, 1 H, H–C(6)(T)); 4.53–4.59 (*m*, 1 H, H–C(6)(T1)); 5.81 (*dd*, *J* = 4.4, 7.8, 1 H, H–C(1')); 5.87 (*dd*, *J* = 4.6, 7.0, 1 H, H–C(1')); 5.98 (*dd*, *J* = 4.4, 7.3, 1 H, H–C(1')); 6.01 (*dd*, *J* = 4.4, 7.3, 1 H, H–C(1')); 6.81–6.84 (*m*, *J* = 8.8, 4 H, H_m of Ar₂PhC); 6.91, 7.06, 7.09, 7.19 (4*s*, 4 H, H–C(6)(T)); 7.20–7.25 (*m*, 1 H, (MeO)₂Tr); 7.26–7.31 (*m*, 6 H, (MeO)₂ Tr); 7.37–7.43 (*m*, 4 H, H_o of Ar₂PhC, 2 H of Bz); 7.53–7.56 (*m*, 1 H, Bz); 7.99–8.01 (*m*, 2 H, Bz). ¹³C-NMR (CDCl₃/CD₃OD 4 : 1, 75 MHz): 12.05 (*q*, Me–C(5)(T)); 25.86 (2*t*, CH₂–C(3')(T4), CH₂–C(3')(T4)); 29.09 (*t*, C(6')(T3)); 32.37, 33.14, 33.99 (3*t*, C(5')(T)); 35.80, 36.21 (2*d*, C(3')(T)); 37.43, 37.59 (2*t*, C(2')(T)); 42.41 (*d*, C(3')(T)); 50.38 (*t*, CH₂–C(3')(T)); 54.37 (*t*, C(6')(T)); 54.92 (*s*, MeO); 61.64 (*t*, C(6')(T1)); 66.04 (*s*, Ar₂PhC); 80.85, 81.90, 81.93 (3*d*, C(4)(T)); 84.58, 84.62, 85.03, 85.07 (4*d*, C(1')(T)); 110.89 (*s*, C(5)(T)); 112.97 (*d*, C_m(Ar₂PhC)); 126.50 (*s*, C_p(Ar₂PhC)); 127.70 (*d*, C_o(Ar₂PhC)); 128.18 (*d*, C_m(Bz)); 129.01 (*d*, C_m(Ar₂PhC)); 129.22 (*d*, C_o(Bz)); 129.46 (*s*, C_{ipso}(Bz)); 130.34 (*d*, C_o(Ar₂PhC)); 133.01 (*d*, C_p(Bz)); 135.49, 135.64 (2*d*, C(6)(T)); 136.40 (*s*, C_{ipso}(Ar₂PhC)); 144.64 (*s*, C_{ipso}(Ar₂PhC)); 150.36 (*s*, C(2)(T)); 157.89 (*s*, C_p(Ar₂PhC)); 164.26 (*s*, C(4)(T)); 166.54 (*s*, PhCO). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10 : 1, *B* = 0.5M 2,4,6-THA in EtOH/0.1M DAC (diammonium citrate) in H₂O 2 : 1, *C* = *A/B* 3 : 1): 1561.1 (*M*[–]).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[*(trityloxy)methyl*]thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenethiomethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-trideoxy-N⁴-(*o*-toluoyl)cytidine (*d*(TrOCH₂-Tso₂Tso₂Tso₂¹⁰C-CH₂OTBDPS); **20b**). As described for **8b**, with **19b** (63 mg, 37 μmol), MeOH/THF 2 : 1 (24 ml), Oxone (90 mg, 146 μmol), NaOAc · 3 H₂O (65 mg, 480 μmol), and deionized H₂O (4 ml) (→ opaque suspension; conversion to sulfoxides within 5 min, then to sulfone within 2.5 h). Workup with sat. Na₂S₂O₃ soln. (3 ml) and CH₂Cl₂ (40 ml), then extraction with CH₂Cl₂ (4 × 15 ml) (no washing with brine): **20b** (58 mg, 90%). Colorless foam. UV (CH₂Cl₂/MeOH 20 : 1): 229, 260, 308. ¹H-NMR ((D₆)DMSO, 500 MHz): 1.00 (*s*, *t*-Bu); 1.76, 1.78, 1.79 (6*s*, 9 H, Me–C(5)(T)); 1.84–1.92 (*m*, 1 H, H–C(5')); 1.99–2.18 (*m*, 5 H, H–C(5')); 2.20–2.38 (*m*, 11 H, 8 H–C(2'), 3 H–C(5')); 2.36 (*s*, Me (to)); 2.49–2.56 (*m*, partly under DMSO, 1 H, H–C(3')); 2.63–2.73 (*m*, 2 H, H–C(3')); 3.02–3.08 (*m*, 2 H, CH₂SO₂CH₂); 3.16–3.39 (*m*, partly under H₂O, 9 H, CH₂SO₂CH₂, 2 H–C(6')(T1)); 3.42–3.48 (*m*, 1 H, CH₂–C(3')); 3.50–3.56 (*m*, 2 H, CH₂–C(3')); 3.67–3.74 (*m*, 4 H, 2 H–C(4'), CH₂–C(3')(C)); 3.75–3.80 (*dt*, 1 H, H–C(4')); 4.00–4.05 (*dt*, 1 H, H–C(4')); 5.97–6.00 (*dd*, 1 H, H–C(1')); 6.01–6.07 (*m*, 3 H, H–C(1')); 7.21, 7.23, 7.24 (3*s*, 3 H, H–C(6)(T)); 7.25–7.32 (*m*, 8 arom. H); 7.33–7.40 (*m*, 9 arom. H); 7.41–7.50 (*m*, 9 H); 7.60–7.63 (*m*, 4 H, Ph₂Si); 8.15 (*d*, *J* = 7.5, H–C(6)(C)); 11.25 (*br.*, 4 H, NH). ¹³C-NMR ((D₆)DMSO, 125 MHz): 12.03, 12.04, 12.10 (3*q*, Me–C(5)(T)); 18.74 (*s*, Me₃C); 19.46 (*q*, Me (to)); 24.43, 26.12 (*t*, C(5')); 26.65 (*q*, Me₃C); 33.00, 35.06 (2*t*, C(5')); 35.85, 36.24 (2*d*, C(3')); 36.62, 36.67, 36.81 (3*t*, C(2')); 43.94 (*d*, C(3')(C)); 49.54, 49.62, 49.82 (3*t*, C(6')(T2), C(6')(T3), C(6')(T4)); 53.62, 54.87 (*t*, CH₂–C(3')(T1), CH₂–C(3')(T3)); 60.65 (*t*, C(6)(T1)); 63.71 (*t*, CH₂–C(3')(T4)); 80.36, 81.30, 81.73 (3*d*, C(4')); 83.62, 83.68 (2*d*, C(1')); 86.04 (*s*, Tr); 86.10

(*d*, C(1')(C)); 96.09 (*d*, C(5)(C)); 109.63, 109.90 (2*s*, C(5)(T)); 125.53 (*d*, to); 126.94 (*d*, Tr); 127.58 (*d*, Ph₂Si); 127.82 (*d*, Tr); 127.96 (*d*, Ph₂Si); 128.17 (*d*, Tr); 129.91 (*d*, Ph₂Si); 130.51, 130.63 (2*d*, to); 132.61, 132.68 (2*s*, Ph₂Si); 135.08, 135.09 (2*d*, Ph₂Si); 135.22, 135.85 (*s*, C₆(to)); 135.99, 136.29, 136.35 (3*d*, C(6)(T)); 143.87 (*s*, Tr); 145.12 (*d*, C(6)(C)); 150.29, 150.34 (*s*, C(2)(T)); 154.37 (*d*, C(2)(C)); 162.69 (*s*, C=O); 163.70 (*s*, C(4)(T)); 169.75 (*s*, C(4)(C)). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10:1, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50:45:5, *C* = *A/B* 1:1): 1754.1 (*M*⁻).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[[(tert-butyl)diphenylsilyloxy]methyl]-3',5'-dideoxythymidine (*d*(TrOCH₂-Tso₂Tso₂Tso₂T-CH₂OTBDPS); **20a**). As described for **8b**, with **19a** (63 mg, 39 μmol), MeOH/THF 2:1 (24 ml), Oxone (123 mg, 200 μmol), NaOAc·3 H₂O (90 mg, 660 μmol), and deionized H₂O (4 ml) (→ opaque suspension; conversion to sulfoxides within 5 min, then to sulfone within 2 h). Workup with sat. Na₂S₂O₃ soln. (5 ml) and CH₂Cl₂ (40 ml), then extraction with CH₂Cl₂ (4 × 15 ml) (no washing with brine): **20a** (59 mg, 92%). Colorless foam. ¹H-NMR ((D₆)DMSO, 500 MHz): 1.00 (*s*, *t*-Bu); 1.75, 1.76, 1.78, 1.80 (4*s*, 12 H, Me-C(5)(T)); 1.81–1.92 (*m*, 2 H, H-C(5')); 2.01–2.18 (*m*, 6 H, H-C(5')); 2.20–2.32 (*m*, 8 H); 2.41–2.48 (*m*, 1 H); 2.50–2.55 (*m*, partly under DMSO, 1 H); 2.65–2.70 (*m*, 2 H); 3.03–3.08 (*m*, 2 H, CH₂SO₂CH₂); 3.17–3.38 (*m*, partly under HDO, 8 H, 2 H-C(6')(T1), CH₂SO₂CH₂); 3.43–3.56 (*m*, 4 H, CH₂SO₂CH₂); 3.65–3.72 (*m*, 4 H, 2 H-C(4'), CH₂-C(3')(T4)); 3.74–3.79 (*dt*, 1 H, H-C(4')); 3.85–3.89 (*dt*, 1 H, H-C(4')); 5.99 (*dd*, *J* = 4.6, 7.3, 1 H, H-C(1')); 6.02–6.07 (*m*, 3 H, H-C(1')); 7.22, 7.23, 7.24 (3*s*, 3 H, H-C(6)(T)); 7.28–7.31 (*m*, 5 arom. H); 7.33–7.38 (*m*, 7 arom. H); 7.42–7.50 (*m*, 10 arom. H); 7.59–7.63 (*m*, 4 H, Ph₂Si); 11.27 (br., 4 H, NH). ¹³C-NMR ((D₆)DMSO, 125 MHz): 12.04, 12.10 (2*q*, Me-C(5)(T)); 18.73 (*s*, Me₃C); 19.59, 24.38, 25.94, 28.98 (4*t*, C5'), CH₂-C(3')(T2), C(6')(T3)); 26.66 (*q*, Me₃C); 33.01, 33.20 (2*d*, C(3')(T2), C(3')(T3)); 35.88, 36.24, 36.63, 36.81 (4*t*, C(2')); 44.33 (*d*, C(3')(T1)); 45.65 (*d*, C(3')(T4)); 49.55, 49.65 (2*t*, C(6')(T2), C(6')(T4)); 53.69 (*t*, CH₂-C(3')(T1), CH₂-C(3')(T3)); 60.65 (*t*, C(6')(T1)); 64.09 (*t*, CH₂-C(3')(T4)); 80.34, 81.31 (2*d*, C(4')); 83.50, 83.63, 83.66, 83.69 (4*d*, C(4'), C(1')); 86.04 (*d*, C(1')); 95.21 (*s*, Tr); 109.46, 109.63, 109.86, 109.90 (4*s*, C(5)(T)); 125.46 (*d*, Tr); 126.94, 127.82, 127.95 (3*d*, Ph₂Si); 128.17 (*d*, Tr); 129.91, 130.59 (2*d*, Ph₂Si); 132.64, 132.68 (2*s*, Ph₂Si); 135.09 (*d*, Ph₂Si); 135.84, 136.00, 136.16, 136.31 (4*d*, C(6)(T)); 143.88 (*s*, Tr); 150.29, 150.33, 150.38 (3*s*, C(2)(T)); 163.41, 163.69 (2*s*, C(4)(T)). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10:1, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50:45:5, *C* = *A/B* 1:1): 1651.6 (*M*⁻).

6. Functionalization of the Tetramers. 5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3'-[[(tert-butyl)diphenylsilyloxy]methyl]-3',5'-dideoxythymidine (*d*(HOCH₂-Tso₂Tso₂Tso₂T-CH₂OTBDPS); **21**). As described for **9b** (Method 2), with **20a** (49 mg, 30 μmol), CH₂Cl₂ (2 ml), and 2.2M ZnCl₂·Et₂O in CH₂Cl₂ (153 μl, 337 μmol) (yellow solid after ca. 100 μl). FC (silica gel, CH₂Cl₂/MeOH 20:1, 10:1, 5:1) yielded **21** (42 mg, quant.). Colorless foam. ¹H-NMR ((D₆)DMSO, 500 MHz): 1.00 (*s*, *t*-Bu); 1.79, 1.79, 1.79, 1.80 (4*s*, 12 H, Me-C(2)(T)); 1.71–1.85 (*m*, 2 H, H-C(5')); 1.87–1.93 (*m*, 1 H, H-C(5')); 1.98–2.19 (*m*, 5 H, H-C(5')); 2.21–2.32 (*m*, 8 H, H-C(2')); 2.40–2.47 (*m*, 1 H, H-C(3')); 2.52–2.58 (*m*, 1 H, H-C(3')); 2.62–2.71 (*m*, 2 H, H-C(3')); 3.15–3.33 (*m*, partly under HDO, 9 H, 2 H-C(6')(T1), CH₂SO₂CH₂); 3.37–3.41 (*dd*, *J* = 3.1, 13.9, 1 H, CH₂-C(3')); 3.43–3.60 (*m*, 4 H, CH₂SO₂CH₂); 3.65–3.74 (*m*, 5 H, 3 H-C(4'), CH₂-C(3')(T4)); 3.85–3.90 (*dt*, *J* = 3.1, 8.3, 1 H, H-C(4')); 5.99 (*dd*, *J* = 4.9, 7.1, 1 H, H-C(1')); 6.02–6.06 (*m*, 3 H, H-C(1')); 7.41–7.49 (*m*, 10 H, 4 H-C(6)(T), 6 H of Ph₂Si); 7.59–7.63 (*m*, 4 H, Ph₂Si); 11.25, 11.26, 11.28 (3 br., 4 H, NH). ¹³C-NMR ((D₆)DMSO, 125 MHz): 12.04, 12.09, 12.10 (3*q*, Me-C(4)(T)); 18.74 (*s*, Me₃C); 24.38, 24.58, 25.94 (3*t*, CH₂-C(3')(T2), C(6')(T3)); 26.67 (*q*, Me₃C); 33.21 (*t*, C(5')); 35.75, 35.91 (2*d*, C(3')(T1), C(3')(T2), C(3')(T3)); 36.64, 36.99 (2*t*, C(2')); 44.33 (*d*, C(3')); 49.57, 49.66 (2*t*, C(6')(T2), C(6')(T4)); 53.66, 54.87 (2*t*, CH₂-C(3')(T1), CH₂-C(3')(T3)); 57.76 (*t*, C(6')(T1)); 64.09 (*t*, CH₂-C(3')(T4)); 80.34, 80.46, 81.30 (3*d*, C(4')); 83.52, 83.61, 83.67 (3*d*, C(4'), C(1')); 109.46, 109.63, 109.86, 109.90 (4*s*, C(5)(T)); 127.95, 129.91, 130.01 (3*d*, Ph₂Si); 132.65, 132.69 (2*s*, Ph₂Si); 135.04, 135.09 (2*d*, Ph₂Si); 135.82, 136.09, 136.16, 136.34 (4*d*, C(6)(T)); 150.31, 150.33, 150.39 (3*s*, C(2)(T)); 163.70, 163.73 (2*s*, C(4)(T)). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10:3, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50:45:5, *C* = *A/B* 1:1): 1408.6 (*M*⁻).

5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-[(4,4'-dimethoxytrityl)thio]methyl]thymidine (*d*(HOCH₂-Tso₂Tso₂T-CH₂STR(OMe)₂); **22**). Compound **18** (120 mg, 77 μmol) was suspended in MeOH/THF 3:1 (8 ml), and 2M NaOH (2.0 ml) was added slowly. Even in the presence of base, **18** did not dissolve completely. The deprotection was terminated after 30 min with acetate buffer (3M AcOH/1M AcONa 3:1;

1.95 ml). The mixture was poured immediately into sat. NaHCO_3 soln. (10 ml) containing ice (10 g). CH_2Cl_2 (40 ml) was added and the org. phase washed with brine (1 \times 15 ml). The aq. phases were re-extracted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1 (3 \times 15 ml). The combined org. phase was dried (MgSO_4) and evaporated. FC (silica gel (column loaded with 1% Et_3N), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100:0, 20:1, 15:1, 10:1) yielded **22** (107 mg, 96%). Colorless foam. UV ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1): 227 (34700), 266 (46100). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz): 1.75–1.83 (*m*, 1 H); 1.83–1.97 (*m*, 3 H); 1.92, 1.93, 1.93, 1.94 (4s, 6 H, Me–C(5)(T)); 1.98–2.20 (*m*, 6 H); 2.23–2.42 (*m*, 8 H); 2.43–2.51 (*m*, 2 H); 2.62–2.73 (*m*, 5 H, CH_2SCH_2 , H–C(3')(T3)); 2.77–2.84 (*m*, 1 H, H–C(3')(T1)); 3.04–3.14 (*m*, 3 H, CH_2 –C(3'), 1 H–C(6')); 3.18–3.25 (*m*, 3 H, 1 H of CH_2 –C(3'), 2 H–C(6')); 3.29–3.39 (*m*, 2 H, 1 H of CH_2 –C(3'), 1 H–C(6')); 3.54–3.59 (*dt*, 1 H, H–C(4')); 3.72–3.77 (*m*, 1 H, H–C(6')(T1)); 3.81 (*s*, 2 MeO), 3.79–3.90 (*m*, 4 H, 1 H–C(6')(T1), 3 H–C(4')); 5.92 (*dd*, $J=4.0, 7.4$, 1 H, H–C(1')); 6.00 (*dd*, $J=4.6, 7.3$, 1 H, H–C(1')); 6.05 (*dd*, $J=4.4, 7.3$, 1 H, H–C(1)); 6.07 (*dd*, $J=4.4, 7.3$, 1 H, H–C(1)); 6.82–6.85 (*m*, $J=8.8$, 4 H, H_m of Ar_2PhC); 7.04, 7.19, 7.22 (3 \cdot 2s, 3 H, H–C(6)(T)); 7.21–7.24 (*m*, 1 H, (MeO) $_2$ Tr); 7.26 (2s, 1 H, H–C(6)(T)); 7.28–7.32 (*m*, 6 H, (MeO) $_2$ Tr), 7.38–7.43 (*m*, 4 H, H_o of Ar_2PhC). $^{13}\text{C-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 4:1, 125 MHz): 9.64, 12.06 (*q*, Me–C(5)(T)); 28.91 (*t*, CH_2 –C(3')(T4)); 33.11, 34.02 (2*t*, C(5')(T)); 35.81 (*d*, C(3')(T)); 35.99, 36.21 (2*d*, CH_2SCH_2); 37.41, 37.78 (*t*, C(2')(T)); 42.32 (*d*, C(3')(T)); 45.99, 50.29 (2*t*, CH_2 –C(3)); 54.28 (*t*, C(6')(T)); 54.88 (*q*, MeO); 58.46 (*t*, C(6')(T1)); 65.91, 66.04 (2*s*, Ar_2PhC); 80.78, 81.61, 82.04, 82.11 (4*d*, C(4')(T)); 83.98, 84.91, 85.32, 85.60 (4*d*, C(1')(T)); 110.88, 110.93 (2*s*, C(5)(T)); 113.11 (*d*, $C_m(\text{Ar}_2\text{PhC})$); 126.33 (*s*, $C_p(\text{Ar}_2\text{PhC})$); 127.83 (*d*, $C_o(\text{Ar}_2\text{PhC})$); 129.04 (*d*, $C_m(\text{Ar}_2\text{PhC})$); 130.30 (*d*, $C_o(\text{Ar}_2\text{PhC})$); 135.79 (*d*, C(6)(T)); 136.35 (*s*, $C_{\text{ipso}}(\text{Ar}_2\text{PhC})$); 143.89, 144.29 (2*s*, $C_{\text{ipso}}(\text{Ar}_2\text{PhC})$); 150.31 (*s*, C(2)(T)); 157.98 (*s*, $C_p(\text{Ar}_2\text{PhC})$); 164.22 (*s*, C(4)(T)); MALDI-TOF MS ($A = \text{dimer in } \text{CH}_2\text{Cl}_2/\text{MeOH } 10:1$, $B = 0.5\text{M } 2,4,6\text{-THA in EtOH}/0.1\text{M DAC in H}_2\text{O } 2:1$, $C = A/B$ 3:1): 1456.2 (M^-).

5'-(Bromomethyl)-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' \rightarrow 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' \rightarrow 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' \rightarrow 5')-3',5'-dideoxy-3'-[[4,4'-dimethoxytrityl]thio]methyl]thymidine (*d*($\text{BrCH}_2\text{-Tso}_2\text{Tso}_2\text{T-CH}_2\text{STr}(\text{OMe})_2$); **23**). As described for **17**, with **22** (105 mg, 72 μmol), PPh_3 (38 mg, 144 μmol), 1,2-dichloroethane/MeCN 4:1 (10 ml), CBr_4 (48 mg, 144 μmol), and 1,2-dichloroethane (1 ml). Quenching with $\text{Et}_3\text{N}/\text{MeOH}$ 1:1 (2 ml) and FC (silica gel (column loaded with 1% Et_3N), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 40:1, 20:0, 15:1, 10:1, 5:1) yielded **22** (20 mg, 19%) and **23** (84 mg, 77%; 95% based on recovered **22**) as colorless foams. $^1\text{H-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 4:1, 500 MHz): 1.78–1.98 (*m*, 3 H); 1.93, 1.93, 1.93, 1.94 (4s, 6 H, Me–C(5)(T)); 2.03–2.20 (*m*, 6 H); 2.21–2.52 (*m*, 11 H); 2.63–2.73 (*m*, 5 H, CH_2SCH_2 , H–C(3')(T3)); 2.78–2.85 (*m*, 1 H, H–C(3')(T1)); 3.06–3.16 (*m*, 3 H, CH_2 –C(3), 1 H–C(6')); 3.19–3.27 (*m*, 4 H, CH_2 –C(3), 2 H–C(6')); 3.29–3.36 (*m*, 1 H, 1 H–C(6')); 3.50–3.62 (*m*, 3 H, H–C(4'), 2 H–C(6')(T1)); 3.81 (*s*, 2 MeO); 3.81–3.93 (*m*, 3 H, 3 H–C(4')); 5.92 (*dd*, $J=4.4, 7.3$, 1 H, H–C(1')); 6.01 (*dd*, 1 H, H–C(1')); 6.04–6.07 (*m*, 2 H, 2 H–C(1')); 6.82–6.85 (*m*, $J=8.8$, 4 H, H_m of Ar_2PhC); 7.03, 7.18, 7.19, 7.20 (4 \times 2s, 4 H, H–C(6)(T)); 7.21–7.25 (*m*, 1 H, (MeO) $_2$ Tr) 7.28–7.33 (*m*, 6 H, (MeO) $_2$ Tr); 7.39–7.42 (*m*, 4 H, H_o of Ar_2PhC). $^{13}\text{C-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 4:1, 75 MHz): 9.64, 12.06 (2*q*, Me–C(5)(T)); 25.83 (*t*, CH_2 –C(3')(T4)); 29.11 (*t*, C(6)(T1)); 33.13, 33.92 (2*t*, C(5')(T)); 35.73 (*d*, C(3')(T)); 36.22, 37.52 (2*t*, C(2')(T)); 42.38 (*d*, C(3')(T)); 50.34 (*t*, CH_2 –C(3')(T)); 54.34 (*t*, C(6')(T)); 54.90 (*q*, MeO); 66.04 (*s*, Ar_2PhC); 81.32, 81.89 (*d*, C(4')(T)); 84.53, 84.87 (2*d*, C(1')(T)); 110.91 (*s*, C(5)(T)); 112.93 (*d*, $C_m(\text{Ar}_2\text{PhC})$); 126.48 (*s*, $C_p(\text{Ar}_2\text{PhC})$); 127.68 (*d*, $C_o(\text{Ar}_2\text{PhC})$); 128.98 (*d*, $C_m(\text{Ar}_2\text{PhC})$); 130.32 (*d*, $C_o(\text{Ar}_2\text{PhC})$); 135.54 (*d*, C(6)(T)); 136.38 (*s*, $C_{\text{ipso}}(\text{Ar}_2\text{PhC})$); 144.63 (*s*, $C_{\text{ipso}}(\text{Ar}_2\text{PhC})$); 150.37 (*s*, C(2)(T)); 157.85 (*s*, $C_p(\text{Ar}_2\text{PhC})$); 164.28 (*s*, C(4)). MALDI-TOF MS ($A = \text{dimer in } \text{CH}_2\text{Cl}_2/\text{MeOH } 10:1$, $B = 0.5\text{M } 2,4,6\text{-THA in EtOH}/0.1\text{M DAC in H}_2\text{O } 2:1$, $C = A/B$ 3:1): 1519.9 (M^-).

5'-(Bromomethyl)-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' \rightarrow 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' \rightarrow 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' \rightarrow 5')-3'-[[tert-butyl]diphenylsilyloxy]methyl]-3',5'-dideoxythymidine (*d*($\text{BrCH}_2\text{-Tso}_2\text{Tso}_2\text{Tso}_2\text{T-CH}_2\text{OTBDPS}$); **24**). Tetramer **21** (38 mg, 27 μmol), monomer **25** (50 mg, 98 μmol), and PPh_3 (66 mg, 250 μmol) were dried overnight under high vacuum at 45° and then dissolved in 1,2-dichloroethane/MeCN 4:1 (10 ml). CBr_4 (83 mg, 250 μmol) in 1,2-dichloroethane (1 ml) was added, and the mixture was stirred for 90 min. Quenching with $\text{Et}_3\text{N}/\text{MeOH}$ 1:1 (1 ml), evaporation, (30° water-bath temp.), and FC (silica gel, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100:0, 40:1, 20:1) yielded tetramer **24** (36 mg, 91%) and monomer **4a** (54 mg, 96%) as colorless foams. **24**: $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$, 500 MHz): 1.00 (*s*, *t*-Bu); 1.79, 1.79, 1.80, 1.80 (4s, 12 H, Me–C(5)(T)); 1.99–2.15 (*m*, 5 H, H–C(5')); 2.16–2.33 (*m*, 11 H, 3 H–C(5'), 8 H–C(2')); 2.41–2.48 (*m*, partly under DMSO, 1 H, H–C(3')); 2.60–2.72 (*m*, 3 H, H–C(3')); 3.12–3.18 (*m*, 2 H, $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.19–3.35 (*m*, partly under H $_2\text{O}$, 7 H, 2 H–C(6')(T1), $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.42–3.55 (*m*, 4 H, $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.59–3.64 (*m*, 1 H, $\text{CH}_2\text{SO}_2\text{CH}_2$); 3.67–3.76 (*m*, 5 H, 3 H–C(4'), CH_2 –C(3')(T4)); 3.85–3.89 (*dt*, $J=3.2, 8.3$, 1 H,

H–C(4')); 6.01–6.06 (*m*, 4 H, H–C(1')); 7.41–7.49 (*m*, 10 H, 4 H–C(6)(T), 6 H of Ph₂Si); 7.58–7.63 (*m*, 4 H, Ph₂Si); 11.25, 11.27 (br., 4 H, NH). ¹³C-NMR ((D₆)DMSO, 125 MHz): 12.05, 12.09 (2*q*, Me–C(5)(T)); 18.74 (*s*, Me₃C); 23.03 (*t*, C(6')(T1)); 26.67 (*q*, Me₂C); 31.19, 33.17 (2*t*, C(5')); 35.59, 35.79, 35.88 (3*d*, C(3')(T1), C(3')(T2), C(3')(T3)); 36.62, 36.71 (2*t*, C(2')); 44.32 (*d*, C(3')); 49.59 (*t*, C(6')); 53.48, 53.64 (2*t*, CH₂–C(3')(T1), CH₂–C(3')(T3)); 57.51 (*t*); 64.08 (*t*, CH₂–C(3')(T4)); 80.33, 81.01, 81.29 (3*d*, C(4')); 83.49, 83.65, 83.72 (3*d*, C(4'), C(1')); 109.81, 109.85, 109.90 (3*s*, C(5)(T)); 127.95, 128.67, 129.91, 129.87 (4*d*, Ph₂Si); 132.64, 132.68 (*s*, Ph₂Si); 135.04, 135.09 (2*d*, Ph₂Si); 135.92, 136.17, 136.28, 136.34 (4*d*, C(6)(T6)); 150.30, 150.33, 150.38 (3*s*, C(2)(T)); 163.69 (*s*, C(4)(T)). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10 : 4, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50 : 45 : 5, *C* = *A/B* 1 : 1): 1472.2 (*M*⁺).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylmethylenesulfonylmethylene-(3' → 5')-2',3',5'-trideoxy-N⁴-(*o*-toluoyl)cytidine (*d*(TrOCH₂-Tso₂-Tso₂-Tso₂¹⁰C-CH₂OH); **27**). Compound **20b** (25 mg, 14.2 μmol) was dissolved in THF (5 ml) at r.t., and a soln. of 1M Bu₄NF in THF (42.7 μl, 42.7 μmol; pH 5–6, adjusted with acetate buffer) was added. The mixture was stirred for 2 h and quenched with Me₃SiOMe (50 μl, 36 μmol). Stirring was continued for 10 min and then the soln. evaporated (30° water-bath temp.). The residue was purified by FC (silica gel, CH₂Cl₂/MeOH 20 : 1, 10 : 1): **27** (20 mg, 93%). Colorless foam. UV (CH₂Cl₂/MeOH 20 : 1): 229, 262, 308. ¹H-NMR ((D₆)DMSO, 500 MHz): 1.76, 1.78, 1.80 (6*s*, 9 H, Me–C(5)(T)); 1.86–1.93 (*m*, 1 H, H–C(5')); 1.98–2.20 (*m*, 6 H, H–C(5')); 2.21–2.36 (*m*, 10 H, H–C(2'), H–C(5'), H–C(3')); 2.36 (*s*, Me(to)); 2.49–2.57 (*m*, partly under DMSO, 1 H, H–C(3')); 2.64–2.72 (*m*, 2 H, H–C(3')); 3.02–3.09 (*m*, 2 H, CH₂SO₂CH₂); 3.13–3.40 (*m*, partly under HDO, 9 H, CH₂SO₂CH₂, 2 H–C(6')(T1)); 3.43–3.49 (*m*, 2 H, CH₂–C(3')); 3.50–3.58 (*m*, 3 H, 1 H of CH₂–C(3'), 2 H of CH₂–C(3')(C)); 3.68–3.80 (*m*, 3 H, 3 H–C(4')); 3.90–3.94 (*dt*, *J* = 3.4, 8.4, 1 H, H–C(4')); 5.97–6.00 (*m*, 2 H, 2 H–C(1')); 6.03–6.07 (*m*, 2 H, 2 H–C(1')); 7.21, 7.23, 7.24 (3*s*, 3 H, H–C(6)(T)); 7.25–7.32 (*m*, 8 H, 6 H of Tr, 2 H_{*m*}(to)); 7.33–7.40 (*m*, 9 H, Tr); 7.45–7.50 (*m*, 3 H, H–C(5)(C), H_{*p*}(to), H_{*o*}(to)); 8.14 (*d*, *J* = 7.5, H–C(6)(C)); 11.27 (br., 4 H, NH). ¹³C-NMR ((D₆)DMSO, 125 MHz): 12.03, 12.05, 12.09 (3*q*, Me–C(5)(T)); 19.45 (*q*, Me(to)); 24.45, 26.19 (2*t*, C(5')); 33.01, 35.39 (2*t*, C(5')); 35.86, 36.23 (2*d*, C(3')); 36.61, 36.81 (2*t*, C(2')); 44.22 (*d*, C(3')(C)); 49.54, 49.63, 49.87 (3*t*, C(6')(T2, T3, T4)); 53.50, 53.62, 53.68 (3*t*, CH₂–C(3')(T1, T2, T3)); 60.65 (*t*, C(6)(T1)); 60.98 (*t*, CH₂–C(3')(C)); 80.37, 81.29, 81.80 (3*d*, C(4')); 83.64, 83.70 (2*d*, C(1')); 86.04 (*s*, Tr); 86.10 (*d*, C(1')(C)); 96.01 (*d*, C(5)(C)); 109.62, 109.89 (2*s*, C(5)(T)); 125.52 (*d*, to); 126.93, 127.81, 128.17 (3*d*, Tr); 130.51, 130.62 (2*d*, to); 135.19, 135.85 (2*s*, C_{*o*}(to)); 135.99, 136.30, 136.38 (3*d*, C(6)(T)); 143.87 (*s*, Tr); 145.06 (*s*, C(6)(C)); 150.28, 150.33 (*s*, C(2)(T)); 154.37 (*d*, C(2)(C)); 162.61 (*s*, C=O); 163.69 (*s*, C(4)(T)); 169.72 (*s*, C(4')(C)). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10 : 4, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50 : 45 : 5, *C* = *A/B* 1 : 1): 1515.5 (*M*⁺).

5'-Deoxy-3'-de(phosphinicooxy)-5'-[(trityloxy)methyl]thymidylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylmethylenesulfonylmethylene-(3' → 5')-3'[(acetylthio)methyl]-2',3',5'-trideoxy-N⁴-(*o*-toluoyl)cytidine (*d*(TrOCH₂-Tso₂-Tso₂¹⁰C-CH₂SAC); **28**). PPh₃ (62 mg, 235 μmol) was dried under high vacuum at 45° for 3 h and was dissolved in THF (1 ml). The soln. was cooled to 0°, and DIAD (33.7 μl, 174 μmol) was slowly added dropwise. The soln. was stirred for 30 min at 0° (→ milky suspension after 15 min). Thioacetic acid (12.4 μl, 174 μmol), the tetramer **27** (13 mg, 8.6 μmol) and monomer **25** (30 mg, 58.5 μmol), which were dried overnight under high vacuum at r.t., were dissolved in THF (1 ml) and alternately added dropwise starting with the acid. The mixture was allowed to warm to r.t., stirred for 2 h, and quenched with Et₃N/MeOH 2 : 1 (0.5 ml). The mixture was evaporated and purified by FC (silica gel, CH₂Cl₂/MeOH 20 : 1, 10 : 1, 5 : 1): tetramer **28** (12.7 mg, 94%) and monomer (32.5 mg, 97%) as colorless foams. **28**: ¹H-NMR ((D₆)DMSO, 500 MHz): 1.76, 1.78, 1.80 (6*s*, 9 H, Me–C(5)(T)); 1.85–1.93 (*m*, 1 H, H–C(5')); 1.98–2.36 (*m*, 16 H, 7 H–C(5'), 8 H–C(2'), H–C(3')); 2.34, 2.36 (2*s*, 6 H, AcS, Me(to)); 2.49–2.57 (*m*, partly under DMSO, 1 H, H–C(3')); 2.65–2.74 (*m*, 2 H, H–C(3')); 2.94 (*dd*, *J* = 7.1, 13.7, 1 H, CH₂–C(3')(C)); 3.02–3.11 (*m*, 3 H, 1 H of CH₂–C(3')(C), CH₂SO₂CH₂); 3.16–3.40 (*m*, partly under HDO, 9 H, CH₂SO₂CH₂, 2 H–C(6')(T1)); 3.43–3.48 (*m*, 1 H, CH₂–C(3')); 3.50–3.57 (*m*, 2 H, CH₂–C(3')); 3.68–3.79 (*m*, 3 H, 3 H–C(4')); 3.90–3.94 (*dt*, *J* = 3.4, 8.3, 1 H, H–C(4')); 5.97–6.00 (*m*, 2 H, 2 H–C(1')); 6.03–6.08 (*m*, 2 H, 2 H–C(1')(C)); 7.21, 7.23, 7.24 (3*s*, 3 H, H–C(6)(T)); 7.25–7.32 (*m*, 8 H, 6 H of Tr, 2 H_{*m*}(to)); 7.33–7.40 (*m*, 9 H, Tr); 7.46 (*d*, *J* = 7.6, H–C(5)(C)); 7.47–7.50 (*m*, 2 H, H_{*p*}(to), H_{*o*}(to)); 8.11 (*d*, *J* = 7.5, 1 H, H–C(6)(C)); 11.16 (br., 1 H, NH); 11.28 (br., 3 H, NH). ¹³C-NMR ((D₆)DMSO, 125 MHz): 12.03, 12.06, 12.09 (3*q*, Me–C(5)(T)); 19.45 (*q*, Me(to)); 24.46, 25.49 (*t*, C(5')); 29.20 (*t*, CH₂–C(3')(C)); 30.52 (*q*, MeCO); 33.01 (*t*, C(5')); 35.86, 36.24 (2*d*, C(3')); 36.62, 36.82 (*t*, C(2')); 37.80 (*t*); 41.61 (*d*, C(3')(C)); 49.54, 49.62, 49.77 (3*t*, C(6')(T2, T3, T4)); 53.50, 53.62, 53.69 (3*t*, CH₂–C(3')(T1, T2, T3)); 60.65 (*t*, C(6)(T1)); 80.37, 81.30, 82.70 (3*d*, C(4')); 83.63, 83.69, 83.74 (3*d*, C(1'));

86.04 (s, Tr); 86.10 (d, C(1')(C)); 96.01 (d, C(5)(C)); 109.64, 109.89, 109.90 (2s, C(5)(T)); 125.53 (d, to); 126.94, 127.82, 128.17 (3d, Tr); 130.53, 130.63 (2d, to); 135.18, 135.86 (2s, C_o(to)); 136.00, 136.31, 136.39 (d, C(6)(T)); 143.88 (s, Tr); 145.15 (d, C(6)(C)); 150.29, 150.34 (2s, C(2)(T)); 154.31 (d, C(2)(C)); 162.67 (s, C=O); 163.70 (s, C(4)(T)); 169.72 (s, C(4)(C)); 195.03 (s, MeCO). MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10:4, *B* = 0.1M CCA in MeCN/EtOH/H₂O 50:45:5, *C* = *A/B* 1:1): 1573.5 (*M*⁻).

3',3''-[Dithiobis(methylene)]bis[5'-deoxy-3'-de(phosphinicooxy)-5'-I(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-2',3',5'-trideoxy-N⁴-(o-toluoyl)cytidine (*d*(TrOCH₂-Tso₂Tso₂Tso₂¹⁰C-CH₂S)₂; **29**). *Method 1*: Compound **28** (10 mg, 6.5 μmol) was dissolved in degassed pyridine/EtOH 3:2 (2.5 ml, 1 h Ar). The soln. was degassed a second time by performing two freeze-pump cycles. At r.t., 2M NaOH (5 μl, 10 μmol) was added, and the reaction was quenched after 40 min with acetate buffer (3M AcOH/1M AcONa; 470 μl). The crude product was filtered through a layer of silica gel (CH₂Cl₂/MeOH 10:1): **29** (9 mg, 98%). Colorless glass.

Method 2: Compound **28** (39 mg, 24.8 μmol) was dissolved in degassed MeOH (5 ml, 1 h Ar). The soln. was degassed a second time by performing two freeze-pump cycles and cooled to 0°. Ammonia was bubbled through the soln. for 15 min and stirring continued for 105 min. The mixture was carefully evaporated at a water-bath temp. of 0° and dried under high vacuum at r.t. The crude product was filtered through a layer of silica (CH₂Cl₂/MeOH 10:1): **28** (37 mg, 98%). Colorless foam. MALDI-TOF MS (*A* = dimer in CH₂Cl₂/MeOH 10:1, *B* = 0.03M anthracene-1,8,9-triol in MeCN/EtOH/H₂O 50:45:5, *C* = *A/B* 1:2): 3062.9 (*M*⁻).

5'-Deoxy-3'-de(phosphinicooxy)-5'-I(trityloxy)methyl]thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-2',3',5'-trideoxy-3'-(mercaptomethyl)-N⁴-(o-toluoyl)cytidine (*d*(TrOCH₂-Tso₂Tso₂Tso₂¹⁰C-CH₂SH); **30**). *Method 1*: Disulfide **29** (5 mg, 1.63 μmol) was dissolved in degassed THF/H₂O 15:1 (1.6 ml, 1 h Ar) and PBu₃ (4.2 μl, 16.7 μmol) was added. The mixture was stirred for 2 h at r.t. The crude product was evaporated and filtered through a layer of silica gel with AcOEt (20 ml) followed by CH₂Cl₂/MeOH 10:1 (20 ml): **30** (5 mg, quant.). Colorless glass.

Method 2: Disulfide **29** (9 mg, 2.94 μmol) was dried under high vacuum at 45° for 24 h. *Ekathiol* resin (58 mg, 29.4 μmol; *Peptide International*, Louisville, Kentucky) was added and the mixture suspended in degassed THF (1.0 ml, 1 h Ar). The mixture was shaken for 4 h at r.t. The resin was treated with CH₂Cl₂/MeOH 10:1 (10 ml), then filtered, and the org. phase was evaporated: **30** (9 mg, quant.). Colorless glass.

5'-I(Benzoyloxy)methyl]-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenethiomethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(mercaptomethyl)thymidine (*d*(BzOCH₂-Tso₂TsTso₂T-CH₂SH); **26**). To **18** (34 mg, 21.8 μmol), 10% CF₃COOH, 5% ethane-1,2-dithiol, 2% (i-Pr)₃SiH and 1% H₂O in CH₂Cl₂ (3 ml) were added. The soln. turned red-orange immediately. The mixture was stirred at r.t. for 5 min and poured into sat. NaHCO₃ soln. (10 ml) containing ice (10 g). The org. phase was washed with brine (1 × 10 ml). The aq. phases were reextracted with CH₂Cl₂/EtOH 10:1 (2 × 10 ml). The combined org. phase was evaporated. FC (silica gel, CH₂Cl₂/MeOH 20:1, 15:1, 10:1) yielded **18** (2 mg, 6%) and **26** (22 mg, 80%; 85% based on recovered **18**) as colorless glasses. UV (CH₂Cl₂/MeOH 20:1): 227 (30000), 267 (42200). ¹H-NMR (CDCl₃/CD₃OD 4:1, 500 MHz): 1.88–2.01 (*m*, 1 H, H–C(5')); 1.91, 1.92, 1.92, 1.93 (4s, 12 H, Me–C(5)(T)); 2.05–2.19 (*m*, 4 H, H–C(5')); 2.22–2.43 (*m*, 13 H, 3 H–C(5'), 8 H–C(2'), 2 H–C(3')); 2.61–2.76 (*m*, 7 H, CH₂SO₂CH₂, CH₂–C(3')(T4), H–C(3')); 2.78–2.85 (*m*, 1 H); 3.11–3.38 (*m*, partly under MeOD, 8 H, CH₂SO₂CH₂); 3.79–3.87 (*m*, 2 H, H–C(4')); 3.89–3.97 (*m*, 2 H, H–C(4')); 4.43–4.50 (*m*, 1 H, H–C(6')(T1)); 4.56–4.62 (*m*, 1 H, H–C(6')(T1)); 5.98–6.03 (*m*, 2 H, H–C(1')); 6.03–6.08 (*m*, 2 H, H–C(1')); 7.18, 7.19, 7.26 (3s, 4 H, H–C(6)(T)); 7.43–7.48 (*m*, 2 H, Bz); 7.57–7.62 (*m*, 1 H, Bz); 8.01–8.03 (*m*, 2 H, Bz). ¹³C-NMR (CDCl₃/CD₃OD 4:1, 75 MHz): 8.45, 12.04 (2*q*, Me–C(5)(T)); 25.48, 26.12 (2*t*, CH₂–C(3')(T2), CH₂–C(3')(T4)); 29.02 (*t*, C(6')(T3)); 32.39, 33.32, 33.92 (3*t*, C(5)(T)); 35.73 (*d*, C(3')(T)); 36.34, 36.57, 37.18, 37.62 (4*t*, C(2')(T)); 42.51 (*d*, C(3')(T)); 45.79 (*d*, C(3')(T)); 50.43 (*t*, CH₂–C(3')(T)); 54.48 (*t*, C(6')(T)); 61.66 (*t*, C(6')(T1)); 80.93, 81.43, 81.89, 81.98 (4*d*, C(4)(T)); 84.61, 85.08 (2*d*, C(1')(T)); 111.01 (*s*, C(5)(T)); 128.22, 129.25 (2*d*, Bz); 129.75 (*s*, Bz); 133.05 (*d*, Bz); 135.54, 135.81 (2*d*, C(6)(T)); 150.39 (*s*, C(2)(T)); 164.31 (*s*, C(4)(T)). ESI-MS (pos.): 1281.4 ([*M*+Na]⁺), 1155.2 ([*M*–TH+Na]⁺).

7. Octamers: Coupling and Oxidation. d(TrOCH₂-Tso₂Tso₂Tso₂¹⁰CsTso₂Tso₂Tso₂T-CH₂OTBDPS) (**32a**). Disulfide **29** (20 mg, 6.53 μmol) was dissolved in degassed DMF/H₂O 5:1 (1 ml, 1 h Ar). PBu₃ (6.5 μl, 26.1 μmol) was added, and the soln. was stirred for 2 h at r.t. The soln. was transferred under Ar pressure through a capillary to a soln. of bromide **24** (20 mg, 13.58 μmol) and Cs₂CO₃ (20 mg, 61.4 μmol) in degassed

DMF/H₂O 5:1 (1 ml, 1 h Ar). The mixture was again degassed by three freeze-pump cycles. The soln. was warmed to 45° and stirred for 6 h during which a white precipitate was formed (TLC monitoring). The reaction was terminated by neutralization with acetate buffer (3M AcOH/1M AcONa; 35 µl), after which the mixture turned clear again. CH₂Cl₂ (20 ml) and brine (5 ml) were added. The aq. phase was extracted with CH₂Cl₂/EtOH 6:1 (5 × 10 ml) and the combined org. phase evaporated at max. 40°. Phosphorus compounds and salts were removed by filtration through a layer of sea sand with AcOEt (20 ml) and deionized H₂O (15 ml). Crude **32a** (39 mg) was then dissolved in CH₂Cl₂/MeOH 15:1 (50 ml). Crude **32a** was used for the following oxidation without further purification. UV (H₂O/MeCN): 266, 309. MALDI-TOF MS (*A* = octamer in CH₂Cl₂/MeOH 5:1, *B* = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, *C* = *A/B* 3:1): 2922.5 (*M*⁻).

TrOCH₂-Tso₂Tso₂Tso₂^{so}CsTso₂TsTso₂T-CH₂STr(OMe)₂ (**32b**). As described for **32a**, with **29** (14 mg, 4.58 µmol), DMF/H₂O 5:1 (2 ml, 1 h Ar), PBu₃ (4.6 µl, 18.4 µmol), **23** (13.9 mg, 9.15 µmol), Cs₂CO₃ (15 mg, 45.8 µmol) (at 45° overnight), and acetate buffer (3M AcOH/1M AcONa; 30 µl) (washing with 3 ml of brine). Crude **32b** (31 mg) was dissolved in CH₂Cl₂/MeOH 15:1 (50 ml). Crude **32b** was used for the following oxidation without further purification. UV (H₂O/MeCN): 266, 309. MALDI-TOF MS (*A* = octamer in CH₂Cl₂/MeOH 10:1, *B* = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, *C* = *A/B* 3:1): 2968.3 (*M*⁻).

d(BzOCH₂-Tso₂TsTso₂TsTso₂T-SCH₂STr(OMe)₂) (**32c**). Thiol **26** (19 mg, 15.1 µmol), bromide **23** (23 mg, 15.1 µmol), and Cs₂CO₃ (20 mg, 60 µmol) were dissolved in degassed DMF (2 ml), 1 h Ar). The soln. was degassed a second time by three freeze-pump cycles. The mixture was warmed to 45° and stirred for 7 h, during which a white precipitate was formed. The reaction was terminated by neutralization with acetate buffer (3M AcOH/1M AcONa; 50 µl), after which the mixture turned clear again. CH₂Cl₂ (20 ml) and brine (5 ml) were added, the aq. phase was extracted with CH₂Cl₂/EtOH 6:1 (5 × 10 ml), and the combined org. phase was evaporated at max. 40°. Phosphorus compounds and salts were removed by filtration through a layer of sea sand with AcOEt (25 ml) and deionized H₂O (20 ml). Crude **32c** (31 mg) was dissolved in CH₂Cl₂/MeOH 10:1 (50 ml). Crude **32c** was used for the following deprotection without further purification. UV (H₂O/MeCN): 267. ¹H-NMR (CDCl₃/CD₃OD 4:1, 300 MHz): 1.67–2.00 (*m*, 5 H); 1.91 (*s*, 24 H, Me–C(5)(T)); 2.01–2.19 (*m*, 10 H); 2.21–2.52 (*m*, 22 H); 2.57–2.84 (*m*, 14 H, CH₂S); 3.04–3.36 (*m*, 16 H, CH₂SO₂CH₂); 3.52–3.58 (*m*, 1 H, H–C(4')); 3.80 (*s*, 2 MeO); 3.81–3.97 (*m*, 7 H, H–C(4')); 4.40–4.49 (*m*, 1 H, H–C(6')(T1)); 4.53–4.61 (*m*, 1 H, H–C(6')(T1)); 5.91 (*dd*, *J* = 4.0, 7.2, 1 h, H–C(1')); 5.96–6.09 (*m*, 7 H, H–C(1')); 6.81–6.85 (*m*, *J* = 9.0, 4 H, H_m of Ar₂PhC); 7.02 (*s*, 1 H, H–C(6)(T)); 7.17–7.24 (*m*, 6 H, 5 H–C(6)(T), (MeO)₂Tr); 7.26–7.34 (*m*, 7 H, 2 H–C(6)(T, (MeO)₂Tr); 7.37–7.47 (*m*, 5 H, 3 H of (MeO)₂Tr, 2 H of Bz); 7.55–7.60 (*m*, 1 H, Bz); 7.99–8.02 (*m*, 2 H, Bz). MALDI-TOF MS (*A* = octamer in CH₂Cl₂/MeOH 10:1; *B* = 0.5M 2,4,6-THA (2,4,6-trihydroxyacetophenone) in EtOH/0.1M DAC in H₂O 2:1, *C* = *A/B* 3:1): 2695.2 (*M*⁻).

d(TrOCH₂-Tso₂Tso₂Tso₂^{so}Cso₂Tso₂Tso₂T-CH₂OTBDPS) (**33a**). Crude **32a** (35 mg) was dissolved in THF/H₂O 25:1 (5.2 ml). The addition of MeOH (8 ml) caused a slight precipitation of **32a**. Oxone (37 mg, 60 µmol) and NaOAc · 3 H₂O (27 mg, 198 µmol) were dissolved in deionized H₂O (0.8 ml) and added under vigorous stirring (→ milky suspension). The mixture was stirred for 20 h at r.t. Excess Oxone was reduced with sat. Na₂S₂O₃ soln. (0.5 ml). The mixture was concentrated to 50% of the original volume, CH₂Cl₂ (30 ml) and deionized H₂O (5 ml) were added, and the aq. phase was extracted with CH₂Cl₂/EtOH 10:1 (5 × 10 ml). The combined org. phase was evaporated. Crude **33a** (32 mg) was used for the following deprotection without further purification. UV (H₂O/MeCN): 266, 309. MALDI-TOF MS (*A* = octamer in CH₂Cl₂/MeOH 5:1, *B* = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, *C* = *A/B* 3:1): 2953.8 (*M*⁻).

d(TrOCH₂-Tso₂Tso₂Tso₂^{so}Cso₂Tso₂Tso₂T-CH₂SO₃⁻) (**33b**). Crude **32b** (16 mg) was dissolved in THF/H₂O 8:1 (4.5 ml). The addition of MeOH (6 ml) formed a slight precipitation of **32b**. Oxone (33 mg, 54 µmol) and NaOAc · 3 H₂O (24 mg, 178 µmol) were dissolved in deionized H₂O (1.0 ml) and added under vigorous stirring (→ milky suspension). After 1 h, TLC monitoring showed the complete oxidation of the terminal CH₂STr(OMe)₂ group to CH₂SO₃⁻. Stirring continued for 3 h at r.t. Excess Oxone was reduced with sat. Na₂S₂O₃ soln. (0.2 ml). The org. solvents were evaporated (30° water-bath temp.), and the remaining soln. was lyophilized. The residual solid was suspended in deionized H₂O (3 × 1 ml) and centrifuged, the supernatant removed, and the combined aq. phase desalted with a SepPak-C₁₈ cartridge (H₂O/MeCN 100:0 (10 ml), 100:10 (5 ml), 100:100 (10 ml), 0:100 (10 ml); detection by UV spectroscopy) to recover the small amount of dissolved **33b**. Crude **33b** (16 mg) was obtained as a colorless powder. UV (H₂O/MeCN): 266, 309. MALDI-TOF MS (*A* = octamer in H₂O/MeCN 1:1, *B* = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, *C* = *A/B* 2:1): 2777.9 (*M*⁻).

8. Deprotection of Octamers. 5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-2',5'-dideoxy-3'-de(phosphini-

cooxycytidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(hydroxymethyl)thymidine (*d*(HOCH₂-Tso₂Tso₂Tso₂Cso₂Tso₂Tso₂T-CH₂OH); **35a**). Crude **33a** (6 mg) was suspended in an Eppendorf in CH₂Cl₂ (0.4 ml), and 2.2M ZnCl₂·Et₂O in CH₂Cl₂ (200 μl, 440 μmol) was added (→ orange soln.). The suspension was centrifuged after 10 min, and the orange supernatant was removed. The precipitate was washed with Et₂O/CH₂Cl₂ 1:1 (4 × 1 ml) and centrifuged, and the supernatant was removed. The crude, slightly orange product consisted of detritylated **34a** (ca. 80%) and starting **33a** (ca. 20%) (by HPLC). Prolongation of the reaction time as well as repeated detritylation with ZnCl₂·Et₂O solution showed no further deprotection. Crude detritylated **34a** with residual **33a** was dissolved in THF/MeOH/H₂O 3:4:2 (0.9 ml), and 2M NaOH (0.2 ml) was added. The soln. was warmed to 45° and stirred for 18 h. The deprotection was monitored by HPLC. Acetate buffer (3M AcOH/1M AcONa; 0.2 ml) was added, and the mixture was desalted twice with a SepPak-C₁₈ cartridge (H₂O/MeCN 100:0 (10 ml), 100:20 (5 ml), 100:10 (5 ml), 100:100 (5 ml), 0:100 (5 ml); detection by UV spectroscopy). The crude product was purified by HPLC (Shodex-RSpak-D18-613 column (6 × 150 mm); flow 1.0 ml/min, A = 0.1M Et₃N, B = MeCN, 5 min 100% A, 0–60% B in 30 min, elution of **35a** after 21.5 min): **35a** (1.5 mg, 31%).

Crude Detritylated **34a**: MALDI-TOF MS (A = octamer in CH₂Cl₂/MeOH 5:1, B = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, C = A/B 5:1): 2711.4 (*M*⁻).

Data of **35a**: UV (H₂O): 264. ¹H-NMR ((D₆)DMSO, 500 MHz): 1.72–1.81 (*m*, 1 H, H-C(5'')); 1.80 (*s*, 21 H, Me-C(5)(T)); 1.84–1.95 (*m*, 2 H, H-C(5'')); 1.97–2.13 (*m*, 9 H, H-C(2''), H-C(5'')); 2.15–2.38 (*m*, 21 H, H-C(2''), H-C(3''), H-C(5'')); 2.48–2.58 (*m*, partly under DMSO, 2 H, H-C(3'')); 2.63–2.72 (*m*, 5 H, H-C(3'')); 3.20–3.37 (*m*, partly under H₂O, 20 H, CH₂-C(3''), H-C(6'')); 3.39–3.59 (*m*, 12 H, CH₂-C(3''), H-C(6'')); 3.66–3.75 (*m*, 7 H, H-C(4'')); 3.75–3.80 (*m*, 1 H, H-C(4'')); 4.55 (*m*, 1 H, OH); 4.81 (*t*, *J* = 5.0, 1 H, OH); 5.75 (*m*, 1 H, H-C(5)(C)); 5.98–6.01 (*m*, 2 H, H-C(1'')); 6.03–6.07 (*m*, 6 H, H-C(1'')); 7.12 (*br.*, 2 H, NH); 7.44 (*2s*, 1 H, H-C(6)(T)); 7.46 (*2s*, 1 H, H-C(6)(T)); 7.49 (*m*, 5 H, H-C(6)(T)); 7.60–7.62 (*d*, *J* = 8.0, 1 H, H-C(6)(C)). MALDI-TOF MS (A = octamer in CH₂Cl₂/MeOH 5:1, B = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, C = A/B 5:1): 2356.0 (*M*⁻).

5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-2',5'-dideoxy-3'-de(phosphinicooxy)cytidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de (phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(sulfomethyl)thymidine Ion(1-) (*d*(HOCH₂-Tso₂Tso₂Tso₂Cso₂Tso₂Tso₂T-CH₂SO₃⁻); **35b**). Method 1: As described for **35a**, with crude **33b** (5 mg), CH₂Cl₂ (0.4 ml), and 2M ZnCl₂·Et₂O in CH₂Cl₂ (200 μl, 440 μmol) (centrifugation for 5 min): 6'-deprotected **34b** (80%) and **33b** (20%). Then with crude **34b**, THF/MeOH/H₂O 3:4:2 (0.9 ml), and 2M NaOH (0.2 ml) at 40° for 21 h. HPLC (see **35a**; **35b** after 15.5 min) gave **35b** (0.9 mg, 21%).

Method 2: Crude **33b** (5 mg) was dissolved in THF/MeOH/H₂O 3:4:2 (0.9 ml), and 2M NaOH (0.2 ml) was added. The soln. was warmed to 40° (*t*, and stirred for 20 h (HPLC monitoring). Acetate buffer (3M AcOH/1M AcONa; 0.2 ml) was added, and the mixture was desalted twice with a SepPak-C₁₈ cartridge (H₂O/MeCN 100:0 (10 ml), 100:10 (5 ml), 100:20 (5 ml), 100:100 (5 ml), 0:100 (5 ml); detection by UV spectroscopy). The crude product was suspended in a soln. of TsOH·H₂O (0.35 mg) in MeOH (0.5 ml) and shaken for 5.5 h at r.t. (HPLC monitoring). The suspension was desalted five times through a SepPak-C₁₈ cartridge (sat. NaHCO₃ soln. (3 ml), H₂O/MeCN 100:0 (5 ml), 100:10 (5 ml), 100:100 (5 ml), 0:100 (5 ml); detection by UV spectroscopy). HPLC: **35b** (90%), tritylated compound (5%), and by-product (5%). Elongation of the reaction time showed an increased amount of by-product. The crude product was purified by HPLC (see Method 1): **35b** (1.0 mg, 23%), identical to the product from Method 1 (by HPLC and MALDI-TOF MS). UV (H₂O): 264. ¹H-NMR (D₂O/CD₃CN 4:1, 500 MHz): 1.78 (*s*, 21 H, Me-C(5)(T)); 1.99–2.16 (*m*, 11 H, H-C(2''), H-C(5'')); 2.23–2.42 (*m*, 22 H, H-C(2''), H-C(3''), H-C(5'')); 2.45–2.53 (*m*, 1 H, H-C(3'')); 2.56–2.65 (*m*, 6 H, H-C(3'')); 3.15–3.38 (*m*, 21 H, CH₂-C(3''), H-C(6'')); 3.53–3.65 (*m*, 11 H, CH₂-C(3''), H-C(6'')); 3.74–3.95 (*m*, 10 H, H-C(4''), CH₂-C(3'')(T8)); 5.32 (*m*, 1 H, H-C(5)(C)); 5.87–5.94 (*m*, 2 H, H-C(1'')); 5.95–6.01 (*m*, 6 H, H-C(1'')); 7.15–7.18 (*br.*, 2 H, NH); 7.29–7.36 (*m*, 7 H, T); 7.52–7.58 (*m*, 1 H, H-C(6)(C)). MALDI-TOF MS (A = octamer in H₂O/MeCN 1:1, B = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, C = A/B 2:1): 2419.7 (*M*⁻).

5'-Deoxy-3'-de(phosphinicooxy)-5'-(hydroxymethyl)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)-

thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-5'-deoxy-3'-de(phosphinicooxy)thymidylylmethylenesulfonylmethylene-(3' → 5')-3',5'-dideoxy-3'-(sulfomethyl)thymidine Ion(1-) (*d*(HOCH₂-Tso₂Tso₂Tso₂Tso₂Tso₂Tso₂Tso₂T-CH₂SO₃⁻); **34c**). Crude **32c** (6 mg) was suspended in MeOH/THF 4:1 (2.5 ml), and 2M NaOH (0.5 ml) was added. The octamer did not dissolve completely in the basic media. The mixture was stirred for 24 h at r.t. and was neutralized with acetate buffer (3M AcOH/1M AcONa; 0.5 ml), lyophilized, desalted with a SepPak-C₁₈ cartridge (H₂O/MeCN 100:0 (10 ml), 100:10 (5 ml), 100:100 (5 ml), 0:100 (10 ml); detection by UV spectroscopy), and again lyophilized to give the 6'-deprotected **33c** (14 mg) as a colorless powder. Crude **33c** was suspended in THF/MeOH/H₂O 3:4:2 (0.9 ml), and a soln. of Oxone (54.1 mg, 88 μmol) and NaOAc·3 H₂O (39.5 mg, 290 μmol) in deionized H₂O (0.5 ml) was added. The mixture was stirred for 24 h at r.t. Sat. Na₂S₂O₃ soln. (0.5 ml) was added, the suspension centrifuged, and the supernatant removed. The crude product was desalted through a SepPak-C₁₈ cartridge (H₂O/MeCN 100:0 (5 ml), 100:10 (5 ml), 100:100 (5 ml), 0:100 (5 ml); detection by UV spectroscopy) and purified by HPLC (Shodex-RSPak-D18-613 column (6 × 150 mm), flow 1.0 ml/min; A = 0.1M Et₃N, B = MeCN, 5 min 100% A, 0–60% B in 30 min, elution of **38c** after 16.0 min): **38c** (1.8 mg, 33%). UV (H₂O): 267. ¹H-NMR ((D₆)DMSO, 500 MHz): 1.80 (*s*, 24 H, Me-C(5)(T)); 1.87–1.94 (*m*, 1 H); 1.98–2.10 (*m*, 7 H); 2.22–2.37 (*m*, 24 H); 2.48–2.58 (*m*, partly under DMSO, 1 H, H-C(3')); 2.63–2.72 (*m*, 7 H, H-C(3')); 3.20–3.62 (*m*, partly under H₂O, 32 H, CH₂-C(3'), H-C(6')); 3.66–3.84 (*m*, 10 H, 8 H-C(4'), CH₂-C(3')(T8)); 5.96–6.01 (*m*, 2 H, H-C(1')); 6.02–6.09 (*m*, 6 H, H-C(1')); 7.43 (*2s*, 1 H, H-C(6)(T)); 7.44 (*2s*, 1 H, H-C(6)(T)); 7.49 (*m*, 5 H, H-C(6)(T)); 7.52 (*s*, 1 H, H-C(6)(T)). MALDI-TOF MS (A = octamer in H₂O/MeCN 1:1, B = 0.5M 2,4,6-THA in EtOH/0.1M DAC in H₂O 2:1, C = A/B 2:1): 2433.8 (*M*⁻).

9. *Characterization and Purification of SNAs: General Aspects.* Intermediates and products in the synthesis of SNA were characterized first by ¹H- and ¹³C-NMR. As reported earlier by other authors, line broadening occurred in spectra of longer SNA, even in DMSO as solvent [23][28], a phenomenon well known with other non-ionic oligonucleotide analogs, including peptide-linked nucleic acid analogs (PNA) [44]. This may arise from aggregation of the single-stranded oligomers. Line broadening diminished when CD₃OD and D₂O were added.

With longer SNAs, NMR analysis became increasingly difficult. MS Techniques were, therefore, employed. Since these oligomers are a new class of compounds, conditions were explored to enable the detection and analysis by MS, including electronic ionization (EI), electrospray ionization (ESI) [45], fast-atom bombardment (FAB) [46], and matrix-assisted laser desorption and ionization with time-of-flight detection (MALDI TOF) [47]. FAB-MS with a 3-nitrobenzyl alcohol (NOBA) matrix was successful for mass analysis of monomers, dimers, and tetramers when the molecule contained neither Tr nor (MeO)₂Tr groups. FAB-MS frequently showed fragment peaks, e.g., a [*M*-(*t*-Bu)]⁺ ([*M*-57]⁺) fragment for intermediates with a *t*-BuPh₂Si protecting group or fragment peaks of nucleobases ([MeC₆H₄CONHC₄H₈N₂O + H]⁺ at *m/z* 230). FAB-MS of trityl-containing compounds, especially dimers and tetramers, were dominated by the Tr⁺ and (MeO)₂Tr⁺ cations, *M*⁺ could often not be observed under these circumstances.

For compounds containing trityl groups, MALDI-TOF MS was useful [23][28]. The fully protected dimeric SNA **3b** was analyzed by MALDI-TOF MS with a 2,5-dihydroxybenzoic acid (DHB) matrix, in positive mode. Insulin was used as an external standard. The protonated cation [*M*+H]⁺ was only detectable in traces. In most other MALDI-TOF MS obtained in positive mode with either DHB or *α*-cyano-4-hydroxycinnamic acid (CCA) as matrix, [*M*+Na]⁺ was the most-prominent peak followed by [*M*+K]⁺ or [*M*+2 Na]⁺, respectively. However, the best spectra for tetrameric and octameric SNAs were measured in the negative mode, detecting the *M*⁻ derivative.

ESI-MS was also useful for analysis of tritylated compounds. A dimeric SNA in THF was successfully analyzed in both the positive ([*M*+Na]⁺) and negative modes (*M*⁻) by an ESI ion-trap mass spectrometer.

The octameric SNAs were purified by HPLC. All other tetramer products described in this work, were purified by FC (silica gel), which allowed separation of molecules with molecular masses up to ca. 1750 g/mol differing in only one functional group. However, the adsorption of SNAs onto silica gel as a result of their high polarity always rendered this purification difficult. A stepwise gradient with a nonpolar solvent and AcOEt as the polar co-eluent was the preferred FC technique for monomeric SNAs. However, the reduced solubility of dimeric SNAs in AcOEt required their purification in a CH₂Cl₂/MeOH gradient system. The separation of tetramers was considerably improved by the addition of 0.25% H₂O to the eluent mixture, which was also reported for rSNAs [30]. It was essential to commence the FC tetramers with a polar starting eluent (usually 2–5% MeOH and 0.25% H₂O in CH₂Cl₂) to diminish the severe loss of product by irreversible adsorption to silica. The loss of a small amount (2–5%) of large, polar SNA on silica gel was inevitable. Losses of up to 10% product

were reported by Richert during FC (silica gel) for tetrameric rSNAs [30]. Surprisingly, the relative retention of products and reagents on TLC was changed in some cases compared to FC. In extreme cases, the elution order of several compounds were inverted. It appeared that larger compounds, which were less polar, had an increased retention time on FC compared to TLC.

The adsorption of octameric SNAs on silica gel was too high to allow successful separation and recovery by standard liquid chromatography. Therefore, after removal of reagents by *SepPak-C₁₈* cartridges, the protected and unprotected octamers were purified by HPLC on reversed phase *C₁₈* columns. H₂O/MeCN Mixtures were chosen as eluents, as the solubility of the octamers was less-restricted in these mixtures than in H₂O/MeOH mixtures. Buffered aq. phases did not improve the separation of oligomeric SNAs on reversed-phase HPLC. HPLC Separation of protected as well as unprotected, non-ionic and singly charged octamers with MeCN/H₂O gradients showed that SNAs were tightly adsorbed onto the *C₁₈* columns, and even seemed to precipitate on the column. This led to reduced separation, loss of product, and contaminated columns. Nevertheless, acceptably pure compound could be obtained (Fig. 1).

The use of basic eluents was evaluated to improve the solubility of sulfones. At pH 11, N(3) (pK_a ca. 10) of thymine is deprotonated, and therefore confers solubility. The volatile buffer 0.1% Et₃N (pH 11) was used on a *Shodex-RSpak-C₁₈* column, a column with a stability range between pH 2 and 12. The gradient 0.1% Et₃N/MeCN improved separation but did not prevent the irreversible adsorption onto the column. Because of these problems, the HPLC purification steps were minimized as much as possible, and only the fully deprotected octamers were purified by reversed-phase HPLC.

10. *Melting Curves.* The melting curves were measured on a *Varian Cary-1-Bio-UV/VIS* spectrophotometer, heated with a *Cary* temperature controller. The SNA and DNA samples (1–3 μm) were dissolved in buffer, transferred to cuvettes (1 cm × 1 cm), degassed in a sonicator, and heated to 85–95° with a heating rate of 3°/min. The cooling rate as well as the heating rate for the melting experiments were 1°/min or 0.5°/min for duplex or triplex experiments, respectively. A stream of dry N₂ was used to avoid condensation on the cuvetts at temp. below 10°. UV Absorption was measured once per min at λ 258–264 nm. Melting temperatures (T_m) were calculated with the *Cary OS/2* software. The program uses the measured data points to generate a separate continuum from which a first-derivative calculation is performed. The first-derivative plot peaks at the maximal incline(s), which is/are the turning point(s) of the melting curve, yield T_m .

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