

Oligonucleotide Analogues with Integrated Bases and BackbonePart 30¹⁾**Synthesis and Association of a Self-Complementary Thiomethylene-Linked Octanucleoside***Dieter Seebach* mit den besten Glückwünschen zugeeignetby **Lorenz Herdeis**, **Siji Thomas**, **Bruno Bernet***, and **Andrea Vasella***

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The protected G*[s]C*[s]U*[s]A*[s]U*[s]A*[s]G*[s]C* octanucleoside **24** was prepared by *S*-alkylation of the thiolate derived from tetranucleoside **23** with the methanesulfonate **22**, and transformed to the silylated and isopropylidened **25**, and further into the fully deprotected octanucleoside **26**. Compound **22** was derived from the methoxytrityl-protected tetranucleoside **21**, and **21** was obtained by *S*-alkylation of the thiolate derived from the dinucleoside **19** with methanesulfonate **17** derived from **16** by detritylation and mesylation. Similarly, tetranucleoside **23** resulted from *S*-alkylation of the thiolate derived from **18** with the methanesulfonate **20** derived from **19**. Dinucleosides **16** and **18** resulted from *S*-alkylation of the thiolate derived from the known cytidine-derived thioacetate **15** with the *C*(8)-substituted guanosine-derived methanesulfonates **12** and **14**, respectively, that were synthesized from the protected precursors **4** and **7** by formylation, reduction, protection, and mesylation.

The structures of the duplexes of **25** and **26** were calculated using AMBER* modelling and based on the known structure of the core tetranucleoside U*[s]A*[s]U*[s]A*. The former shows a helix with a bent helix axis and strong buckle and propeller twists, whereas the latter is a regular, right-handed, and apparently strain-free helix. In agreement with modelling, the silylated and isopropylidened octanucleoside **25** in (CDCl₂)₂ solution led to a mixture of associated species possessing at most four *Watson–Crick* base pairs, while the fully deprotected octanucleoside **26** in aqueous medium forms a duplex, as evidenced by a decreasing CD absorption upon increasing the temperature and by a UV-melting curve with a melting temperature of *ca.* 10° below the one of the corresponding RNA octamer, indicating cooperativity between base pairing and base-pair stacking.

Introduction. – Our investigation of analogues that replace the contiguous backbone of oligonucleotides by linking elements between pairs of nucleobases (ONIBs)²⁾ has shown that self-complementary and non self-complementary, partially protected di- and tetranucleosides, possessing a variety of linking elements, undergo pairing in CDCl₃ solution [2–9]. Pairing (*i.e.*, the formation of cyclic duplexes) depends on the sequence of the nucleobases, and on the constitution and conformation of the linking elements. Pairing requires a *syn* conformation, and involves *Watson–Crick* and/or *Hoogsteen*-type H-bonds. In some ONIBs, pairing in CDCl₃ is accompanied by stacking of the nucleobases, as evidenced by CD spectroscopy.

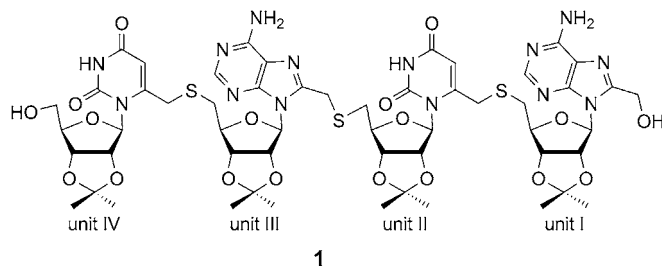
¹⁾ Part 29: [1].

²⁾ Abbreviation of the originally suggested term ‘**O**ligo**N**ucleotides **I**ntegrating **B**ackbone and bases’.

Two types of linkers have proven of special interest for our goal of making ONIBs that pair in aqueous solutions. In the corresponding ONIBs, two adjacent nucleobases are either joined by *S*-methylene-5-thioribosyl units [4–8], or by *S*-methylene-*L*-cysteine-containing dipeptidyl units [1]. We have already shown that octameric ONIBs of the latter type associate in aqueous solution in a sequence-specific, reversible way to form a left-handed double helix with antiparallel strand orientation, characterized by melting temperatures and free enthalpies that are higher than those of natural RNA and DNA of the same sequence [1]. In parallel experiments, we pursued the goal of making H₂O-soluble octameric ONIBs of the former type. Pairing in aqueous solution of two types of ONIBs should conclusively demonstrate that the structural differentiation of oligonucleotides in a contiguous backbone and appended nucleobases is neither a prerequisite for pairing, nor for the formation of defined conformers.

The partially protected thiomethylene-linked U*[s]A*[s]U*[s]A*³) tetranucleoside **1** pairs in CDCl₃ according to a *Watson–Crick* type to form a right-handed antiparallel helix, as determined by NMR spectroscopy and force-field calculations [6]. The fully deprotected tetranucleoside, however, proved too poorly soluble in H₂O to investigate its pairing properties.

The structure of the isopropylidene-protected U*[s]A*[s]U*[s]A* tetranucleoside **1** is characterized by a strong bending of the helix axis that leads to a pronounced inclination of the central base pairs (roll angle of *ca.* 15°) and results in poor base stacking [6]. This bending appears incompatible with helix formation of longer oligonucleosides of this type. However, a self-complementary octanucleoside, obtained by extending the tetranucleoside with cytidine and guanosine derived units, may well be sufficiently soluble in H₂O, considering the H₂O solubility of cytidine-derived di- and tetranucleosides [8], and partial or complete deprotection may confer them a sufficient flexibility to form a full helix turn, in spite of the strong bending of the helix axis of the core U*[s]A*[s]U*[s]A* tetranucleoside.



Results and Discussion. – 1. *Calculation of Fully Paired Duplex Structures of the Target Octanucleosides 25 and 26.* To evaluate the formation of cyclic duplexes with all nucleobases involved in base pairing, we calculated the cyclic duplexes of the target

³) *Conventions for abbreviated notation:* The substitution at C(6) of pyrimidines and C(8) of purines is denoted by an asterisk (*); for example, U* and A* for hydroxymethylated uridine and adenosine derivatives, respectively. U^(*) and A^(*) represent both unsubstituted and hydroxymethylated nucleobases. The moiety linking C(6)CH₂ or C(8)CH₂ of unit II, and C(5') of unit I is indicated in square brackets, *i.e.*, [s] for a S-atom.

G*[s]C*[s]U*[s]A*[s]U*[s]A*[s]G*[s]C* octanucleosides **25** and **26** (see *Scheme 3* below) with the force-field programme *AMBER** implemented in *Macromodel* v.6.0 [10]. The duplex structure of the U*[s]A*[s]U*[s]A* tetranucleoside **1** (**1**·**1** = **26C** in [6]) corresponds to the central units III – VI of **25** and **26**. It was extended on both sides by G*[s]C* moieties possessing also *Watson–Crick* base pairs. This calculation of **25**·**25** resulted, after releasing all constraints, in a left-handed double helix with eight *Watson–Crick* base pairs (H-bond distances of 1.68–1.82 Å; *Fig. 1, a*) in spite of the strong curvature of **1**·**1**. The isopropylidene and silyl protecting groups of **25**·**25** were

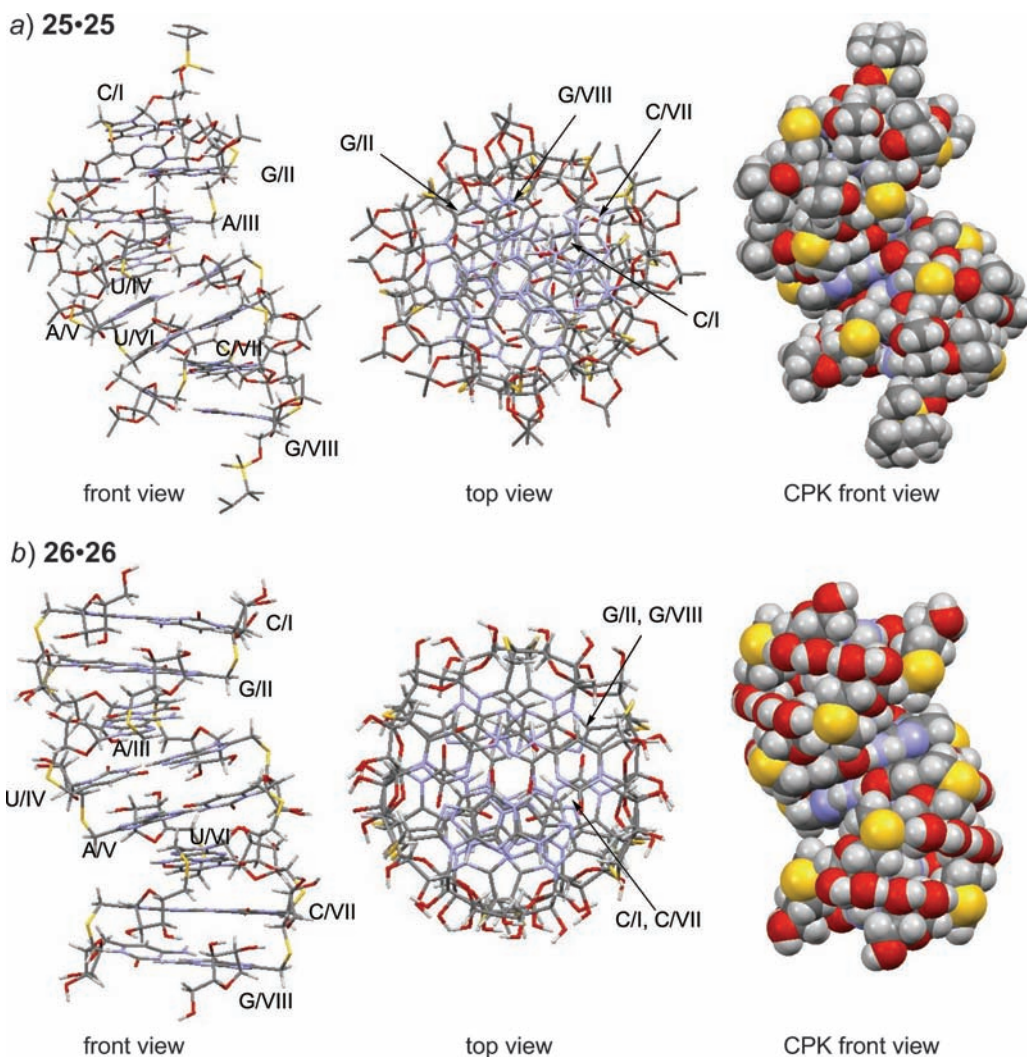


Fig. 1. *AMBER**-calculated structures of the *Watson–Crick* base-paired duplexes a) **25**·**25** and b) **26**·**26**: front and top views (for **25**·**25**: H-atoms of the protecting groups are omitted for enhanced clarity), and space-filling (CPK) front view

removed, and minimisation gave the structure of the duplex **26**·**26** (with shorter H-bond distances of 1.64–1.79 Å) of the fully deprotected octanucleoside **26** (Fig. 1, b).

Both structures **25**·**25** and **26**·**26** contain six units per turn as indicated in the top view (Fig. 1). Despite the isopropylidene groups, the former duplex possesses a deeper major groove than the latter one (see CPK structures). The duplex **26**·**26** shows a regular double helix with a linear helix axis (type-B helix with a small central hole), whereas the duplex **25**·**25** is less regular and possesses a bent helix axis (inclined to the right in the top view) that is, however, less pronounced than for the duplex **1**·**1** of the U*[s]A*[s]U*[s]A* tetranucleoside. In addition, the front view shows a better π -stacking of the nucleobases (distance between base pairs of 3.1–3.5 Å), smaller propeller twists, and smaller roll angles for **26**·**26** than for **25**·**25**. This indicates a severe backbone strain in the cyclic duplex **25**·**25** which may prevent the formation of a completely base-paired cyclic duplex. However, the far more regular structure of **26**·**26**, with little or no backbone strain, suggests an easy formation of this duplex. It appeared worthwhile to synthesize **25** and **26**, and to investigate their pairing properties.

2. *Synthesis of G*[s]C*[s]U*[s]A*[s]U*[s]A*[s]G*[s]C* Octanucleosides.* We aimed at a convergent synthesis of the title octanucleoside rather than following the longer linear strategy underlying the synthesis of the core tetranucleoside **1** [6]. As conceived, the synthesis required the four dinucleosides **17**–**20** (cf. Scheme 3 below), to be coupled to form two tetranucleosides, **21** and **23**. Coupling of tetranucleoside **22** derived from **21** with **23** should provide the fully protected octanucleoside **24**. Thus, each coupling step requires the directed formation of a thioether by alkylating a thiolate anion, generated by cleaving the C(5')–SR group, with a methanesulfonate (or a similar electrophile) derived from a nucleoside possessing a hydroxymethyl substituent at C(6) or C(8). Finally, deprotection of **24** should first provide the isopropylidened and silylated derivative **25**, and then the desired fully deprotected octanucleoside **26**.

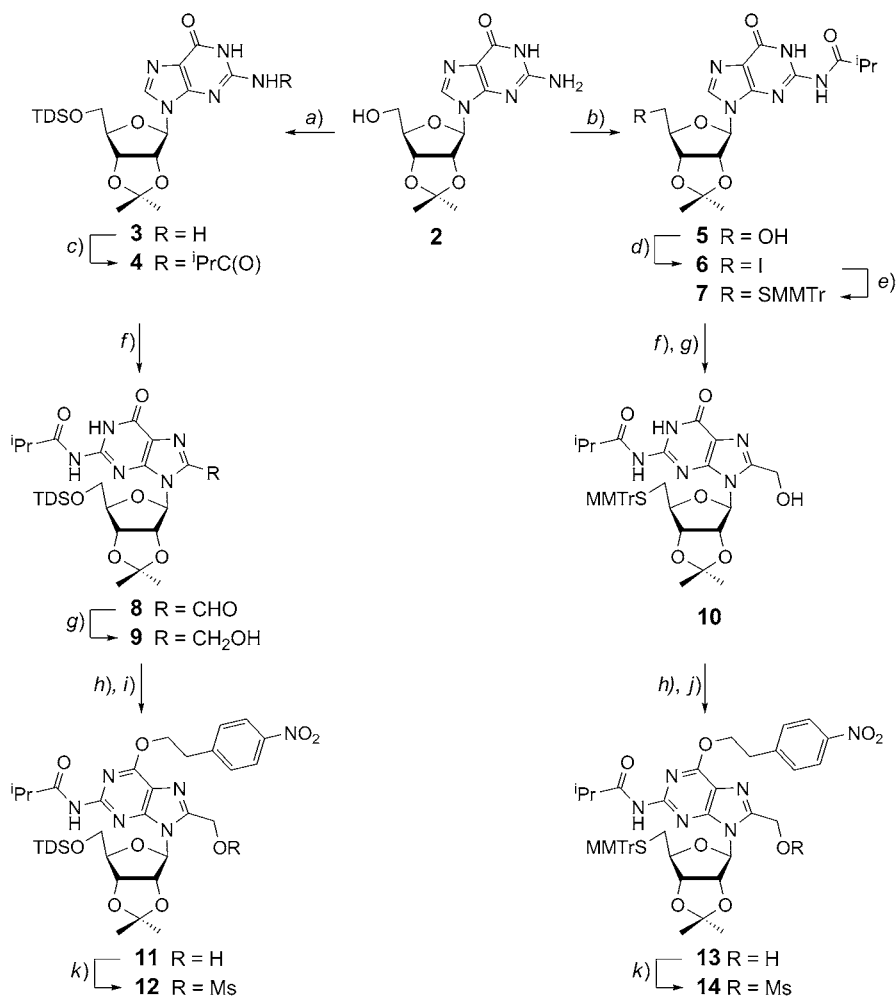
The synthesis started with the preparation of the guanosine-derived methanesulfonate **12** and **14**, corresponding to units VIII and II of the octanucleosides (Scheme 1). For the synthesis of **12**, the known 2',3'-*O*-isopropylidene-guanosine (**2**) [11] was silylated under standard conditions to the hexyl(dimethyl)silyl (= TDS) ether **3**, and then transformed according to [12] to the isobutyramide **4**. For the synthesis of **14**, **2** was directly protected as isobutyramide **5** [13] and further treated with (PhO)₃PMel [14] to provide iodo derivative **6** that was substituted with the sodium salt of monomethoxytritylthiol⁴) to yield 90% of **7**.

The protected nucleosides **4** and **7** differ only by the substituent at C(5'), and were both hydroxymethylated by deprotonation at C(8) with excess LiHMDS, formylation, and reduction. Formyl derivative **8** obtained from **4** was isolated and reduced with NaBH₄ to hydroxymethyl derivative **9**⁵), while the analogous formyl derivative resulting from **7** was reduced *in situ* to hydroxymethyl derivative **10**. The purine moiety of **9** and **10** was further protected by alkylation with 2-(4-nitrophenyl)ethanol under

⁴) Monomethoxytritylthiol was synthesized from the corresponding alcohol according to a procedure developed by *Jahn-Hofmann* and *Engels* [15].

⁵) Compound **9** was synthesized by *Daniel Egli* [16].

Scheme 1



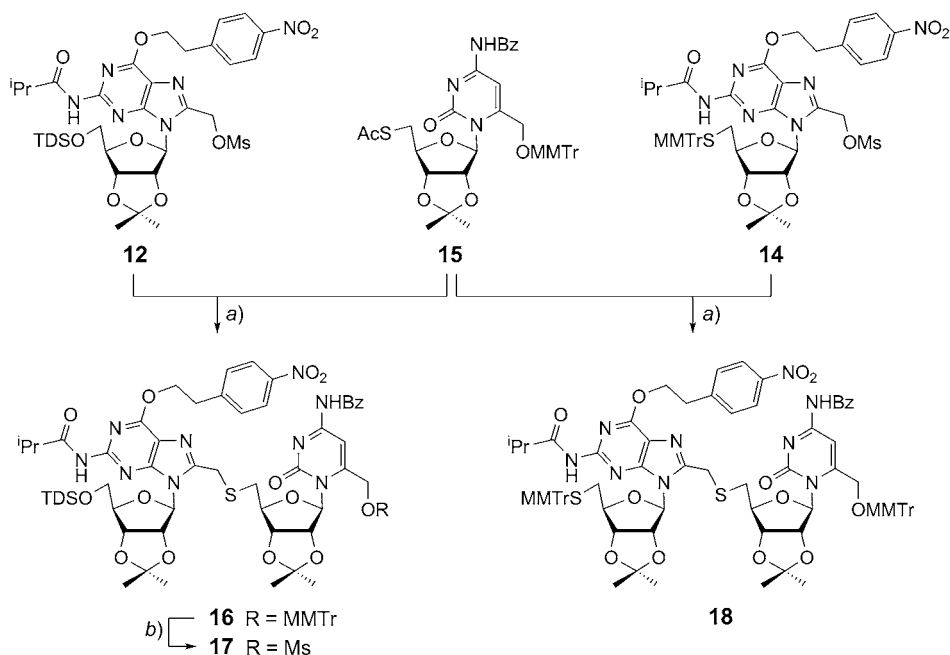
a) TDSiCl, 1*H*-imidazole, DMF; 91%. b) Me_3SiCl , pyridine, CH_2Cl_2 , then iPrCOCl , then MeOH and H_2O ; 84%. c) $(\text{iPrCO})_2\text{O}$, 4-(dimethylamino)pyridine (DMAP), pyridine; 95%. d) $(\text{PhO})_3\text{PMeI}$, MeCN; 93%. e) MMTTrSH, NaH, THF; 90%. f) Lithium bis(trimethylsilyl)amide (LiHMDS), DMF, THF, $-78^\circ \rightarrow \text{r.t.}$; 80% of **8**, 98% of **9**, 74% of **10** from **7**. h) 1-(Trimethylsilyl)-1*H*-imidazole, dioxane, then PPh_3 , diisopropyl azodicarboxylate (DIAD), 2-(4-nitrophenyl)ethanol. i) AcOH/ H_2O 1:1; 88% of **11**. j) NH_4F , MeOH; 78% of **13**. k) MsCl, EtN^iPr_2 , CH_2Cl_2 ; 87% of **12**, 80% of **14**. TDS = *tert*-butyldimethylsilyl (= dimethyl(1,1,2-trimethylpropyl)silyl), MMTTr = (monomethoxy)trityl (= (4-methoxyphenyl)diphenylmethyl).

Mitsunobu conditions [17]. This alkylation required the introduction of a temporary *O*-trimethylsilyl group [18]. It was removed immediately upon alkylation using aqueous AcOH to yield the 2-phenylethyl derivative **11**, while **13** was prepared by desilylation with NH_4F in MeOH to avoid any acid-promoted detritylation. The hydroxymethyl

derivatives **11** and **13** were transformed under standard conditions to the methanesulfonates **12** and **14**, respectively.

For the synthesis of the G*[s]C* dinucleosides, we deacetylated the known cytidine-derived thioacetate **15** [8] to generate the corresponding thiolate (*Scheme 2*). Best results were obtained by treating **15** with MeSNa in MeOH/THF. The crude thiolate was *S*-alkylated with methanesulfonates **12** and **14** to obtain the dinucleosides **16** (71%) and **18** (65%), respectively. Reductive detritylation of **16** with Cl₂CHCOOH in the presence of ⁱPr₃SiH, and reaction of the crude alcohol with MsCl/EtNⁱPr₂ gave methanesulfonate **17**.

Scheme 2



a) 1. MeSNa, THF/MeOH 1:1, -10° ; 2. **12** or **14**, EtNⁱPr₂, DMSO; 71% of **16**, 65% of **18**. b) 1. Cl₂CHCO₂H, ⁱPr₃SiH, CH₂Cl₂; 2. MsCl, EtNⁱPr₂, CH₂Cl₂; 84%. Bz = Benzoyl.

The known U*[s]A* dinucleoside **19** [6] played a key role in the synthesis of the tetranucleosides **21** (→ units V–VIII of **24**) and **23** (→ units I–IV; *Scheme 3*). It was transformed to tetranucleoside **21** (65%) by *S*-deacetylation (best with K₂CO₃ in MeOH/CH₂Cl₂) and treatment of the resulting thiolate with methanesulfonate **17**. For the synthesis of tetranucleoside **23**, dinucleoside **19** was transformed under standard conditions to the methanesulfonate **20**. The thiolate required for the formation of tetranucleoside **23** was generated by reductive *O*- and *S*-detritylation of **18**, followed by deprotonation with *Hünig's* base. Selective *S*-alkylation of the resulting thiolate with **20** provided tetranucleoside **23** (67%).

For the synthesis of octanucleoside **24**, we removed the *O*-MMTr group of **21** similarly as described for **19**, and transformed the resulting alcohol to methanesulfonate **22** (74%). *S*-Deacetylation of **23** was first attempted by treatment with MeSNa in THF/MeOH, but this resulted in low coupling yields, while *S*-deacetylation with NaOH in pyridine/EtOH [19], followed by coupling of the resulting thiolate with **22**, yielded 61% of the desired octanucleoside **24**.

We aimed at a partial deprotection of **24** to obtain the isopropylidened and silylated **25**, as this octanucleoside might well pair in CHCl₃ and conceivably also in aqueous solution. We first attempted at cleaving the 2-(4-nitrophenyl)ethyl groups of **24** with 0.5M DBU (= 1,8-diazabicycloundec-7-ene) in pyridine [18], but the protracted dealkylation was accompanied by the formation of substantial amounts of unidentified side products. However, replacing DBU by 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) [20] led to the desired cleavage of the 2-(4-nitrophenyl)ethyl groups. The resulting crude material was fully debenzoylated by the action of saturated NH₃ in MeOH to yield 50% of **25**. Finally, aqueous HCO₂H led to desilylation and complete deisopropylideneation of **25**, providing octanucleoside **26** (60%) that was purified by filtration through a *C18* functionalized silica column (*Bakerbond C18*). The purity of **26** was determined by HPLC/MS (*Fig. 2*). The high-resolution ESI mass spectrum shows peaks at *m/z* 1151.2740, 1162.2639, and 1085.2514 for $[M + 2 H]^{2+}$, $[M + H + Na]^{2+}$, and the fragment ion $[M - C_5H_9O_4 + 3 H]^{2+}$ resulting from loss of the ribosyl moiety of unit VIII, respectively. These ions show the parent peaks of the isotopomers in the expected ratio (see the *Exper. Part*). Investigations of the duplex formation of **26** were limited to UV and CD spectroscopy due to the small amount (2 mg), and the fact that 1D- and 2D-NMR spectra had to be recorded in H₂O containing 10–20% of an aprotic cosolvent and at 0–5° [21] to detect exchangeable H-atoms.

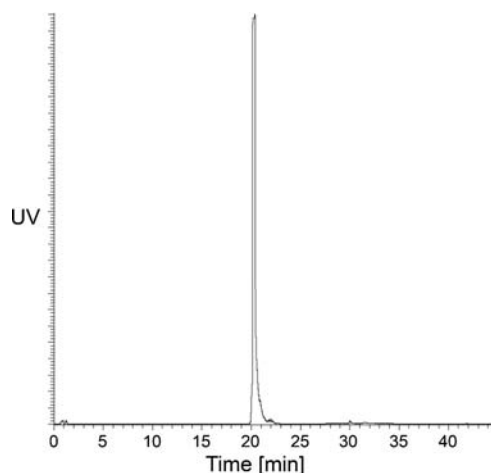
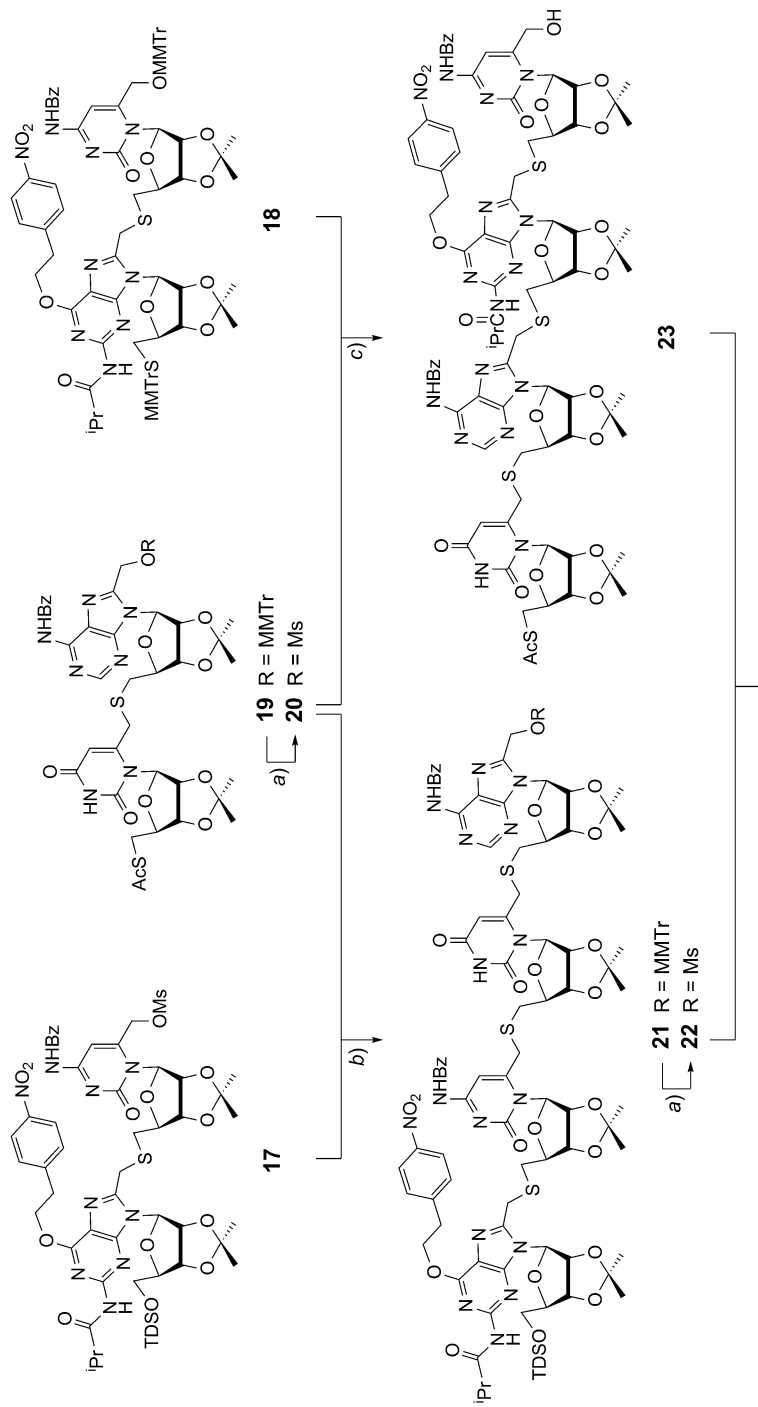
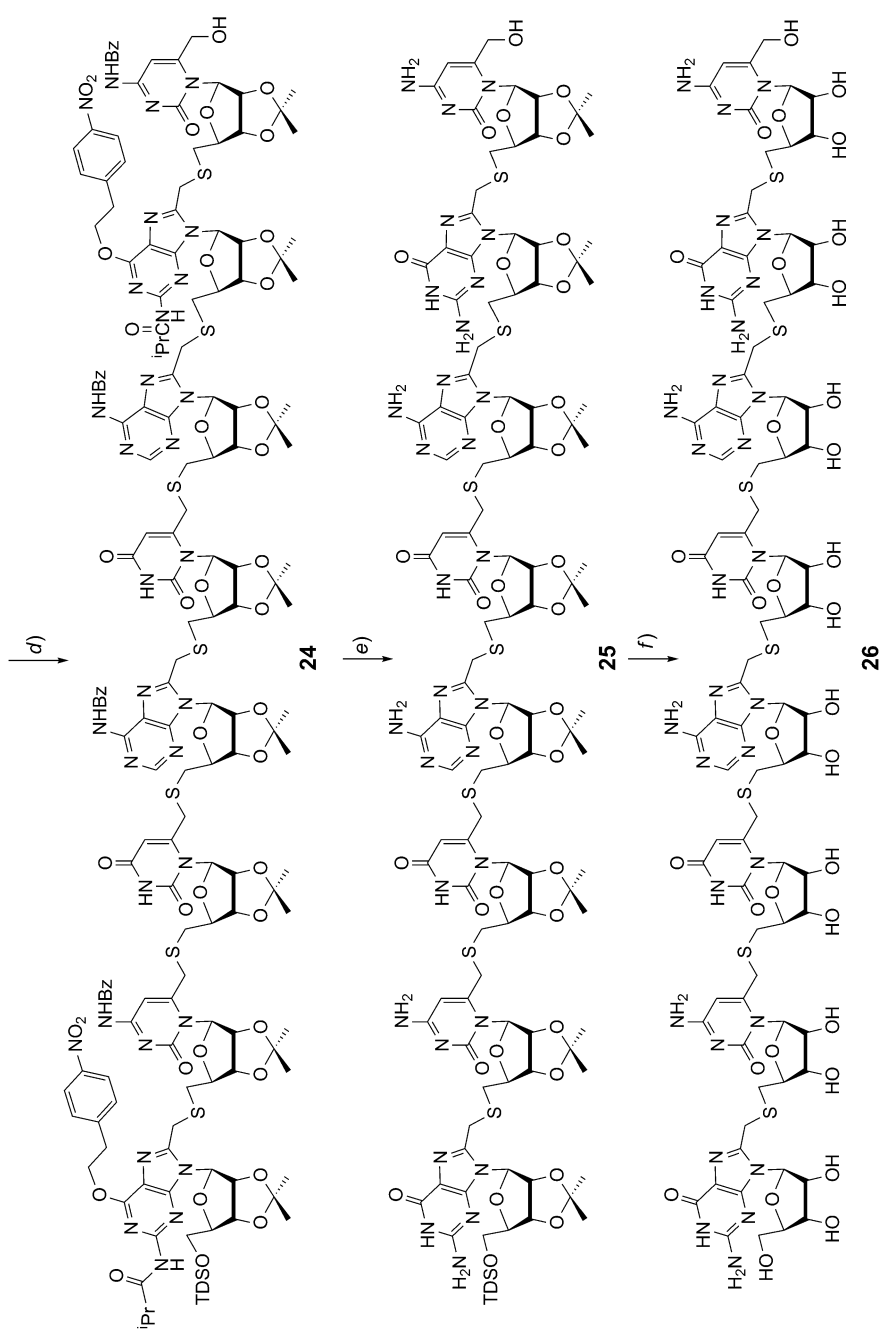


Fig. 2. HPLC Trace of the completely deprotected octamer **26** after purification (UV detection at 260 nm)

3. *Conformation of the 2',3'-O-Isopropylidened Guanosine Mononucleosides 2–14*. The orientation of the guaninyl moiety in 2',3'-*O*-isopropylidene-guanosines should show a similar dependence upon the substituents at C(8) and at C(5') as observed

Scheme 3





a) 1. $\text{Cl}_3\text{CHCO}_2\text{H}$, Pr_3SiH , CH_2Cl_2 ; 2. MsCl , EtNPr_2 , CH_2Cl_2 ; 69% of **20**; 74% of **22**. b) 1. **19**, K_2CO_3 , $\text{MeOH}/\text{CH}_2\text{Cl}_2$ 9:1; 2. **17**, EtNPr_2 , DMSO ; 65%.
 c) 1. **18**, $\text{CF}_3\text{CO}_2\text{H}$, Pr_3SiH , CH_2Cl_2 ; 2. **20**, EtNPr_2 , DMSO ; 67%. d) 1. **23**, Im aq. NaOH , pyridine/ EtOH 2:1; 2. **22**, EtNPr_2 , DMSO ; 61%. e) 1. 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD), pyridine; 2. NH_3/MeOH , CH_2Cl_2 ; 50%. f) $\text{HCO}_2\text{H}/\text{H}_2\text{O}$ 4:1; 60%.

for adenosines (see [4] and refs. cit. therein), and thus lead to a similar influence on the chemical shift of H–C(2'). 8-Unsubstituted and *O*(5')-silylated adenosines ($\delta(\text{H–C}(2')) = 5.20\text{--}5.30$ ppm) in CDCl₃ adopt an *anti* conformation, whereas 8-unsubstituted and *O*(5')-acetylated as well as *C*(5')-sulfanylated derivatives ($\delta(\text{H–C}(2')) \approx 5.50$ ppm) adopt a *syn/anti* equilibrium together with a *gt/tg* orientation of the substituent at C(5') [4]. Both 8-substituted and *O*(5')-unprotected adenosines ($\delta(\text{H–C}(2')) = 5.65\text{--}5.80$ and $5.25\text{--}5.30$ ppm, resp.) adopt a *syn* conformation, the former for steric reasons and the latter as the consequence of a strong intramolecular O(5')–H...N(3) H-bond [4].

In the guanosine series, the chemical shift of H–C(2') is not only influenced by the orientation of the guanosyl moiety, but also by the *N*²- and *O*⁶-protecting groups (see Table 3 in the *Exper. Part*). In CDCl₃, the 5'-*O*-silylated amine **3** and the corresponding isobutyramide **4** adopt an *anti* conformation, as evidenced by the upfield shift for H–C(2') ($\delta(\text{H–C}(2')) = 5.15$ and 4.99 ppm, resp.) and by a prominent population of the *gg* conformation (*gg/gt/tg* 50:25:25 and 60:25:15⁶), resp.). As expected, the trityl thioether **7** adopts a *ca.* 1:1 *gg/gt* equilibrium ($J(4',5'a) = 7.1$, $J(4',5'b) = 6.6$ Hz). The bulky trityl group entails completely an *anti* conformation ($\delta(\text{H–C}(2')) = 5.01$ ppm). The iodo derivative **6** also adopts a *ca.* 1:1 *gg/gt* equilibrium, while the downfield shift for H–C(2') (5.24 ppm) reveals a *syn/anti* equilibrium, similarly to that observed for the corresponding adenosine analogue [14]. The intramolecular O(5')–H...N(3') H-bond of the alcohol **5** [13] in CDCl₃ is evidenced by the *gg* orientation of HO–C(5') ($J(5'a,\text{OH}) = 12.2$ Hz) and by the upfield shift for H–C(2'), resonating at 5.12 ppm, resulting from a combination of the *syn* conformation and a substantial population of the furanose (*S*) conformation, similarly to what we had observed for adenosines [4]. The intramolecular H-bond of **2** in (D₆)DMSO [22] is completely cleaved, as evidenced by $J(5'a,\text{OH}) = J(5'b,\text{OH}) = 5.1$ Hz. The chemical shift $\delta(\text{H–C}(2')) = 5.17$ ppm suggests an exclusive *anti* conformation (as compared with **3** in CDCl₃), assuming the absence of a solvent effect.

The 8-substituted guanosines **8–14** adopt a *syn* conformation, evidenced by the downfield shift for H–C(2'). For *O*⁶-unsubstituted guanosines, $\delta(\text{H–C}(2')) = 5.34$ ppm of **10** may be typical; the weak downfield shift for H–C(2') of **9** (5.42 ppm) is probably due to the change of the solvent from CDCl₃ to (D₆)DMSO, and the weak upfield shift for H–C(2') of **8** (5.18 ppm) to the anisotropy of the CHO group. Protection of *O*⁶ leads to a stronger downfield shift for H–C(2') of **11–14** (5.52–5.69 ppm). Here, $\delta(\text{H–C}(2'))$ is influenced both by the protection of HOCH₂–C(8) (downfield shift for the methanesulfonates) and the substituent at C(5') (downfield shift for MMTr thioethers). The thioethers **10**, **13**, and **14** adopt a *ca.* 1:1 *gt/tg* equilibrium, whereas a significant population of the *gg* conformation is observed for the silyl ethers **8**, **9**, **11**, and **12** (*gg/gt/tg* 10:50:40 for **8**, 15:40:45 for **9**, 41:32:27 for **11**, and 17:45:38 for **12**) hinting at a C(2)–N–H...O(5') H-bond in the *gg* conformer. Force-field calculations (Spartan 08 for Macintosh [23]) confirm the feasibility of such a H-bond.

The furanose ring of **4** and **6–14** prefers a (*N*) conformation as indicated by $J(1',2')/J(3',4') = 0.3\text{--}0.8$, whereas **3** adopts a 1:1 equilibrium between the (*N*) and the (*S*) conformer.

⁶) Calculated from $J(4',5'a)$ and $J(4',5'b)$ with the formulae given in [4].

4. *Conformation of the Di- and Tetranucleosides 16–18 and 20–23.* The expected *syn* conformation of the C(6)- and C(8)-substituted units of the di- and tetranucleosides **16–18** and **20–23** is evidenced by the downfield shift for H–C(2') (uridine units: 5.19–5.24, cytidine units: 5.18–5.30, adenosine units: 5.50–5.74, guanosine units: 5.58–5.89 ppm; see *Table 5* in the *Exper. Part*). The chemical shift $\delta(\text{H–C}(2'))$ of the guanosine units shows a dependence on the substituents at C(5'): 5.87–5.89 ppm for the TDS silyl ethers **16**, **17**, **21**, and **22** (probably due to the intramolecular C(2)–N–H \cdots O(5') H-bond in the *gg* conformer), 5.67 ppm for MMTr thioether **18**, and 5.58 ppm for the internal thioether **23**. The CH₂S linker prefers a *gt/tg ca.* 1:1 conformation, but sometimes also a substantial preference for the *gt* conformation is evidenced (*e.g.*, *gt/tg* 65:35 for U and C of **22**). All furanose rings of **16–18** and **20–22** prefer a (*N*) conformation.

5. *Conformation of the Isopropylidened Octanucleosides 24 and 25.* The isopropylidened octanucleosides **24** and **25** are well soluble in CDCl₃. Whereas the ¹H-NMR signals of **24** with protected C, G, and A nucleobases are well-resolved, those of **25** in CDCl₃, CD₂Cl₂, and (CDCl₂)₂ are broad, preventing the assignment of coupling constants. Neither heating (50° in CDCl₃) nor cooling (–50° in CD₂Cl₂) led to a sharpening of the signals. However, well-resolved NMR signals are observed in (D₆)DMSO where **25** is present as a solvated monoplex, as evidenced by $\delta(\text{H–N}(3/\text{IV,VI})) = 11.4$ and $\delta(\text{H–N}(1/\text{II,VIII})) = 10.8$ ppm (compare with 11.3 and 10.7 ppm, resp., for the corresponding H–N of monouridines and monoguanosines in (D₆)DMSO [6][22]). The octanucleosides **24** in CDCl₃ and **25** in (D₆)DMSO adopt similar conformations (see *Table 7* in the *Exper. Part*) as their di- and tetrameric precursors.

6. *Pairing of the Isopropylidened and Silylated Octanucleoside 25 in Chlorinated Solvents.* The ¹H-NMR spectrum of **25** in (CDCl₂)₂ at room temperature shows broad overlapping signals, and the ¹³C-NMR spectrum exhibits lines for several sets of slowly equilibrating associates⁷⁾. Even the ¹H-NMR signals for the TDS group at *ca.* 0 ppm (SiMe₂) and at 0.7–0.9 ppm (SiCMe₂CHMe₂) evidence a mixture of associates. The

Table 1. NH Signals above 8 ppm of the Silylated and Isopropylidened Octanucleoside **25** in (CDCl₂)₂

δ [ppm] (H equiv.)	Assignment
13.88 (0.4 H)	H–N(1) of G or H–N(3) of U
13.55 (0.4 H)	H–N(1) of G or H–N(3) of U
(0.2 H)	H–N(1) of G or H–N(3) of U
13.35 (0.4 H)	H–N(1) of G or H–N(3) of U
13.32 (0.2 H)	H–N(1) of G or H–N(3) of U
11.72 (0.4 H)	H–N(1) of G
(0.2 H)	H–N(1) of G
13.64, 13.51, 13.42, 13.06 (each < 0.05 H)	H–N(1) of G or H–N(3) of U
9.92 (0.2 H)	H–N(3) of U
8.93, 8.57 (both 0.1 H)	NH of NH ₂
8.34–8.24 (2.4 H)	NH of NH ₂ , H–C(2) of A

7) We investigated the pairing of the easily accessible silyl ether **25**, although the results of parent di- and tetranucleosides [4][6] suggest that the corresponding desilylated alcohol should show a far higher tendency to form cyclic duplexes.

integral for the $\text{SiCMe}_2\text{CHMe}_2$ moiety was used to gauge the H equivalents for the NH signals above 8.0 ppm (Table 1). There are three sets of NH signals with an intensity ratio of 8:4:<1. However, only *ca.* 2.4 of the 4 H-equivalents for H–N(1/II,VIII) of the guanosine and for H–N(3/IV,VI) of the uridine moieties were detected between 9.9 and 13.9 ppm; apparently, 1.6 H equivalents are hidden by coalescence.

Despite the broad ^1H -NMR signals, we could obtain satisfactory sensitivity-enhanced 2D-NMR spectra. The CH assignments are taken from the HSQC spectrum where only cross-peaks for a single associate were observed (Table 9 in the *Exper. Part*). Based on characteristic ^1H and ^{13}C chemical shifts, the HSQC spectrum allowed a rough assignment of the CH (*i.e.*, CH(5) of pyrimidines, and CH(1') to CH(4')) and CH_2 signals ($\text{CH}_2(5')$ and $\text{CH}_2\text{-C}(6/8)$). In the DQF-COSY spectrum, there are cross-peaks between the signals for CH(2') and CH(3') and for CH(4') and $\text{CH}_2(5')$. Additionally, there are cross-peaks between the purine CH(1') and CH(2'), and between the pyrimidine CH(3') and CH(4') signals. This allows to assign sequences CH(1')/CH(2')/CH(3') and CH(4')/CH $_2$ (5') for purine units, and sequences CH(3')–CH $_2$ (5') for pyrimidine units. The NOESY spectrum allows assignment of the CH and CH_2 signals to individual units, especially by cross-peaks between $\text{CH}_2\text{-C}(6/8)$ and $\text{CH}_2(5')$, between H–C(4') and H–C(1'), and between $\text{CH}_2\text{-C}(6)$ and H–C(5) of the pyrimidine units. Thus, the sequence of the nucleobases in **25** is corroborated by the 2D-NMR spectra. The chemical shifts for H–C(2'), resonating at 5.85–6.36 ppm (purine bases) and 4.98–5.41 ppm (pyrimidine bases) evidence a *syn* conformation of all nucleoside units. The signals for H–C(5/IV) (7.32 ppm) and H–C(1'/II) (6.66 ppm) are well separated from other signals, and both integrate only for 0.2 H. This evidences that only 20% of **25** is 'visible' in the 2D-NMR spectra. Therefore, and due to the presence of exchange ROE cross-peaks, we renounced an analysis for the cross-peaks of the NH signals in the ROESY spectrum.

The chemical shifts for H–N(1/II,VIII) and H–N(3/IV,VI) (signals between 9.9 and 13.9 ppm in Table 1) suggest that three duplexes in a ratio of 8:4:<1 are formed with 2–4 units involved in a quite stable base pairing (\rightarrow visible signals) and four units with a loose, rapidly exchanging base pairing (\rightarrow signals hidden by coalescence). According to the chemical shifts⁸⁾, the most abundant duplex ($\delta(\text{HN}) = 13.88, 13.55, 13.35,$ and 11.72 ppm) possesses three stable *Watson–Crick* base pairs and a free guanosine unit⁹⁾, and the least abundant duplex ($\delta(\text{HN}) = 13.64, 13.51, 13.42,$ and 13.06 ppm) even four stable *Watson–Crick* base pairs. Although a cyclic duplex with eight base pairs cannot be excluded, it appears rather improbable. The second abundant duplex visible in the 2D-NMR spectra ($\delta(\text{HN}) = 13.55, 13.32, 11.72,$ and 9.92 ppm) possesses only two stable *Watson–Crick* base pairs, and both a free guanosine and uridine unit. Tentatively, one may postulate that units II and III in all three associates are engaged in *Watson–Crick* base pairing, and that the terminal guanosine unit I of the two major

⁸⁾ H–N(3) of free uridines in CDCl_3 resonates at 10.4–10.8 ppm [5] and of *Watson–Crick* U·A base pairs at 12.5–13.5 ppm [4]. H–N(1) of guanosine in DMSO resonates at 10.6–10.7 ppm [24], of G·G base pairs in CDCl_3 at 12.0–12.1 ppm [25] and in $\text{H}_2\text{O}/\text{D}_2\text{O}$ at 11.0 ppm [24], and of *Watson–Crick* G·C base pairs in H_2O at 12.4–13.7 ppm [26].

⁹⁾ Duplexes possessing both *Watson–Crick*- and *Hoogsteen*-type base pairs cannot be formed due to strongly different radii of the helix [4].

associates is not involved in base pairing, as it is also the case for uridine unit IV of the second associate. This result is consistent with the conclusion from the analysis of the U*[s]A*[s]U*[s]A* tetramer that helical strain prevents the formation of cyclic duplexes of higher analogues with complete base pairing [6]. A more detailed characterization of these three associates requires further analytical and spectroscopic investigations.

Watson–Crick-H-bonded duplexes of U*[s]A* and A*[s]U* dinucleosides show poorly temperature-dependent CD spectra, as due to large distances between the base pairs [4]. Similarly, the cyclic duplexes of C*[s]C* and C*[s]C*[s]C*[s]C* di- and tetranucleosides show no temperature dependence [8]. Hence, a strong CD absorption of **25** is hardly a strong evidence for a cyclic duplex. The CD spectrum of a 0.1 mM solution of **25** in CHCl₃ at 0° shows a negative Cotton effect (negative maximum at 292 and stronger positive maximum at 270 nm; Fig. 3). Upon increasing the temperature to 50°, the negative maximum disappears in favour of a positive shoulder, and the positive maximum at 270 nm is increased. This evidences the disappearance of one stacked associate and a stronger persistence of another one, possibly with stacked hydroxymethylated cytidine units I, similarly as observed in the C*[s]C*[s]C*[s]C* series [8].

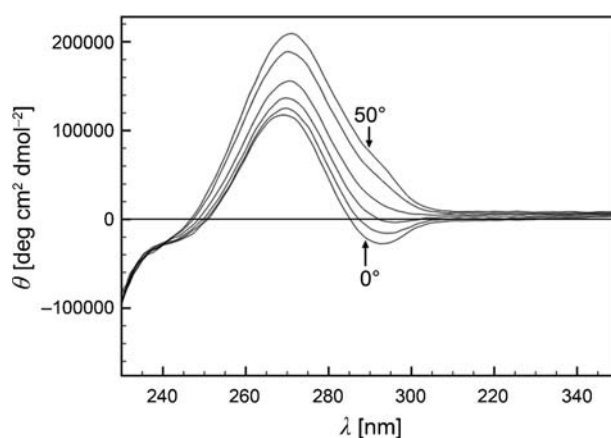


Fig. 3. Temperature-dependent CD spectra in 10° steps from 0 to 50° of the isopropylidened and silylated octanucleoside **25** for a 0.1-mM solution in CHCl₃

7. *Qualitative Pairing Studies of the Fully Deprotected 26 in Aqueous Solutions.* The fully deprotected octanucleoside **26** is sufficiently soluble in H₂O to allow investigating its pairing properties in aqueous solution by CD and UV spectroscopy. The CD absorbance of **26** in an aqueous solution is strong. Hence, a highly diluted solution ($c = 4 \mu\text{M}$, 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0) was analysed, and as a consequence of this high dilution there was disturbing noise (Fig. 4, a). Heating to 90° led to a strong decrease of the negative Cotton effect. This agrees well with the dissociation of the cyclic duplex with increasing temperature.

This dissociation of the cyclic duplex is ascertained by a UV melting curve (Fig. 4, b). The melting temperature and the thermodynamic parameters of the duplex of **26** were calculated according to the *Nearest-Neighbor-Method* and compared to

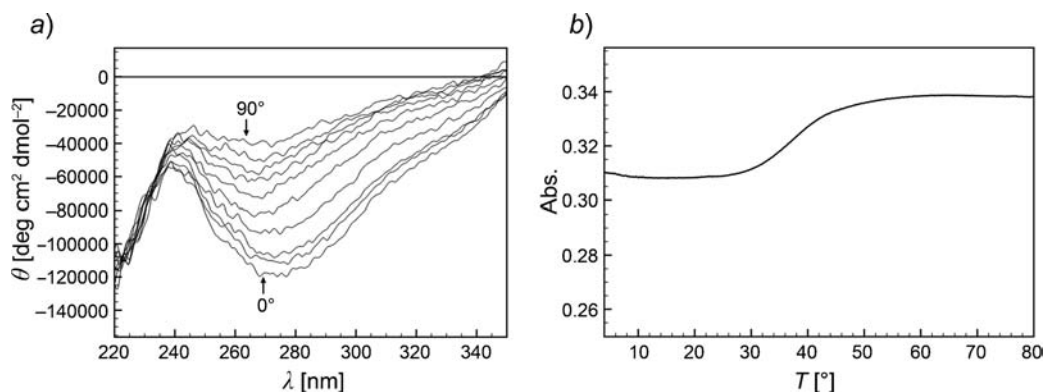


Fig. 4. a) Temperature-dependent CD spectra in 10° steps from 0 to 90° and b) temperature-dependent UV spectrum at 260 nm of the deprotected octanucleoside **26** ($4 \mu\text{M}$ in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0)

those of the parent RNA octanucleotide possessing the same sequence [27]; both duplexes show a similar duplex stability (Table 2). The melting temperature of **26** is lower by 10° than the one of the analogous RNA octanucleotide, and corresponds to a lower $-\Delta H_{298}$ value (60.1 vs. 71.9 kcal/mol), indicating weaker non-bonding interactions, in keeping with a less efficient base stacking of duplexed **26**. The lower $-\Delta H$ correlates with a lower $-\Delta S$ (166.5 vs. 198.6 cal/(mol K)), suggesting a more favourable conformation of the monoplex ('preorganisation'). However, the UV melting was very slowly reversible, showing no hypochromic effect upon cooling from 80 to 4° at a rate of $0.1^\circ/\text{min}$. Only prolonged cooling of the sample (at 4° over 12 h) allowed us to repeat the melting experiment. The reason for the slow re-association remains speculative. It has been reported that nucleoside analogues with non-ionic backbones suffer from low solubility in aqueous media, presumably due to poor solvation, resulting in unspecific self-aggregation [28] as it was observed for peptide-derived analogues (PNA) [29]. For cysteine-derived, peptide-linked ONIBs [1], an ionic linking element was essential for pairing in aqueous solution, favouring the solubility and possibly also an extended conformation [30]. The slow re-association of **26** in aqueous solution may imply that the thermodynamic parameters ΔG , ΔH , and ΔS (calculated from the melting curve) might not reflect the actual stability of the duplex, since the ratio between monoplex and duplex does not express an equilibrium situation.

Table 2. UV Melting Temperature and Thermodynamic Data for the Duplex of **26** and of the Corresponding RNA Analogue rGCUAUAGC

	T_m [$^\circ$]	ΔG_{298} [kcal/mol]	ΔH [kcal/mol]	ΔS [cal/(mol K)]
26	38.5	-10.5	-60.1	-166.5
rGCUAUAGC [27]	48.9	-10.2	-71.9	-198.6

Conclusions. – CD Spectroscopy and a UV melting curve of the duplex of **26** confirm that the structural differentiation of oligonucleotide analogues into a contiguous backbone and attached bases is not required for pairing, and this in spite

of a missing NMR analysis. The release of helical strain upon deisopropylideneation is favorable for the formation of a cyclic duplex with complete base pairing. The association of higher isopropylideneated ONIBs linked by thiomethylene groups in organic solvents is dominated by base pairing, whereas both base pairing and stacking contribute to the stabilisation of the cyclic duplexes of the corresponding deprotected ONIBs in aqueous solution, as suggested by force-field calculations.

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Experimental Part

General. Solvents were distilled, *i.e.*, THF from Na/benzophenone, CH₂Cl₂, MeOH, DMF, pyridine, ³Pr₂NH, and EtN⁺Pr₂ from CaH₂. Reactions were run under Ar or N₂. Qual. TLC: precoated silica-gel plates (*Fluka silica gel 60 F254*). Flash chromatography (FC): silica gel *Merck 60* (0.04–0.063 mm). HPLC-MS (*Waters Atlantis dC18-3*, 100 × 3 mm; MeCN/H₂O/HCO₂H 10:90:0.1–95:5:0.1; flow rate, 0.2 ml/min; *Finnigan LCQ Deca Ion Trap* ESI-MS). Optical rotations: 1-dm cell at 25° and 589 nm. FT-IR Spectra: solid state (ATR). UV Melting-curve experiments: performed by equilibrating a soln. of the oligonucleotide in the given buffer for 12 h at 4°, and heating to 80° at a rate of 0.1°/min; melting curves and thermodynamic parameters were calculated by the ‘Thermal Software Version 02.00’ on a *Cary 100* UV spectrometer. ¹H- and ¹³C-NMR spectra: at 300, 400, 500 or 600 MHz and at 75, 100, 125 or 150 MHz, resp. MS: matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) with 0.05M indol-3-acrylic acid (IAA) in THF, or with 0.05M α -cyano-4-hydroxycinnamic acid (CCA) in MeCN/EtOH/H₂O, and HR-MALDI-MS with 0.05M 2,5-dihydroxybenzoic acid (DHB) in THF.

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-2',3'-O-isopropylidene-2'-deoxy-2'-ribose (3). DMF (50 ml, freshly distilled from CaH₂) was transferred *via* cannula into a 250-ml flat-bottom flask equipped with mechanical stirring and a drying tube (CaCl₂), followed by addition at 26° with **2** [11] (recrystallized from H₂O, finely ground and dried (P₂O₅); 11.91 g, 36.8 mmol), 1*H*-imidazole (7.5 g, 110 mmol), and hexyl(dimethyl)silyl chloride (TDSCl; 7.5 ml, 37.8 mmol, dropwise addn. over 15 min). The mixture was stirred for 4 h, diluted with CH₂Cl₂ (300 ml), and washed with H₂O (100 ml). The aq. layer was extracted with CH₂Cl₂ (2 × 150 ml). The combined org. layers were washed with brine (3 × 150 ml) and evaporated. The residual yellow oil was treated with H₂O (100 ml). The precipitate was filtered off by suction and azeotropically dried with toluene to afford **3** (15.3 g, 91%). White solid. *R*_f (AcOEt/MeOH/H₂O 7:2:1) 0.81. M.p. 254°. FT-IR (ATR): 3313w, 3166w, 2956w, 2867w, 1686m, 1630m, 1595m, 1538m, 1487m, 1373m, 1316w, 1251m, 1211m, 1156m, 1073s, 842s, 778s. ¹H-NMR (300 MHz, CDCl₃): see *Table 3*; additionally, 12.06 (br. s, exchanged with D₂O, H–N(1)); 6.14 (br. s, exchanged with D₂O, H₂N–C(2)); 1.61, 1.39 (2s, Me₂CO₂); 1.59 (*sept.*, *J* ≈ 6.9, Me₂CH); 0.86 (*d*, *J* = 6.8, Me₂CHSi); 0.83 (*s*, Me₂CSi); 0.09, 0.08 (2s, Me₂Si). ¹³C-NMR (75 MHz, CDCl₃): see *Table 4*; additionally, 113.81 (*s*, Me₂CO₂); 34.05 (*d*, Me₂CH); 27.22, 25.51 (2*q*, Me₂CO₂); 25.29 (*s*, Me₂CSi); 18.51 (*q*, Me₂CSi); 20.31 (*q*, Me₂CH); –3.30, –3.43 (2*q*, SiMe₂). HR-ESI-MS: 953.4014 (79, [2*M* + Na]⁺, C₄₂H₇₀N₁₀NaO₁₀Si⁺; calc. 953.4707), 488.2308 (100, [*M* + Na]⁺, C₂₁H₃₅N₅NaO₅Si⁺; calc. 488.2300).

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-2'-deoxy-2'-ribose (4). Iso-butyric anhydride (12 ml, 72.3 mmol) and DMAP (120 mg, 1 mmol) were added at 23° to a stirred soln. of **3** (15.3 g, 32.9 mmol) in pyridine (150 ml) in a 250-ml flat-bottom flask, and the mixture was heated to 80° for 13 h. The pale orange soln. was allowed to reach 23°, diluted with Et₂O (500 ml), and washed with sat. NaHCO₃ soln. (3 × 100 ml). The combined aq. layers were extracted with Et₂O (200 ml). The combined org. layers were washed with 0.001M HCl (5 × 150 ml) and H₂O (2 × 200 ml), dried (MgSO₄), filtered, and evaporated. Remaining traces of pyridine were azeotropically removed with toluene. FC (17.6 g, 150 g of silica; AcOEt/cyclohexane 1:1 → 4:1) of the orange residue afforded **4** (16.8 g, 95%) as a colourless glass, which crystallized from Et₂O/cyclohexane by slow evaporation of Et₂O. White needles. *R*_f (MeOH/CH₂Cl₂ 1:9) 0.89. M.p. 134–136°. [α]_D²⁵ = –100.3 (*c* = 1.0, CHCl₃). UV (CHCl₃): 286 (12630), 264 (14800). FT-IR (ATR): 3512w, 3416w, 3221w, 3016m, 2963m, 2865w, 1697s, 1605s, 1559m, 1471w.

Table 3. Selected $^1\text{H-NMR}$ Chemical Shifts [ppm] and Coupling Constants [Hz] of the Guanosine Mononucleosides **3**, **4**, and **6–14** in CDCl_3

H-Atom	3	4	6	7^{a)}	8^{a)}	9^{b)}	10^{a)}	11^{a)}	12	13^{a)}	14
H–C(8)	7.73	7.90	7.84	7.67	–	–	–	–	–	–	–
CH _a –C(8)	–	–	–	–	9.83 ^{c)}	4.61	4.79	4.93	5.52	4.90	5.49
CH _b –C(8)	–	–	–	–	–	4.56	4.73	4.87	5.50	4.90	5.47
H–C(1')	5.99	5.92	5.99	5.83	6.52	6.27	6.05	6.24	6.15	6.11	6.05
H–C(2')	5.15	4.99	5.24	5.01	5.18	5.42	5.34	5.52	5.69	5.55	5.59
H–C(3')	4.90	4.85	4.93	4.56	5.04	5.25	4.81	5.11	5.15	5.07	5.05
H–C(4')	4.32	4.32	4.31	4.10	4.11	4.05	3.94	4.22	4.26	4.01	3.99
H _a –C(5')	3.81	3.77	3.37	2.48	3.82	3.63	2.52	3.81	3.75	2.48	2.51
H _b –C(5')	3.75	3.72	3.31	2.41	3.77	3.63	2.43	3.75	3.70	2.43	2.46
$J(\text{H}_a, \text{H}_b)$	–	–	–	–	–	14.1	14.0	14.2	13.0	^{d)}	12.9
$J(1', 2')$	2.8	2.7	2.7	2.2	3.1	1.3	2.0	2.9	2.8	1.9	2.1
$J(2', 3')$	6.2	6.0	6.6	6.3	6.9	6.3	6.5	6.7	6.6	6.4	6.5
$J(3', 4')$	2.8	3.4	3.6	3.1	4.7	4.1	4.1	4.0	3.6	3.4	3.3
$J(4', 5'a)$	4.1	3.5	7.2	7.1	5.5	6.0	6.6	4.4	5.3	6.6	6.4
$J(4', 5'b)$	4.3	4.1	5.1	6.6	6.7	6.0	7.9	5.0	6.3	8.0	8.2
$J(5'a, 5'b)$	11.2	11.5	10.6	12.6	10.9	^{d)}	12.6	11.2	11.0	12.8	12.8

^{a)} Assignments based on a HSQC spectrum. ^{b)} In $(\text{D}_6)\text{DMSO}$. ^{c)} CH=O. ^{d)} Not assigned.

Table 4. Selected $^{13}\text{C-NMR}$ Chemical Shifts [ppm] of the Guanosine Mononucleosides **3**, **4**, and **6–14** in CDCl_3

C-Atom	3	4	6	7^{a)}	8^{a)}	9^{b)}
C(2)	153.63	148.11 ^{c)}	148.46 ^{c)}	147.72	149.60	147.65
C(4)	150.85	148.05 ^{c)}	148.26 ^{c)}	147.69	150.34	149.11
C(5)	117.12	121.16	121.82	122.01	122.64	119.03
C(6)	158.73	155.78	156.07	155.55	155.52	155.52
C(8)	135.81	137.25	138.46	137.53	143.47	149.80
CH ₂ –C(8)	–	–	–	–	182.39 ^{d)}	57.68
C(1')	90.20	90.18	90.47	90.18	89.08	89.06
C(2')	84.36	84.85	84.48	84.51	83.94	83.86
C(3')	81.24	80.23	84.09	83.40	81.60	80.25
C(4')	86.92	86.90	86.46	85.44	86.76	86.49
C(5')	63.13	63.24	5.70	34.94	63.34	62.09
C-Atom	10^{a)}	11^{a)}	12	13^{a)}	14	
C(2)	147.46	151.67	152.40	151.37	152.23	
C(4)	148.88	153.77	153.57	153.14	153.25	
C(5)	119.55	114.77	114.61	114.03	117.35	
C(6)	155.63	160.27	161.08	160.11	161.00	
C(8)	150.02	152.56	152.40	152.46	152.23	
CH ₂ –C(8)	57.69	58.38	63.22	57.91	62.77	
C(1')	89.37	89.73	90.31	89.56	90.11	
C(2')	84.06	83.86	83.06	83.91	83.82	
C(3')	83.71	80.45	81.44	83.83	83.62	
C(4')	85.73	86.68	87.55	86.51	86.44	
C(5')	34.71	62.85	62.99	34.87	34.80	

^{a)} Assignments based on a HSQC and a HMBC spectrum. ^{b)} In $(\text{D}_6)\text{DMSO}$. ^{c)} Assignments may be interchanged. ^{d)} CHO–C(8).

1403w, 1377w, 1313w, 1221m, 1135m, 1090m, 968w, 837w. ¹H-NMR (300 MHz, CDCl₃): see Table 3; additionally, 12.23 (br. s, exchanged with D₂O, H–N(1)); 10.07 (br. s, exchanged with D₂O, HN–C(2)); 2.77 (sept., *J* = 6.9, Me₂CHC=O); 1.53 (sept., *J* ≈ 6.9, Me₂CHCMe₂Si); 1.50, 1.28 (2s, Me₂CO₂); 1.21, 1.19 (2d, *J* = 6.6, Me₂CHC=O); 0.80 (d, *J* = 6.9, Me₂CHCMe₂Si); 0.77, 0.76 (2s, Me₂CSi); 0.04, 0.02 (2s, Me₂Si). ¹³C-NMR (75 MHz, CDCl₃): see Table 4; additionally, 179.63 (s, NC=O); 113.92 (s, Me₂CO₂); 35.97 (d, Me₂CHC=O); 33.85 (d, Me₂CHCMe₂Si); 26.99, 25.15 (2q, Me₂CO₂); 25.15 (s, Me₂CSi); 20.17, 20.07 (2q, Me₂CHCMe₂Si); 18.95, 18.89 (2q, Me₂CHC=O); 18.31, 18.27 (2q, Me₂CSi); –3.57, –3.71 (2q, SiMe₂). HR-ESI-MS: 537.2930 (29), 536.2893 (100, *M*⁺, C₂₅H₄₂N₅O₆Si⁺; calc. 536.2899).

N²-Isobutyryl-2',3'-O-isopropylidene-5'-(4-methoxyphenyl)diphenylmethyl-5'-thioguanosine (**5**) [13]. A suspension of **2** (24.6 g, 76.1 mmol) in CH₂Cl₂ (300 ml) and pyridine (75 ml) was cooled to 0°, treated with Me₃SiCl (58.4 ml, 457 mmol), stirred for 6 h at 0°, treated dropwise with isobutyryl chloride (8.8 ml, 83.7 mmol), stirred for 10 min at 0° and for 16 h at r.t., treated with MeOH (100 ml), and stirred for 12 h. After evaporation, a soln. of the residue in CH₂Cl₂ was washed with H₂O (2 ×), dried (MgSO₄), and evaporated. Filtration through a pad of silica gel (CH₂Cl₂/MeOH 95:5) gave **5** (25.2 g, 84%). Colourless solid.

5'-Deoxy-5'-iodo-N²-isobutyryl-2',3'-O-isopropylidene-5'-(4-methoxyphenyl)diphenylmethyl-5'-thioguanosine (**6**). A soln. of **5** (11.8 g, 30 mmol) in MeCN (7 ml) was treated in portions with (PhO)₃PMeI (20.4 g, 45.1 mmol). The mixture was stirred for 60 min, diluted with MeOH (50 ml), stirred for 30 min, and evaporated. A soln. of the residue in AcOEt (200 ml) was washed with 10% Na₂S₂O₃ soln., H₂O, and brine, dried (MgSO₄), and evaporated. FC (CH₂Cl₂/MeOH 99:1 → 39:1 → 9:1) gave **6** (14.0 g, 93%). Pale-yellow foam. *R*_f (CH₂Cl₂/MeOH 19:1) 0.39. [α]_D²⁵ = –24.2 (*c* = 1.0, CHCl₃). UV (CHCl₃): 256 (13940), 284 (10530). IR (ATR): 3143w, 2976w, 2935w, 2874w, 1669s, 1603s, 1556s, 1477m, 1401m, 1374m, 1315w, 1252m, 1208m, 1154s, 1076s, 1019m, 969w, 947w, 872m, 856m, 816m, 782m, 750m, 721m. ¹H-NMR (300 MHz, CDCl₃): see Table 3; additionally, 12.17 (br. s, H–N(1)); 9.31 (br. s, HN–C(2)); 2.71 (sept., *J* = 6.8, Me₂CH); 1.58, 1.36 (2s, Me₂CO₂); 1.27, 1.26 (2d, *J* = 6.8, Me₂CH). ¹³C-NMR (75 MHz, CDCl₃): see Table 4; additionally, 179.91 (s, NHC=O); 114.73 (s, Me₂CO₂); 36.15 (d, Me₂CH); 27.10, 25.28 (2q, Me₂CO₂); 19.10, 19.05 (2q, Me₂CH). HR-MALDI-MS: 504.0744 (100, [*M* + H]⁺, C₁₇H₂₃IN₅O₅⁺; calc. 504.0738). Anal. calc. for C₁₇H₂₂IN₅O₅ (503.30): C 40.57, H 4.41, N 13.91; found: C 40.86, H 4.52, N 13.94.

N²-Isobutyryl-2',3'-O-isopropylidene-5'-S-[(4-methoxyphenyl)diphenylmethyl]-5'-thioguanosine (**7**). A suspension of 60% NaH in mineral oil (96 mg, 2.4 mmol) in THF (4 ml) was cooled to 0°, treated with a soln. of MMTTrSH (735 mg, 2.4 mmol) in THF (4 ml), stirred for 5 min, and treated with **6** (500 mg, 1.0 mmol). The mixture was stirred for 5 min at 0° and for 90 min at r.t., and diluted with sat. NH₄Cl soln. The org. solvents were evaporated, and the aq. phase was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. FC (CH₂Cl₂/MeOH 39:1) gave **7** (613 mg, 90%). Colourless foam. *R*_f (CH₂Cl₂/MeOH 19:1) 0.48. [α]_D²⁵ = +9.9 (*c* = 1.0, CHCl₃). UV (CHCl₃): 254 (18690), 282 (12890). IR (ATR): 3152w, 3058w, 2977w, 2934w, 2835w, 1672s, 1604s, 1557s, 1507m, 1484m, 1467m, 1443m, 1413m, 1402m, 1374m, 1304w, 1250m, 1210m, 1181m, 1155m, 1078s, 1033m, 947w, 909w, 874w, 821w, 783w, 758w, 739w. ¹H-NMR (400 MHz, CDCl₃): see Table 3; additionally, 12.08 (br. s, H–N(1)); 8.84 (br. s, HN–C(2)); 7.36–7.34 (*m*, 4 arom. H); 7.28–7.17 (*m*, 8 arom. H); 6.79–6.75 (*m*, 2 arom. H); 3.77 (*s*, MeO); 2.62 (sept., *J* = 6.8, Me₂CH); 1.50, 1.26 (2s, Me₂CO₂); 1.23, 1.21 (2d, *J* = 6.8, Me₂CH). ¹³C-NMR (100 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 4; additionally, 178.65 (s, NHC=O); 158.39 (*s*); 144.69 (2s); 136.39 (*s*); 130.81, 129.49–126.98 (several *d*); 114.57 (*s*, Me₂CO₂); 113.39 (2d); 66.88 (*s*, Ph₂C); 55.40 (*q*, MeO); 36.60 (*d*, Me₂CH); 27.19, 25.48 (2q, Me₂CO₂); 19.20, 19.07 (2q, Me₂CH). HR-MALDI-MS: 720.2234 (16, [*M* + K]⁺, C₃₇H₃₉KN₅O₆S⁺; calc. 720.2253), 704.2499 (81, [*M* + Na]⁺, C₃₇H₃₉N₅NaO₆S⁺; calc. 704.2513), 273.1274 (100, MMTTr⁺, C₂₀H₁₇O⁺; calc. 273.1274).

5'-O-[(Dimethyl(1,1,2-trimethylpropyl)silyl)-8-formyl-N²-isobutyryl-2',3'-O-isopropylidene-5'-thioguanosine (**8**). A cold (0°) soln. of hexamethyldisilazane (13.4 ml, 63.5 mmol) in THF (150 ml) was treated with a soln. of 1.55M BuLi in hexane (246 ml, 381 mmol) during 30 min, cooled to –78°, and treated dropwise over 1 h with a soln. of **4** (8.5 g, 15.9 mmol) in THF (80 ml). The slightly yellow soln. was treated with DMF (31 ml, 400 mmol), allowed to warm to 24°, and stirred for 2 h. The mixture was diluted with AcOEt (750 ml), extracted with 5% aq. NaH₂PO₄ soln. (2 × 300 ml) and brine (2 × 100 ml), and evaporated. FC (acetone/toluene 1:4) of the residual orange resin afforded **8** (7.15 g, 80%). Colourless glass. *R*_f (AcOEt) 0.78. [α]_D²⁵ = +6.2 (*c* = 1.0, CHCl₃). UV (CHCl₃): 335 (18420), 322 (19300), 290 (6040,

shoulder), 277 (4040, shoulder). FT-IR (CHCl₃): 3411w, 3194w, 2960w, 2868w, 1726m, 1693s, 1604s, 1555s, 1467w, 1429m, 1384w, 1312w, 1091m, 949w, 865w. ¹H-NMR (500 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 3; additionally, 12.29 (br. s, exchanged with D₂O, H–N(1)); 9.02 (br. s, exchanged with D₂O, HN–C(2)); 2.71 (sept., *J* = 6.9, Me₂CHC=O); 1.57 (sept., *J* = 6.8, Me₂CHCMe₂Si); 1.54, 1.32 (2s, Me₂CO₂); 1.28, 1.25 (2d, *J* = 6.9, Me₂CHC=O); 0.85, 0.84 (2d, *J* = 6.8, Me₂CHCMe₂Si); 0.830, 0.825 (2s, Me₂CSi); 0.08, 0.07 (2s, Me₂Si). ¹³C-NMR (125 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 4; additionally, 179.01 (s, HNC=O); 115.05 (s, Me₂CO₂); 36.60 (d, Me₂CHC=O); 34.07 (d, Me₂CHCMe₂Si); 27.23, 25.45 (2q, Me₂CO₂); 25.45 (s, Me₂CSi); 20.33 (q, Me₂CHCMe₂Si); 19.02 (q, Me₂CHC=O); 18.47, 18.45 (2q, Me₂CSi); –3.29, –3.35 (2q, Me₂Si). HR-MALDI-MS (3-HPA): 564.2850 (100, [*M* + H]⁺, C₂₆H₄₂N₅O₇Si⁺; calc. 564.2848). Anal. calc. for C₂₆H₄₁N₅O₇Si (563.72): C 55.40, H 7.33, N 12.42; found: C 55.68, H 7.33, N 12.27.

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-8-(hydroxymethyl)-N²-isobutyryl-2',3'-O-isopropylidene-guanosine (**9**) [16]. A suspension of NaBH₄ (246 mg, 6.5 mmol) in EtOH (7.7 ml) was treated dropwise with a soln. of **8** (3.5 g, 6.2 mmol) in EtOH (40 ml), stirred for 30 min, and treated with sat. aq. NH₄Cl soln. (30 ml) and CH₂Cl₂ (150 ml). The layers were separated, and the aq. layer was extracted with CH₂Cl₂ (50 ml). The combined org. layers were dried (MgSO₄), filtered, evaporated, and dried in high vacuum to afford **9** (3.53 g, quant.), which was used without further purification for the next step. White solid. *R*_f (AcOEt) 0.31. M.p. 180° (dec.). [*α*]_D²⁵ = +15.9 (*c* = 1.0, CHCl₃). UV (CHCl₃): 291 (12150), 262 (14160). FT-IR (CHCl₃): 3550w, 3419w, 3206w, 2975s, 1698s, 1606s, 1561s, 1427m, 1377m, 1257m, 1155m, 1080s, 1046s, 974w, 950w, 876m, 837m. ¹H-NMR (300 MHz, (D₆)DMSO): see Table 3; additionally, 12.09 (br. s, exchanged with D₂O, H–N(1)); 11.37 (br. s, exchanged with D₂O, HN–C(2)); 5.70 (br. s, exchanged with D₂O, OH); 2.78 (sept., *J* = 6.9, Me₂CHC=O); 1.52, 1.33 (2s, Me₂CO₂); 1.47 (sept., *J* = 6.9, Me₂CHCMe₂Si); 1.14 (d, *J* = 6.6, Me₂CHC=O); 0.76, 0.75 (2d, *J* = 6.9, Me₂CHCMe₂Si); 0.71, 0.70 (2s, Me₂CSi); –0.08, –0.10 (2s, Me₂Si). ¹³C-NMR (75 MHz, CDCl₃): see Table 4; additionally, 179.31 (s, NC=O); 114.48 (s, Me₂CO₂); 36.01 (d, Me₂CHC=O); 33.95 (d, Me₂CHCMe₂Si); 27.11, 25.36 (2q, Me₂CO₂); 25.36 (s, Me₂CSi); 20.31, 20.24 (2q, Me₂CHCMe₂Si); 19.14, 18.94 (2q, Me₂CHC=O); 18.42, 18.39 (2q, Me₂CSi); –3.42 (q, Me₂Si). HR-MALDI-MS (3-HPA): 588.2825 (25, [*M* + Na]⁺, C₂₆H₄₃N₅NaO₇Si⁺; calc. 588.2824), 566.3004 (100, [*M* + H]⁺, C₂₆H₄₄N₅O₇Si⁺; calc. 566.3005), 394.2263 (24).

8-(Hydroxymethyl)-N²-isobutyryl-2',3'-O-isopropylidene-5'-S-[(4-methoxyphenyl)diphenylmethyl]-5'-thioguanosine (**10**). A soln. of hexamethyldisilazane (9.04 g, 56 mmol) in THF (100 ml) was cooled to 0°, treated dropwise with 1.6M BuLi in hexane (35 ml, 56 mmol), stirred for 30 min, and cooled to –70°. The cold soln. was transferred via a Teflon canula to a cold (–78°) soln. of **7** (7.64 g, 11.2 mmol) in THF (100 ml), keeping the temp. of the mixture below –68°. The mixture was stirred for 1 h at –78°, treated with DMF (22 ml, 280 mmol), stirred for 10 min, warmed to 0°, stirred for 1 h, and poured into a mixture of 2M HCl (56 ml), ice, and H₂O (100 ml). The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with 0.1M HCl, H₂O (2 ×) and brine, dried (MgSO₄), and evaporated. A soln. of the residue in EtOH (120 ml) was added dropwise to a suspension of NaBH₄ (424 mg, 11.2 mmol) in EtOH (100 ml). The mixture was stirred for 30 min and poured into a mixture of 0.1M HCl and ice. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (2 ×) and brine, dried (MgSO₄), and evaporated. FC (CH₂Cl₂/MeOH 24:1) gave **10** (5.90 g, 74%). Colourless foam. *R*_f (toluene/acetone 1:1) 0.50. [*α*]_D²⁵ = +74.9 (*c* = 1.0, CHCl₃). IR (ATR): 3550w, 3160w, 2970w, 2931w, 1736m, 1710m, 1676s, 1603s, 1556s, 1507m, 1489m, 1425m, 1373s, 1301w, 1248s, 1216m, 1181m, 1155m, 1075s, 1032s, 949w, 907w, 862m, 820w, 790m, 758w, 725s, 699s. ¹H-NMR (400 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 3; additionally, 12.09 (br. s, H–N(1)); 9.44 (br. s, HN–C(2)); 7.33–7.15 (m, 12 arom. H); 6.78–6.74 (m, 2 arom. H); 4.14 (br. s, OH); 3.77 (s, MeO); 2.65 (sept., *J* = 6.9, Me₂CH); 1.48, 1.25 (2s, Me₂CO₂); 1.23, 1.21 (2d, *J* = 6.9, Me₂CH). ¹³C-NMR (100 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 4; additionally, 179.02 (s, NHC=O); 158.27 (s); 144.81, 144.74, 136.55 (3s); 130.73–126.81 (several d); 114.54 (s, Me₂CO₂); 113.39 (2d); 66.56 (s, Ph₂C); 55.34 (q, MeO); 36.32 (d, Me₂CH); 27.20, 25.55 (2q, Me₂CO₂); 19.11, 19.05 (2q, Me₂CH). HR-MALDI-MS: 750.2374 (24, [*M* + K]⁺, C₃₈H₄₁KN₅O₇S⁺; calc. 750.2358), 734.2606 (52, [*M* + Na]⁺, C₃₈H₄₁N₅NaO₇S⁺; calc. 734.2619), 712.2815 (32, [*M* + H]⁺, C₃₈H₄₂N₅O₇S⁺; calc. 712.2799), 273.1270 (100, MMT⁺, C₂₀H₁₇O⁺; calc. 273.1274).

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-8-(hydroxymethyl)-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**11**). A soln. of **9** (3.97 g, 7.01 mmol) in dioxane (40 ml) was treated with 1-(trimethylsilyl)-1H-imidazole (1.35 ml, 9.11 mmol) and stirred for 90 min. The mixture was treated with PPh₃ (3.67 g, 14 mmol), 2-(4-nitrophenyl)ethanol (2.34 g, 14 mmol), and dropwise with diisopropyl azodicarboxylate (DIAD, 2.8 ml, 14 mmol), stirred for 2 h, diluted with brine/H₂O 1:1, and extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue in AcOH/H₂O 1:1 (160 ml) was stirred for 1 h and neutralized carefully with 5M NaOH. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with sat. NaHCO₃ soln., H₂O, and brine, dried (MgSO₄), and evaporated. FC (pentane/AcOEt 2:3 → 1:2) gave **11** (4.42 g, 88%). Yellow foam. *R*_f (pentane/AcOEt 2:3) 0.30. $[\alpha]_D^{25} = +23.4$ (*c* = 1.0, CHCl₃). UV (CHCl₃): 274 (27890). IR (ATR): 3296w, 2959w, 2871w, 1721w, 1610m, 1591m, 1517s, 1457m, 1423m, 1381m, 1342s, 1248m, 1216m, 1183m, 1155m, 1071s, 974w, 906w, 828s, 794m, 777m, 745m. ¹H-NMR (400 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 3; additionally, 8.18–8.14 (*m*, 2 arom. H); 7.80 (*br. s*, HN–C(2)); 7.51–7.48 (*m*, 2 arom. H); 4.79 (*t*, *J* = 6.9, CH₂CH₂O); 3.59 (*br. t*, *J* = 5.9, OH); 3.31 (*t*, *J* = 6.9, CH₂CH₂O); 3.15–3.01 (*br. s*, Me₂CHC=O); 1.61, 1.39 (2s, Me₂CO₂); 1.58 (*sept.*, *J* = 6.9, Me₂CH); 1.28, 1.27 (2*d*, *J* = 6.9, Me₂CHC=O); 0.83 (*d*, *J* = 6.9, Me₂CH); 0.81, 0.80 (2s, Me₂CSi); 0.05, 0.04 (2s, Me₂Si). ¹³C-NMR (100 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 4; additionally, 175.56 (*s*, NHC=O); 146.92, 145.58 (2*s*); 129.96 (2*d*); 123.77 (2*d*); 116.50 (*s*, Me₂CO₂); 66.83 (*t*, CH₂CH₂O); 35.73 (*d*, Me₂CHC=O); 35.09 (*t*, CH₂CH₂O); 34.03 (*d*, Me₂CH); 27.20, 25.41 (2*q*, Me₂CO₂); 25.46 (*s*, Me₂CSi); 20.33, 20.28 (2*q*, Me₂CSi); 19.39, 19.27 (2*q*, Me₂CHC=O); 18.41, 18.39 (2*q*, Me₂CH); –3.47, –3.49 (2*q*, Me₂Si). HR-MALDI-MS: 737.3289 (59, [M + Na]⁺, C₃₄H₅₀N₆NaO₉Si⁺; calc. 737.3301), 715.3482 (100, [M + H]⁺, C₃₄H₅₁N₆O₉Si⁺; calc. 715.3481). Anal. calc. for C₃₄H₅₀N₆O₉Si (714.89): C 57.12, H 7.05, N 11.76; found: C 56.85, H 7.19, N 11.48.

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-8-[[methylsulfonyl]oxy]methyl]-O⁶-[2-(4-nitrophenyl)ethyl]guanosine (**12**). A soln. of **11** (6.31 g, 8.83 mmol) in CH₂Cl₂ (150 ml) was cooled to 0°, treated with EtN³Pr₂ (1.70 ml, 9.7 mmol) and dropwise with MsCl (0.75 ml, 9.7 mmol), stirred for 30 min, and poured into H₂O. The phases were separated, and the aq. phase was extracted with CH₂Cl₂ (2 ×). The combined org. phases were washed with H₂O (2 ×) and brine, dried (MgSO₄), and evaporated. FC (pentane/AcOEt 3:1 → 1:1) gave **12** (6.08 g, 87%). Yellow foam. *R*_f (pentane/AcOEt 1:2) 0.66. $[\alpha]_D^{25} = +12.6$ (*c* = 1.0, CHCl₃). UV (CHCl₃): 275 (27360). IR (ATR): 3300w (*br.*), 2960w, 2871w, 1721w, 1610m, 1590m, 1518m, 1459m, 1427m, 1342s, 1246m, 1214m, 1174s, 1155m, 1074s, 1040m, 1000w, 946m, 828w, 796m, 776m, 737m. ¹H-NMR (300 MHz, CDCl₃): see Table 3; additionally, 8.20–8.15 (*m*, 2 arom. H); 7.73 (*br. s*, HN–C(2)); 7.52–7.47 (*m*, 2 arom. H); 4.80 (*t*, *J* = 6.9, CH₂CH₂O); 3.32 (*t*, *J* = 6.9, CH₂CH₂O); 3.08 (*s*, MsO); 2.99–2.88 (*br. s*, Me₂CHC=O); 1.55, 1.41 (2s, Me₂CO₂); 1.53 (*sept.*, *J* = 6.8, Me₂CH); 1.29, 1.27 (2*d*, *J* = 6.9, Me₂CHC=O); 0.81 (*d*, *J* = 6.8, Me₂CH); 0.77, 0.76 (2s, Me₂CSi); 0.03 (*s*, Me₂Si). ¹³C-NMR (75 MHz, CDCl₃): see Table 4; additionally, 175.04 (*s*, NHC=O); 145.71, 147.50 (2*s*); 130.06 (2*d*); 123.90 (2*d*); 117.41 (*s*, Me₂CO₂); 67.14 (*t*, CH₂CH₂O); 38.54 (*q*, MsO); 36.19 (*d*, Me₂CHC=O); 35.15 (*t*, CH₂CH₂O); 34.18 (*d*, Me₂CH); 27.33, 25.62 (2*q*, Me₂CO₂); 25.37 (*s*, Me₂CSi); 20.39, 20.37 (2*q*, Me₂CSi); 19.51, 19.37 (2*q*, Me₂CHC=O); 18.54, 18.51 (2*q*, Me₂CH); –3.38, –3.41 (2*q*, Me₂Si). HR-MALDIMS: 793.3271 (100, [M + H]⁺, C₃₅H₅₃N₆O₁₁SSi⁺; calc. 793.3257). Anal. calc. for C₃₅H₅₂N₆O₁₁SSi (792.98): C 53.01, H 6.61, N 10.60; found: C 53.28, H 6.58, N 10.55.

8-(Hydroxymethyl)-N²-isobutyryl-2',3'-O-isopropylidene-5'-S-[(4-methoxyphenyl)diphenylmethyl]-O⁶-[2-(4-nitrophenyl)ethyl]-5'-thioguanosine (**13**). A soln. of **10** (6.18 g, 8.68 mmol) in dioxane (50 ml) was treated with 1-(trimethylsilyl)-1H-imidazole (1.34 g, 9.55 mmol) and stirred for 2 h. The mixture was treated with PPh₃ (3.42 g, 13 mmol), 2-(4-nitrophenyl)ethanol (2.18 g, 13 mmol), and dropwise with DIAD (2.63 ml, 13 mmol), stirred for 4 h, diluted with H₂O (100 ml), and extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue in MeOH (50 ml) was treated with NH₄F (1.60 g, 43.4 mmol) and stirred for 2 h. The mixture was poured into H₂O and extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. FC (pentane/AcOEt 2:3) gave **13** (5.82 g, 78%). Yellow foam. *R*_f (pentane/AcOEt 2:3) 0.22. $[\alpha]_D^{25} = +71.6$ (*c* = 1.0, CHCl₃). UV (CHCl₃): 274 (29340). IR (ATR): 3300w (*br.*), 3054w, 2975w, 2931w, 1719m, 1607m, 1592m, 1515s, 1508s, 1458m, 1425m, 1381m,

1343s, 1299w, 1246s, 1217s, 1180s, 1155m, 1071s, 1033s, 1000m, 906w, 856m, 822w, 793m, 741m, 724m. ¹H-NMR (300 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 3; additionally, 8.19–8.15 (*m*, 2 arom. H); 7.68 (br. *s*, HN–C(2)); 7.52–7.49 (*m*, 2 arom. H); 7.30–7.11 (*m*, 12 arom. H); 6.73–6.68 (*m*, 2 arom. H); 4.86–4.74 (*m*, CH₂CH₂O); 3.76 (*s*, MeO); 3.33 (*t*, *J* = 6.9, CH₂CH₂O); 2.92 (*t*, *J* = 6.2, OH); 2.86 (br. *sept.*, *J* = 6.5, Me₂CH); 1.53, 1.34 (2*s*, Me₂CO₂); 1.23 (*d*, *J* = 6.9, Me₂CH). ¹³C-NMR (75 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 4; additionally, 174.66 (*s*, NHC=O); 157.91 (*s*); 146.78, 145.57, 144.70, 144.68, 136.44 (5*s*); 130.60–123.73 (several *d*); 116.60 (*s*, Me₂CO₂); 113.06 (2*d*); 66.98 (*t*, CH₂CH₂O); 66.32 (*s*, Ph₂C); 55.30 (*q*, MeO); 36.18 (*d*, Me₂CH); 35.21 (*t*, CH₂CH₂O); 27.22, 25.58 (2*q*, Me₂CO₂); 19.56, 19.50 (2*q*, Me₂CH). HR-MALDI-MS: 883.3100 (100, [M + Na]⁺, C₄₆H₄₈N₆NaO₉S⁺; calc. 883.3096). Anal. calc. for C₄₆H₄₈N₆O₉S (860.99): C 64.17, H 5.62, N 9.76; found: C 63.93, H 5.72, N 9.56.

N²-Isobutyryl-2',3'-O-isopropylidene-8-[[methylsulfonyl]oxy]methyl]-5'-S-[4-methoxyphenyl]diphenylmethyl]-O⁶-[2-(4-nitrophenyl)ethyl]-5'-thioguanosine (**14**). A soln. of **13** (5.25 g, 6.1 mmol) in CH₂Cl₂ (40 ml) was cooled to 0°, treated with EtⁿPr₂ (1.17 ml, 6.7 mmol) and dropwise with MsCl (0.52 ml, 6.7 mmol), stirred for 45 min, and poured on ice. The phases were separated, and the aq. phase was extracted with CH₂Cl₂ (2 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. FC (pentane/AcOEt 1:1 → 2:3) gave **14** (4.58 g, 80%). Yellow foam. R_f (pentane/AcOEt 2:3) 0.35. [α]_D²⁵ = +35.1 (*c* = 1.0, CHCl₃). UV (CHCl₃): 276 (27030). IR (ATR): 3400–3200w (br.), 2974w, 2934w, 1720w, 1606m, 1594m, 1508s, 1458m, 1427m, 1342s, 1300w, 1247m, 1213s, 1174s, 1154s, 1088m, 1073m, 1033m, 1000m, 969m, 947m, 856m, 822m, 793m, 759w, 742m, 699s. ¹H-NMR (300 MHz, CDCl₃): see Table 3; additionally, 8.21–8.16 (*m*, 2 arom. H); 7.68 (br. *s*, HN–C(2)); 7.53–7.49 (*m*, 2 arom. H); 7.29–7.13 (*m*, 12 arom. H); 6.74–6.69 (*m*, 2 arom. H); 4.85–4.77 (*m*, CH₂CH₂O); 3.76 (*s*, MeO); 3.34 (*t*, *J* = 6.9, CH₂CH₂O); 3.04 (*s*, MsO); 2.85 (br. *sept.*, *J* = 6.7, Me₂CH); 1.55, 1.35 (2*s*, Me₂CO₂); 1.24 (*d*, *J* = 6.9, Me₂CH). ¹³C-NMR (75 MHz, CDCl₃): see Table 4; additionally, 174.75 (*s*, NHC=O); 158.12 (*s*); 147.03, 145.52, 145.49, 144.85, 136.56 (5*s*); 130.71–126.60 (several *d*); 123.87 (2*d*); 114.39 (*s*, Me₂CO₂); 113.17 (2*d*); 67.15 (*t*, CH₂CH₂O); 66.31 (*s*, Ph₂C); 55.28 (*q*, MeO); 38.40 (*q*, MsO); 36.16 (*d*, Me₂CH); 35.11 (*t*, CH₂CH₂O); 27.10, 25.50 (2*q*, Me₂CO₂); 19.43, 19.36 (2*q*, Me₂CH). HR-MALDI-MS: 961.2873 (100, [M + Na]⁺, C₄₇H₅₀N₆NaO₁₁S₂; calc. 961.2871). Anal. calc. for C₄₇H₅₀N₆O₁₁S₂ (939.06): C 60.11, H 5.37, N 8.95; found: C 59.84, H 5.61, N 9.04.

5'-O-[[Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-2',3'-O-isopropylidene-6-[[4-methoxyphenyl]diphenylmethoxy]methyl]-5'-thiocytidine (**16**). A soln. of **15** [8] (3.78 g, 5.05 mmol) in THF/MeOH 1:1 (60 ml) was cooled to –10°, treated dropwise with a 1M MeSNa soln. in MeOH (10.1 ml), stirred for 2 h at –10°, and poured into 0.1M HCl (50 ml). After adding brine, the aq. phase was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue and **12** (4.00 g, 5.05 mmol) in DMSO (15 ml) was degassed, treated with EtⁿPr₂ (2.64 ml, 15.2 mmol), and stirred for 6 h. Sat. NH₄Cl soln. was added, and the aq. phase was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (3 ×) and brine, dried (MgSO₄), and evaporated. FC (toluene/acetone 5:1 → 4:1) gave **16** (5.01 g, 71%). Yellow foam. R_f (toluene/acetone 4:1) 0.14. [α]_D²⁵ = –46.7 (*c* = 1.0, CHCl₃). UV (CHCl₃): 267 (47610). IR (ATR): 3500–3200w (br.), 2959w, 2936w, 2870w, 1679m, 1610s, 1587m, 1570m, 1517m, 1511m, 1457m, 1422m, 1372m, 1344s, 1300m, 1249s, 1212s, 1181m, 1154m, 1063s, 1033m, 1000m, 900w, 871w, 829s, 794m, 777m, 745m, 726w. ¹H-NMR (500 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 5; additionally, 9.00 (br. *s*, BzNH); 8.14–8.11 (*m*, 2 arom. H); 7.93 (br. *d*, *J* = 7.2, 2 arom. H); 7.71 (br. *s*, NHC=O/II); 7.62–7.23 (*m*, 17 arom. H, H–C(5/I)); 6.87–6.84 (*m*, 2 arom. H); 4.74 (*t*, *J* = 7.0, CH₂CH₂O); 3.77 (*s*, MeO); 3.25 (*t*, *J* = 7.0, CH₂CH₂O); 3.01–2.89 (br. *s*, Me₂CHC=O); 1.60, 1.42 (6 H), 1.27 (3*s*, 2 Me₂CO₂); 1.51 (*sept.*, *J* = 6.9, Me₂CHCMe₂); 1.26 (*d*, *J* = 6.9, Me₂CHC=O); 0.79, 0.78 (2*d*, *J* = 6.9, Me₂CHCMe₂); 0.74, 0.73 (2*s*, Me₂CSi); –0.08, –0.09 (2*s*, Me₂Si). ¹³C-NMR (125 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 174.96 (*s*, NHC=O/II); 166.15 (*s*, NHC=O/I); 159.05 (*s*); 146.89, 145.55, 143.41, 143.31, 134.34 (5*s*); 133.11 (*d* and *s*); 130.56–127.36 (several *d*); 123.74 (2*d*); 113.83, 113.46 (2*s*, 2 Me₂CO₂); 113.46 (2*d*); 88.52 (*s*, Ph₂C); 66.83 (*t*, CH₂CH₂O); 55.23 (*q*, MeO); 35.91 (*d*, Me₂CHC=O); 35.10 (*t*, CH₂CH₂O); 34.07 (*d*, Me₂CHCMe₂); 27.23, 27.11, 25.53, 25.34 (4*q*, 2 Me₂CO₂); 25.22 (*s*, Me₂CSi); 20.29, 20.26 (2*q*, Me₂CSi); 19.42, 19.29 (2*q*, Me₂CHC=O); 18.44, 18.39 (2*q*,

Table 5. Selected ¹H-NMR Chemical Shifts [ppm] and Coupling Constants [Hz] of the Di- and Tetranucleosides **16–18** and **20–23** in CDCl₃^a)

H-Atom	16	17	18	20	21	22	23
<i>Adenosine unit</i>							
H–C(2)				8.78	8.79	8.81	8.67
CH _a –C(8)				5.67	4.60	5.60	4.14
CH _b –C(8)				5.64	4.55	5.60	4.05
H–C(1')				6.28	6.27	6.28	6.22
H–C(2')				5.50	5.55	5.66	5.74
H–C(3')				5.18	5.12	5.12	5.17
H–C(4')				4.48	4.34–4.30	4.42	4.40–4.34
H _a –C(5')				3.00	2.97–2.89	2.914	2.85–2.81
H _b –C(5')				2.86	2.97–2.89	2.87	2.85–2.81
<i>J</i> (H _a ,H _b)				13.1	12.1	^b)	15.3
<i>J</i> (1',2')				1.8	2.0	2.3	1.6
<i>J</i> (2',3')				6.4	6.2	6.4	6.3
<i>J</i> (3',4')				4.3	4.7	4.0	3.7
<i>J</i> (4',5'a)				8.3	^b)	5.8	^b)
<i>J</i> (4',5'b)				4.3	^b)	7.4	^b)
<i>J</i> (5'a,5'b)				14.8	^b)	14.3	^b)
<i>Uridine unit</i>							
H–C(5)				5.03	5.26 ^c)	5.38 ^c)	5.35
CH _a –C(6)				3.61	3.58	3.56 ^c)	3.53 ^c)
CH _b –C(6)				3.45	3.47	3.56 ^c)	3.53 ^c)
H–C(1')				5.74	5.77	5.79	5.78
H–C(2')				5.24	5.21	5.20	5.19
H–C(3')				4.78	4.93	4.92	4.82
H–C(4')				4.05	4.20	4.26	4.11
H _a –C(5')				3.13	2.97–2.89	2.97	3.22–3.15
H _b –C(5')				3.13	2.97–2.89	2.86	3.22–3.15
<i>J</i> (H _a ,H _b)				14.9	14.6	^b)	^b)
<i>J</i> (1',2')				< 1.0	< 1.0	0.9	< 1.0
<i>J</i> (2',3')				6.4	6.4	6.4	6.4
<i>J</i> (3',4')				3.9	4.2	4.3	4.0
<i>J</i> (4',5'a)				7.2	6.5	8.0	^b)
<i>J</i> (4',5'b)				7.2	6.5	5.8	^b)
<i>J</i> (5'a,5'b)				^b)	^b)	14.4	^b)
<i>Cytidine unit</i>							
H–C(5)	^d)	7.65 ^c)	^d)		^d)	7.42 ^c)	^d)
CH _a –C(6)	4.19 ^c)	5.26	4.19 ^c)		3.72	3.75	4.75
CH _b –C(6)	4.19 ^c)	5.19	4.19 ^c)		3.65	3.69	4.63
H–C(1')	5.88	5.71	5.89		5.90	5.92	5.68
H–C(2')	5.24	5.28	5.27		5.29	5.30	5.18
H–C(3')	4.82	5.01	4.98		5.04	5.06–5.03	4.94
H–C(4')	4.19	4.31	4.23		4.29	4.30	4.40–4.34
H _a –C(5')	2.93	2.96	2.95		2.97–2.89	2.96	3.17
H _b –C(5')	2.89	2.94	2.92		2.97–2.89	2.918	2.85–2.81
<i>J</i> (H _a ,H _b)	^b)	13.4	^b)		14.6	14.5	14.5
<i>J</i> (1',2')	0.7	1.0	< 1.0		< 1.0	< 1.0	< 1.0
<i>J</i> (2',3')	6.4	6.5	6.1		6.3	6.3	6.0
<i>J</i> (3',4')	4.0	3.7	3.9		3.7	3.7	4.2

Table 5 (cont.)

H-Atom	16	17	18	20	21	22	23
$J(4',5'a)$	6.9	7.1	6.9		6.3	8.4	9.7
$J(4',5'b)$	7.4	7.6	7.6		6.3	6.5	^{b)}
$J(5'a,5'b)$	13.3	13.5	13.4		^{b)}	13.4	13.9
<i>Guanosine unit</i>							
CH _a -C(8)	4.03	4.08	4.03		4.08	4.08	4.07 ^{c)}
CH _b -C(8)	3.97	3.99	3.95		3.97	3.98	4.07 ^{c)}
H-C(1')	6.27	6.30	6.20		6.28	6.24	6.43
H-C(2')	5.87	5.89	5.67		5.87	5.87	5.58
H-C(3')	5.20	5.20	5.11		5.18	5.18	5.36–5.34
H-C(4')	4.22	4.23	4.00		4.23–4.21	4.22	4.40–4.34
H _a -C(5')	3.61	3.63	2.42		3.63–3.61	3.62	2.88
H _b -C(5')	3.59	3.60	2.39		3.60–3.57	3.60	2.74
$J(H_a, H_b)$	14.3	14.3	14.3		14.3	14.4	^{b)}
$J(1',2')$	2.0	2.1	1.6		2.0	2.0	< 1
$J(2',3')$	6.2	6.3	6.3		6.3	6.3	5.9
$J(3',4')$	3.4	3.5	3.1		3.4	3.6	^{b)}
$J(4',5'a)$	5.9	5.8	6.9		^{b)}	5.9	6.3
$J(4',5'b)$	6.4	6.4	8.0		^{b)}	6.4	6.3
$J(5'a,5'b)$	10.9	10.9	12.8		^{b)}	10.9	13.9

^{a)} Assignments based on a HSQC and a HMBC spectrum. ^{b)} Not assigned. ^{c)} Broad signals. ^{d)} Hidden by aromatic signals.

Me_2CHCMe_2); -3.53 (*q*, Me_2Si). HR-MALDI-MS: 1427.5812 (14), 1426.5776 (47), 1425.5756 (92), 1424.5731 (100, $[M + Na]^+$, $C_{74}H_{87}N_9NaO_{15}SSi^+$; calc. 1424.5704), 1404.6024 (10), 1403.5985 (27), 1402.5953 (31, $[M + H]^+$, $C_{74}H_{88}N_9O_{15}SSi^+$; calc. 1402.5884), 886.3877 (27), 885.3844 (55, $[M - C_{32}H_{26}N_3O_4]^+$, $C_{42}H_{61}N_6O_{11}SSi^+$; calc. 885.3883).

5'-O-[(Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-2',3'-O-isopropylidene-6-(methanesulfonyloxymethyl)-5'-thiocytidine (**17**). A soln. of **16** (625 mg, 446 μ mol) in CH_2Cl_2 (3 ml) was treated with Cl_2CHCO_2H (300 μ l) and iPr_3SiH (274 μ l, 1.34 mmol), stirred for 1 h, and poured into sat. $NaHCO_3$ soln. The phases were separated, and the aq. phase was extracted with CH_2Cl_2 (2 \times). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue in CH_2Cl_2 (5 ml) was cooled to 0°, treated with EtN^iPr_2 (81 μ l, 491 μ mol) and $MsCl$ (38 μ l, 491 μ mol), stirred for 30 min, and poured into ice/water. After extraction with $AcOEt$ (3 \times), the combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC ($AcOEt$ /pentane 5:2 → 1:0) gave **17** (450 mg, 84%). Yellow foam. R_f ($AcOEt$ /pentane 2:1) 0.34. $[\alpha]_D^{25} = -29.7$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 270 (52180). IR (ATR): 3400–3150w (br.), 2958w, 2936w, 2865w, 1682m, 1612m, 1585m, 1570m, 1519m, 1460m, 1422m, 1370m, 1343s, 1237m, 1212m, 1177m, 1155m, 1067s, 1012m, 1000m, 970m, 948m, 900w, 869w, 829m, 792m, 779m, 735w, 699m. ¹H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see Table 5; additionally, 8.79 (br. s, BzNH); 8.16–8.13 (*m*, 2 arom. H); 7.90 (br. *d*, $J = 7.6$, 2 arom. H); 7.73 (br. s, NHC=O/II); 7.64–7.61 (*m*, 1 arom. H); 7.53–7.46 (*m*, 4 arom. H); 4.77 (*t*, $J = 6.9$, CH_2CH_2O); 3.29 (*t*, $J = 6.9$, CH_2CH_2O); 3.20 (*s*, MsO); 2.98–2.90 (br. s, $Me_2CHC=O$); 1.60, 1.50, 1.42, 1.32 (4s, 2 Me_2CO_2); 1.51 (*sept.*, $J = 6.9$, Me_2CHCMe_2); 1.27, 1.26 (2*d*, $J = 6.9$, $Me_2CHC=O$); 0.78 (*d*, $J = 6.9$, Me_2CHCMe_2); 0.74, 0.73 (2s, Me_2CSi); -0.07 , -0.08 (2s, Me_2Si). ¹³C-NMR (125 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 174.92 (*s*, NHC=O/II); 166.09 (*s*, NHC=O/I); 146.88, 145.59 (2*s*); 133.52 (*d*); 132.55 (*s*); 130.01–127.71 (several *d*); 123.78 (2*d*); 113.85, 113.84 (2*s*, 2 Me_2CO_2); 66.84 (*t*, CH_2CH_2O); 38.63 (*q*, MsO); 35.98 (*d*, $Me_2CHC=O$);

Table 6. Selected ^{13}C -NMR Chemical Shifts [ppm] of the Di- and Tetranucleosides **16–18** and **20–23** in CDCl_3^{a}

C-Atom	16	17	18	20	21	22	23
<i>Adenosine unit</i>							
C(2)				152.68	152.26	153.38	152.35
C(4)				152.58	152.26	152.26	152.47
C(5)				124.15	123.06	122.73	122.31
C(6)				151.37	149.84	150.47	149.53
C(8)				146.77	151.45	146.78	152.86
$\text{CH}_2\text{-C}(8)$				62.59	59.64	62.24	28.23
C(1')				90.07	89.72	90.05	89.93
C(2')				84.10	83.71	83.46	83.43
C(3')				84.52	84.09	83.86	83.86
C(4')				90.29	88.49 ^{b)}	87.73	87.73
C(5')				33.05	33.07	33.51	32.56
<i>Uridine unit</i>							
C(2)				151.16	151.06 ^{c)}	150.94 ^{c)}	150.55
C(4)				162.65	161.64	161.49	162.57
C(5)				104.04	104.11	104.16	104.17
C(6)				151.64	150.97	150.91 ^{c)}	151.08
$\text{CH}_2\text{-C}(6)$				32.66	32.80	33.11	32.78
C(1')				91.71	91.65	91.71	91.68
C(2')				84.98	84.97	84.92	84.97
C(3')				84.42	83.62	83.62	84.28
C(4')				88.29	89.68	89.95	88.20
C(5')				31.53	33.57	33.61	31.33
<i>Cytidine unit</i>							
C(2) ^{b)}	155.43	154.82	155.14		155.27	155.23	155.09
C(4)	162.56	162.46	162.39		161.83	161.10	162.31
C(5) ^{b)}	97.51	98.31	^{d)}		97.85	97.98	95.21
C(6)	157.38	152.12	157.47		157.93 ^{b)}	158.05 ^{b)}	160.65
$\text{CH}_2\text{-C}(6)$	62.84	64.26	62.83		34.15	34.19	60.10
C(1')	93.05	93.01	93.12		92.56	92.67	91.68
C(2')	84.54	84.53	84.70		84.73	84.74	84.58
C(3')	84.72	84.71	84.86		84.84	84.89	84.53
C(4')	88.24	88.92	88.53		88.92	88.92	91.51
C(5')	33.81	33.57	34.83		33.50	33.29	34.32
<i>Guanosine unit</i>							
C(2)	151.24	151.26	151.12		151.23 ^{c)}	151.27	151.41
C(4)	153.73	153.75	153.52		153.74	153.79	153.16
C(5)	117.04	117.01	117.06		117.00	117.03	116.90
C(6)	160.00	159.97	159.97		159.95	159.98	159.78
C(8)	150.67	150.48	150.36		150.61	150.65	150.57
$\text{CH}_2\text{-C}(8)$	28.33	28.29	28.29		28.19	28.19	29.04
C(1')	89.94	89.91	89.76		89.89	89.95	89.75
C(2')	82.86	82.87	83.69		82.86	82.88	83.92
C(3')	81.67	81.57	83.92		81.60	81.66	83.86
C(4')	87.79	87.76	86.57		87.71	87.73	88.04
C(5')	63.17	63.12	33.75		63.13	63.17	34.55

^{a)} Assignments based on a HSQC and a HMBC spectrum. ^{b)} Broad signals. ^{c)} Assignments may be interchanged. ^{d)} Not assigned.

35.10 (*t*, CH₂CH₂O); 34.05 (*d*, Me₂CHCMe₂); 27.21, 27.02, 25.50, 25.26 (4*q*, 2 Me₂CO₂); 25.22 (*s*, Me₂CSi); 20.28, 20.25 (2*q*, Me₂CSi); 19.40, 19.30 (2*q*, Me₂CHC=O); 18.43, 18.38 (2*q*, Me₂CHCMe₂); – 3.53, – 3.54 (2*q*, Me₂Si). HR-MALDI-MS: 1247.4035 (28), 1246.4010 (42, [M + K]⁺, C₅₅H₇₃KN₉O₁₆S₂Si⁺; calc. 1246.4018), 1232.4312 (34), 1231.4324 (60), 1230.4301 (84, [M + Na]⁺, C₅₅H₇₃N₉NaO₁₆S₂Si⁺; calc. 1230.4278), 887.3911 (23), 886.3910 (55), 885.3871 (100, [M – C₁₃H₁₂N₃O₅S]⁺, C₄₂H₆₁N₆O₁₁SSi⁺; calc. 885.3883). Anal. calc. for C₅₅H₇₃N₉O₁₆S₂Si (1208.45): C 54.67, H 6.09, N 10.43; found: C 54.94, H 6.05, N 10.30.

N²-Isobutyryl-2',3'-O-isopropylidene-5'-S-[(4-methoxyphenyl)diphenylmethyl]-O⁶-[2-(4-nitrophenyl)ethyl]-5'-thioguanosine-8-methyl-(8^l → 5'-S)-N⁴-benzoyl-2',3'-O-isopropylidene-6-[[4-(4-methoxyphenyl)diphenylmethoxy)methyl]-5'-thiocytidine (**18**). A soln. of **15** (550 mg, 735 μmol) in THF/MeOH 1 : 1 (8 ml) was cooled to – 10° and treated dropwise with a 1M soln. of MeSNa in MeOH (1.47 ml), stirred for 3 h at – 10°, and poured on 0.1M HCl (15 ml). Brine was added, and the aq. phase was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue and **14** (690 mg, 735 μmol) in DMSO (1.5 ml) was degassed, treated with EtNⁱPr₂ (385 μl, 2.21 mmol), and stirred for 4 h. After addition of sat. NH₄Cl soln., the aq. phase was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (3 ×) and brine, dried (MgSO₄), and evaporated. FC (toluene/acetone 5 : 1 → 4 : 1) gave **18** (745 mg, 65%). Yellow foam. R_f (toluene/acetone 4 : 1) 0.18. [α]_D²⁵ = – 18.5 (*c* = 0.5, CHCl₃). UV (CHCl₃): 266 (49030). IR (ATR): 3500–3200w (br.), 3054w, 2958w, 2930w, 2852w, 1693m, 1676m, 1608s, 1508s, 1484m, 1457m, 1447m, 1422m, 1372m, 1344s, 1300m, 1249s, 1213s, 1180s, 1155m, 1086s, 1063s, 1033s, 1000m, 900w, 869w, 855w, 827m, 793m, 743m, 724m, 698s. ¹H-NMR (500 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 5; additionally, 8.60 (br. *s*, BzNH); 8.14–8.11 (*m*, 2 arom. H); 7.89 (br. *d*, *J* = 6.5, 2 arom. H); 7.62 (br. *s*, NHC=O/II); 7.62–7.59 (*m*, 2 arom. H); 7.52–7.45 (*m*, 9 arom. H, H–C(5/I)); 7.38–7.30 (*m*, 7 arom. H); 7.26–7.24 (*m*, 2 arom. H); 7.17–7.08 (*m*, 9 arom. H); 6.87–6.83 (*m*, 2 arom. H); 6.70–6.67 (*m*, 2 arom. H); 4.78 (*t*, *J* = 7.0, CH₂CH₂O); 3.76, 3.74 (2*s*, 2 MeO); 3.28 (*t*, *J* = 7.0, CH₂CH₂O); 2.89–2.80 (br. *s*, Me₂CH); 1.54, 1.43, 1.35, 1.28 (4*s*, 2 Me₂CO₂); 1.22, 1.21 (2*d*, *J* = 6.9, Me₂CH). ¹³C-NMR (125 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 174.73 (*s*, NHC=O/II); 159.06, 158.01 (2*s*); 146.92, 145.58, 144.83, 144.81, 143.41, 143.33, 136.59, 134.32 (8*s*); 133.18 (*d*); 132.16 (*s*); 130.63–126.52 (several *d*); 123.78 (2*d*); 113.86, 113.43 (2*s*, 2 Me₂CO₂); 113.46 (2*d*); 113.07 (2*d*); 88.56 (*s*, Ph₂C/I); 66.92 (*t*, CH₂CH₂O); 66.20 (*s*, Ph₂C/II); 55.23, 55.19 (2*q*, 2 MeO); 35.92 (*d*, Me₂CH); 35.15 (*t*, CH₂CH₂O); 27.10 (2 *C*), 25.48, 25.31 (3*q*, 2 Me₂CO₂); 19.36, 19.32 (2*q*, Me₂CH); NHC=O/I and C(5/I) not assigned due to broadening of the signals. HR-MALDI-MS: 1573.5557 (26), 1572.5548 (61), 1571.5542 (98), 1570.5533 (100, [M + Na]⁺, C₈₆H₈₅N₉NaO₁₅S₂⁺; calc. 1570.5499).

5'-S-Acetyl-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6^l → 5'-S)-N⁶-benzoyl-2',3'-O-isopropylidene-8-[(methylsulfonyl)oxy)methyl]-5'-thioadenosine (**20**). A soln. of **19** [6] (1.10 g, 1 mmol) in CH₂Cl₂ (6 ml) was treated with Cl₂CHCO₂H (0.6 ml) and ³Pr₃SiH (0.62 ml, 3 mmol), stirred for 90 min, and poured into ice and sat. NaHCO₃ soln. The mixture was extracted with CH₂Cl₂ (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue in CH₂Cl₂ (6 ml) at 0° was treated with EtNⁱPr₂ (0.19 ml, 1.1 mmol) and dropwise with MsCl (86 μl, 1.1 mmol), stirred for 30 min, and poured into ice/water. The mixture was extracted with CH₂Cl₂ (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. FC (AcOEt/pentane 4 : 1 → AcOEt) gave **20** (617 mg, 69%). Colourless foam. R_f (AcOEt/Et₂O 2 : 3) 0.20. [α]_D²⁵ = – 41.1 (*c* = 1.0, CHCl₃). UV (CHCl₃): 279 (25440). IR (ATR): 3200w (br.), 2987w, 2936w, 1687s, 1610m, 1579w, 1531w, 1502w, 1487m, 1448m, 1430w, 1371m, 1298w, 1243s, 1211m, 1173s, 1156m, 1083s, 1073s, 954m, 856m, 800m, 766m, 705m, 626w. ¹H-NMR (400 MHz, CDCl₃; assignments based on a HSQC spectrum): see Table 5; additionally, 10.99 (br. *s*, H–N(3/II)); 10.05 (br. *s*, BzNH); 8.07–8.05 (*m*, 2 arom. H); 7.53–7.51 (*m*, 3 arom. H); 3.06 (*s*, MsO); 2.28 (*s*, AcS); 1.63, 1.51, 1.38, 1.32 (4*s*, 2 Me₂C). ¹³C-NMR (100 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 194.84 (*s*, SC=O); 165.45 (*s*, NHC=O); 132.98 (*d*); 132.85 (*s*); 128.88 (2*d*); 128.56 (2*d*); 115.21, 113.85 (2*s*, 2 Me₂C); 38.63 (*q*, MsO); 30.66 (*q*, MeC=O); 27.31, 27.26, 25.51, 25.37 (4*q*, 2 Me₂C). HR-MALDI-MS: 928.1746 (46, [M + K]⁺, C₃₇H₄₃KN₇O₁₃S₃⁺; calc. 928.1713), 912.1995 (100, [M + Na]⁺, C₃₇H₄₃N₇NaO₁₃S₃⁺; calc. 912.1973), 890.2163 (98, [M + H]⁺, C₃₇H₄₄N₇O₁₃S₃⁺; calc. 890.2154).

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-2',3'-O-isopropylidene-5'-thiocytidine-6-methyl-(6' → 5'-S)-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-N⁶-benzoyl-2',3'-O-isopropylidene-8-[(4-methoxyphenyl)diphenylmethoxymethyl]-5'-thioadenosine (**21**). A soln. of **19** (1.09 g, 1 mmol) in MeOH/CH₂Cl₂ 9:1 (20 ml) was treated with powdered K₂CO₃ (279 mg, 2 mmol), stirred for 35 min, and poured into sat. NH₄Cl soln. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (2 ×) and brine, dried (MgSO₄), and evaporated. A soln. of the residue and **17** (1.22 g, 1 mmol) in DMSO (6 ml) was degassed, treated with EtNⁱPr₂ (0.53 ml, 3 mmol), stirred for 6 h, and poured into sat. NH₄Cl soln. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (3 ×) and brine, dried (MgSO₄), and evaporated. FC (toluene/acetone 3:1 → 2:1) gave **21** (1.41 g, 65%). Yellow foam. *R*_f (toluene/acetone 5:2) 0.25. [α]_D²⁵ = -74.0 (*c* = 0.5, CHCl₃). UV (CHCl₃): 272 (65010). IR (ATR): 3200_w (br.), 2934_w, 2865_w, 1692_s, 1607_w, 1510_m, 1448_m, 1421_m, 1380_m, 1344_s, 1248_s, 1211_s, 1180_m, 1155_m, 1068_s, 1000_m, 980_m, 902_w, 867_m, 830_m, 794_w, 779_w, 766_w, 746_w, 730_w, 700_m. ¹H-NMR (500 MHz, CDCl₃; assignments based on a DQF-COSY and a HSQC spectrum): see Table 5; additionally, 10.10 (br. s, H-N(3/II)); 9.35, 8.70 (2 br. s, BzNH); 8.11–8.09 (*m*, 2 arom. H); 8.04, 7.89 (2*d*, *J* = 7.3, 4 arom. H); 7.76 (br. s, NHC=O/IV); 7.58–7.38 (*m*, 15 arom. H, H-C(5/III)); 7.32–7.21 (*m*, 7 arom. H); 4.76–4.71 (*m*, CH₂CH₂O); 3.76 (*s*, MeO); 3.27 (*t*, *J* = 7.1, CH₂CH₂O); 3.00–2.92 (br. s, Me₂CHC=O); 1.52 (*sept.*, *J* = 6.9, Me₂CHCMe₂); 1.59, 1.54, 1.53, 1.48, 1.40, 1.36, 1.33, 1.32 (8*s*, 4 Me₂CO₂); 1.25 (*d*, *J* = 6.9, Me₂CHC=O); 0.79, 0.78 (2*d*, *J* = 6.9, Me₂CHCMe₂); 0.74, 0.73 (2*s*, Me₂CSi); -0.07, -0.09 (2*s*, Me₂Si). ¹³C-NMR (125 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 175.02 (*s*, NHC=O/IV); 166.37, 164.69 (2*s*, NHC=O/I,III); 158.91 (*s*); 146.83, 145.57, 143.43, 143.35, 134.25 (5*s*); 133.42 (*d* and *s*); 132.67 (*d*); 132.45 (*s*); 130.57–127.28 (several *d*); 123.72 (2*d*); 114.73, 113.92, 113.83, 113.47 (4*s*, 4 Me₂CO₂); 113.40 (2*d*); 88.21 (*s*, Ph₂C); 66.82 (*t*, CH₂CH₂O); 55.24 (*q*, MeO); 35.85 (*d*, Me₂CHC=O); 35.09 (*t*, CH₂CH₂O); 34.04 (*d*, Me₂CHCMe₂); 27.24, 27.23, 27.15, 27.07, 25.54, 25.44, 25.21 (2 *C*) (7*q*, 4 Me₂CO₂); 25.20 (*s*, Me₂CSi); 20.28, 20.34 (2*q*, Me₂CSi); 19.41, 19.29 (2*q*, Me₂CHC=O); 18.43, 18.38 (2*q*, Me₂CHCMe₂); -3.54 (*q*, Me₂Si). HR-MALDI-MS: 2179.7910 (24), 2178.7855 (51), 2177.7845 (82), 2176.7841 (100), 2175.7851 (71, [M + Na]⁺, C₁₀₈H₁₂₄N₁₆NaO₂₄S₃Si⁺; calc. 2175.7798), 886.3902 (74), 885.3870 (74, [M - C₆₆H₆₃N₁₀O₁₃S₂]⁺, C₄₂H₆₁N₆O₁₁SSi⁺; calc. 885.3883). Anal. calc. for C₁₀₈H₁₂₄N₁₆O₂₄S₃Si (2154.54): C 60.21, H 5.80, N 10.40; found: C 59.95, H 5.88, N 10.12.

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-2',3'-O-isopropylidene-5'-thiocytidine-6-methyl-(6' → 5'-S)-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-N⁶-benzoyl-2',3'-O-isopropylidene-8-[(methylsulfonyl)oxy]methyl]-5'-thioadenosine (**22**). A soln. of **21** (90 mg, 42 μmol) in CH₂Cl₂ (2 ml) was treated with Cl₂CHCO₂H (0.2 ml) and ⁱPr₃SiH (28 μl, 126 μmol), stirred for 1 h, and poured into sat. NaHCO₃ soln. The mixture was extracted with CH₂Cl₂ (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue in CH₂Cl₂ (1.5 ml) at 0° was treated with EtNⁱPr₂ (8 μl, 46 μmol) and MsCl (3.6 μl, 46 μmol), stirred for 30 min, and poured into H₂O. The mixture was extracted with CH₂Cl₂ (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. FC (toluene/acetone 2:1) gave **22** (61 mg, 74%). Pale-yellow foam. *R*_f (toluene/acetone 1:1) 0.54. [α]_D²⁵ = -90.7 (*c* = 0.5, CHCl₃). UV (CHCl₃): 272 (72450). IR (ATR): 3200_w (br.), 2936_w, 2865_w, 1693_s, 1606_s, 1519_m, 1469_m, 1457_m, 1420_m, 1370_s, 1344_s, 1239_s, 1211_s, 1175_m, 1155_m, 1071_s, 1000_m, 979_m, 846_m, 855_m, 829_m, 793_m, 779_m, 702_m. ¹H-NMR (600 MHz, CDCl₃; assignments based on a DQF-COSY and a HSQC spectrum): see Table 5; additionally, 9.70 (br. s, H-N(3/II)); 9.18, 8.85 (2 br. s, 2 BzNH); 8.12–8.10 (*m*, 2 arom. H); 8.02, 7.92 (2*d*, *J* = 7.4, 4 arom. H); 7.76 (br. s, NHC=O/IV); 7.61–7.42 (*m*, 8 arom. H); 4.78–4.72 (*m*, CH₂CH₂O); 3.28–3.23 (*m*, CH₂CH₂O); 3.14 (*s*, MsO); 2.98–2.93 (br. s, Me₂CHC=O); 1.50 (*sept.*, *J* = 6.9, Me₂CHCMe₂); 1.63, 1.60, 1.55, 1.50, 1.41, 1.40, 1.33 (6 H) (7*s*, 4 Me₂CO₂); 1.25 (*d*, *J* = 6.9, Me₂CHC=O); 0.79 (*d*, *J* = 6.9, Me₂CHCMe₂); 0.74, 0.73 (2*s*, Me₂CSi); -0.07, -0.08 (2*s*, Me₂Si). ¹³C-NMR (150 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 175.05 (*s*, NHC=O/IV); 164.66, 161.10 (2*s*, C(4/III), NHC=O); 146.88, 145.62 (2*s*); 133.42, 132.98 (2*d*); 133.30, 132.55 (2*s*); 129.97–127.86 (several *d*); 123.74 (2*d*); 115.21, 114.02, 113.86, 113.50 (4*s*, 4 Me₂CO₂); 66.86 (*t*, CH₂CH₂O); 38.46 (*q*, MsO); 35.87 (*d*, Me₂CHC=O); 35.12 (*t*, CH₂CH₂O); 34.08 (*d*, Me₂CHCMe₂); 27.27,

27.17, 27.14, 27.11, 25.56, 25.38, 25.24 (2 C) (7q, 4 Me₂CO₂); 25.24 (s, Me₂CSi); 20.31, 20.27 (2q, Me₂CSi); 19.41, 19.30 (2q, Me₂CHC=O); 18.45, 18.40 (2q, Me₂CHCMe₂); – 3.51 (q, Me₂Si); signal of C(4/III) hidden by the noise. HR-ESI-MS: 1963.6585 (26), 1962.6582 (46), 1961.6549 (82), 1960.6581 (100), 1959.6564 (100, [M + H]⁺, C₈₉H₁₁₁N₁₆O₂₅Si⁺; calc. 1959.6553).

5'-S-Acetyl-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-N⁶-benzoyl-2',3'-O-isopropylidene-5'-thioadenosine-8-methyl-(8' → 5'-S)-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]-5'-thioguanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-6-(hydroxymethyl)-2',3'-O-isopropylidene-5'-thiocytidine (**23**). A soln. of **18** (190 mg, 123 μmol) in CH₂Cl₂ (2 ml) was treated with CF₃CO₂H (50 μl) and ³Pr₃SiH (151 μl, 738 μmol), stirred for 45 min, and poured into ice and sat. NaHCO₃ soln. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (2 ×) and brine, dried (MgSO₄), and evaporated. A soln. of the residue and **20** (109 mg, 123 μmol) in DMSO (300 μl) was degassed, treated with Et₃NPr₂ (64 μl, 369 μmol), stirred for 3 h, and poured into sat. NH₄Cl soln. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (3 ×) and brine, dried (MgSO₄), and evaporated. FC (toluene/acetone 2:1 → 1:1) gave **23** (148 mg, 67%). Pale-yellow foam. R_f (toluene/acetone 5:3) 0.27. [α]_D²⁵ = – 6.3 (c = 0.5, CHCl₃). UV (CHCl₃): 267 (66670). IR (ATR): 3230w (br.), 2984w, 2931w, 1689s, 1607s, 1519m, 1455m, 1421m, 1380s, 1373s, 1343s, 1237s, 1211s, 1155m, 1069s, 998m, 981m, 899w, 865m, 793w, 732w, 702m. ¹H-NMR (500 MHz, CDCl₃; assignments based on a DQF-COSY and a HSQC spectrum): see Table 5; additionally, 10.27 (br. s, H–N(3/IV)); 9.55 (br. s, BzNH); 8.83 (br. s, BzNH, HN–C(2/II)); 8.11–8.09 (m, 2 arom. H); 8.02, 7.87 (2d, J = 7.5, 4 arom. H); 7.61–7.38 (m, 8 arom. H, H–C(5/I)); 5.60 (br. s, OH); 4.66 (t, J = 6.8, CH₂CH₂O); 3.21 (t, J = 6.8, CH₂CH₂O); 3.03–2.94 (br. s, Me₂CHC=O); 2.33 (s, AcS); 1.61, 1.52, 1.51, 1.47, 1.38, 1.33, 1.31, 1.24 (8s, 4 Me₂CO₂); 1.22, 1.20 (2d, J = 6.9, Me₂CHC=O). ¹³C-NMR (125 MHz, CDCl₃; assignments based on a HSQC and a HMBC spectrum): see Table 6; additionally, 194.76 (s, SC=O); 166.07, 165.83 (2s, 2 NHC=O); 146.82, 145.55 (2s); 133.70 (2s); 133.28, 132.52 (2d); 129.85–127.72 (several d); 123.74 (2d); 114.74, 113.80, 113.76, 113.40 (4s, 4 Me₂CO₂); 66.70 (t, CH₂CH₂O); 35.67 (d, Me₂CHC=O); 35.01 (t, CH₂CH₂O); 30.60 (q, MeC=O); 27.15 (3 C), 26.94, 25.32, 25.28, 25.20, 25.02 (6q, 4 Me₂CO₂); 19.47, 19.33 (2q, Me₂CHC=O). HR-MALDI-MS: 1822.5327 (31), 1821.5295 (72), 1820.5280 (100), 1819.5258 (100, [M + Na]⁺, C₈₂H₉₂N₁₆NaO₂₃S₄⁺; calc. 1819.5296), 983.2866 (40), 982.2833 (84, [M – C₃₈H₄₀N₉O₁₀S]⁺, C₄₄H₅₂N₇O₁₃S₃⁺; calc. 982.2780).

5'-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-2',3'-O-isopropylidene-5'-thiocytidine-6-methyl-(6' → 5'-S)-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-N⁶-benzoyl-2',3'-O-isopropylidene-5'-thioadenosine-8-methyl-(8' → 5'-S)-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-N⁶-benzoyl-2',3'-O-isopropylidene-5'-thioadenosine-8-methyl-(8' → 5'-S)-N²-isobutyryl-2',3'-O-isopropylidene-O⁶-[2-(4-nitrophenyl)ethyl]-5'-thioguanosine-8-methyl-(8' → 5'-S)-N⁴-benzoyl-6-(hydroxymethyl)-2',3'-O-isopropylidene-5'-thiocytidine (**24**). A soln. of **23** (110 mg, 61 μmol) in degassed pyridine/EtOH 2:1 (600 μl) was cooled to 0°, treated dropwise with a cooled aq. 1M NaOH soln. (300 μl), and stirred for 30 min. The mixture was treated with 2M HCl (150 μl), poured onto ice, and extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (2 ×) and brine, dried (MgSO₄), and evaporated. Excess pyridine was removed by co-evaporation with toluene. A soln. of the residue and **22** (120 mg, 61 μmol) in DMSO (600 μl) was degassed, treated with Et₃NPr₂ (32 μl, 183 μmol), stirred for 6 h, and poured into ice and sat. NH₄Cl soln. The mixture was extracted with AcOEt (3 ×). The combined org. phases were washed with H₂O (3 ×) and brine, dried (MgSO₄), and evaporated. FC (diol phase; AcOEt) gave **24** (135 mg, 61%). Yellow foam. R_f (diol phase; AcOEt) 0.45. [α]_D²⁵ = – 39.2 (c = 0.5, CHCl₃). UV (CHCl₃): 268 (89780). IR (ATR): 3400–3200w (br.), 2984w, 2937w, 1690s, 1607s, 1519m, 1458m, 1421m, 1380s, 1344s, 1240s, 1212s, 1156m, 1071s, 981m, 866m, 833m, 794w, 704w. ¹H-NMR (600 MHz, CDCl₃; assignments based on a DQF-COSY and a HSQC spectrum): see Table 7; additionally, 12.11, 10.22 (2 br. s, H–N(3/IV,VI)); 9.77, 9.64, 9.52, 8.95 (4 br. s, 4 BzNH); 8.66, 7.80 (2 br. s, NH–C(2/II,VIII)); 8.12–8.07 (m, 4 arom. H); 8.02, 7.97, 7.91, 7.88 (4d, J = 7.5, 8 arom. H); 7.59–7.37 (m, 16 arom. H, H–C(5/I,VII)); 4.76–4.73 (m, CH₂CH₂O); 4.65–4.63 (covered, CH₂CH₂O); 3.25 (t, J = 7.0, CH₂CH₂O); 3.21–3.18 (m, CH₂CH₂O); 2.98–2.72 (m, 2 H–C(5'/II–VII), 2 Me₂CHC=O); 1.51 (sept., J = 6.9, Me₂CHCMe₂); 1.60, 1.59 (6 H), 1.54, 1.49 (9 H), 1.39, 1.37, 1.32, 1.31 (6 H), 1.26 (12 H) (9s, 8 Me₂CO₂); 1.26–1.25 (m, 2 Me₂CHC=O); 0.78 (d, J = 6.9, Me₂CH); 0.74, 0.73 (2s, Me₂CSi); – 0.07, – 0.08 (2s, Me₂Si). ¹³C-NMR

Table 7. Selected $^1\text{H-NMR}$ Chemical Shifts [ppm] and Coupling Constants [Hz] of the Octanucleosides **24** and **25**^{a)}

H-Atom	24 (CDCl_3)		25 ((D_6) DMSO)	
	A(III)	A(V)	A(III)	A(V)
H-C(5)	b)	b)	5.80	5.67–5.64
$\text{CH}_a\text{-C}(6)$	4.72	3.76	4.37	3.79–3.72
$\text{CH}_b\text{-C}(6)$	4.64	3.68	4.30–4.24	3.65–3.59
H-C(1')	5.73	5.92	5.80	5.67–5.64
H-C(2')	5.20–5.16	5.30	5.18–5.14	5.18–5.14
H-C(3')	4.96–4.93	5.04	4.83	4.81
H-C(4')	4.42–4.34	4.33–4.28	4.15–3.98	4.15–3.98
$\text{H}_a\text{-C}(5')$	3.15–3.10	2.98–2.72	2.90–2.65	2.90–2.65
$\text{H}_b\text{-C}(5')$	3.15–3.10	2.98–2.72	2.90–2.65	2.90–2.65
$J(\text{H}_a, \text{H}_b)$	13.2	14.6	14.1	c)
$J(1', 2')$	< 1	< 1	< 1	c)
$J(2', 3')$	c)	6.6	6.3	6.4
$J(3', 4')$	c)	3.8	3.5	3.7
$J(4', 5'a)$	c)	c)	c)	c)
$J(4', 5'b)$	c)	c)	c)	c)
$J(5'a, 5'b)$	c)	c)	c)	c)
	G(II)	G(VIII)	G(II)	G(VIII)
$\text{CH}_a\text{-C}(8)$	4.11	4.09	3.91	3.91
$\text{CH}_b\text{-C}(8)$	4.06	3.97	3.85	3.83
H-C(1')	6.40	6.28	6.05	6.12
H-C(2')	5.81	5.87	5.39	5.49
H-C(3')	5.35–5.33	5.20–5.16	5.00	5.11
H-C(4')	4.42–4.34	4.26–4.17	4.15–3.98	4.15–3.98
$\text{H}_a\text{-C}(5')$	2.98–2.72	3.62	2.90–2.65	3.67
$\text{H}_b\text{-C}(5')$	2.98–2.72	3.60	2.90–2.65	3.62
$J(\text{H}_a, \text{H}_b)$	13.2	14.5	14.2	14.2
$J(1', 2')$	< 1	1.9	1.1	1.1
$J(2', 3')$	5.8	6.3	6.5	6.2
$J(3', 4')$	c)	c)	3.8	3.9
$J(4', 5'a)$	c)	5.9	c)	7.1
$J(4', 5'b)$	c)	6.4	c)	6.0
$J(5'a, 5'b)$	c)	10.9	c)	11.3
	A(III)	A(V)	A(III)	A(V)
H-C(2)	8.63	8.70	8.15	8.15
$\text{CH}_a\text{-C}(8)$	4.09	4.10	4.15–3.98	4.15–3.98
$\text{CH}_b\text{-C}(8)$	4.04	4.02	4.15–3.98	4.15–3.98
H-C(1')	6.23	6.25	6.21	6.21
H-C(2')	5.71	5.73	5.67–5.64	5.67–5.64
H-C(3')	5.12	5.20–5.16	5.04–5.01	5.04–5.01
H-C(4')	4.33–4.28	4.42–4.34	4.30–4.24	4.30–4.24
$\text{H}_a\text{-C}(5')$	2.98–2.72	2.98–2.72	2.90–2.65	2.90–2.65
$\text{H}_b\text{-C}(5')$	2.98–2.72	2.98–2.72	2.90–2.65	2.90–2.65
$J(\text{H}_a, \text{H}_b)$	13.2	14.7	c)	c)
$J(1', 2')$	1.5	< 1	< 1	< 1
$J(2', 3')$	6.5	6.5	c)	c)
$J(3', 4')$	3.9	c)	c)	c)

Table 7 (cont.)

H-Atom	24 (CDCl ₃)		25 ((D ₆)DMSO)	
	A(III)	A(V)	A(III)	A(V)
<i>J</i> (4',5'a)	°)	°)	°)	°)
<i>J</i> (4',5'b)	°)	°)	°)	°)
<i>J</i> (5'a,5'b)	°)	°)	°)	°)
	U(IV)	U(VI)	U(IV)	U(VI)
H–C(5)	5.33	5.35	5.72 ^{d)}	5.63 ^{d)}
CH _a –C(6)	3.54	3.53	3.79–3.72	3.79–3.72
CH _b –C(6)	3.50	3.48	3.65–3.59	3.65–3.59
H–C(1')	5.77	5.73	5.75	5.74
H–C(2')	5.20–5.16	5.11	5.18–5.14	5.18–5.14
H–C(3')	4.91	4.96–4.93	4.75–4.72	4.75–4.72
H–C(4')	4.26–4.17	4.26–4.17	4.15–3.98	4.15–3.98
H _a –C(5')	2.98–2.72	2.98–2.72	2.90–2.65	2.90–2.65
H _b –C(5')	2.98–2.72	2.98–2.72	2.90–2.65	2.90–2.65
<i>J</i> (H _a ,H _b)	14.4	14.5	°)	°)
<i>J</i> (1',2')	< 1	< 1	°)	°)
<i>J</i> (2',3')	6.4	5.1	°)	°)
<i>J</i> (3',4')	4.5	°)	°)	°)
<i>J</i> (4',5'a)	°)	°)	°)	°)
<i>J</i> (4',5'b)	°)	°)	°)	°)
<i>J</i> (5'a,5'b)	°)	°)	°)	°)

a) Assignment based on a DQF-COSY and a HSQC spectrum. b) Hidden by aromatic signals. c) Not assigned. d) Broad signal.

(150 MHz, CDCl₃; assignments based on a HMBC and a HSQC spectrum): see Table 8; additionally, 175.72, 175.09 (2s, NHC=O/II, VIII); 165.76, 162.60 (2s, 4 NHC=O, C(4I)); 146.87, 146.85, 145.65, 145.57 133.83 (2 C), 133.75 (2 C) (6s); 133.40, 133.23, 132.64, 132.54 (4d); 129.99–127.82 (several d); 123.73 (4d); 114.70, 114.60, 114.00, 113.86, 113.81, 113.70, 113.50, 113.46 (8s, 8 Me₂CO₂); 66.86, 66.81 (2t, 2 CH₂CH₂O); 35.88 (br. d, 2 Me₂CHC=O); 35.13, 35.02 (2t, 2 CH₂CH₂O); 34.08 (d, Me₂CHCMe₂); 27.27–27.13, 25.56–25.44 (several q, 8 Me₂CO₂); 25.09 (s, Me₂CSi); 20.30, 20.27 (2q, Me₂CSi); 19.47, 19.41, 19.33, 19.29 (4q, 2 Me₂CHC=O); 18.45, 18.41 (2q, Me₂CHCMe₂); – 3.51 (q, Me₂Si). HR-MALDI-MS: 3644.1560 (61), 3643.1538 (97), 3642.1424 (100), 3641.1466 (89), 3640.1718 (50, [M + Na]⁺, C₁₆₈H₁₉₆N₃₂NaO₄₄S₇Si⁺; calc. 3640.1790).

5'-O-[(Dimethyl(1,1,2-trimethylpropyl)silyl)-2',3'-O-isopropylidene-8-methyl-(8' → 5'-S)-2',3'-O-isopropylidene-5'-thiocytidine-6-methyl-(6' → 5'-S)-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-2',3'-O-isopropylidene-5'-thioadenosine-8-methyl-(8' → 5'-S)-2',3'-O-isopropylidene-5'-thiouridine-6-methyl-(6' → 5'-S)-2',3'-O-isopropylidene-5'-thioadenosine-8-methyl-(8' → 5'-S)-2',3'-O-isopropylidene-5'-thioguanosine-8-methyl-(8' → 5'-S)-6-(hydroxymethyl)-2',3'-O-isopropylidene-5'-thiocytidine (25)]. Octanucleoside **24** (82 mg, 23 μmol) was dissolved in a 0.5M soln. of 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) in pyridine (1 ml). The mixture was stirred for 30 min, poured into 0.1M aq. HCl, and extracted with CH₂Cl₂ (3 ×). The combined org. phases were washed with H₂O and brine, dried (MgSO₄), and evaporated. A soln. of the residue in CH₂Cl₂ (0.4 ml) in a pressure tube was treated with sat. methanolic NH₃ (2 ml). The pressure tube was sealed, and the soln. was stirred for 60 h. After evaporation, a soln. of the residue in CHCl₃ was washed with H₂O and brine, dried (MgSO₄), and evaporated. FC (NH₂ phase; CHCl₃/MeOH 4 : 1) gave **25** (31 mg, 50%). Colourless solid. R_f (NH₂ phase; CHCl₃/MeOH 4 : 1) 0.21. [α]_D²⁵ = – 84.6 (c = 0.25, CHCl₃). UV (CHCl₃): 268 (73110). IR (ATR): 3332w (br.), 3173w (br.), 2984w, 2925w, 2854w, 1689s, 1641s, 1532w, 1454w, 1434w, 1373s, 1331w, 1300w, 1259w,

Table 8. Selected ^{13}C -NMR Chemical Shifts [ppm] of the Octanucleosides **24** and **25**^{a)}

C-Atom	24 (CDCl ₃)		25 ((D ₆)DMSO)	
	C(I)	C(VII)	C(I)	C(VII)
C(2)	155.06 ^{b)}	155.36 ^{b)}	155.64	155.64
C(4)	162.60 ^{d)}	^{c)} ^{d)}	165.65	165.05
C(5)	98.14 ^{b)} ^{e)}	95.05 ^{b)} ^{e)}	93.13	95.80
C(6)	160.52	157.94	155.94	152.07
CH ₂ -C(6)	34.15	34.15	59.08	32.11
C(1')	91.79 ^{f)}	92.64	91.00	90.74
C(2')	84.89	84.74	84.72	84.49 ^{d)}
C(3')	84.64	84.89	84.39 ^{d)}	84.27 ^{d)}
C(4')	91.27	88.90	87.52	87.28
C(5')	34.40	33.62–32.98	33.18 ^{e)}	33.39 ^{e)}
	G(II)	G(VIII)	G(II)	G(VIII)
C(2)	151.28	151.28	153.75 ^{f)}	153.66 ^{f)}
C(4)	153.18	153.78	151.23	151.63
C(5)	117.01 ^{g)}	116.94 ^{g)}	115.40 ^{g)}	115.37 ^{g)}
C(6)	159.84	159.84	156.53 ^{h)}	156.51 ^{h)}
C(8)	150.78	150.68	143.23	143.17
CH ₂ -C(8)	28.20 ^{h)}	28.06 ^{h)}	26.75	26.75
C(1')	89.75	89.94	88.44	88.27
C(2')	84.00–83.87	82.88	83.57	83.23
C(3')	84.00–83.87	81.68	83.50 ⁱ⁾	81.13
C(4')	88.53 ^{b)}	87.73	86.26	87.92
C(5')	33.62–32.98	33.62–32.98	33.11 ^{e)}	63.35
	A(III)	A(V)	A(III)	A(V)
C(2)	152.31	152.31	152.47 ^{b)}	152.47 ^{b)}
C(4)	152.50	152.23	149.63	149.63
C(5)	122.39 ⁱ⁾	121.17 ⁱ⁾	117.75 ^{k)}	117.64 ^{k)}
C(6)	149.55	149.39	155.46 ^{l)}	155.41 ^{l)}
C(8)	152.67	151.70	148.04 ^{m)}	147.86 ^{m)}
CH ₂ -C(8)	29.07 ^{h)}	28.20 ^{h)}	27.47 ⁿ⁾	27.25 ⁿ⁾
C(1')	89.78	89.69	88.82	88.76
C(2')	83.45	83.60	82.75 ^{o)}	82.63 ^{o)}
C(3')	84.00–83.87	84.00–83.87	83.36 ⁱ⁾	83.36 ⁱ⁾
C(4')	87.45	87.84	85.67 ^{p)}	85.37 ^{p)}
C(5')	33.62–32.98	33.62–32.98	32.67 ^{e)}	32.52 ^{e)}
	U(IV)	U(VI)	U(IV)	U(VI)
C(2)	150.92	150.75	150.83	150.78
C(4)	161.97 ^{j)}	161.65 ^{j)}	161.98 ^{q)}	161.94 ^{q)}
C(5)	104.06	104.27	103.54 ^{r)}	103.43 ^{r)}
C(6)	150.95	150.83	151.13 ^{s)}	151.09 ^{s)}
CH ₂ -C(6)	32.80 ^{k)}	32.68 ^{k)}	31.55	31.55
C(1')	91.66	91.37 ^{f)}	90.81 ^{t)}	90.61 ^{t)}
C(2')	84.53	84.99	84.27 ^{d)}	84.22 ^{d)}
C(3')	83.63	84.00–83.87	83.71	83.71
C(4')	89.86	88.79	88.20	87.41
C(5')	33.62–32.98	33.62–32.98	32.52 ^{e)}	32.46 ^{e)}

^{a)} Assignment based on a HSQC and a HMBC spectrum. ^{b)} Broad signals. ^{c)} Not assigned. ^{d)–t)} Assignments may be interchanged.

1209m, 1155m, 1063w, 983m, 930w, 865m, 831m, 796w, 781w, 719w, 682w. $^1\text{H-NMR}$ (600 MHz, (D_6) DMSO; assignments based on a DQF-COSY and a HSQC spectrum): see Table 7; additionally, 11.4 (br. s, H–N(3/IV,VI)); 10.8 (br. s, H–N(1/II,VIII)); 7.29, 7.27, 7.22, 7.21 (4 br. s, H₂N–C(4/I,VII), H₂N–C(6/III,V)); 6.64, 6.55 (2 br. s, H₂N–C(2/II,VIII)); 1.51 (sept., $J = 6.9$, Me₂CHCMe₂); 1.52, 1.51 (6 H), 1.46, 1.44, 1.43, 1.42, 1.40, 1.32, 1.30 (6 H), 1.26 (6 H), 1.25, 1.23 (6 H) (12s, 8 Me₂CO₂); 0.78, 0.77 (2d, $J = 6.9$, Me₂CHCMe₂); 0.73, 0.72 (2s, Me₂CSi); – 0.06, – 0.09 (2s, Me₂Si). See also Table 9. $^{13}\text{C-NMR}$ (150 MHz, (D_6) DMSO; assignments based on a DQF-COSY, HSQC and a HMBC spectrum): see Table 8; additionally, 113.19 (2 C), 112.77, 112.68, 112.58, 112.41, 111.99, 111.96 (7s, 8 Me₂CO₂); 33.51 (d, Me₂CHCMe₂); 26.91 (2 C), 26.90, 26.86, 26.82 (2 C), 26.81, 26.71, 25.26, 25.03, 25.02, 24.94 (3 C), 24.92, 24.82 (12q, 8 Me₂CO₂); 24.63 (s, Me₂CSi); 20.10, 20.01 (2q, Me₂CSi); 18.22, 18.14 (2q, Me₂CHCMe₂); – 3.66, – 3.72 (2q, Me₂Si); signals of C(4/I,VII) and C(6/II,VIII) hidden by the noise. HR-MALDI-MS: 2789.9050 (41), 2788.9020 (71), 2787.9023 (97), 2786.9044 (100), 2785.9017 (59, $[M + \text{Na}]^+$, C₁₁₆H₁₅₄N₃₀NaO₃₄S₇Si⁺; calc. 2785.8959).

Table 9. ^1H - and ^{13}C -NMR Chemical Shifts [ppm] for the CH and CH₂ Groups of the Isopropylidened and Silylated Octanucleoside **25** in $(\text{CDCl}_3)_2$ as Obtained from a HSQC Spectrum (assignments based on DQF-COSY, TOCSY, and NOESY spectra)

	G(VIII)	C(VII)	U(VI)	A(V)	U(IV)	A(III)	G(II)	C(I)
H–C(5)	–	5.49	5.59	–	7.32	–	–	5.71
C(5)	–	99.9	105.9	–	104.8	–	–	95.6
H–C(1')	6.10	5.70	5.75	6.41	5.89	6.47	6.66	5.70
C(1')	89.6	91.6 ^a	93.0	91.0	89.5	90.0	89.1	92.1 ^a
H–C(2')	6.36	4.98	5.28	5.85	5.41	6.10	6.36	5.32
C(2')	80.3	86.0	85.4	82.3	84.6	80.1	79.5	85.3
H–C(3')	4.94	4.94	4.91 ^a	5.16	4.98	4.70	4.82	4.90 ^a
C(3')	82.6	82.6	84.9 ^a	83.7	83.8	83.9	83.6	85.1 ^a
H–C(4')	4.29	4.46	4.28	4.42	4.31	3.68	4.55	4.49
C(4')	86.0	90.4	88.0	83.8	92.9	84.2	84.8	93.8
2 H–C(5')	3.80/3.59	3.18/2.85	3.02/2.71	2.71/2.65	3.02/2.71	2.56/2.51	2.97	3.15/2.91
C(5')	63.9	35.7	35.8 ^a	32.1	30.3 ^a	34.1	34.6	33.3
CH ₂ –C(6/8)	3.86	4.00/3.50	3.79	4.32/3.93	4.42/3.77	4.13/3.83	4.37/4.15	4.52/4.44
CH ₂ –C(6/8)	29.5	30.5	32.6	29.4	33.2	28.6	30.1	60.0

^a) Assignments in rows may be interchanged.

Guanosine-8-methyl-(8' → 5'-S)-5'-thiocytidine-6-methyl-(6' → 5'-S)-5'-thiouridine-6-methyl-(6' → 5'-S)-5'-thioadenosine-8-methyl-(8' → 5'-S)-5'-thiouridine-6-methyl-(6' → 5'-S)-5'-thioadenosine-8-methyl-(8' → 5'-S)-5'-thioguanosine-8-methyl-(8' → 5'-S)-6-(hydroxymethyl)-5'-thiocytidine (26). A soln. of **25** (4 mg, 1.5 μmol) in HCO₂H/H₂O 4 : 1 (0.3 ml) was stirred for 18 h and evaporated at 23°. A soln. of the residue in 25% aq. NH₃ (1 ml) was lyophilized. A soln. of the residue in a mixture of H₂O and 25% aq. NH₃ was applied on a *Bakerbond C18* column (conditioned with H₂O). The column was washed with ca. 5 ml H₂O. The product was obtained by elution with H₂O/MeCN/NH₄OH 4 : 1 : 1. Evaporation of the solvents gave **26** (2 mg, 60%). Colourless powder. R_f (NH₂ phase; MeOH/CHCl₃/H₂O 3 : 1 : 1) 0.65. t_R (HPLC: *Waters Atlantis dC18-3*, 100 × 3 mm; MeCN/H₂O/HCO₂H 10 : 90 : 0.1 → 95 : 5 : 0.1; flow rate, 0.2 ml/min) 20.37 min. HR-ESI-MS: 1163.2620 (17), 1162.7634 (19), 1162.2639 (16, $[M + H + \text{Na}]^{2+}$, C₈₄H₁₀₅N₃₀NaO₃₄S₇²⁺; calc. 1162.2671), 1153.7745 (12), 1153.2764 (22), 1152.7762 (62), 1152.2748 (92), 1151.7717 (93), 1151.2740 (100, $[M + 2 H]^{2+}$, C₈₄H₁₀₆N₃₀O₃₄S₇²⁺; calc. 1151.2761), 1086.7526 (17), 1086.2519 (23), 1085.7535 (31), 1085.2514 (30, $[M - \text{C}_3\text{H}_9\text{O}_4 + 3 H]^{2+}$, C₇₉H₉₈N₃₀O₃₀S₇²⁺; calc. 1085.2550).

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