Oligonucleotide Analogues with Integrated Bases and Backbone

Part 30[1])

Synthesis and Association of a Self-Complementary Thiomethylene-Linked Octanucleoside

Dieter Seebach mit den besten Glückwünschen zugeeignet

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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 isopropylidenated **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 isopropylidenated octanucleoside **25** in $(CDCl_2)_2$ 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 $CDCl_3$ 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 $CDCl_3$ is accompanied by stacking of the nucleobases, as evidenced by CD spectroscopy.

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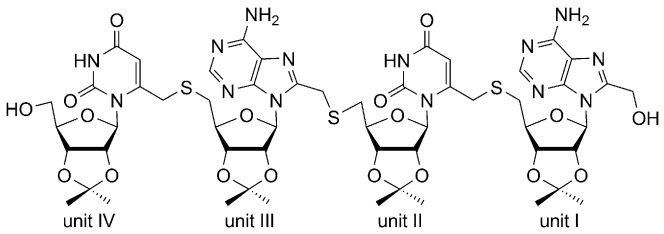
[1]) Part 29: [1].

[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 H_2O-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 $CDCl_3$ 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 H_2O 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 H_2O, considering the H_2O 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.



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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 $C(6)CH_2$ or $C(8)CH_2$ 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 extented 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

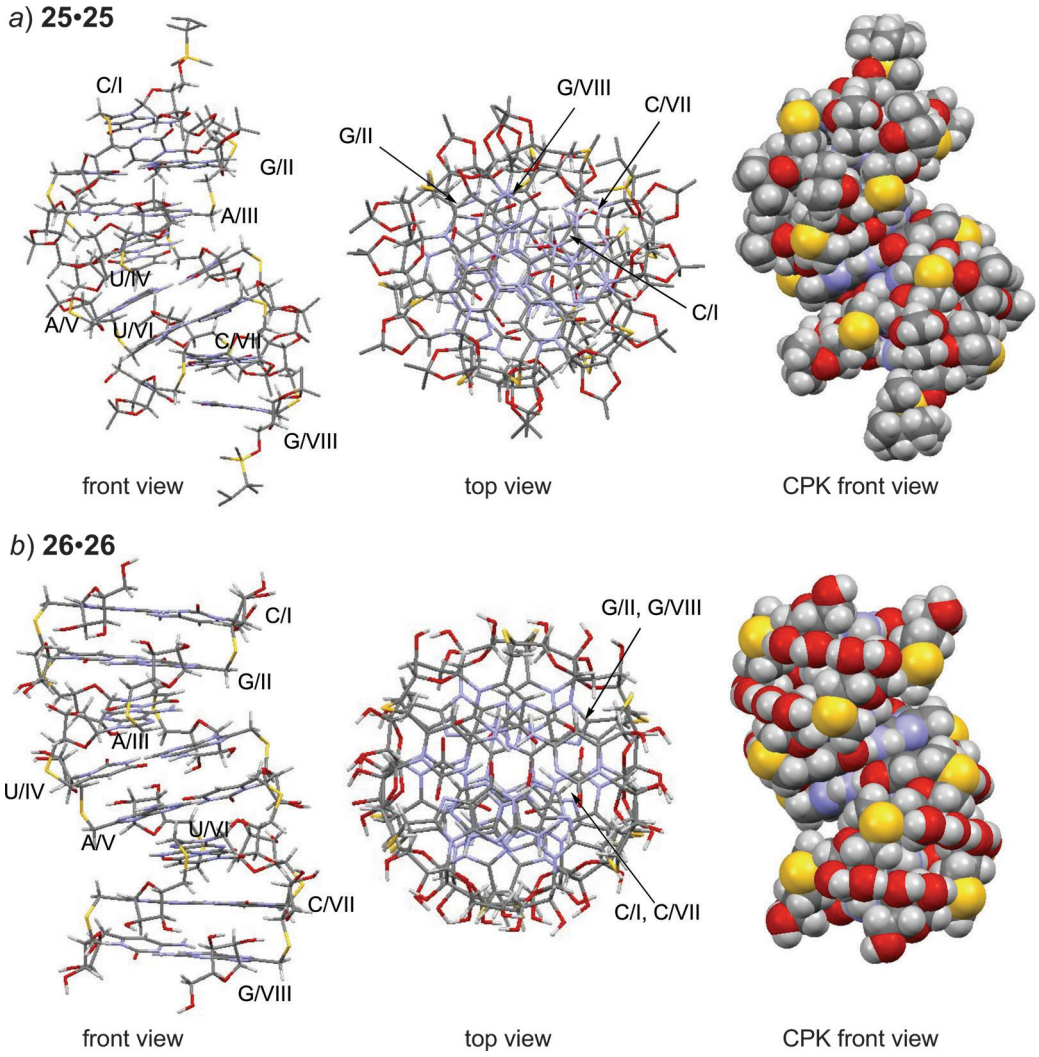

Fig. 1. *AMBER*-calculated structures of the* Watson–Crick *base-paired duplexes* a) **25** · **25** *and* b) **26** • **26**: *front and top views* (for **25** • **25**: H-atoms of the protecting groups are omitted for enhanced clarity), *and space-filling* (CPK) *front view*

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 isopropylidenated 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*-isopropylideneguanosine (**2**) [11] was silylated under standard conditions to the thexyl(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 $(PhO)_3PMeI$ [14] to provide iodo derivative **6** that was substituted with the sodium salt of monomethoxytritylthiol[4]) 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 $NaBH_4$ 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

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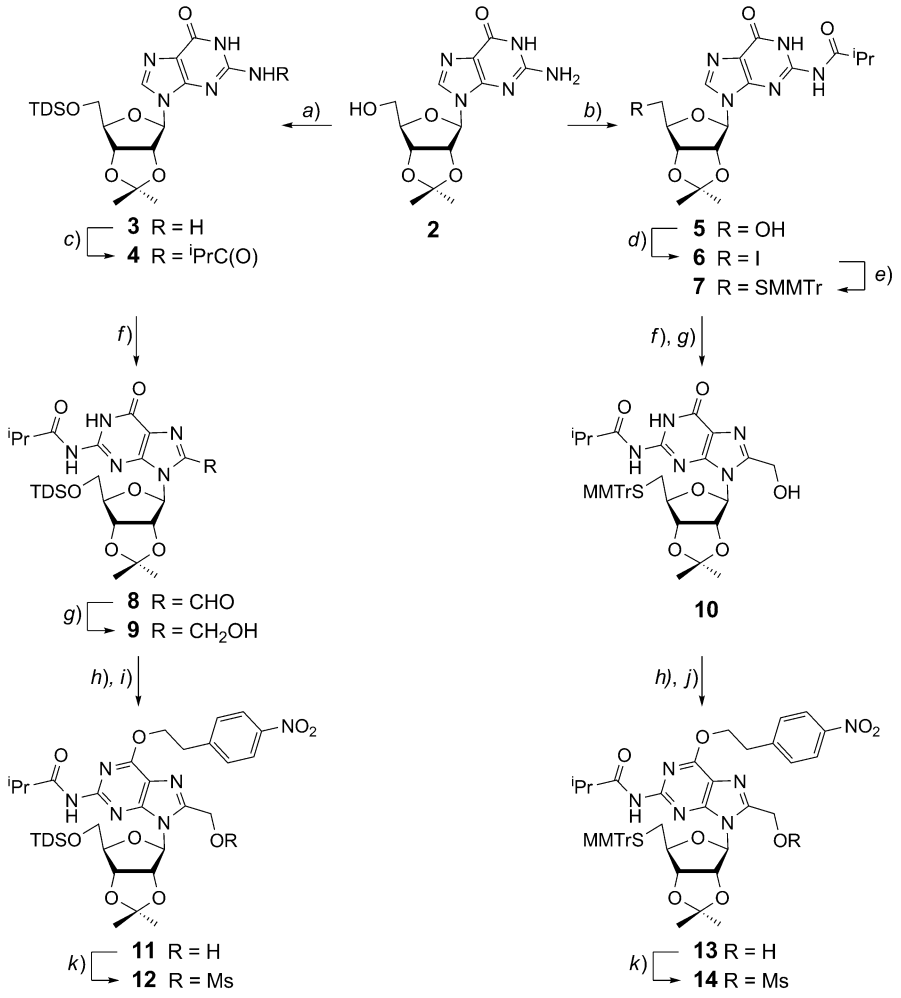
[4]) Monomethoxytritylthiol was synthesized from the corresponding alcohol according to a procedure developed by *Jahn-Hofmann* and *Engels* [15].

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

Scheme 1



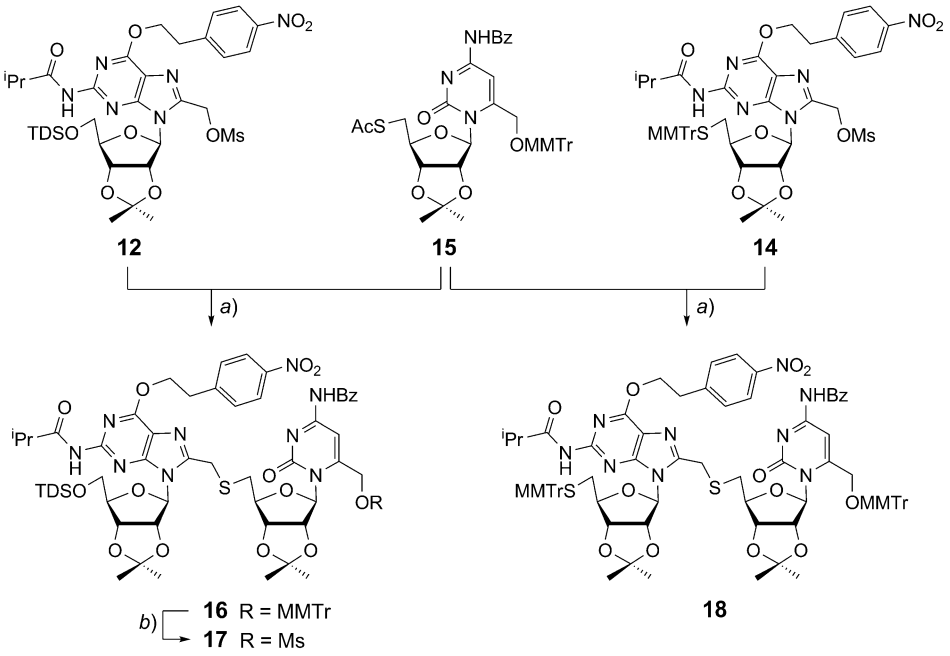
a) TDSCl, 1*H*-imidazole, DMF; 91%. *b*) Me_3SiCl, pyridine, CH_2Cl_2, then ^iPrCOCl, then MeOH and H_2O; 84%. *c*) $(^iPrCO)_2O$, 4-(dimethylamino)pyridine (DMAP), pyridine; 95%. *d*) $(PhO)_3PMeI$, MeCN; 93%. *e*) MMTrSH, NaH, THF; 90%. *f)* Lithium bis(trimethylsilyl)amide (LiHMDS), DMF, THF, $-78° \rightarrow$ r.t; 80% of **8**. *g*) $NaBH_4$, EtOH; < 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 = thexyl(dimethyl)silyl (= dimethyl(1,1,2-trimethylpropyl)silyl), MMTr = (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 $Cl_2CHCOOH$ in the presence of $^iPr_3SiH$, and reaction of the crude alcohol with MsCl/EtN^iPr_2 gave methanesulfonate **17**.

Scheme 2



a) 1. MeSNa, THF/MeOH 1:1, –10°; 2. **12** or **14**, EtN^iPr_2, DMSO; 71% of **16**, 65% of **18**. *b*) 1. Cl_2CHCO_2H, $^iPr_3SiH$, CH_2Cl_2; 2. MsCl, EtN^iPr_2, CH_2Cl_2; 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 K_2CO_3 in MeOH/CH_2Cl_2) 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 isopropylidenated and silylated **25**, as this octanucleoside might well pair in $CHCl_3$ 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 NH_3 in MeOH to yield 50% of **25**. Finally, aqueous HCO_2H led to desilylation and complete deisopropylidenation 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 H_2O containing 10–20% of an aprotic cosolvent and at 0–5° [21] to detect exchangeable H-atoms.

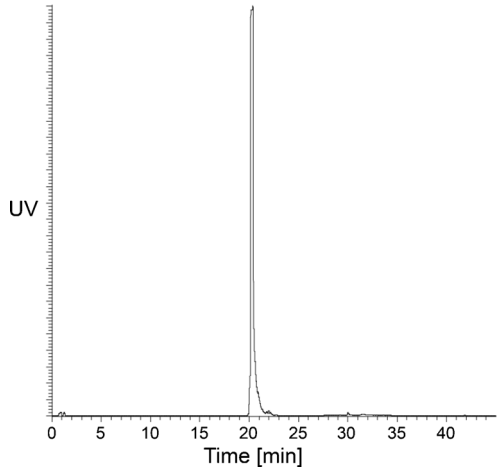
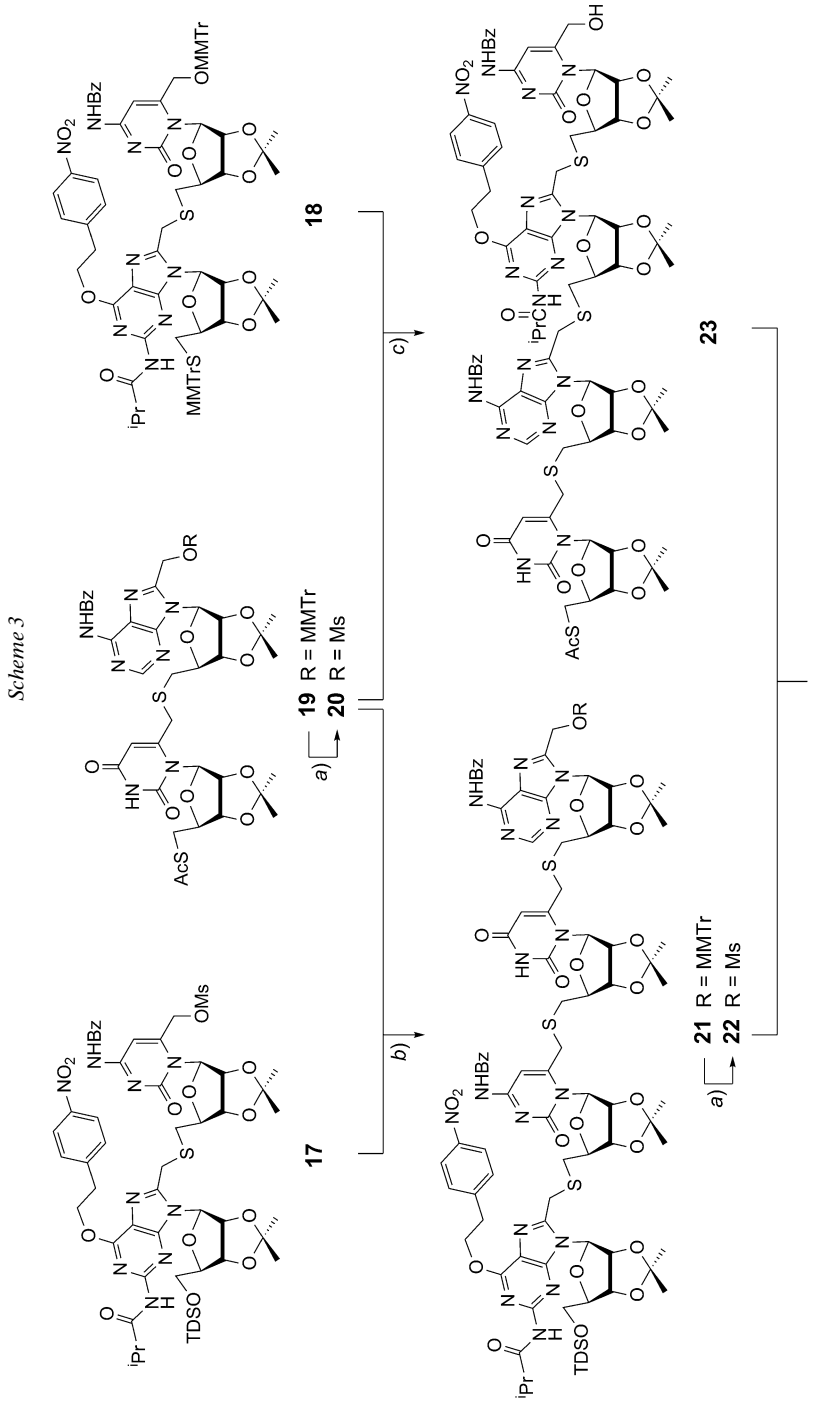
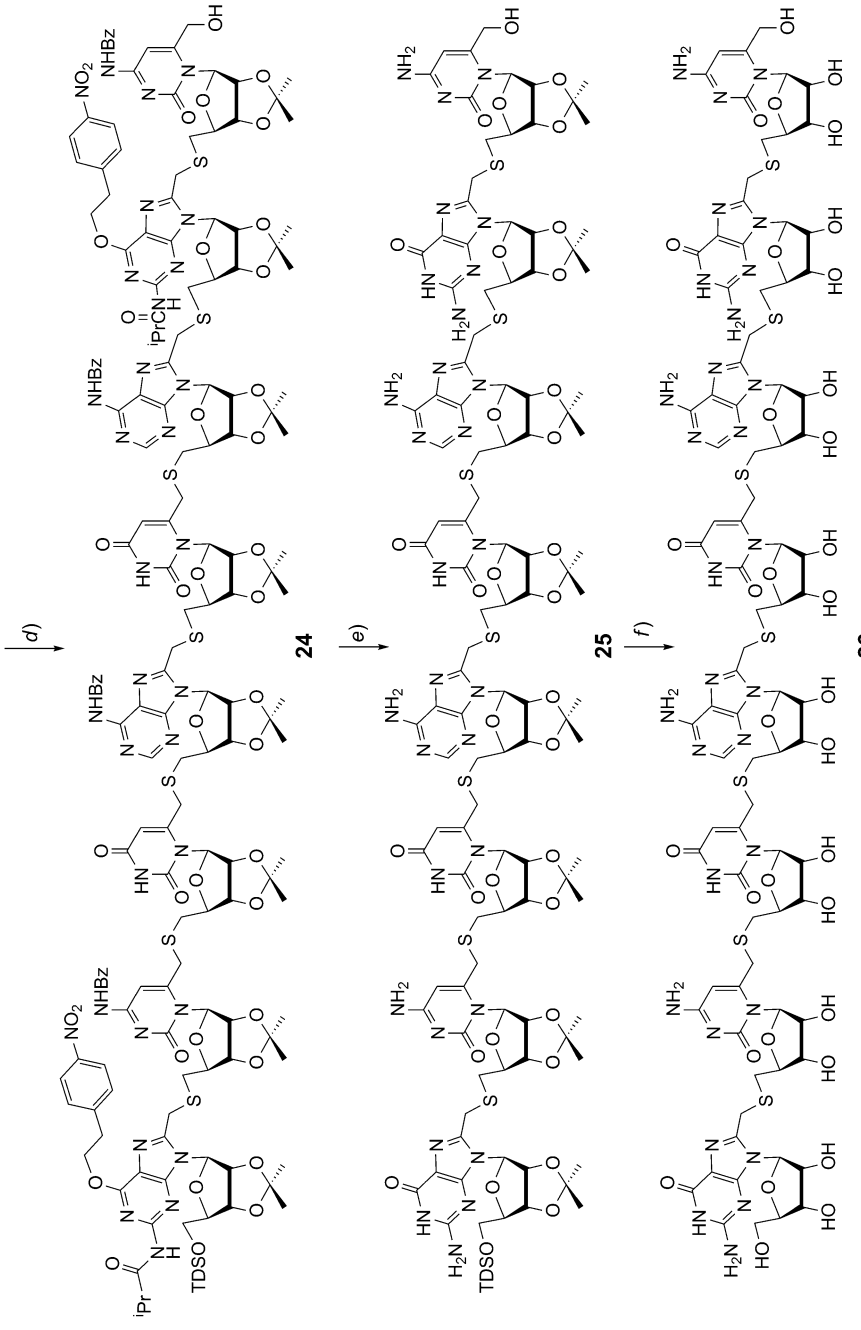


Fig. 2. *HPLC Trace of the completely deprotected octamer* **26** *after purification* (UV detection at 260 nm)

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

Scheme 3





a) 1. Cl_2CHCO_2H, $^iPr_3SiH$, CH_2Cl_2; 2. MsCl, EtN^iPr_2, CH_2Cl_2; 69% of **20**; 74% of **22**. *b*) 1. **19**, K_2CO_3, $MeOH/CH_2Cl_2$ 9 : 1; 2. **17**, EtN^iPr_2, DMSO; 65%. *c*) 1. **18**, CF_3CO_2H, $^iPr_3SiH$, CH_2Cl_2; 2. **20**, EtN^iPr_2, DMSO; 67%. *d*) 1. **23**, 1M aq. NaOH, pyridine/EtOH 2 : 1; 2. **22**, EtN^iPr_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*) HCO_2H/H_2O 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 (δ(H–C(2′)) = 5.20 – 5.30 ppm) in $CDCl_3$ adopt an *anti* conformation, whereas 8-unsubstituted and *O(5′)*-acetylated as well as *C(5′)*-sulfanylated derivatives (δ(H–C(2′)) ≈ 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 (δ(H–C(2′)) = 5.65 – 5.80 and 5.25 – 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 $CDCl_3$, 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′) (δ(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 : 15[6]), 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 (δ(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 $CDCl_3$ is evidenced by the *gg* orientation of HO–C(5′) (J(5′a,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 (D_6)DMSO [22] is completely cleaved, as evidenced by J(5′a,OH) = J(5′b,OH) = 5.1 Hz. The chemical shift δ(H–C(2′)) = 5.17 ppm suggests an exclusive *anti* conformation (as compared with **3** in $CDCl_3$), 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, δ(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 $CDCl_3$ to (D_6)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, δ(H–C(2′)) is influenced both by the protection of $HOCH_2$–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 – 0.8, whereas **3** adopts a 1 : 1 equilibrium between the (*N*) and the (*S*) conformer.

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[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 δ(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 ⋯ 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 CH_2S 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 Isopropylidenated Octanucleosides* **24** *and* **25**. The isopropylidenated octanucleosides **24** and **25** are well soluble in $CDCl_3$. Whereas the ^{1}H-NMR signals of **24** with protected C, G, and A nucleobases are well-resolved, those of **25** in $CDCl_3$, CD_2Cl_2, and $(CDCl_2)_2$ are broad, preventing the assignment of coupling constants. Neither heating (50° in $CDCl_3$) nor cooling (−50° in CD_2Cl_2) led to a sharpening of the signals. However, well-resolved NMR signals are observed in (D_6)DMSO where **25** is present as a solvated monoplex, as evidenced by δ(H–N(3/IV,VI)) = 11.4 and δ(H–N(1/II,VIII)) = 10.8 ppm (compare with 11.3 and 10.7 ppm, resp., for the corresponding H–N of monouridines and monoguanosines in (D_6)DMSO [6][22]). The octanucleosides **24** in $CDCl_3$ and **25** in (D_6)DMSO adopt similar conformations (see *Table 7* in the *Exper. Part*) as their di- and tetrameric precursors.

6. *Pairing of the Isopropylidenated and Silylated Octanucleoside* **25** *in Chlorinated Solvents*. The ^{1}H-NMR spectrum of **25** in $(CDCl_2)_2$ at room temperature shows broad overlapping signals, and the ^{13}C-NMR spectrum exhibits lines for several sets of slowly equilibrating associates[7]). Even the ^{1}H-NMR signals for the TDS group at *ca.* 0 ppm ($SiMe_2$) and at 0.7–0.9 ppm ($SiCMe_2CHMe_2$) evidence a mixture of associates. The

Table 1. *NH Signals above 8 ppm of the Silylated and Isopropylidenated Octanucleoside* **25** *in* $(CDCl_2)_2$

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 SiC*Me*$_2$CH*Me*$_2$ 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 ^{1}H-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 ^{1}H 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 (CH_2(5′) and CH_2–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 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 CH_2–C(6/8) and CH_2(5′), between H–C(4′) and H–C(1′), and between CH_2–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 (→ visible signals) and four units with a loose, rapidly exchanging base pairing (→ signals hidden by coalescence). According to the chemical shifts[8]), the most abundant duplex (δ(HN) = 13.88, 13.55, 13.35, and 11.72 ppm) possesses three stable *Watson–Crick* base pairs and a free guanosine unit[9]), and the least abundant duplex (δ(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 (δ(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

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[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 H_2O/D_2O 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 $CHCl_3$ 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].

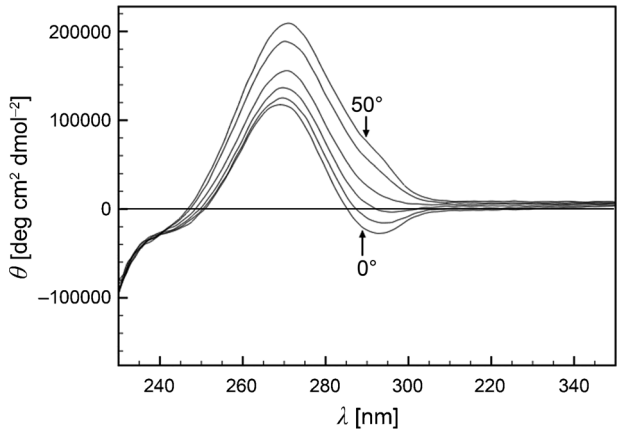

Fig. 3. *Temperature-dependent CD spectra in 10° steps from 0 to 50° of the isopropylidenated and silylated octanucleoside* **25** *for a 0.1-m*M *solution in* $CHCl_3$

7. *Qualitative Pairing Studies of the Fully Deprotected* **26** *in Aqueous Solutions.* The fully deprotected octanucleoside **26** is sufficiently soluble in H_2O 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 μM, 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

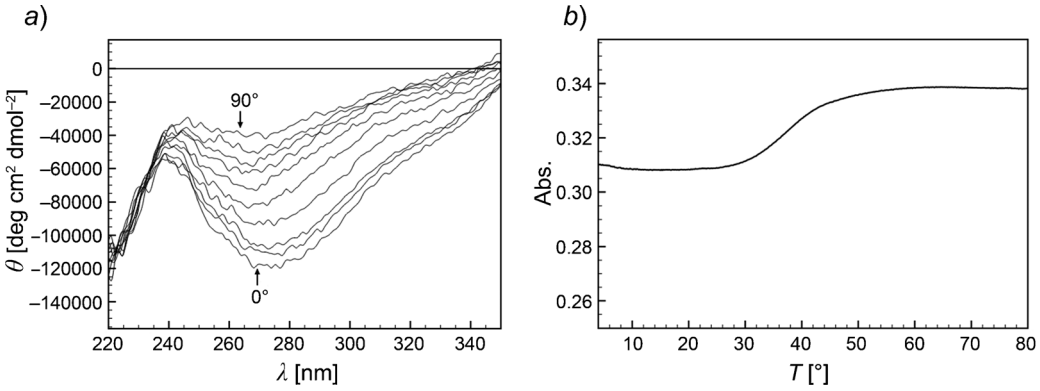

Fig. 4. a) *Temperature-dependent CD spectra in 10° steps from 0 to 90° and* b) *temperature-dependent UV spectrum at 260 nm of the deprotected octanucleoside* **26** (4 μM in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0)

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 °/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 [°]	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 deisopropylidenation is favorable for the formation of a cyclic duplex with complete base pairing. The association of higher isopropylidenated 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.

We thank Prof. Dr. *Bernhard Jaun* and Dr. *Marc-Olivier Ebert* for helpful discussions and the *Swiss National Science Foundation* and *Syngenta AG*, Basel, for financial support.

Experimental Part

General. Solvents were distilled, *i.e.*, THF from Na/benzophenone, CH_2Cl_2, MeOH, DMF, pyridine, $^iPr_2NH$, and EtN^iPr_2 from CaH_2. Reactions were run under Ar or N_2. 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/H_2O/HCO_2H 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. ^{1}H- and ^{13}C-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/H_2O, 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-isopropylideneguanosine (**3**). DMF (50 ml, freshly distilled from CaH_2) was transferred *via* cannula into a 250-ml flat-bottom flask equipped with mechanical stirring and a drying tube ($CaCl_2$), followed by addition at 26° with **2** [11] (recrystallized from H_2O, finely ground and dried (P_2O_5); 11.91 g, 36.8 mmol), 1*H*-imidazole (7.5 g, 110 mmol), and thexyl(dimethyl)silyl chloride (TDSCl; 7.5 ml, 37.8 mmol, dropwise addn. over 15 min). The mixture was stirred for 4 h, diluted with CH_2Cl_2 (300 ml), and washed with H_2O (100 ml). The aq. layer was extracted with CH_2Cl_2 (2 × 150 ml). The combined org. layers were washed with brine (3 × 150 ml) and evaporated. The residual yellow oil was treated with H_2O (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/H_2O 7:2:1) 0.81. M.p. 254°. FT-IR (ATR): 3313*w*, 3166*w*, 2956*w*, 2867*w*, 1686*m*, 1630*m*, 1595*m*, 1538*m*, 1487*m*, 1373*m*, 1316*w*, 1251*m*, 1211*m*, 1156*m*, 1073*s*, 842*s*, 778*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 3*; additionally, 12.06 (br. *s*, exchanged with D_2O, H–N(1)); 6.14 (br. *s*, exchanged with D_2O, H_2N–C(2)); 1.61, 1.39 (2*s*, Me_2CO_2); 1.59 (*sept.*, $J \approx 6.9$, Me_2CH); 0.86 (*d*, $J = 6.8$, Me_2CHSi); 0.83 (*s*, Me_2CSi); 0.09, 0.08 (2*s*, Me_2Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 4*; additionally, 113.81 (*s*, Me_2CO_2); 34.05 (*d*, Me_2CH); 27.22, 25.51 (2*q*, Me_2CO_2); 25.29 (*s*, Me_2CSi); 18.51 (*q*, Me_2CSi); 20.31 (*q*, Me_2CH); −3.30, −3.43 (2*q*, $SiMe_2$). HR-ESI-MS: 953.4014 (79, $[2M+Na]^+$, $C_{42}H_{70}N_{10}NaO_{10}Si^+$; calc. 953.4707), 488.2308 (100, $[M+Na]^+$, $C_{21}H_{35}N_5NaO_5Si^+$; calc. 488.2300).

5′-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N^2-isobutyryl-2′,3′-O-isopropylideneguanosine (**4**). Isobutyric 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 Et_2O (500 ml), and washed with sat. $NaHCO_3$ soln. (3 × 100 ml). The combined aq. layers were extracted with Et_2O (200 ml). The combined org. layers were washed with 0.001M HCl (5 × 150 ml) and H_2O (2 × 200 ml), dried ($MgSO_4$), 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 Et_2O/cyclohexane by slow evaporation of Et_2O. White needles. R_f (MeOH/CH_2Cl_2 1:9) 0.89. M.p. 134–136°. $[\alpha]_D^{25} = -100.3$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 286 (12630), 264 (14800). FT-IR (ATR): 3512*w*, 3416*w*, 3221*w*, 3016*m*, 2963*m*, 2865*w*, 1697*s*, 1605*s*, 1559*m*, 1471*w*,

Table 3. *Selected ^{1}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
$J(H_a,H_b)$	–	–	–	–	–	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 (D_6)DMSO. [c]) CH=O. [d]) Not assigned.

Table 4. *Selected ^{13}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 (D_6)DMSO. [c]) Assignments may be interchanged. [d]) CHO–C(8).

1403*w*, 1377*w*, 1313*w*, 1221*m*, 1135*m*, 1090*m*, 968*w*, 837*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 3*; additionally, 12.23 (br. *s*, exchanged with D_2O, H–N(1)); 10.07 (br. *s*, exchanged with D_2O, HN–C(2)); 2.77 (*sept.*, $J = 6.9$, $Me_2CHC{=}O$); 1.53 (*sept.*, $J \approx 6.9$, Me_2CHCMe_2Si); 1.50, 1.28 (2*s*, Me_2CO_2); 1.21, 1.19 (2*d*, $J = 6.6$, $Me_2CHC{=}O$); 0.80 (*d*, $J = 6.9$, Me_2CHCMe_2Si); 0.77, 0.76 (2*s*, Me_2CSi); 0.04, 0.02 (2*s*, Me_2Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 4*; additionally, 179.63 (*s*, NC=O); 113.92 (*s*, Me_2CO_2); 35.97 (*d*, $Me_2CHC{=}O$); 33.85 (*d*, Me_2CHCMe_2Si); 26.99, 25.15 (2*q*, Me_2CO_2); 25.15 (*s*, Me_2CSi); 20.17, 20.07 (2*q*, Me_2CHCMe_2Si); 18.95, 18.89 (2*q*, $Me_2CHC{=}O$); 18.31, 18.27 (2*q*, Me_2CSi); −3.57, −3.71 (2*q*, $SiMe_2$). HR-ESI-MS: 537.2930 (29), 536.2893 (100, M^+, $C_{25}H_{42}N_5O_6Si^+$; calc. 536.2899).

N^2-*Isobutyryl-2′,3′*-O-*isopropylideneguanosine* (**5**) [13]. A suspension of **2** (24.6 g, 76.1 mmol) in CH_2Cl_2 (300 ml) and pyridine (75 ml) was cooled to 0°, treated with Me_3SiCl (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 CH_2Cl_2 was washed with H_2O (2×), dried ($MgSO_4$), and evaporated. Filtration through a pad of silica gel (CH_2Cl_2/MeOH 95:5) gave **5** (25.2 g, 84%). Colourless solid.

5′-Deoxy-5′-iodo-N^2-*isobutyryl-2′,3′*-O-*isopropylideneguanosine* (**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% $Na_2S_2O_3$ soln., H_2O, and brine, dried ($MgSO_4$), and evaporated. FC (CH_2Cl_2/MeOH 99:1 → 39:1 → 9:1) gave **6** (14.0 g, 93%). Pale-yellow foam. R_f (CH_2Cl_2/MeOH 19:1) 0.39. $[\alpha]_D^{25} = -24.2$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 256 (13940), 284 (10530). IR (ATR): 3143*w*, 2976*w*, 2935*w*, 2874*w*, 1669*s*, 1603*s*, 1556*s*, 1477*m*, 1401*m*, 1374*m*, 1315*w*, 1252*m*, 1208*m*, 1154*s*, 1076*s*, 1019*m*, 969*w*, 947*w*, 872*m*, 856*m*, 816*m*, 782*m*, 750*m*, 721*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 3*; additionally, 12.17 (br. *s*, H–N(1)); 9.31 (br. *s*, HN–C(2)); 2.71 (*sept.*, $J = 6.8$, Me_2CH); 1.58, 1.36 (2*s*, Me_2CO_2); 1.27, 1.26 (2*d*, $J = 6.8$, Me_2CH). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 4*; additionally, 179.91 (*s*, NHC=O); 114.73 (*s*, Me_2CO_2); 36.15 (*d*, Me_2CH); 27.10, 25.28 (2*q*, Me_2CO_2); 19.10, 19.05 (2*q*, Me_2CH). HR-MALDI-MS: 504.0744 (100, $[M + H]^+$, $C_{17}H_{23}IN_5O_5^+$; calc. 504.0738). Anal. calc. for $C_{17}H_{22}IN_5O_5$ (503.30): C 40.57, H 4.41, N 13.91; found: C 40.86, H 4.52, N 13.94.

N^2-*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 MMTrSH (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. NH_4Cl soln. The org. solvents were evaporated, and the aq. phase was extracted with AcOEt (3×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (CH_2Cl_2/MeOH 39:1) gave **7** (613 mg, 90%). Colourless foam. R_f (CH_2Cl_2/MeOH 19:1) 0.48. $[\alpha]_D^{25} = +9.9$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 254 (18690), 282 (12890). IR (ATR): 3152*w*, 3058*w*, 2977*w*, 2934*w*, 2835*w*, 1672*s*, 1604*s*, 1557*s*, 1507*m*, 1484*m*, 1467*m*, 1443*m*, 1413*m*, 1402*m*, 1374*m*, 1304*w*, 1250*m*, 1210*m*, 1181*m*, 1155*m*, 1078*s*, 1033*m*, 947*w*, 909*w*, 874*w*, 821*w*, 783*w*, 758*w*, 739*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 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.*, $J = 6.8$, Me_2CH); 1.50, 1.26 (2*s*, Me_2CO_2); 1.23, 1.21 (2*d*, $J = 6.8$, Me_2CH). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 4*; additionally, 178.65 (*s*, NHC=O); 158.39 (*s*); 144.69 (2*s*); 136.39 (*s*); 130.81, 129.49–126.98 (several *d*); 114.57 (*s*, Me_2CO_2); 113.39 (2*d*); 66.88 (*s*, Ph_2C); 55.40 (*q*, MeO); 36.60 (*d*, Me_2CH); 27.19, 25.48 (2*q*, Me_2CO_2); 19.20, 19.07 (2*q*, Me_2CH). HR-MALDI-MS: 720.2234 (16, $[M + K]^+$, $C_{37}H_{39}KN_5O_6S^+$; calc. 720.2253), 704.2499 (81, $[M + Na]^+$, $C_{37}H_{39}N_5NaO_6S^+$; calc. 704.2513), 273.1274 (100, $MMTr^+$, $C_{20}H_{17}O^+$; calc. 273.1274).

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]-8-formyl*-N^2-*isobutyryl-2′,3′*-O-*isopropylideneguanosine* (**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. NaH_2PO_4 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. $[\alpha]_D^{25} = +6.2$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 335 (18420), 322 (19300), 290 (6040,

shoulder), 277 (4040, shoulder). FT-IR ($CHCl_3$): 3411*w*, 3194*w*, 2960*w*, 2868*w*, 1726*m*, 1693*s*, 1604*s*, 1555*s*, 1467*w*, 1429*m*, 1384*w*, 1312*w*, 1091*m*, 949*w*, 865*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 3*; additionally, 12.29 (br. *s*, exchanged with D_2O, H–N(1)); 9.02 (br. *s*, exchanged with D_2O, HN–C(2)); 2.71 (*sept.*, $J = 6.9$, Me_2CHC=O); 1.57 (*sept.*, $J = 6.8$, Me_2CHCMe_2Si); 1.54, 1.32 (2*s*, Me_2CO_2); 1.28, 1.25 (2*d*, $J = 6.9$, Me_2CHC=O); 0.85, 0.84 (2*d*, $J = 6.8$, Me_2CHCMe_2Si); 0.830, 0.825 (2*s*, Me_2CSi); 0.08, 0.07 (2*s*, Me_2Si). ^{13}C-NMR (125 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 4*; additionally, 179.01 (*s*, HNC=O); 115.05 (*s*, Me_2CO_2); 36.60 (*d*, Me_2CHC=O); 34.07 (*d*, Me_2CHCMe_2Si); 27.23, 25.45 (2*q*, Me_2CO_2); 25.45 (*s*, Me_2CSi); 20.33 (*q*, Me_2CHCMe_2Si); 19.02 (*q*, Me_2CHC=O); 18.47, 18.45 (2*q*, Me_2CSi); −3.29, −3.35 (2*q*, Me_2Si). HR-MALDI-MS (3-HPA): 564.2850 (100, $[M+H]^+$, $C_{26}H_{42}N_5O_7Si^+$; calc. 564.2848). Anal. calc. for $C_{26}H_{41}N_5O_7Si$ (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-isopropylideneguanosine (**9**) [16]. A suspension of $NaBH_4$ (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. NH_4Cl soln. (30 ml) and CH_2Cl_2 (150 ml). The layers were separated, and the aq. layer was extracted with CH_2Cl_2 (50 ml). The combined org. layers were dried ($MgSO_4$), 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.). $[\alpha]_D^{25} = +15.9$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 291 (12150), 262 (14160). FT-IR ($CHCl_3$): 3550*w*, 3419*w*, 3206*w*, 2975*s*, 1698*s*, 1606*s*, 1561*s*, 1427*m*, 1377*m*, 1257*m*, 1155*m*, 1080*s*, 1046*s*, 974*w*, 950*w*, 876*m*, 837*m*. ^{1}H-NMR (300 MHz, (D_6)DMSO: see *Table 3*; additionally, 12.09 (br. *s*, exchanged with D_2O, H–N(1)); 11.37 (br. *s*, exchanged with D_2O, HN–C(2)); 5.70 (br. *s*, exchanged with D_2O, OH); 2.78 (*sept.*, $J = 6.9$, Me_2CHC=O); 1.52, 1.33 (2*s*, Me_2CO_2); 1.47 (*sept.*, $J = 6.9$, Me_2CHCMe_2Si); 1.14 (*d*, $J = 6.6$, Me_2CHC=O); 0.76, 0.75 (2*d*, $J = 6.9$, Me_2CHCMe_2Si); 0.71, 0.70 (2*s*, Me_2CSi); −0.08, −0.10 (2*s*, Me_2Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 4*; additionally, 179.31 (*s*, NC=O); 114.48 (*s*, Me_2CO_2); 36.01 (*d*, Me_2CHC=O); 33.95 (*d*, Me_2CHCMe_2Si); 27.11, 25.36 (2*q*, Me_2CO_2); 25.36 (*s*, Me_2CSi); 20.31, 20.24 (2*q*, Me_2CHCMe_2Si); 19.14, 18.94 (2*q*, Me_2CHC=O); 18.42, 18.39 (2*q*, Me_2CSi); −3.42 (*q*, Me_2Si). HR-MALDI-MS (3-HPA): 588.2825 (25, $[M+Na]^+$, $C_{26}H_{43}N_5NaO_7Si^+$; calc. 588.2824), 566.3004 (100, $[M+H]^+$, $C_{26}H_{44}N_5O_7Si^+$; 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 H_2O (100 ml). The mixture was extracted with AcOEt (3×). The combined org. phases were washed with 0.1M HCl, H_2O (2×) and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue in EtOH (120 ml) was added dropwise to a suspension of $NaBH_4$ (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 H_2O (2×) and brine, dried ($MgSO_4$), and evaporated. FC (CH_2Cl_2/MeOH 24:1) gave **10** (5.90 g, 74%). Colourless foam. R_f (toluene/acetone 1:1) 0.50. $[\alpha]_D^{25} = +74.9$ ($c = 1.0$, $CHCl_3$). IR (ATR): 3550*w*, 3160*w*, 2970*w*, 2931*w*, 1736*m*, 1710*m*, 1676*s*, 1603*s*, 1556*s*, 1507*m*, 1489*m*, 1425*m*, 1373*s*, 1301*w*, 1248*s*, 1216*m*, 1181*m*, 1155*m*, 1075*s*, 1032*s*, 949*w*, 907*w*, 862*m*, 820*w*, 790*m*, 758*w*, 725*s*, 699*s*. ^{1}H-NMR (400 MHz, $CDCl_3$; 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.*, $J = 6.9$, Me_2CH); 1.48, 1.25 (2*s*, Me_2CO_2); 1.23, 1.21 (2*d*, $J = 6.9$, Me_2CH). ^{13}C-NMR (100 MHz, $CDCl_3$; 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 (3*s*); 130.73–126.81 (several *d*); 114.54 (*s*, Me_2CO_2); 113.39 (2*d*); 66.56 (*s*, Ph_2C); 55.34 (*q*, MeO); 36.32 (*d*, Me_2CH); 27.20, 25.55 (2*q*, Me_2CO_2); 19.11, 19.05 (2*q*, Me_2CH). HR-MALDI-MS: 750.2374 (24, $[M+K]^+$, $C_{38}H_{41}KN_5O_7S^+$; calc. 750.2358), 734.2606 (52, $[M+Na]^+$, $C_{38}H_{41}N_5NaO_7S^+$; calc. 734.2619), 712.2815 (32, $[M+H]^+$, $C_{38}H_{42}N_5O_7S^+$; calc. 712.2799), 273.1270 (100, $MMTr^+$, $C_{20}H_{17}O^+$; 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)-1*H*-imidazole (1.35 ml, 9.11 mmol) and stirred for 90 min. The mixture was treated with PPh_3 (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/H_2O 1:1, and extracted with AcOEt (3×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue in AcOH/H_2O 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. $NaHCO_3$ soln., H_2O, and brine, dried ($MgSO_4$), 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. $[\alpha]_D^{25} = +23.4$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 274 (27890). IR (ATR): 3296*w*, 2959*w*, 2871*w*, 1721*w*, 1610*m*, 1591*m*, 1517*s*, 1457*m*, 1423*m*, 1381*m*, 1342*s*, 1248*m*, 1216*m*, 1183*m*, 1155*m*, 1071*s*, 974*w*, 906*w*, 828*s*, 794*m*, 777*m*, 745*m*. ^{1}H-NMR (400 MHz, $CDCl_3$; 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*, $J = 6.9$, CH_2CH_2O); 3.59 (br. *t*, $J = 5.9$, OH); 3.31 (*t*, $J = 6.9$, CH_2CH_2O); 3.15–3.01 (br. *s*, $Me_2CHC{=}O$); 1.61, 1.39 (2*s*, Me_2CO_2); 1.58 (*sept.*, $J = 6.9$, Me_2CH); 1.28, 1.27 (2*d*, $J = 6.9$, $Me_2CHC{=}O$); 0.83 (*d*, $J = 6.9$, Me_2CH); 0.81, 0.80 (2*s*, Me_2CSi); 0.05, 0.04 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$; 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*, Me_2CO_2); 66.83 (*t*, CH_2CH_2O); 35.73 (*d*, $Me_2CHC{=}O$); 35.09 (*t*, CH_2CH_2O); 34.03 (*d*, Me_2CH); 27.20, 25.41 (2*q*, Me_2CO_2); 25.46 (*s*, Me_2CSi); 20.33, 20.28 (2*q*, Me_2CSi); 19.39, 19.27 (2*q*, $Me_2CHC{=}O$); 18.41, 18.39 (2*q*, Me_2CH); −3.47, −3.49 (2*q*, Me_2Si). HR-MALDI-MS: 737.3289 (59, $[M + Na]^+$, $C_{34}H_{50}N_6NaO_9Si^+$; calc. 737.3301), 715.3482 (100, $[M + H]^+$, $C_{34}H_{51}N_6O_9Si^+$; calc. 715.3481). Anal. calc. for $C_{34}H_{50}N_6O_9Si$ (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 CH_2Cl_2 (150 ml) was cooled to 0°, treated with EtN^iPr_2 (1.70 ml, 9.7 mmol) and dropwise with MsCl (0.75 ml, 9.7 mmol), stirred for 30 min, and poured into H_2O. The phases were separated, and the aq. phase was extracted with CH_2Cl_2 (2×). The combined org. phases were washed with H_2O (2×) and brine, dried ($MgSO_4$), 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. $[\alpha]_D^{25} = +12.6$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 275 (27360). IR (ATR): 3300*w* (br.), 2960*w*, 2871*w*, 1721*w*, 1610*m*, 1590*m*, 1518*m*, 1459*m*, 1427*m*, 1342*s*, 1246*m*, 1214*m*, 1174*s*, 1155*m*, 1074*s*, 1040*m*, 1000*w*, 946*m*, 828*w*, 796*m*, 776*m*, 737*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 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*, $J = 6.9$, CH_2CH_2O); 3.32 (*t*, $J = 6.9$, CH_2CH_2O); 3.08 (*s*, MsO); 2.99–2.88 (br. *s*, $Me_2CHC{=}O$); 1.55, 1.41 (2*s*, Me_2CO_2); 1.53 (*sept.*, $J = 6.8$, Me_2CH); 1.29, 1.27 (2*d*, $J = 6.9$, $Me_2CHC{=}O$); 0.81 (*d*, $J = 6.8$, Me_2CH); 0.77, 0.76 (2*s*, Me_2CSi); 0.03 (*s*, Me_2Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 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*, Me_2CO_2); 67.14 (*t*, CH_2CH_2O); 38.54 (*q*, MsO); 36.19 (*d*, $Me_2CHC{=}O$); 35.15 (*t*, CH_2CH_2O); 34.18 (*d*, Me_2CH); 27.33, 25.62 (2*q*, Me_2CO_2); 25.37 (*s*, Me_2CSi); 20.39, 20.37 (2*q*, Me_2CSi); 19.51, 19.37 (2*q*, $Me_2CHC{=}O$); 18.54, 18.51 (2*q*, Me_2CH); −3.38, −3.41 (2*q*, Me_2Si). HR-MALDIMS: 793.3271 (100, $[M + H]^+$, $C_{35}H_{53}N_6O_{11}SSi^+$; calc. 793.3257). Anal. calc. for $C_{35}H_{52}N_6O_{11}SSi$ (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)-1*H*-imidazole (1.34 g, 9.55 mmol) and stirred for 2 h. The mixture was treated with PPh_3 (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 H_2O (100 ml), and extracted with AcOEt (3×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue in MeOH (50 ml) was treated with NH_4F (1.60 g, 43.4 mmol) and stirred for 2 h. The mixture was poured into H_2O and extracted with AcOEt (3×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (pentane/AcOEt 2:3) gave **13** (5.82 g, 78%). Yellow foam. R_f (pentane/AcOEt 2:3) 0.22. $[\alpha]_D^{25} = +71.6$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 274 (29340). IR (ATR): 3300*w* (br.), 3054*w*, 2975*w*, 2931*w*, 1719*m*, 1607*m*, 1592*m*, 1515*s*, 1508*s*, 1458*m*, 1425*m*, 1381*m*,

1343*s*, 1299*w*, 1246*s*, 1217*s*, 1180*s*, 1155*m*, 1071*s*, 1033*s*, 1000*m*, 906*w*, 856*m*, 822*w*, 793*m*, 741*m*, 724*m*. ^{1}H-NMR (300 MHz, $CDCl_3$; 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*, CH_2CH_2O); 3.76 (*s*, MeO); 3.33 (*t*, J = 6.9, CH_2CH_2O); 2.92 (*t*, J = 6.2, OH); 2.86 (br. *sept.*, J = 6.5, Me_2CH); 1.53, 1.34 (2*s*, Me_2CO_2); 1.23 (*d*, J = 6.9, Me_2CH). ^{13}C-NMR (75 MHz, $CDCl_3$; 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*, Me_2CO_2); 113.06 (2*d*); 66.98 (*t*, CH_2CH_2O); 66.32 (*s*, Ph_2C); 55.30 (*q*, MeO); 36.18 (*d*, Me_2CH); 35.21 (*t*, CH_2CH_2O); 27.22, 25.58 (2*q*, Me_2CO_2); 19.56, 19.50 (2*q*, Me_2CH). HR-MALDI-MS: 883.3100 (100, $[M + Na]^+$, $C_{46}H_{48}N_6NaO_9S^+$; calc. 883.3096). Anal. calc. for $C_{46}H_{48}N_6O_9S$ (860.99): C 64.17, H 5.62, N 9.76; found: C 63.93, H 5.72, N 9.56.

N^2-*Isobutyryl-2′,3′*-O-*isopropylidene-8-{[(methylsulfonyl)oxy]methyl}-5′*-S-*[(4-methoxyphenyl)diphenylmethyl]*-O^6-*[2-(4-nitrophenyl)ethyl]-5′-thioguanosine* (**14**). A soln. of **13** (5.25 g, 6.1 mmol) in CH_2Cl_2 (40 ml) was cooled to 0°, treated with EtN^{i}Pr$_2$ (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 CH_2Cl_2 (2×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), 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. $[\alpha]_D^{25} = +35.1$ (c = 1.0, $CHCl_3$). UV ($CHCl_3$): 276 (27030). IR (ATR): 3400–3200*w* (br.), 2974*w*, 2934*w*, 1720*w*, 1606*m*, 1594*m*, 1508*s*, 1458*m*, 1427*m*, 1342*s*, 1300*w*, 1247*m*, 1213*s*, 1174*s*, 1154*s*, 1088*m*, 1073*m*, 1033*m*, 1000*m*, 969*m*, 947*m*, 856*m*, 822*m*, 793*m*, 759*w*, 742*m*, 699*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 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*, CH_2CH_2O); 3.76 (*s*, MeO); 3.34 (*t*, J = 6.9, CH_2CH_2O); 3.04 (*s*, MsO); 2.85 (br. *sept.*, J = 6.7, Me_2CH); 1.55, 1.35 (2*s*, Me_2CO_2); 1.24 (*d*, J = 6.9, Me_2CH). ^{13}C-NMR (75 MHz, $CDCl_3$): 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*, Me_2CO_2); 113.17 (2*d*); 67.15 (*t*, CH_2CH_2O); 66.31 (*s*, Ph_2C); 55.28 (*q*, MeO); 38.40 (*q*, MsO); 36.16 (*d*, Me_2CH); 35.11 (*t*, CH_2CH_2O); 27.10, 25.50 (2*q*, Me_2CO_2); 19.43, 19.36 (2*q*, Me_2CH). HR-MALDI-MS: 961.2873 (100, $[M + Na]^+$, $C_{47}H_{50}N_6NaO_{11}S_2$; calc. 961.2871). Anal. calc. for $C_{47}H_{50}N_6O_{11}S_2$ (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]*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8^1 → 5′*-S*)*-N^4-*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 H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **12** (4.00 g, 5.05 mmol) in DMSO (15 ml) was degassed, treated with EtN^{i}Pr$_2$ (2.64 ml, 15.2 mmol), and stirred for 6 h. Sat. NH_4Cl soln. was added, and the aq. phase was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (3×) and brine, dried ($MgSO_4$), 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. $[\alpha]_D^{25} = -46.7$ (c = 1.0, $CHCl_3$). UV ($CHCl_3$): 267 (47610). IR (ATR): 3500–3200*w* (br.), 2959*w*, 2936*w*, 2870*w*, 1679*m*, 1610*s*, 1587*m*, 1570*m*, 1517*m*, 1511*m*, 1457*m*, 1422*m*, 1372*m*, 1344*s*, 1300*m*, 1249*s*, 1212*s*, 1181*m*, 1154*m*, 1063*s*, 1033*m*, 1000*m*, 900*w*, 871*w*, 829*s*, 794*m*, 777*m*, 745*m*, 726*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 5*; additionally, 9.00 (br. *s*, BzN*H*); 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, CH_2CH_2O); 3.77 (*s*, MeO); 3.25 (*t*, J = 7.0, CH_2CH_2O); 3.01–2.89 (br. *s*, Me_2CHC=O); 1.60, 1.42 (6 H), 1.27 (3*s*, 2 Me_2CO_2); 1.51 (*sept.*, J = 6.9, Me_2CHCMe_2); 1.26 (*d*, J = 6.9, Me_2CHC=O); 0.79, 0.78 (2*d*, J = 6.9, Me_2CHCMe_2); 0.74, 0.73 (2*s*, Me_2CSi); −0.08, −0.09 (2*s*, Me_2Si). ^{13}C-NMR (125 MHz, $CDCl_3$; 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 Me_2CO_2); 113.46 (2*d*); 88.52 (*s*, Ph_2C); 66.83 (*t*, CH_2CH_2O); 55.23 (*q*, MeO); 35.91 (*d*, Me_2CHC=O); 35.10 (*t*, CH_2CH_2O); 34.07 (*d*, Me_2CHCMe_2); 27.23, 27.11, 25.53, 25.34 (4*q*, 2 Me_2CO_2); 25.22 (*s*, Me_2CSi); 20.29, 20.26 (2*q*, Me_2CSi); 19.42, 19.29 (2*q*, Me_2CHC=O); 18.44, 18.39 (2*q*,

Table 5. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Di- and Tetranucleosides* **16**–**18** *and* **20**–**23** *in* $CDCl_3$[a])

H-Atom	**16**	**17**	**18**	**20**	**21**	**22**	**23**
Adenosine unit							
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
$J(H_a,H_b)$				13.1	12.1	[b])	15.3
J(1′,2′)				1.8	2.0	2.3	1.6
J(2′,3′)				6.4	6.2	6.4	6.3
J(3′,4′)				4.3	4.7	4.0	3.7
J(4′,5′a)				8.3	[b])	5.8	[b])
J(4′,5′b)				4.3	[b])	7.4	[b])
J(5′a,5′b)				14.8	[b])	14.3	[b])
Uridine unit							
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
$J(H_a,H_b)$				14.9	14.6	[b])	[b])
J(1′,2′)				< 1.0	< 1.0	0.9	< 1.0
J(2′,3′)				6.4	6.4	6.4	6.4
J(3′,4′)				3.9	4.2	4.3	4.0
J(4′,5′a)				7.2	6.5	8.0	[b])
J(4′,5′b)				7.2	6.5	5.8	[b])
J(5′a,5′b)				[b])	[b])	14.4	[b])
Cytidine unit							
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
$J(H_a,H_b)$	[b])	13.4	[b])		14.6	14.5	14.5
J(1′,2′)	0.7	1.0	< 1.0		< 1.0	< 1.0	< 1.0
J(2′,3′)	6.4	6.5	6.1		6.3	6.3	6.0
J(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
Guanosine unit							
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$_2$CHCMe$_2$); −3.53 (*q*, Me$_2$Si). 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]*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8′ → 5′*-S*)*-N^4-*benzoyl-2′,3′*-O-*isopropylidene-6-(methanesulfonyloxymethyl)-5′-thiocytidine* (**17**). A soln. of **16** (625 mg, 446 µmol) in CH_2Cl_2 (3 ml) was treated with Cl_2CHCO_2H (300 µl) and $^iPr_3SiH$ (274 µl, 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 ×). 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 µl, 491 µmol) and MsCl (38 µl, 491 µmol), stirred for 30 min, and poured into ice/water. After extraction with AcOEt (3 ×), 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 – 3150*w* (br.), 2958*w*, 2936*w*, 2865*w*, 1682*m*, 1612*m*, 1585*m*, 1570*m*, 1519*m*, 1460*m*, 1422*m*, 1370*m*, 1343*s*, 1237*m*, 1212*m*, 1177*m*, 1155*m*, 1067*s*, 1012*m*, 1000*m*, 970*m*, 948*m*, 900*w*, 869*w*, 829*m*, 792*m*, 779*m*, 735*w*, 699*m*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 5*; additionally, 8.79 (br. *s*, BzN*H*); 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$_2$C*H*C=O); 1.60, 1.50, 1.42, 1.32 (4*s*, 2 Me$_2$CO$_2$); 1.51 (*sept.*, J = 6.9, Me$_2$C*H*CMe$_2$); 1.27, 1.26 (2*d*, J = 6.9, *Me*$_2$CHC=O); 0.78 (*d*, J = 6.9, *Me*$_2$CHCMe$_2$); 0.74, 0.73 (2*s*, Me$_2$CSi); −0.07, −0.08 (2*s*, Me$_2$Si). ^{13}C-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$_2$*C*O$_2$); 66.84 (*t*, CH_2CH_2O); 38.63 (*q*, MsO); 35.98 (*d*, Me$_2$*C*HC=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**
Adenosine unit							
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
CH_2–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
Uridine unit							
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
CH_2–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
Cytidine unit							
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
CH_2–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
Guanosine unit							
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
CH_2–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*, *C*H_2CH$_2$O); 34.05 (*d*, Me$_2$*C*HCMe$_2$); 27.21, 27.02, 25.50, 25.26 (4*q*, 2 *Me*$_2$CO$_2$); 25.22 (*s*, Me$_2$*C*Si); 20.28, 20.25 (2*q*, *Me*$_2$CSi); 19.40, 19.30 (2*q*, *Me*$_2$CHC=O); 18.43, 18.38 (2*q*, *Me*$_2$CHCMe$_2$); −3.53, −3.54 (2*q*, Me$_2$Si). HR-MALDI-MS: 1247.4035 (28), 1246.4010 (42, $[M + K]^+$, $C_{55}H_{73}KN_9O_{16}S_2Si^+$; calc. 1246.4018), 1232.4312 (34), 1231.4324 (60), 1230.4301 (84, $[M + Na]^+$, $C_{55}H_{73}N_9NaO_{16}S_2Si^+$; calc. 1230.4278), 887.3911 (23), 886.3910 (55), 885.3871 (100, $[M - C_{13}H_{12}N_3O_5S]^+$, $C_{42}H_{61}N_6O_{11}SSi^+$; calc. 885.3883). Anal. calc. for $C_{55}H_{73}N_9O_{16}S_2Si$ (1208.45): C 54.67, H 6.09, N 10.43; found: C 54.94, H 6.05, N 10.30.

N^2-*Isobutyryl-2′,3′*-O-*isopropylidene-5′*-S-*[(4-methoxyphenyl)diphenylmethyl]*-O^6-*[2-(4-nitrophenyl)-ethyl]-5′-thioguanosine-8-methyl-(8^1 → 5′*-S*)*-N^4-*benzoyl-2′,3′*-O-*isopropylidene-6-{[(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 H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **14** (690 mg, 735 μmol) in DMSO (1.5 ml) was degassed, treated with Et*N*^{i}Pr$_2$ (385 μl, 2.21 mmol), and stirred for 4 h. After addition of sat. NH_4Cl soln., the aq. phase was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (3×) and brine, dried ($MgSO_4$), and evaporated. FC (toluene/acetone 5:1 → 4:1) gave **18** (745 mg, 65%). Yellow foam. R_f (toluene/acetone 4:1) 0.18. $[\alpha]_D^{25} = -18.5$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 266 (49030). IR (ATR): 3500–3200*w* (br.), 3054*w*, 2958*w*, 2930*w*, 2852*w*, 1693*m*, 1676*m*, 1608*s*, 1508*s*, 1484*m*, 1457*m*, 1447*m*, 1422*m*, 1372*m*, 1344*s*, 1300*m*, 1249*s*, 1213*s*, 1180*s*, 1155*m*, 1086*s*, 1063*s*, 1033*s*, 1000*m*, 900*w*, 869*w*, 855*w*, 827*m*, 793*m*, 743*m*, 724*m*, 698*s*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 5*; additionally, 8.60 (br. *s*, BzN*H*); 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*, $J = 7.0$, CH_2CH_2O); 3.76, 3.74 (2*s*, 2 MeO); 3.28 (*t*, $J = 7.0$, CH_2CH_2O); 2.89–2.80 (br. *s*, Me$_2$C*H*); 1.54, 1.43, 1.35, 1.28 (4*s*, 2 Me$_2$CO$_2$); 1.22, 1.21 (2*d*, $J = 6.9$, *Me*$_2$CH). ^{13}C-NMR (125 MHz, $CDCl_3$; 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 Me$_2$*C*O$_2$); 113.46 (2*d*); 113.07 (2*d*); 88.56 (*s*, Ph$_2$*C*/I); 66.92 (*t*, CH_2CH_2O); 66.20 (*s*, Ph$_2$*C*/II); 55.23, 55.19 (2*q*, 2 MeO); 35.92 (*d*, Me$_2$*C*H); 35.15 (*t*, *C*H_2CH$_2$O); 27.10 (2 C), 25.48, 25.31 (3*q*, 2 *Me*$_2$CO$_2$); 19.36, 19.32 (2*q*, *Me*$_2$CH); 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, $[M + Na]^+$, $C_{86}H_{85}N_9NaO_{15}S_2^+$; calc. 1570.5499).

5′-S-*Acetyl-2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6^1 → 5′*-S*)*-N^6-*benzoyl-2′,3′*-O-*isopropylidene-8-{[(methylsulfonyl)oxy]methyl}-5′-thioadenosine* (**20**). A soln. of **19** [6] (1.10 g, 1 mmol) in CH_2Cl_2 (6 ml) was treated with Cl_2CHCO_2H (0.6 ml) and ^{i}Pr$_3$SiH (0.62 ml, 3 mmol), stirred for 90 min, and poured into ice and sat. $NaHCO_3$ soln. The mixture was extracted with CH_2Cl_2 (3×). 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 (6 ml) at 0° was treated with Et*N*^{i}Pr$_2$ (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 CH_2Cl_2 (3×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/pentane 4:1 → AcOEt) gave **20** (617 mg, 69%). Colourless foam. R_f (AcOEt/Et$_2$O 2:3) 0.20. $[\alpha]_D^{25} = -41.1$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 279 (25440). IR (ATR): 3200*w* (br.), 2987*w*, 2936*w*, 1687*s*, 1610*m*, 1579*w*, 1531*w*, 1502*w*, 1487*m*, 1448*m*, 1430*w*, 1371*m*, 1298*w*, 1243*s*, 1211*m*, 1173*s*, 1156*m*, 1083*s*, 1073*s*, 954*m*, 856*m*, 800*m*, 766*m*, 705*m*, 626*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 5*; additionally, 10.99 (br. *s*, H–N(3/II)); 10.05 (br. *s*, BzN*H*); 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 Me$_2$C). ^{13}C-NMR (100 MHz, $CDCl_3$; 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 Me$_2$*C*); 38.63 (*q*, MsO); 30.66 (*q*, *Me*C=O); 27.31, 27.26, 25.51, 25.37 (4*q*, 2 *Me*$_2$C). HR-MALDI-MS: 928.1746 (46, $[M + K]^+$, $C_{37}H_{43}KN_7O_{13}S_3^+$; calc. 928.1713), 912.1995 (100, $[M + Na]^+$, $C_{37}H_{43}N_7NaO_{13}S_3^+$; calc. 912.1973), 890.2163 (98, $[M + H]^+$, $C_{37}H_{44}N_7O_{13}S_3^+$; calc. 890.2154).

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8^1 → 5′*-S*)*-N^4-*benzoyl-2′,3′*-O-*isopropylidene-5′-thiocytidine-6-methyl-(6^1 → 5′*-S*)-2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6^1 → 5′*-S*)*-N^6-*benzoyl-2′,3′*-O-*isopropylidene-8-{[(4-methoxyphenyl)diphenylmethoxy]methyl}-5′-thioadenosine* (**21**). A soln. of **19** (1.09 g, 1 mmol) in MeOH/CH_2Cl_2 9:1 (20 ml) was treated with powdered K_2CO_3 (279 mg, 2 mmol), stirred for 35 min, and poured into sat. NH_4Cl soln. The mixture was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (2×) and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **17** (1.22 g, 1 mmol) in DMSO (6 ml) was degassed, treated with EtN^iPr_2 (0.53 ml, 3 mmol), stirred for 6 h, and poured into sat. NH_4Cl soln. The mixture was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (3×) and brine, dried ($MgSO_4$), 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. $[\alpha]_D^{25} = -74.0$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 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*. ^{1}H-NMR (500 MHz, $CDCl_3$; 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*, BzN*H*); 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*, CH_2CH_2O); 3.76 (*s*, MeO); 3.27 (*t*, $J = 7.1$, CH_2CH_2O); 3.00–2.92 (br. *s*, Me_2CHC=O); 1.52 (*sept.*, $J = 6.9$, Me_2CHCMe_2); 1.59, 1.54, 1.53, 1.48, 1.40, 1.36, 1.33, 1.32 (8*s*, 4 Me_2CO_2); 1.25 (*d*, $J = 6.9$, Me_2CHC=O); 0.79, 0.78 (2*d*, $J = 6.9$, Me_2CHCMe_2); 0.74, 0.73 (2*s*, Me_2CSi); −0.07, −0.09 (2*s*, Me_2Si). ^{13}C-NMR (125 MHz, $CDCl_3$; 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 Me_2CO_2); 113.40 (2*d*); 88.21 (*s*, Ph_2C); 66.82 (*t*, CH_2CH_2O); 55.24 (*q*, MeO); 35.85 (*d*, Me_2CHC=O); 35.09 (*t*, CH_2CH_2O); 34.04 (*d*, Me_2CHCMe_2); 27.24, 27.23, 27.15, 27.07, 25.54, 25.44, 25.21 (2 C) (7*q*, 4 Me_2CO_2); 25.20 (*s*, Me_2CSi); 20.28, 20.34 (2*q*, Me_2CSi); 19.41, 19.29 (2*q*, Me_2CHC=O); 18.43, 18.38 (2*q*, Me_2CHCMe_2); −3.54 (*q*, Me_2Si). HR-MALDI-MS: 2179.7910 (24), 2178.7855 (51), 2177.7845 (82), 2176.7841 (100), 2175.7851 (71, $[M + Na]^+$, $C_{108}H_{124}N_{16}NaO_{24}S_3Si^+$; calc. 2175.7798), 886.3902 (74), 885.3870 (74, $[M - C_{66}H_{63}N_{10}O_{13}S_2]^+$, $C_{42}H_{61}N_6O_{11}SSi^+$; calc. 885.3883). Anal. calc. for $C_{108}H_{124}N_{16}O_{24}S_3Si$ (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]*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8^1 → 5′*-S*)*-N^4-*benzoyl-2′,3′*-O-*isopropylidene-5′-thiocytidine-6-methyl-(6^1 → 5′*-S*)-2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6^1 → 5′*-S*)*-N^6-*benzoyl-2′,3′*-O-*isopropylidene-8-{[(methylsulfonyl)oxy]methyl}-5′-thioadenosine* (**22**). A soln. of **21** (90 mg, 42 μmol) in CH_2Cl_2 (2 ml) was treated with Cl_2CHCO_2H (0.2 ml) and $^iPr_3SiH$ (28 μl, 126 μmol), stirred for 1 h, and poured into sat. $NaHCO_3$ soln. The mixture was extracted with CH_2Cl_2 (3×). 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 (1.5 ml) at 0° was treated with EtN^iPr_2 (8 μl, 46 μmol) and MsCl (3.6 μl, 46 μmol), stirred for 30 min, and poured into H_2O. The mixture was extracted with CH_2Cl_2 (3×). The combined org. phases were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (toluene/acetone 2:1) gave **22** (61 mg, 74%). Pale-yellow foam. R_f (toluene/acetone 1:1) 0.54. $[\alpha]_D^{25} = -90.7$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 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*. ^{1}H-NMR (600 MHz, $CDCl_3$; 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 BzN*H*); 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*, CH_2CH_2O); 3.28–3.23 (*m*, CH_2CH_2O); 3.14 (*s*, MsO); 2.98–2.93 (br. *s*, Me_2CHC=O); 1.50 (*sept.*, $J = 6.9$, Me_2CHCMe_2); 1.63, 1.60, 1.55, 1.50, 1.41, 1.40, 1.33 (6 H) (7*s*, 4 Me_2CO_2); 1.25 (*d*, $J = 6.9$, Me_2CHC=O); 0.79 (*d*, $J = 6.9$, Me_2CHCMe_2); 0.74, 0.73 (2*s*, Me_2CSi); −0.07, −0.08 (2*s*, Me_2Si). ^{13}C-NMR (150 MHz, $CDCl_3$; 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 Me_2CO_2); 66.86 (*t*, CH_2CH_2O); 38.46 (*q*, MsO); 35.87 (*d*, Me_2CHC=O); 35.12 (*t*, CH_2CH_2O); 34.08 (*d*, Me_2CHCMe_2); 27.27,

27.17, 27.14, 27.11, 25.56, 25.38, 25.24 (2 C) (7*q*, 4 *Me*$_2$CO$_2$); 25.24 (*s*, Me$_2$*C*Si); 20.31, 20.27 (2*q*, *Me*$_2$CSi); 19.41, 19.30 (2*q*, *Me*$_2$CHC=O); 18.45, 18.40 (2*q*, *Me*$_2$CHCMe$_2$); −3.51 (*q*, Me$_2$Si); 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, $[M+H]^+$, $C_{89}H_{111}N_{16}O_{25}S_4Si^+$; calc. 1959.6553).

5′-S-*Acetyl-2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6*^{1} → *5′*-S*)*-N^6-*benzoyl-2′,3′*-O-*isopropylidene-5′-thioadenosine-8-methyl-(8*^{1} → *5′*-S*)*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)-ethyl]-5′-thioguanosine-8-methyl-(8*^{1} → *5′*-S*)*-N^4-*benzoyl-6-(hydroxymethyl)-2′,3′*-O-*isopropylidene-5′-thiocytidine* (**23**). A soln. of **18** (190 mg, 123 μmol) in CH_2Cl_2 (2 ml) was treated with CF_3CO_2H (50 μl) and ^{i}Pr$_3$SiH (151 μl, 738 μmol), stirred for 45 min, and poured into ice and sat. $NaHCO_3$ soln. The mixture was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (2×) and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **20** (109 mg, 123 μmol) in DMSO (300 μl) was degassed, treated with EtN^{i}Pr$_2$ (64 μl, 369 μmol), stirred for 3 h, and poured into sat. NH_4Cl soln. The mixture was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (3×) and brine, dried ($MgSO_4$), 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. $[\alpha]_D^{25} = -6.3$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 267 (66670). IR (ATR): 3230*w* (br.), 2984*w*, 2931*w*, 1689*s*, 1607*s*, 1519*m*, 1455*m*, 1421*m*, 1380*s*, 1373*s*, 1343*s*, 1237*s*, 1211*s*, 1155*m*, 1069*s*, 998*m*, 981*m*, 899*w*, 865*m*, 793*w*, 732*w*, 702*m*. ^{1}H-NMR (500 MHz, $CDCl_3$; 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*, BzN*H*); 8.83 (br. *s*, BzN*H*, HN–C(2/II)); 8.11–8.09 (*m*, 2 arom. H); 8.02, 7.87 (2*d*, $J = 7.5$, 4 arom. H); 7.61–7.38 (*m*, 8 arom. H, H–C(5/I)); 5.60 (br. *s*, OH); 4.66 (*t*, $J = 6.8$, CH_2CH_2O); 3.21 (*t*, $J = 6.8$, CH_2CH_2O); 3.03–2.94 (br. *s*, Me$_2$C*H*C=O); 2.33 (*s*, AcS); 1.61, 1.52, 1.51, 1.47, 1.38, 1.33, 1.31, 1.24 (8*s*, 4 Me_2CO_2); 1.22, 1.20 (2*d*, $J = 6.9$, *Me*$_2$CHC=O). ^{13}C-NMR (125 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 6*; additionally, 194.76 (*s*, SC=O); 166.07, 165.83 (2*s*, 2 NHC=O); 146.82, 145.55 (2*s*); 133.70 (2*s*); 133.28, 132.52 (2*d*); 129.85–127.72 (several *d*); 123.74 (2*d*); 114.74, 113.80, 113.76, 113.40 (4*s*, 4 Me$_2$*C*O$_2$); 66.70 (*t*, CH_2CH_2O); 35.67 (*d*, Me$_2$*C*HC=O); 35.01 (*t*, *C*H$_2$CH$_2$O); 30.60 (*q*, *Me*C=O); 27.15 (3 C), 26.94, 25.32, 25.28, 25.20, 25.02 (6*q*, 4 *Me*$_2$CO$_2$); 19.47, 19.33 (2*q*, *Me*$_2$CHC=O). HR-MALDI-MS: 1822.5327 (31), 1821.5295 (72), 1820.5280 (100), 1819.5258 (100, $[M+Na]^+$, $C_{82}H_{92}N_{16}NaO_{23}S_4^+$; calc. 1819.5296), 983.2866 (40), 982.2833 (84, $[M - C_{38}H_{40}N_9O_{10}S]^+$, $C_{44}H_{52}N_7O_{13}S_3^+$; calc. 982.2780).

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine-8-methyl-(8* → *5′*-S*)*-N^4-*benzoyl-2′,3′*-O-*isopropylidene-5′-thiocytidine-6-methyl-(6*^{1} → *5′*-S*)-2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6*^{1} → *5′*-S*)*-N^6-*benzoyl-2′,3′*-O-*isopropylidene-5′-thioadenosine-8-methyl-(8*^{1} → *5′*-S*)-2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6*^{1} → *5′*-S*)*-N^6-*benzoyl-2′,3′*-O-*isopropylidene-5′-thioadenosine-8-methyl-(8*^{1} → *5′*-S*)*-N^2-*isobutyryl-2′,3′*-O-*isopropylidene*-O^6-*[2-(4-nitrophenyl)ethyl]-5′-thioguanosine-8-methyl-(8*^{1} → *5′*-S*)*-N^4-*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 H_2O (2×) and brine, dried ($MgSO_4$), 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 EtN^{i}Pr$_2$ (32 μl, 183 μmol), stirred for 6 h, and poured into ice and sat. NH_4Cl soln. The mixture was extracted with AcOEt (3×). The combined org. phases were washed with H_2O (3×) and brine, dried ($MgSO_4$), and evaporated. FC (diol phase; AcOEt) gave **24** (135 mg, 61%). Yellow foam. R_f (diol phase; AcOEt) 0.45. $[\alpha]_D^{25} = -39.2$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 268 (89780). IR (ATR): 3400–3200*w* (br.), 2984*w*, 2937*w*, 1690*s*, 1607*s*, 1519*m*, 1458*m*, 1421*m*, 1380*s*, 1344*s*, 1240*s*, 1212*s*, 1156*m*, 1071*s*, 981*m*, 866*m*, 833*m*, 794*w*, 704*w*. ^{1}H-NMR (600 MHz, $CDCl_3$; 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 BzN*H*); 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 (4*d*, $J = 7.5$, 8 arom. H); 7.59–7.37 (*m*, 16 arom. H, H–C(5/I,VII)); 4.76–4.73 (*m*, CH_2CH_2O); 4.65–4.63 (covered, CH_2CH_2O); 3.25 (*t*, $J = 7.0$, CH_2CH_2O); 3.21–3.18 (*m*, CH_2CH_2O); 2.98–2.72 (*m*, 2 H–C(5′/II–VII), 2 Me$_2$C*H*C=O); 1.51 (*sept.*, $J = 6.9$, Me$_2$C*H*CMe$_2$); 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) (9*s*, 8 Me$_2$CO$_2$); 1.26–1.25 (*m*, 2 *Me*$_2$CHC=O); 0.78 (*d*, $J = 6.9$, *Me*$_2$CH); 0.74, 0.73 (2*s*, Me$_2$CSi); −0.07, −0.08 (2*s*, Me$_2$Si). ^{13}C-NMR

Table 7. *Selected ^{1}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
CH_a–C(6)	4.72	3.76	4.37	3.79–3.72
CH_b–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
H_a–C(5′)	3.15–3.10	2.98–2.72	2.90–2.65	2.90–2.65
H_b–C(5′)	3.15–3.10	2.98–2.72	2.90–2.65	2.90–2.65
$J(H_a,H_b)$	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)
CH_a–C(8)	4.11	4.09	3.91	3.91
CH_b–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
H_a–C(5′)	2.98–2.72	3.62	2.90–2.65	3.67
H_b–C(5′)	2.98–2.72	3.60	2.90–2.65	3.62
$J(H_a,H_b)$	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
CH_a–C(8)	4.09	4.10	4.15–3.98	4.15–3.98
CH_b–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
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
$J(H_a,H_b)$	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)
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])
	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
$J(H_a,H_b)$	14.4	14.5	[c])	[c])
J(1′,2′)	< 1	< 1	[c])	[c])
J(2′,3′)	6.4	5.1	[c])	[c])
J(3′,4′)	4.5	[c])	[c])	[c])
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])

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

(150 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): see *Table 8*; additionally, 175.72, 175.09 (2*s*, NHC=O/II,VIII); 165.76, 162.60 (2*s*, 4 NHC=O, C(4/I)); 146.87, 146.85, 145.65, 145.57 133.83 (2 C), 133.75 (2 C) (6*s*); 133.40, 133.23, 132.64, 132.54 (4*d*); 129.99–127.82 (several *d*); 123.73 (4*d*); 114.70, 114.60, 114.00, 113.86, 113.81, 113.70, 113.50, 113.46 (8*s*, 8 Me_2CO_2); 66.86, 66.81 (2*t*, 2 CH_2CH_2O); 35.88 (br. *d*, 2 Me_2CHC=O); 35.13, 35.02 (2*t*, 2 CH_2CH_2O); 34.08 (*d*, Me_2CHCMe_2); 27.27–27.13, 25.56–25.44 (several *q*, 8 Me_2CO_2); 25.09 (*s*, Me_2CSi); 20.30, 20.27 (2*q*, Me_2CSi); 19.47, 19.41, 19.33, 19.29 (4*q*, 2 Me_2CHC=O); 18.45, 18.41 (2*q*, Me_2CHCMe_2); −3.51 (*q*, Me_2Si). HR-MALDI-MS: 3644.1560 (61), 3643.1538 (97), 3642.1424 (100), 3641.1466 (89), 3640.1718 (50, $[M + Na]^+$, $C_{168}H_{196}N_{32}NaO_{44}S_7Si^+$; calc. 3640.1790).

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylideneguanosine-8-methyl-(8*^{1} → 5′-S)-*2′,3′*-O-*isopropylidene-5′-thiocytidine-6-methyl-(6*^{1} → 5′-S)-*2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6*^{1} → 5′-S)-*2′,3′*-O-*isopropylidene-5′-thioadenosine-8-methyl-(8*^{1} → 5′-S)-*2′,3′*-O-*isopropylidene-5′-thiouridine-6-methyl-(6*^{1} → 5′-S)-*2′,3′*-O-*isopropylidene-5′-thioadenosine-8-methyl-(8*^{1} → 5′-S)-*2′,3′*-O-*isopropylidene-5′-thioguanosine-8-methyl-(8*^{1} → 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 CH_2Cl_2 (3×). 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 (0.4 ml) in a pressure tube was treated with sat. methanolic NH_3 (2 ml). The pressure tube was sealed, and the soln. was stirred for 60 h. After evaporation, a soln. of the residue in $CHCl_3$ was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (NH_2 phase; $CHCl_3$/MeOH 4:1) gave **25** (31 mg, 50%). Colourless solid. R_f (NH_2 phase; $CHCl_3$/MeOH 4:1) 0.21. $[\alpha]_D^{25} = -84.6$ ($c = 0.25$, $CHCl_3$). UV ($CHCl_3$): 268 (73110). IR (ATR): 3332*w* (br.), 3173*w* (br.), 2984*w*, 2925*w*, 2854*w*, 1689*s*, 1641*s*, 1532*w*, 1454*w*, 1434*w*, 1373*s*, 1331*w*, 1300*w*, 1259*w*,

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.

1209*m*, 1155*m*, 1063*w*, 983*m*, 930*w*, 865*m*, 831*m*, 796*w*, 781*w*, 719*w*, 682*w*. ^{1}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*, H_2N–C(4/I,VII), H_2N–C(6/III,V)); 6.64, 6.55 (2 br. *s*, H_2N–C(2/II,VIII)); 1.51 (*sept.*, J = 6.9, Me_2CHCMe_2); 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) (12*s*, 8 Me_2CO_2); 0.78, 0.77 (2*d*, J = 6.9, Me_2CHCMe_2); 0.73, 0.72 (2*s*, Me_2CSi); −0.06, −0.09 (2*s*, Me_2Si). See also *Table 9*. ^{13}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 (7*s*, 8 Me_2CO_2); 33.51 (*d*, Me_2CHCMe_2); 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 (12*q*, 8 Me_2CO_2); 24.63 (*s*, Me_2CSi); 20.10, 20.01 (2*q*, Me_2CSi); 18.22, 18.14 (2*q*, Me_2CHCMe_2); −3.66, −3.72 (2*q*, Me_2Si); 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, $[M + Na]^+$, $C_{116}H_{154}N_{30}NaO_{34}S_7Si^+$; calc. 2785.8959).

Table 9. *^{1}H- and ^{13}C-NMR Chemical Shifts* [ppm] *for the CH and CH_2 Groups of the Isopropylidenated and Silylated Octanucleoside* **25** *in ($CDCl_2$)$_2$ 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-(8^1 → 5′-S)-5′-thiocytidine-6-methyl-(6^1 → 5′-S)-5′-thiouridine-6-methyl-(6^1 → 5′-S)-5′-thioadenosine-8-methyl-(8^1 → 5′-S)-5′-thiouridine-6-methyl-(6^1 → 5′-S)-5′-thioadenosine-8-methyl-(8^1 → 5′-S)-5′-thioguanosine-8-methyl-(8^1 → 5′-S)-6-(hydroxymethyl)-5′-thiocytidine (**26**). A soln. of **25** (4 mg, 1.5 μmol) in HCO_2H/H_2O 4:1 (0.3 ml) was stirred for 18 h and evaporated at 23°. A soln. of the residue in 25% aq. NH_3 (1 ml) was lyophilized. A soln. of the residue in a mixture of H_2O and 25% aq. NH_3 was applied on a *Bakerbond C18* column (conditioned with H_2O). The column was washed with *ca.* 5 ml H_2O. The product was obtained by elution with H_2O/MeCN/NH_4OH 4:1:1. Evaporation of the solvents gave **26** (2 mg, 60%). Colourless powder. R_f (NH_2 phase; MeOH/$CHCl_3$/H_2O 3:1:1) 0.65. t_R (HPLC: *Waters Atlantis dC18-3*, 100 × 3 mm; MeCN/H_2O/HCO_2H 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, $[M + H + Na]^{2+}$, $C_{84}H_{105}N_{30}NaO_{34}S_7^{2+}$; calc. 1162.2671), 1153.7745 (12), 1153.2764 (22), 1152.7762 (62), 1152.2748 (92), 1151.7717 (93), 1151.2740 (100, $[M + 2\,H]^{2+}$, $C_{84}H_{106}N_{30}O_{34}S_7^{2+}$; calc. 1151.2761), 1086.7526 (17), 1086.2519 (23), 1085.7535 (31), 1085.2514 (30, $[M - C_5H_9O_4 + 3\,H]^{2+}$, $C_{79}H_{98}N_{30}O_{30}S_7^{2+}$; calc. 1085.2550).

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