Oligonucleotide Analogues with Integrated Bases and Backbone

Part 27

Synthesis and Association of Thiomethylene-Linked Cytidine-Derived Dinucleosides and Tetranucleosides

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The (chloromethyl)cytidine **7** was obtained from alcohol **4** that was synthesized from the protected cytidine **3** by *C(6)*-formylation and reduction. Thioacetate **10** was obtained from the cytidine **2**, and thioacetate **8** from a *Mitsunobu* reaction of alcohol **6**. The thiomethylene-linked dinucleoside **11** was synthesized by thioether formation between the 6-(chloromethyl)cytidine **7** and the thiolate generated by *S*-deacetylating and *N*-debenzoylating the cytidine-5′-thioacetate **10**. Dinucleoside **11** was desilylated to **12**, and fully deprotected to **13**. Similarly to **11**, the *C(6)*-substituted analogue **14** was obtained from **7** and the *C(6)*-substituted **8**. Stepwise deprotection of **14** provided **15** – **17**, and complete deprotection gave **18**. The thioacetylated and *N*-benzoylated dinucleoside **21** was obtained from the methanesulfonate **9** and the thiolate that was generated from thioacetate **8**. Similarly, **7** and **8** yielded **19** that was transformed into the methanesulfonate **20**. The tetranucleoside **23** was synthesized from the methanesulfonate **20** and the thiol derived from **21**. It was debenzoylated to **23** and completely deprotected to **24**.

The partially protected dinucleosides **11**, **14**, and **15**, and the tetranucleoside **23** pair strongly in $CDCl_3$. The crystal structure of **11** · MeOH shows the formation of an antiparallel cyclic duplex possessing nearly orthogonal base pairs due to MeOH acting as H-acceptor from one base pair and H-donor to the other base pair. A large distance of *ca.* 6 Å between the base pairs of the cyclic duplexes was predicted by *Maruzen* modeling. It is corroborated by the absence of base stacking in $CHCl_3$ solution of the duplexes formed by the (self-complementary) dinucleosides **11**, **14**, and **15**, as evidenced by a weak temperature dependence of the CD spectra. The association constants for **11**, **14**, **15**, and **23** were calculated from the concentration dependence of the chemical shift of H_2N–C(4). No concentration dependence of the H_2N–C(4) signals was observed for solutions of **23** in $CDCl_3$, (D_6)acetone, CD_3CN, (D_8)THF, (D_5)pyridine, and $CDCl_3$/(D_6)DMSO 4:1. As a consequence of the strong association, the association constant for **23** had to be determined in CD_3CN/(D_6)DMSO 4:1. The temperature dependence of the CD spectra of the fully deprotected **18** und **24**, but not of **13**, in H_2O is rationalized by base stacking of the hydroxymethylated cytosine moieties that associate by intermolecular H-bonds of $HOCH_2$–C(6/I) to an acceptor of unit I. The ^{1}H-NMR spectrum of **18** and **24**, but not of **13**, shows a 9:1 mixture of the monoplex and the base-stacked duplex.

Introduction. – We have described a novel type of oligoribonucleotide analogues (ONIBs)[2]) that possess linking elements between nucleobases instead of a contiguous

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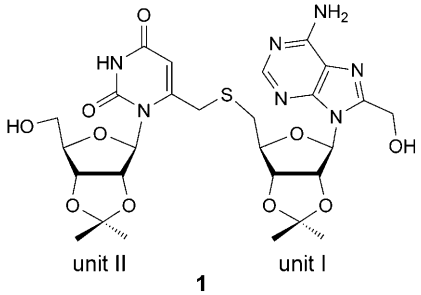
[2]) Abbreviation of the originally suggested term '**O**ligo**N**ucleotides **I**ntegrating **B**ackbone and bases'.

backbone. Non-self-complementary and self-complementary partially protected thiomethylene-linked dinucleosides [1], self-complementary ethynylene- [2], ethenylene- [3], oxymethylene- [1][4], aminomethylene- [5][6], sulfonyl- and sulfinylmethylene-linked [7] dinucleosides pair in $CDCl_3$ by forming *Watson–Crick*- and/or *Hoogsteen*-type H-bonds between the nucleobases. Temperature-dependent CD spectra evidence that formation of these H-bonds is accompanied by base stacking. The structure of the duplexes possessing oxymethylene, thiomethylene, or ethynylene linkers was analyzed [8]. The *syn*-conformation (strongly favoured by substitution at C(6) of uridine and C(8) of adenosine) was always required for pairing, *i.e.,* for the formation of cyclic duplexes. The thiomethylene-linked, partially protected ONIBs are particularly attractive. They pair well, adopting a *gt* conformation about the C(4′)–C(5′) bond in unit I (see **1** for the numbering of the units), and are most readily accessible [1]. The strongest pairing (in $CDCl_3$) is shown by the thiomethylene-linked U*[s]A*[3]) dinucleoside **1** ($K_{ass} = 2.8 \cdot 10^4$ M^{-1}). More recently, the pairing of a partially protected uridine- and adenosine-derived self-complementary thiomethylene-linked tetranucleoside and the structure of the duplex have been analysed by a detailed NMR study [9]. Unfortunately, the fully deprotected thiomethylene-linked uridine- and adenosine-derived tetranucleosides are poorly soluble in H_2O. This poor solubility did not allow determination of their pairing in H_2O, and is a significant disadvantage for investigating their biological properties.



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So far, we only synthesised adenosine- and uridine-derived nucleosides. It is to be expected that cytidine- and guanosine-derived analogues pair more strongly, as they involve three rather than two H-bonds in a base pair. Interest in cytidine- and guanosine-derived analogues, and the prospect that their polar character would lead to a higher solubility in H_2O prompted us to prepare and analyse their structure and properties, beginning with cytidine-derived C*[s]C$^{(*)}$ dinucleosides and C*[s]C*[s]C*[s]C* tetranucleosides.

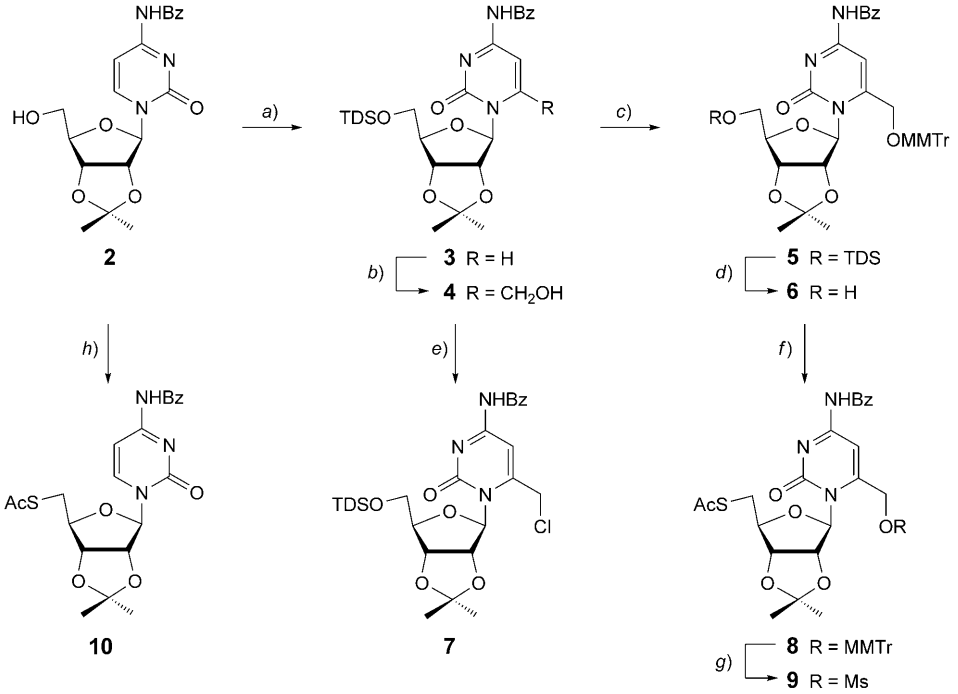
Results and Discussion. – *Synthesis of C*[s]C$^{(*)}$ Dinucleosides and C*[s]C*[s]C*[s]C* Tetranucleosides.* We considered two ways to C*[s]C$^{(*)}$ dinucleo-

[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.

sides, *viz.* the transformation of U*[s]U* dinucleosides [10] and thioether formation between cytidine mononucleosides. We initially prepared the C*[s]C* dinucleoside **14** (*cf. Scheme 2*) by substitution of the bis(O^4-*o*-nitrophenyl) ether of the corresponding U*[s]U* dinucleoside by NH_3 in MeOH, but obtained better results by thioether formation between protected cytidine monomers, as discussed below.

The electrophilic mononucleosides **7** and **9**, required for the synthesis of the thioethers, were obtained from the intermediate alcohol **4** that was readily prepared from the known 4-*N*-benzoyl-2′,3′-*O*-isopropylidenecytidine (**2**) [11] (*Scheme 1*).

Scheme 1



TDS = Thexyl(dimethyl)silyl (= dimethyl(1,1,2-trimethylpropyl)silyl), MMTr = (monomethoxy)trityl (= (4-methoxyphenyl)(diphenyl)methyl). *a*) TDSCl, 1*H*-imidazole, DMF; 91%. *b*) 1. Lithium diisopropylamide (LDA), −78°, THF, then DMF; 2. AcOH; 3. $NaBH_4$, EtOH; 86%. *c*) MMTrCl, $^iPr_2NEt$, 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2; 87%. *d*) $(HF)_3 \cdot NEt_3$, THF; 93%. *e*) Ms_2O, $^iPr_2NEt$, CH_2Cl_2, addition of LiCl in DMF; 80%. *f*) PPh_3, diisopropyl azodicarboxylate (DIAD), AcSH, THF; 99%. *g*) 1. Cl_2CHCO_2H, CH_2Cl_2, $^iPr_3SiH$; 2. Ms_2O, $^iPr_2NEt$, CH_2Cl_2; 69%. *h*) 1. NaH, 1-tosyl-1*H*-imidazole, THF; 2. AcSK, DMF; 69%.

Protection of OH at C(5′) by standard silylation [12] gave the thexyl(dimethyl)silyl ether **3**. Deprotonation of **3** with excess LDA [13], followed by the addition of DMF, hydrolysis, and reduction of the resulting aldehyde, yielded 86% of the hydroxymethylated cytidine **4**. To obtain **9** from this common intermediate, we monomethoxytritylated **4** to yield 87% of **5** that was desilylated [14] to **6**. Substitution of this alcohol with AcSH under *Mitsunobu* conditions [15] resulted in the *C(5′)-S*-acetate **8**. It was

detritylated [16] and directly transformed to the methanesulfonate **9**, which possesses an electrophilic and a protected nucleophilic site, as it is required for the synthesis of longer oligonucleosides. Chloro derivative **7** was obtained in a yield of 80% by mesylation of **4**, followed by treatment with LiCl. The *C(5′)-S*-acetate **10**, which acts as protected nucleophile, was prepared by substitution with excess AcSK of the crude *C(5′)-p*-toluenesulfonate obtained from **2**.

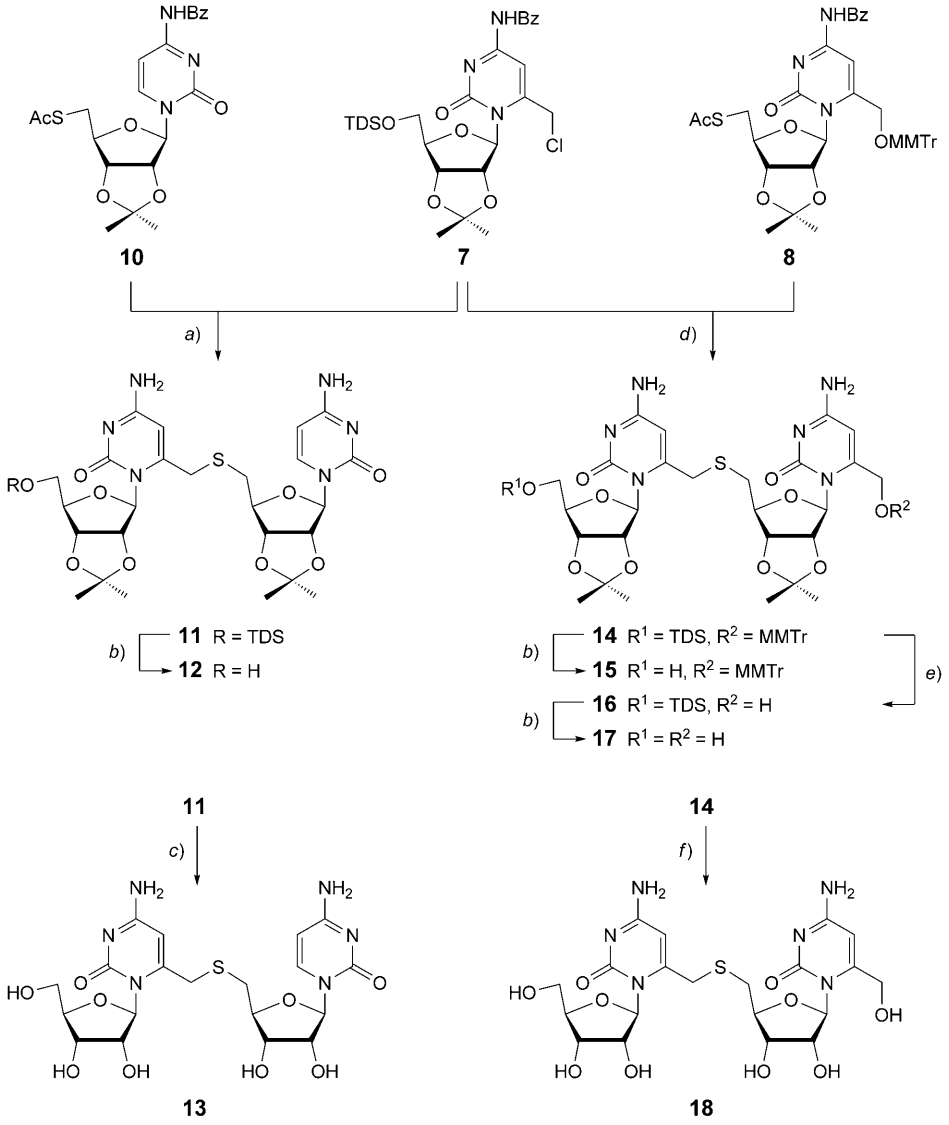
Exposing the thioacetates **8** and **10** to NH_3 or K_2CO_3 in MeOH [17] led both to the desired *S*-deacetylation and *N*-debenzoylation (*Scheme 2*). This was immediately followed by the addition of **7** to yield the C*[s]C(*) dinucleosides **11** (93%) and **14** (62%), respectively. Desilylation of these dinucleosides with $(HF)_3 \cdot NEt_3$ in THF led to the corresponding alcohols **12** (94%) and **15** (88%), while detritylation of **14** yielded the silyl ether **16** (83%) that was desilylated to diol **17** (95%). Finally, treating **11** with 50% aqueous CF_3COOH yielded the unprotected C*[s]C dinucleoside **13** (86%). Similarly, **14** was transformed by treatment with $^{i}Pr_3SiH$ in aqueous CF_3COOH to the unprotected C*[s]C* **18** (75%).

To synthesise the protected C*[s]C*[s]C*[s]C* tetranucleoside **22**, we treated thioacetate **8** with MeSNa in THF/MeOH 1:1 at $-10°$ [18]. These conditions led to *S*-deacetylation without concomitant *N*-debenzoylation (*Scheme 3*). The crude deacetylation product reacted with the chloromethylated **7** in the presence of Cs_2CO_3 in DMF to yield 60% of the protected dinucleoside **19**, and, similarly, with the methanesulfonate **9** to yield 62% of **21**. Detritylation of **19** and reaction of the crude alcohol with Ms_2O/$^{i}Pr_2NEt$ in CH_2Cl_2 provided the methanesulfonate **20** (79%). Similarly to the mononucleoside **8**, dinucleoside **21** was deacetylated by treatment with MeSNa in THF/MeOH 1:1. Addition of **20** to the resulting crude thiolate yielded 51% of **22**. *N*-Debenzoylation of **22** with a saturated solution of NH_3 in MeOH/CH_2Cl_2 gave tetranucleoside **23** (77%) that was fully deprotected to the H_2O-soluble tetranucleoside **24** (69%) by treatment with $^{i}Pr_3SiH$ in 80% aqueous HCOOH.

Conformation of the Cytidine Monomers. As expected by comparison to analogous uridine derivatives [1][2][19], the *C(6)*-unsubstituted cytidine derivative **3** adopts an *anti*-conformation, evidenced by the chemical shift of H–C(2′) resonating at 4.76 ppm (see *Table 4* in the *Exper. Part*). The small *J*(4′,5′a) and *J*(4′,5′b) values (2.5 and 3.6 Hz, resp.) evidence a predominant *gg*-conformation (*gg*/*gt*/*tg* 74:22:4; calculated according to [1]). Similarly to the *C(6)*-unsubstituted uridine *C(5′)-S*-acetate [1], the cytidine *C(5′)-S*-acetate **10** exists as a mixture of (mostly) *syn*- and *anti*-conformers, as evidenced by the downfield shift for H–C(2′), resonating at 5.13 ppm, and a distinctly lower population of the *gg*-conformer (*gg*/*gt*/*tg* 10:44:46; *cf. Table 4* in the *Exper. Part*). The thioacetate **10** shows a stronger preference for the (*N*)-conformation than the silyl ether **3** (*J*(1′,2′)/*J*(3′,4′) of 0.4 *vs.* 0.7).

The chemical shift (5.30–5.36 ppm) for H–C(2′) of the *C(6)*-substituted cytidines **4**–**9** confirms the *syn*-conformation. The thioacetates **8** and **9** prefer exclusively a *gt*/*tg*-conformation ($J(4',5'a) + J(4',5'b) = 14.0–14.4$ Hz), whereas **4**, **5**, and **7** ($J(4',5'a) + J(4',5'b) = 12.5–12.8$ Hz) populate also the *gg*-conformation, if only to a small extent (*ca.* 5–10%). The conformation of the CH_2OH group of **6** could not be determined in detail, as the signals of H_a–C(5′), H_b–C(5′), and HO–C(5′) overlap. Similarly to the analogous *C(6)*-substituted uridine derivatives [1], **3**–**5** and **7**–**9** show a stronger preference for the (*N*)-conformer than alcohol **6** (*J*(1′,2′)/*J*(3′,4′) of 0.25 *vs.* 0.6).

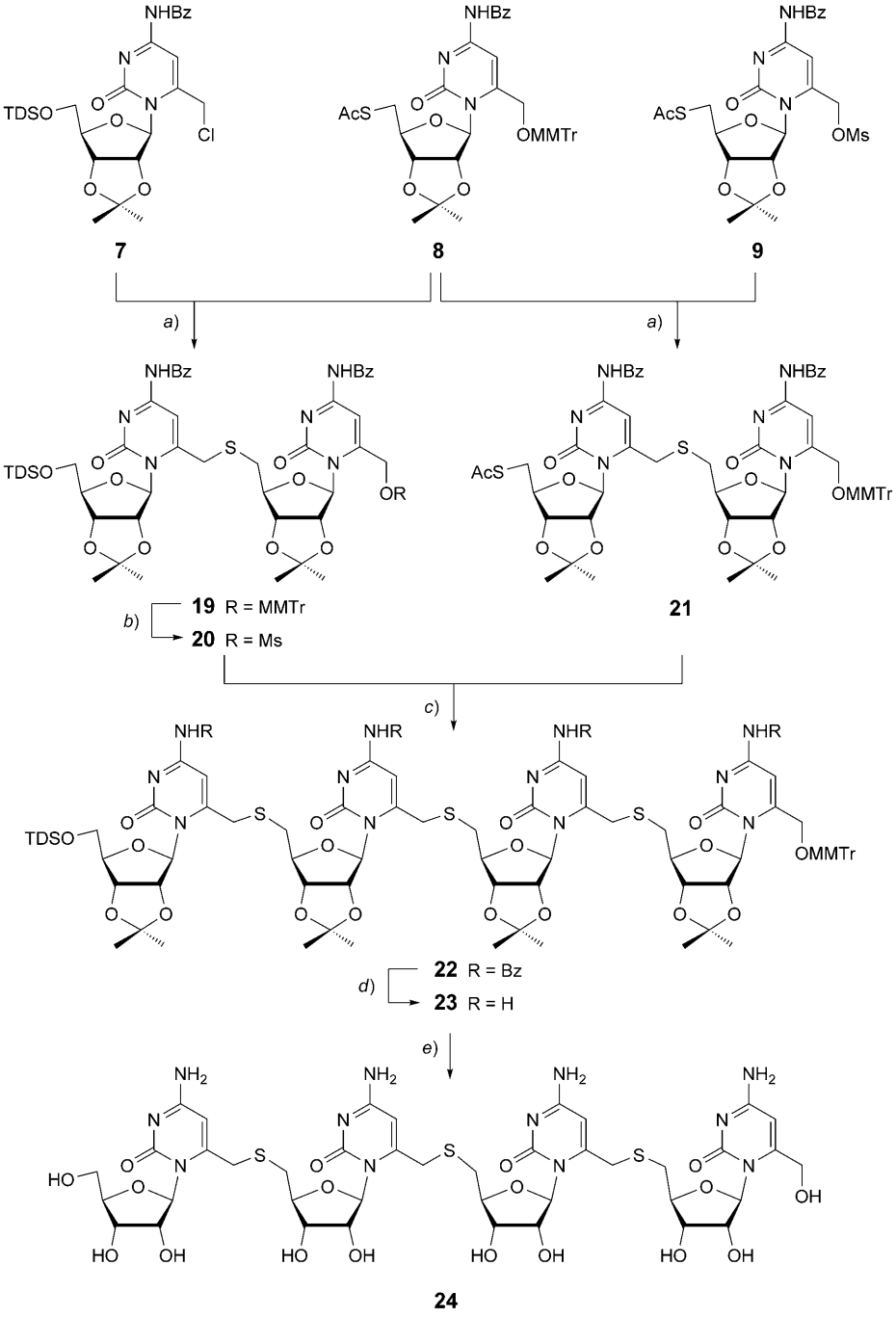
Scheme 2



a) NH_3, MeOH; 93%. *b*) $(HF)_3 \cdot NEt_3$, THF; 94% of **12**; 88% of **15**; 95% of **17**. *c*) CF_3CO_2H/H_2O 1:1; 86%. *d*) K_2CO_3, MeOH; 62%. *e*) Cl_2CHCO_2H, $^iPr_3SiH$, CH_2Cl_2; 83%. *f*) $^iPr_3SiH$, CF_3CO_2H/H_2O 1:1; 75%.

The benzoates **2**–**9** show broad ^{1}H-NMR signals for H–C(5), and broad ^{13}C-NMR signals at *ca.* 96, 155, and 166 ppm. This evidences an equilibrium between the benzamide **T1** (weak C(5)–H···O=C H-bond) and its imino tautomer **T2** (strong N(3)–H···O=C H-bond), which is exclusively observed for 5-substituted cytidines [20]

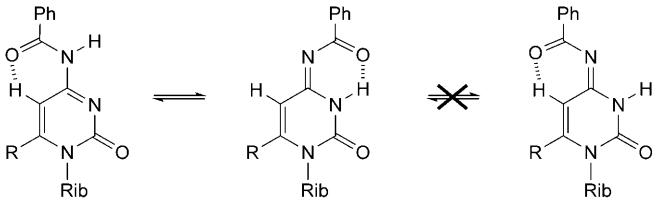
Scheme 3



a) 1. **8**, MeSNa, MeOH/THF 1:1, −10°; 2. **7** or **9**, LiBr, Cs_2CO_3, DMF; 60% of **19**; 62% of **21**. *b*) 1. Cl_2CHCO_2H, $^iPr_3SiH$, CH_2Cl_2; 2. Ms_2O, $^iPr_2NEt$, CH_2Cl_2; 79%. *c*) Analogous to *a*, with **21** and **20**; 51%. *d*) NH_3, MeOH/CH_2Cl_2; 77%. *e*) $^iPr_3SiH$, HCO_2H/H_2O 4:1; 69%.

(*Scheme 4*). For the **T1** and **T2** isomers, the ^{13}C-NMR data of *Herdewijn* and co-workers [20] evidence large $\Delta\delta$ values for C(2) (7 ppm), C(5) (8 ppm), and the C=O group of the benzoyl moiety (11 ppm; *cf.* [6] and refs. cit. therein), but small $\Delta\delta$ values for C(4) and C(6) (< 2 ppm). Hence, the broad signals at *ca.* 96, 155, and 166 ppm are assigned to C(5), C(2), and Ph*C*=O, respectively, and the rather sharp signals at 162–163 and 145–160 ppm to C(4) and C(6), respectively. This assignment is corroborated by the HMBC spectrum of the *p*-toluenesulfonate derived from **2** (data not given), especially by cross-peaks between the again rather sharp signal of C(4) at 162 ppm and both the H–C(5) and H–C(6) signals. We differ from the opinion of *Sekine* and co-workers [21] by assuming that tautomer **T3** (weaker C(5)–H···O=C H-bond than in **T1**) does not contribute significantly to the tautomeric equilibrium. The relatively weak downfield shift of Ph*C*=O (166 ppm) suggests an equilibrium between **T1** and only one imino tautomer.

Scheme 4



Structure and Association of the C[s]C(*) Dinucleosides.* 1. *Homopairing of Cytidines.* There is only one pairing pattern for unprotonated cytidines, forming two H-bonds between N(3) and H_2N–C(4) (*Fig. 1,a*). The homoassociation of lipophilic cytidines in $CHCl_3$ was studied by IR [22], *Raman* [23], and ^{1}H-NMR spectroscopy [24], and by calorimetry [25]. An association constant $K_{ass} = 40-42\ M^{-1}$ at 25° was determined, assuming a 1:1 association, with $-\Delta H^\circ = 4.9$ kcal/mol[4]) [23][24], similar to the self-association of adenosines and uridines in $CHCl_3$ [26][27]. *Ab initio* calculations of the C_i-symmetric CC base pair [28] evidence a length of 2.05 Å for the NH···N H-bond and a stabilisation energy of 17.35 kcal/mol for one base pair in the gas phase.

A priori, C*[s]C(*) dinucleosides may form parallel and antiparallel cyclic duplexes. Depending on the orientation of the base pairs, C*[s]C(*) dinucleosides can form three antiparallel cyclic duplexes, **CC1**–**CC3** (*Fig. 1,b*) and four parallel ones, **CC3**–**CC7** (*Fig. 1,c*). **CC1** is C_1-symmetric, the other duplexes are C_2-symmetric. These cyclic duplexes were evaluated with the help of *Maruzen* models. A *gt*-conformation of the linker was set in agreement with the consistently observed conformation of thiomethylene-linked A- and U-derived dinucleosides. This conformation leads to a large distance between the base pairs of all duplexes (*ca.* 6 Å) that would even allow an isomerization of the *C(6/1)*-unsubstituted isomers **CC4** and **CC6** to **CC5** and **CC7**,

[4]) $-\Delta H^\circ$ resulting from calorimetric measurements [25] depends strongly upon the concentration, and is significantly lower (1.7 kcal/mol).

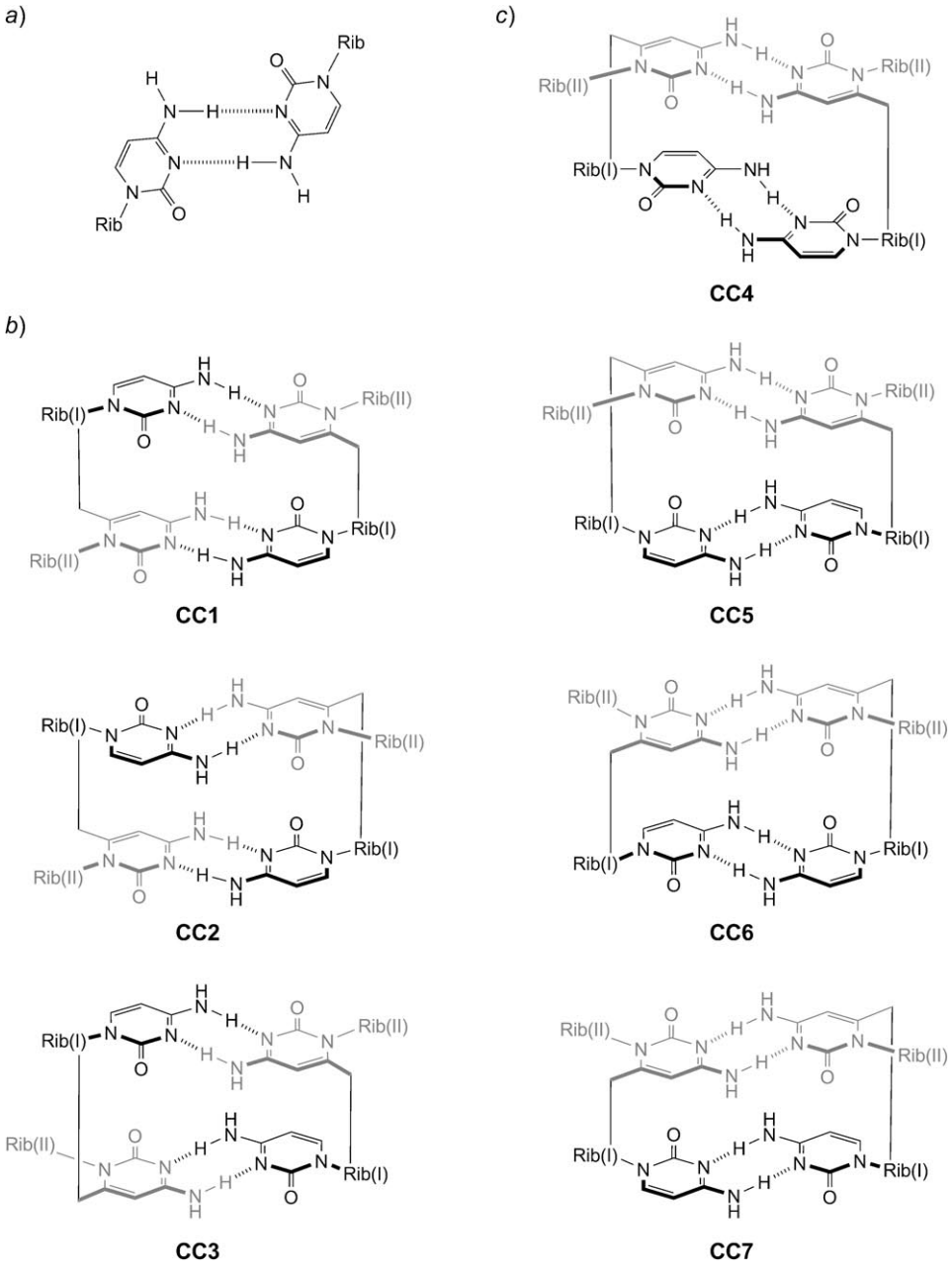


Fig. 1. a) *Self-association of cytidine mononucleotides.* b) *and* c) *Schematic representation of the possible antiparallel* (*i.e.*, **CC1**–**CC3**) *and parallel* (*i.e.*, **CC4**–**CC7**) *cyclic duplexes obtained from C*[s]C(*) dinucleosides.* Conformational analysis based on *Maruzen* models.

respectively, by rotating the base pair between units I without breaking the H-bonds. The χ angles of unit I are listed in *Table 1*. Surprisingly, the cytosine unit of unit I appears to adopt not only a *syn* (**CC4** and **CC6**) or a high-*syn* (**CC1** and **CC2**) conformation, but also an *anti* (**CC1** and **CC3**) and even a nonclassic *anti* (**CC5** and **CC7**) conformation. Some duplexes appear to be disfavoured by steric interactions of the substituent at C(6/I) and of the bulky TDS group. The parallel duplex **CC4** appeared to be the most favourable one; among the desilylated dinucleosides, the most favourable ones were the parallel duplexes **CC4** and **CC6**, and the antiparallel duplex **CC2**.

Table 1. Maruzen *Modeling of the C*[s]C Cyclic Duplexes* **CC1**–**CC7** *and Their 6-Substituted Analogues*

	χ/I	Steric interaction of $ROCH_2$–C(6/I)[a])	Steric interaction of TDSO–C(5′/II)[b])
Antiparallel duplexes			
CC1	$-60°$ (*anti*),	strongly disturbing	disturbing
	$+120°$ (high *syn*)	not disturbing	not disturbing
CC2	$+120°$ (high *syn*)	not disturbing	disturbing
CC3	$-60°$ (*anti*)	strongly disturbing	not disturbing
Parallel duplexes			
CC4	$+100°$ (*syn*)	not disturbing	not disturbing
CC5	$-50°$ (nonclassic *anti*)	disturbing	not disturbing
CC6	$+80°$ (*syn*)	not disturbing	disturbing
CC7	$-30°$ (nonclassic *anti*)	disturbing	not disturbing

[a]) H–C(6/I) is at worst weakly disturbing. [b]) HO–C(5′/II) shows no disturbing interactions.

Syn- and *anti*-configured cyclic duplexes should be easily identified by strong ROESY cross-peaks between H–C(6/I) (or CH_2–C(6/I)), and either H–C(1′/I) or H–C(2′/I) [29]. Antiparallel cyclic duplexes should be easily identified by a ROESY cross-peak between the two H_aN–C(6/I) and H_aN–C(6/II) involved in base pairing and thus resonating at low field. No cross-peaks are expected for parallel cyclic duplexes (interaction with the identical partner).

2. *Crystal Structure of* **11**·*MeOH*. Crystals of **11**·MeOH suitable for X-ray analysis[5]) were obtained by slow evaporation of a solution of **11** in MeOH. The crystals are orthorhombic (space group $P2_12_12_1$), and they reveal an antiparallel duplex comprising two dinucleoside units, **A** and **B**, which possess a slightly different geometry (*Fig. 2* and *Table 2*). As expected for a cyclic duplex, the nucleobases adopt a *syn*-conformation ($\chi = 65.9-69.3°$). The linker is characterised by a *gt*-conformation ($\eta_1 =$ 50.9° and 56.11°, $\eta_2 = 170.9°$ and 175.3°), a distorted *gauche*-conformation θ ($-99.7°$ and $-99.5°$), and two *gauche* angles ι and κ ($-76.2°$, $-74.6°$ and $-73.1°$, $-75.1°$ resp.). The base pairs are arranged almost orthogonally to each other, and do not stack. This is due to the interaction with MeOH. Although the H-atom of the OH group could

[5]) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-782324. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

not be located, it is evident that MeOH acts a H-bond acceptor from HN–C(4/II) of one base pair (C(4/II)$NH^{B}\cdots$OMe distance of 2.07 Å) and as H-donor to O=C(2/I) of the other base pair ($MeO\cdots O^{B}$=C(2/I) distance of 2.673 Å; *Fig. 2,b*). All furanose rings adopt a shallow (*N*)-conformation. The base pairs are characterized by $N\cdots H$ distances of 2.11 – 2.22 Å. The bridging by MeOH and the strong buckle (23 and 35°) and propeller twists (11 and 16°) suggest that the solid state structure of **11** · MeOH is hardly a good model for the solution structure of the cyclic duplex of **11**. The

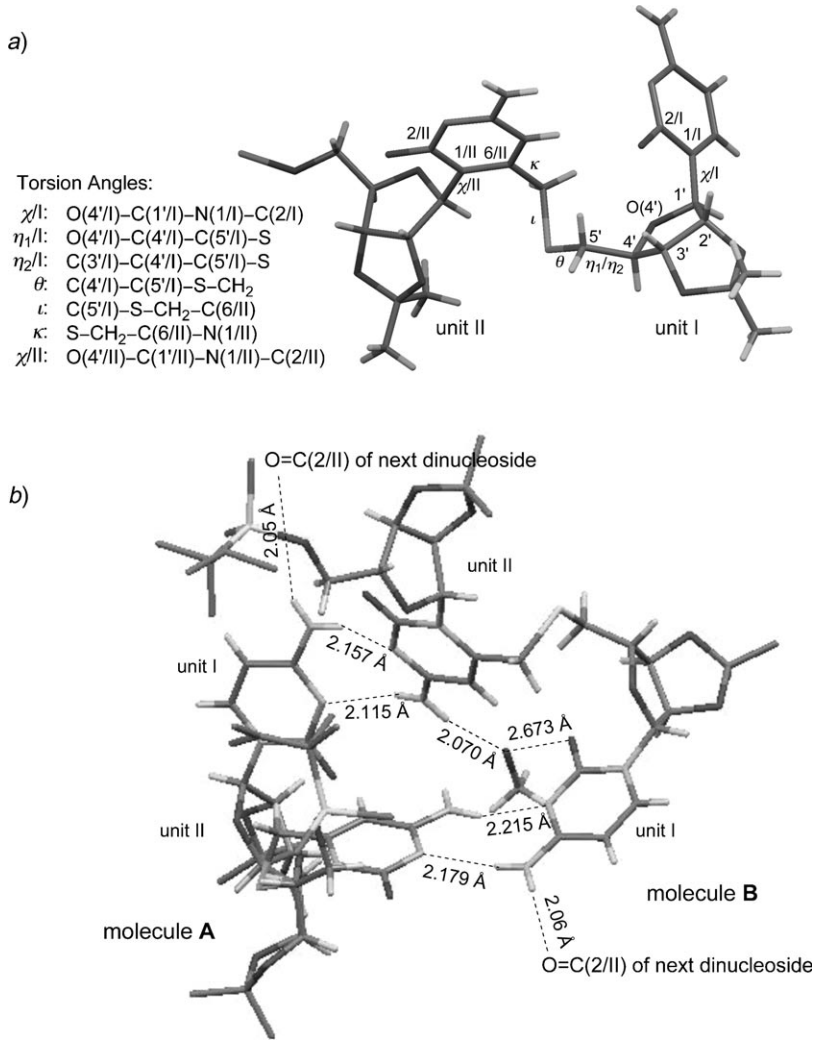


Fig. 2. a) *Capped-sticks representation of the crystal structure of the dinucleoside* **11** · *MeOH with definitions of the torsion angles* (substituents at Si omitted for clarity). b) *Cyclic duplex of* **11** · *MeOH with intra- and intermolecular H-bonds* (H-atoms of the silyl and the isopropylidene groups omitted for clarity; MeO*H* not located).

Table 2. *Selected Distances, Bond Angles, and Torsion Angles of Crystalline* **11** · *MeOH*

H···N Distance and N–H···N bond angle		Distance [Å]	Bond angle [°]
N(4/I)–H^{A}···N^{B}(3/II)		2.115	152.9
N^{A}(3/I)···H^{B}–N(4/II)		2.157	155.8
N(4/I)–H^{A}···H^{B}–N(4/II)		2.555	
N(4/I)–H^{B}···N^{A}(3/II)		2.215	159.8
N^{B}(3/I)···H^{A}–N(4/II)		2.179	163.8
N(4/I)–H^{B}···H^{A}–N(4/II)		2.394	
N(4/I)–H^{B}···OMe		2.070	168.8
C(2/I)=O^{B}···OMe		2.673	
Torsion angle	Short notation	Molecule **A** [°]	Molecule **B** [°]
O(4′/I)–C(1′/I)–N(1/I)–C(2/I)	χ/I	69.3	65.9
C(1′/I)–C(2′/I)–C(3′/I)–C(4′/I)		9.8	8.7
C(2′/I)–C(3′/I)–C(4′/I)–O(4′/I)		– 9.0	– 11.1
C(3′/I)–C(4′/I)–O(4′/I)–C(1′/I)		5.0	9.7
O(4′/I)–C(4′/I)–C(5′/I)–S	η_1	50.9	56.1
C(3′/I)–C(4′/I)–C(5′/I)–S	η_2	170.9	175.3
C(4′/I)–C(5′/I)–S–CH$_2$	Θ	– 99.7	– 99.5
C(5′/I)–S–CH$_2$–C(6/II)	ι	– 76.2	– 74.6
S–CH$_2$–C(6/II)–N(1/II)	κ	– 73.1	– 75.1
O(4′/II)–C(1′/II)–N(1/II)–C(2/II)	χ/II	66.9	68.1
C(1′/II)–C(2′/II)–C(3′/II)–C(4′/II)		8.2	– 2.4
C(2′/II)–C(3′/II)–C(4′/II)–O(4′/II)		– 13.0	– 2.7
C(3′/II)–C(4′/II)–O(4′/II)–C(1′/II)		13.3	7.2

conformation of the cyclic duplex of **11** · MeOH did not change upon minimisation of the solid-state structure by the MM3* force field programme [30]. However, removing MeOH led to a profound distortion of the structure, and the CC base pairs were replaced by single H-bonds to N(3) or O=C(2) as H-acceptors.

3. *Association of the C*[s]C(*) Dinucleosides.* The C*[s]C* dinucleosides **16** and **17** are not soluble in $CDCl_3$, and their association was analysed in (D_6)DMSO. The chemical shifts for the NH_2 groups of **16** and **17** in (D_6)DMSO (7.24 – 7.67 ppm; *Table 6* in the *Exper. Part*) are similar to those of corresponding monocytidines (7.09 – 7.35 ppm [31]) and reveal mostly solvated monoplexes. The C*[s]C alcohol **12** is sufficiently soluble in $CDCl_3$ to record the ^{1}H- and ^{13}C-NMR spectra, but not well enough to follow the concentration dependence of the NH chemical shifts.

The more extensively protected C*[s]C(*) dinucleosides **11**, **14**, and **15** are fairly well soluble in $CDCl_3$. Their association in this solvent was studied by vapour pressure osmometry (VPO) of the apparent molecular weight, and by ^{1}H-NMR and CD spectroscopy. The unambiguous assignments of the H- and C-signals are based on DQF-COSY spectra of **11**, **14**, and **15**, HSQC spectra of **11** and **14**, and HMBC spectra of **11** and **14**. ROESY Cross-peaks between the NH_2 groups and H–C(5) allowed an unambiguous assignment of the NH signals of **11** and **14** (see below for a detailed discussion). Unfortunately, the broad NH signals of **12** and **15** do not show any ROESY cross-peaks, and the assignment of their NH signals is based on a comparison with the spectra of **11** and **14**. The association constants K_{ass} of **11**, **14**, and **15** were determined

on the basis of the concentration dependence of the chemical shifts of HN–C(4) involved in base pairing (shift concentration curve (SCC)).

The formation of a cyclic duplex of **11** in $CHCl_3$ was evidenced by determining the molecular weight by VPO at a concentration of 1 and 5 mM, revealing an apparent molecular weight of 1454.19 and 1489.75 g/mol, *i.e.*, 1.97 and 2.02 times the molecular weight of the monoplex, respectively.

The SCCs of **11**, **14**, and **15** were determined by stepwise dilution of 50 mM solutions in $CDCl_3$ to 0.2 mM and following the chemical shift for H_aN–C(4/I) and H_aN–C(4/II) that are involved in base pairing and, therefore, resonate at low field (> 9 ppm for 10 mM solutions), leading to two curves for each dinucleoside (*Fig. 3*,*a*). The plateau reached by the SCCs of **11**, **14**, and **15** for concentrations > 10 mM and the steep ascent of the SCCs evidence a ready formation of cyclic duplexes. The chemical shifts of H_aN–C(4/I) and H_aN–C(4/II) of **12** (5 mM) are similar to those of **15**, suggesting similar SCCs for **12** as for **15**. Whereas the plateau for H_aN–C(4/II) of the four dinucleosides **11**, **12**, **14**, and **15** is found within a narrow range at 9.3 – 9.4 ppm, the plateau for H_aN–C(4/I) of the alcohols **12** (9.8 ppm) and **15** (10.2 ppm) is at a distinctly lower field than the plateau for the silyl ethers **11** (10.8 ppm) and **14** (10.6 ppm; *Table 6* in the *Exper. Part*). Thus, cleavage of the TDS group of **11** and **14** leads to an upfield shift for H_aN–C(4/I) of **12** and **15**, and this hints at the presence of antiparallel duplexes. The SCCs of H_aN–C(4/I) of **11** and **15** do not form an ideal plateau, as a weak and constant decrease is observed for concentrations above 5 mM (see SCCs of **11** in *Fig. 3*,*b*). Increasing the concentration of **11** from 15 to 176 mM leads to an upfield shift for H_aN–C(4/I) of 0.4 ppm[6]). This phenomenon was rationalised by assuming that the duplexes associate further at high concentrations. Indeed, H_bN–C(4/I) of **11**, but not H_bN–C(4/II), shows a significant downfield shift upon increasing the concentration (5.47 ppm for a 14 mM and 5.96 ppm for a 176 mM solution). This suggests that the duplexes associate at higher concentration by an interduplex H-bond from H_bN–C(4/I) to an O=C(2), similarly as in the crystal of **11** · MeOH (*Fig. 2*,*b*). It appears reasonable to assume that this H-bond of H_bN–C(4/I) weakens the H-bond of H_aN–C(4/I), and thus leads to an upfield shift of H_aN–C(4/I).

The equilibrium constants were calculated according to a method of *Gutowsky* and *Saika* [32], assuming an equilibrium between monoplex and duplex, and including a value of 5.60 ppm for a 0.0001 mM solution. This value was obtained by extrapolating the SCC of the hydroxymethylated cytidine monomer obtained by debenzoylation of **4**[7]). The association constants K_{ass} were calculated from the SCCs of H_aN–C(4′/I) and H_aN–C(4′/II) in *Fig. 3*. The concentration range considered for H_aN–C(4/I) of **11** and **15** was restricted to below 15 mM, *i.e.,* to the increasing section of the SCC. The association constant K_{ass} increases from **14** (25000/31000 M^{-1}; *Table 3*) *via* **11** (39000/52000 M^{-1}) to **15** (96000/120000 M^{-1}), corresponding to $-\Delta G_{295}$ values of 5.9 – 6.9 kcal/mol. While identical K_{ass} values should be obtained from the SCCs of H_aN–C(4′/I) and H_aN–C(4′/II), K_{ass} value obtained from the SCCs of H_aN–C(4′/I) is *ca.* 1.3 times larger

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[6]) See [27] for a similar observation for duplexes of U*[s]U* dinucleosides.

[7]) There are two NH signals at higher concentration that coalesce at lower concentration. Therefore, the value of 5.60 ppm derived from the SCC of the more deshielded NH may be somewhat too small, although the K_{ass} and $-\Delta G$ values agree well with the results of *Sartorius* and *Schneider* [24].

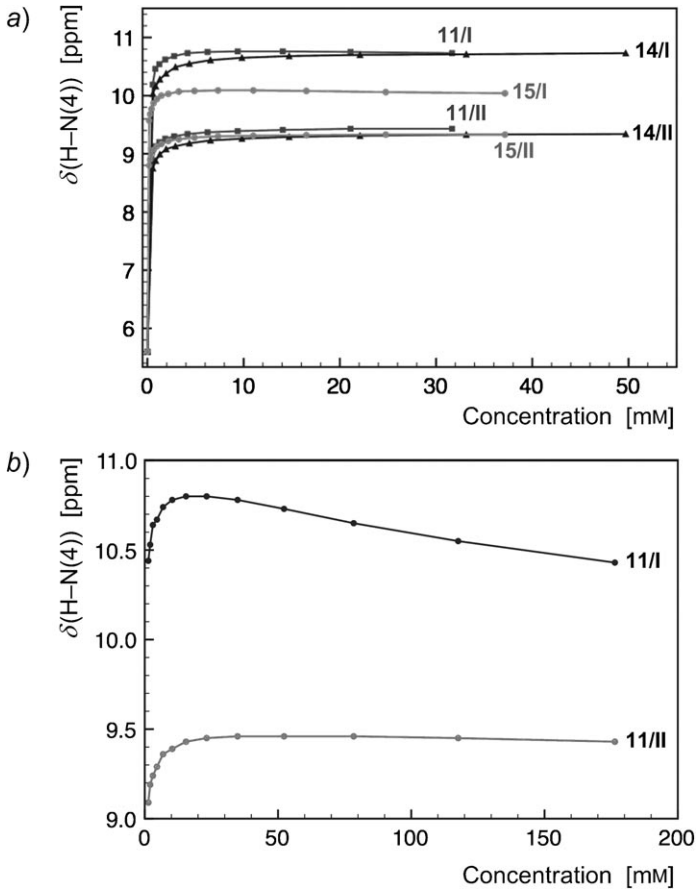

Fig. 3. a) *Shift/concentration curves* (SCCs) *of the more deshielded HN–C(4/I and II) of the C*[s]C(*) dinucleosides* **11**, **14**, *and* **15** *for 0.19–49.7 mM solutions in* $CDCl_3$ (including a value of 5.60 ppm for a 0.0001 mM solution). b) *SCCs of the more deshielded HN–C(4/I and II) of* **11** *for 0.19–176 mM solutions in* $CDCl_3$.

than K_{ass} obtained from the SCCs of H_aN–C(4′/II), but the difference is within the error limits.

Thermodynamic parameters for the association of **11**, **14**, and **15** were determined by *van't Hoff* analysis of the ^{1}H-NMR spectra obtained from 1–2 mM solutions in $CDCl_3$ in a temperature range from 10 to 50° and in 10° intervals (*Table 3*). The $-\Delta H$ values evidence a similar strength for the CC base pair as for the *WC*-type base pairs of U*[s]A(*) dinucleosides [1].

The linker of the paired C*[s]C(*) dinucleosides **11**, **12**, **14**, and **15** adopts a *gt*-conformation, as evidenced by *J*(4′,5′a/I) in the range of 10.9–11.1 Hz and *J*(4′,5′b/I) in the range of 1.5–2.1 Hz, whereas the solvated monoplexes of **16** and **17** (*J*(4′,5′a/I) in the range of 6.3–6.7 Hz and (*J*(4′,5′b/I) in the range of 7.7–7.9 Hz; *Table 6* in the *Exper. Part*) prefer a *ca.* 1:1 *gt*/*tg*-equilibrium. The *gt*-conformation of **11**, **12**, **14**, and **15**

Table 3. *^{1}H-NMR Chemical Shifts of H_aN–C(4) of the Monoplex ($c = 0$ mM) and the Cyclic Duplexes ($c = \infty$), and Association Constant K_{ass} as Calculated from the SCCs of the C*[s]C(*) Dinucleosides* **11**, **14**, *and* **15** *in* Fig. 3. Thermodynamic parameters by *van't Hoff* analysis for 1–2 mM solutions in $CDCl_3$ at 10–50°.

Dinucleoside	K_{ass} [M^{-1}]	$\delta_{monoplex}$ [a]) [ppm]	δ_{duplex} [b]) [ppm]	$-\Delta G_{295}$ [c]) [kcal/mol]	$-\Delta H$ [kcal/mol]	$-\Delta S$ [cal/mol·K]
H_aN–C(4/I) [d])						
11	52000 ± 24000	5.55 ± 0.13	10.96 ± 0.10	6.4	12.6	21.3
14	31000 ± 3000	5.57 ± 0.04	10.85 ± 0.08	6.1	14.7	26.8
15	120000 ± 20000	5.49 ± 0.05	10.22 ± 0.03	6.9	17.9 [d])	38.6 [d])
H_aN–C(4/II)						
11	39000 ± 16000	5.57 ± 0.10	9.54 ± 0.06	6.2	14.1	26.9
14	25000 ± 2000	5.58 ± 0.05	9.43 ± 0.02	5.9	14.0	25.5
15	96000 ± 7000	5.53 ± 0.03	9.40 ± 0.01	6.7	15.2 [d])	29.9 [d])

[a]) Extrapolated for $c = 0$. [b]) Extrapolated for $c = \infty$. [c]) Calculated from K_{ass}. [d]) Only increasing δ values are used for the numerical analysis (**11**: up to 9 mM, **14**: up to 50 mM, **15**: up to 11 mM). [d]) Temperature range 20–50°.

is corroborated by ROESY cross-peaks between H–C(3′/I) and both H_a–C(5′/I) and H_b–C(5′/I). As expected, the cross-peaks with H_a–C(5′/I), possessing the larger coupling with H–C(4′/I), are more intensive. The proximity of H_a–C(5′/I) = $H_{pro\text{-}R}$–C(5′/I) to the nucleobase leads to an opposite relative shielding of $H_{pro\text{-}R}$–C(5′/I) and $H_{pro\text{-}S}$–C(5′/I) (*cf.* [33]), as it was already observed in the U*[s]A(*) and A*[s]U(*) series [1]. Both furanose units of **11**, **12**, **14**, and **15** prefer an (*N*)-conformation.

A slight upfield shift for H–C(2′/I) relative to H–C(2′/II) ($\Delta\delta$ 0.05–0.14 ppm) suggests an incomplete preference for the *syn*-conformation of unit I. *syn*/*anti*-Equilibria are analysed more precisely by ROESY spectra (*cf.* [29][1]). The exclusive *syn*-conformation of unit II of **11**, **12**, **14**, and **15** is evidenced by a strong cross-peak between the more strongly shielded H_bC–C(6/II) and H–C(1′/II), and the absence of a cross-peak between this H-atom and H–C(2′/II). H_bC–C(6/II) shows also a TOCSY cross-peak (same phase as the signals on the diagonal) with H–C(5/II), whereas CH_a–C(6/II) shows both a cross-peak with H–C(5/II) and a TOCSY cross-peak with H–C(1′/II), confirming the same, rigid conformation of the CH_2SCH_2 linker of all four dinucleosides. H–C(6/I) of the paired C*[s]C dinucleosides **11** and **12** show strong cross-peaks with H–C(5/I) and H–C(1′/I), and a weaker one with H–C(2′/I). The intensity ratio of the cross-peaks with H–C(1′/I) and H–C(2′/I) is *ca.* 7:3. Since the distance C(6/I)H···HC(2′/I) in the *anti*-conformers is distinctly shorter than the distance C(6/I)H···HC(1′/I) in the *syn*-conformers (as deduced from *Maruzen* modeling), the *syn*/*anti*-equilibrium must be distinctly larger than 7:3. Nevertheless, the cross-peaks evidence that significant amounts of **11** and **12** adopt an *anti*-conformation in the cyclic duplex. Both H-atoms of C(6/I)–CH_2 of the C*[s]C* dinucleosides **14** and **15** show strong cross-peaks with H–C(5/I) and H–C(1′/I), and only **14** shows also a weaker cross-peak with H–C(2′/I). This evidences free rotation around the C(6/I)–CH_2 bond, the exclusive *syn*-conformation of unit I of **15**, and a *syn*/*anti*-equilibrium of **14**, implying that **14** adopts partially the *anti*-conformation in the cyclic duplex, and this in spite of the *C(6/*

I)-substitution. The analysis of δ(H–C(2′/I)) does not fit well with these ROESY data, indicating that other factors must also affect the chemical shift of H–C(2′/I).

Antiparallel and parallel cyclic duplexes should be easily identified by the presence or absence of a cross-peak between the two NH groups engaged in base pairing. This is so, because the two NH groups engaged in base pairing of parallel duplexes involve homotopic units. All four NH of **11** show strong cross-peaks with H–C(5) of the corresponding unit, *i.e.*, there are cross-peaks between the H–C(5/I) signal at 5.64 ppm and the NH signals at 10.80 and 5.42 ppm, and between the H–C(5/II) signal at 5.81 ppm and the NH signals at 9.41 and 7.14 ppm. Weak EXSY cross-peaks (same phase as the diagonal) were observed between the NH signals at 10.80 and 5.42 ppm and between the NH signals at 9.41 and 7.14 ppm. This evidences that the base pairs break apart within the mixing time of the ROESY measurement, allowing exchange of the position of the NH groups by rotation about the C(4)–NH_2 bond. The presence of weak ECSY cross-peaks and the absence of a (strong) ROESY cross-peak between the two NH at low field strongly suggest parallel duplexes of **11**. The NH signals of **14** at 10.64, 9.30, and 7.22 ppm show strong ROESY cross-peaks with H–C(5), whereas the NH signal at 5.40 ppm is too close to the H–C(5/I) signal at 5.42 ppm to detect a cross-peak. Due to broader NH signals, no EXSY cross-peaks were observed. The absence of a (strong) cross-peak between the signals at 10.64 and 9.30 ppm is a strong indication for parallel duplexes of **14**.

Unfortunately, the NH signals of **12** and **15** are too broad to show any ROESY cross-peaks, and do not allow assignment of the direction of the duplexes. There is a striking difference between the silyl ethers **11** and **14**, and the corresponding alcohols **12** and **15**; deprotection of TDSO–C(5′/II) moiety leads to a strong upfield shift of H_aN–C(4/I) ($\Delta\delta = 0.97$ and 0.48 ppm, resp.; *Table 6* in the *Exper. Part* and *Fig. 3*), whereas the chemical shift of H_aN–C(4/II) is hardly affected ($\Delta\delta \leq 0.07$ ppm). For parallel cyclic duplexes, HO–C(5′/II) must have a close interaction with the base pair between units I to explain the effect of deprotecting TDSO–C(5′/II) on the chemical shift characterising the plateau of the SCC of unit I (*Fig. 3*,*a*). This is not the case for **CC4**, where HO–C(5′/II) points away from this base pair (*see Fig. 1*), but could be realised for **CC6**, where HO–C(5′/II) may have a π-contact close to C(5/I). We do, however, not see why this interaction should be sufficient to favour **CC6**. It is more likely that HO–C(5′/II) of **CC6** forms an intramolecular H-bond to O=C(2/II) (in $CDCl_3$ solution). The antiparallel duplex **CC2** may well be favoured, as pairing of both bases is strengthened by cooperative H-bonding involving HO–C(5′/II).

The above ^{1}H-NMR analysis suggests an equilibrium between the parallel duplexes **CC4** and **CC5** of the silyl ethers **11** and **14**, with **CC4** predominating. The isomerisation of **CC4** to **CC5** of **11** could take place by rotation of the intact base pair between units I, while the analogous isomerisation of **14** requires breaking this base pair apart and reorienting the cytosine moieties. The alcohols **12** and **15**, however, appear to form (most probably) antiparallel duplexes. For **12**, there may be an equilibrium between **CC2** and **CC3**, with **CC2** predominating, while **15** forms only **CC2**. This difference agrees well with the expectation, since the $MMTrOCH_2$ substituent of **15** strongly disfavours an *anti*-orientation of the nucleobase.

The CD spectra of 1 mM solutions of the dinucleosides **11**, **14**, and **15** in $CHCl_3$ (*Fig. 4*) show a very weak dependence of the ellipticity on the temperature, evidencing

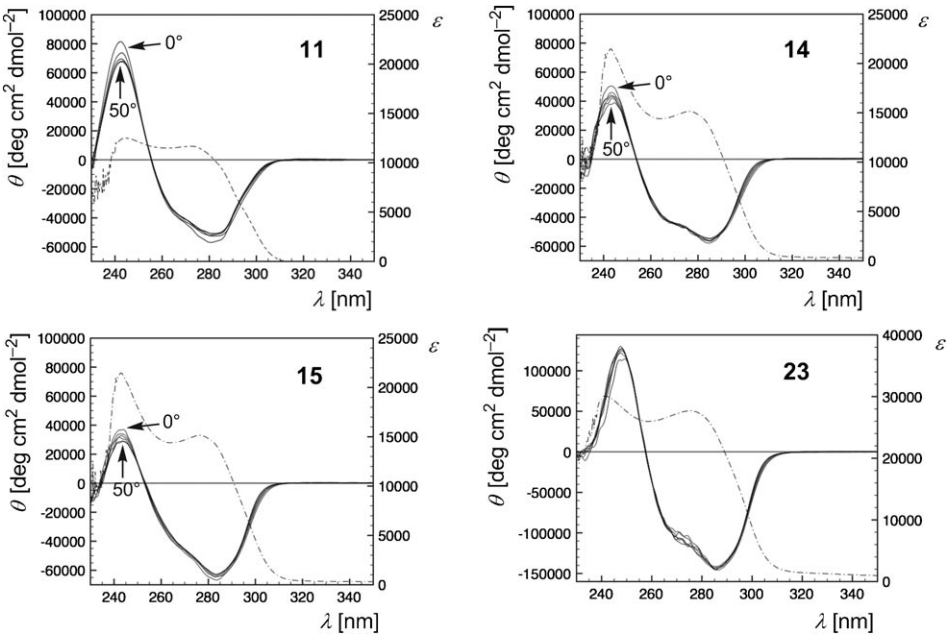

Fig. 4. *Temperature-dependent CD spectra* (solid lines, in 10° steps from 0 to 50°) *and UV spectra* (dashed lines) *of the dinucleosides* **11**, **14**, *and* **15**, *and the tetranucleoside* **23** *for 1 mM solutions in* $CHCl_3$

the absence of stacking in the cyclic duplexes, in agreement with the large distance of *ca.* 6 Å between the base pairs found by *Maruzen* modeling (see above). All spectra show negative *Cotton* effects characterised by a maximum around 245 nm and a minimum around 280 nm.

Association of the 2′,3′-O-Isopropylidene-Protected C[s]C*[s]C*[s]C* Tetranucleoside* **23**. The association of the isopropylidene-protected and thus lipophilic tetranucleoside **23** was studied in several solvents and solvent mixtures by ^{1}H-NMR spectroscopy, VPO of the apparent molecular weight, and temperature-dependent CD spectroscopy. The DQF-COSY ^{1}H-NMR spectrum of **23** in $CDCl_3$ shows signals for four H_2N–C(4′) groups, each with a signal at low and high field (10.78/5.35, 10.08/7.29, 10.04/7.29, and 9.43/7.01 ppm), evidencing that all NH_2 groups are involved in base pairing. ^{1}H-NMR Dilution experiments with solutions of **23** in $CDCl_3$, (D_6)acetone, CD_3CN, (D_8)THF, (D_5)pyridine, and $CDCl_3$/(D_6)DMSO 4 : 1 showed no concentration dependence of the NH signals, suggesting that the association in these solvents is too strong. ^{1}H-NMR Spectra in $CDCl_3$/(D_6)DMSO < 4 : 1 showed broad NH signals.

The mixture CD_3CN/(D_6)DMSO 4 : 1 proved suitable for a dilution experiment of **23**. The solubility limited the concentration to a maximum of 10 mM so that the chemical shift of the broad signal for H_aN–C(4/I – IV) was followed in the concentration range between 10 and 0.39 mM (*Fig. 5*). The dilution experiment resulted in a SCC, which shows a strong bending at concentrations between 1 and 5 mM, evidencing the formation of cyclic duplexes. Unfortunately, the limited concentration range did not allow checking the formation of a plateau. Calculation led to chemical shifts of 6.98 ±

0.10 and 9.97 ± 0.09 ppm for H_aN–C(4/I – IV) of the monoplex and duplex, respectively, and to a K_{ass} value of 6100 ± 1600, corresponding to a $-\Delta G_{295}$ value of 5.1 kcal/mol. For comparison, the concentration dependence of δ(NH) of **11** was studied in the same solvent mixture. A 0.8 mM solution showed 3 NH signals at 6.55, 6.78, and 6.98 ppm (2 NH) that were shifted downfield to 7.08, 7.12, 7.30, and 7.46 ppm upon increasing the concentration to 31 mM. Overlapping of the signals was observed at intermediate concentrations. The SCC of the most strongly deshielded NH of **11** is depicted in *Fig. 5*. The weak downfield shift (< 0.5 ppm) of all NH signals of **11** evidences some unspecific and weakly persistent intermolecular interactions at higher concentrations.

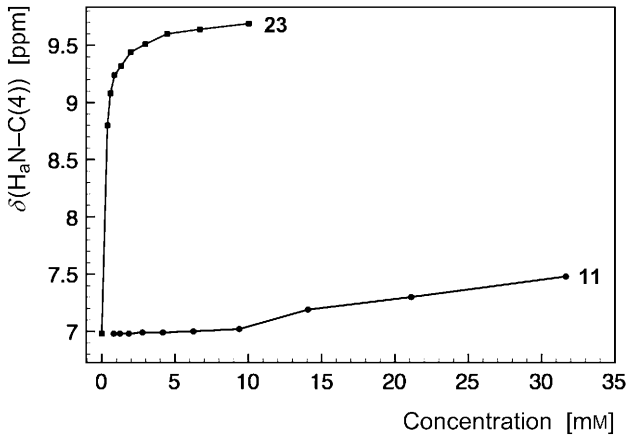

Fig. 5. *Shift/concentration curves* (SCCs) *for H_aN–C(4/I – IV) of the tetranucleoside* **23** (including a value of 6.98 ppm for a 0.0001 mM solution) *and for the most deshielded H_aN–C(4) of the dinucleoside* **11** *in $CD_3CN/(D_6)DMSO$ 4:1*

The conformation of the cyclic duplex of the tetranucleoside **23** in $CDCl_3$ was investigated by NOESY ^{1}H-NMR spectroscopy. The amino groups of the terminal units I and IV of **23** show the same NOESY pattern as units I and II of the dinucleosides **11** and **14**; *i.e.*, strong cross-peaks between H–C(5) and both NH, and an EXSY cross-peak between the two NH signals (only visible for unit I). The amino groups of the central units II and III of **23** show a different pattern, *viz.*, cross-peaks between the two NH, and between the more strongly shielded NH and H–C(5), and a TOCSY cross-peak between the more deshielded NH and H–C(5). This evidences that only the terminal base pairs were broken within the mixing time of the NOESY measurement to allow rotation about the C(4)–NH_2 bond. The absence of any cross-peak between the signals at 10.78 and 9.43 ppm, and between the signals at 10.08 and 10.04 ppm is a strong evidence for parallel duplexes. The three linkers adopt the same conformation, as revealed by large J(4′,5′a/I – III) of *ca.* 11 Hz and small J(4′,5′b/I – III) ≤ 1.5 Hz, evidencing the *gt*-conformation, and strong ROESY cross-peaks between H–C(1′/II – IV) and the more strongly shielded CH–C(6/II – IV), and between H–C(5/II – IV) and the less shielded CH–C(6/II – IV). Units II – IV prefer completely the *syn*-conformation, as indicated by strong cross-peaks between H–C(1′/II – IV) and the more shielded

CH–C(6/II – IV), and by a weak cross-peak between H–C(2′/II – IV) and the more shielded CH–C(6/II – IV). Unit I, however, adopts a *syn*/*anti*-equilibrium. This is evidenced by strong cross-peaks of both CH–C(6/I) with both H–C(1′/I) and H–C(2′/I). The cross-peaks with H–C(1′/I) are twice as large as those with H–C(2′/I). Thus, **23** shows the same conformational equilibrium as the parent dinucleoside **14** (*i.e.,* **CC4** and some **CC5**).

As expected, the CD spectrum of **23** in $CHCl_3$ (*Fig. 4*) is similar to the CD spectra of the dinucleosides **11**, **14**, and **15**. It shows no base stacking, confirming the large distance between the base pairs in the cyclic duplex.

Association of the Unprotected C[*s*]C(*) Dinucleosides* **13** *and* **18**, *and of the C*[*s*]C*[*s*]C*[*s*]C* Tetranucleoside* **24**. CD Spectroscopy is used to evidence base stacking of dinucleosides in aqueous solutions [34 – 36]. We recorded CD spectra for 1 mM solutions of **13**, **18**, and **24** in H_2O in steps of 10° between 0 and 90° (*Fig. 6*). The spectrum of the *C(6/I)*-unsubstituted **13** shows no dependence of the ellipticity on the temperature, evidencing the absence of stacking in H_2O, as it is expected for solvated monoplexes and linear duplexes, and also for the cyclic duplexes with a large distance between the base pairs. The CD spectra of the *C(6/I)*-hydroxymethylated **18** and **24**, however, show a temperature-dependent negative *Cotton* effect with a exciton interaction at 270 nm, leading to two CD bands and evidencing base-stacking. This observation is rationalized by stacking of the hydroxymethylated cytosine moieties

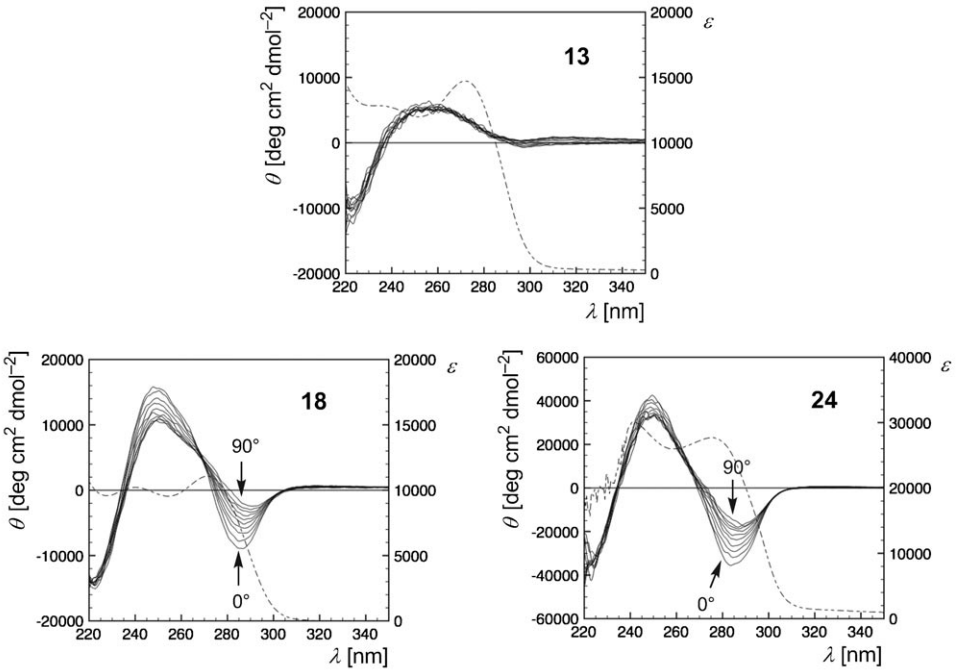

Fig. 6. *Temperature-dependent CD spectra* (solid lines, in 10° steps from 0 to 50°) *and UV spectra* (dashed lines) *of the dinucleosides* **13** *and* **18**, *and the tetranucleoside* **24** *for 1 mM solutions in* H_2O

promoted by intermolecular H-bonds of $HOCH_2$–C(6/I) to an unidentified acceptor of unit I.

^{1}H-NMR Spectra of **13**, **18**, and **24** in D_2O (*Table 8* in the *Exper. Part*) were recorded at a concentration of 10 mM for **13** and **18**, and of 2.6 mM for **24**. Whereas **13** shows a single set of signals, **18** and **24** are revealed as 9 : 1 mixtures of isomers. Double sets of signals of unit I and of the linking unit only of **18** are in agreement with a 9 : 1 mixture of the solvated monoplex and the solvated base-stacked duplex, evidenced by CD spectroscopy, as discussed above. These two species do not equilibrate on the NMR time scale. For **24**, only the H–C(1′/I) signal of the minor component is visible, whereas the other signals are hidden by signals of the other units. The same downfield shift for H–C(1′/I) of the minor components of **18** and **24** ($\Delta\delta = 0.06$ ppm relative to the major component) suggests a solvated base-stacked duplex as the minor component also for **24**. To detect the chemical shifts of the NH_2 groups, ^{1}H-NMR spectra of **13** and **18** were recorded in H_2O/D_2O 9 : 1 at 23°. They show weak NH signals at 6.65 – 7.6 ppm, with only 10% of the expected intensity. Similar chemical shifts as the NH_2 group of cytidine monophosphate (200 mM solution: NH_2 signals at 7.35 and 6.85 ppm) [37] evidence – as expected – the absence of base pairing.

In the D_2O spectrum of **24**, the signals for the corresponding H-atoms of the four units overlap except for H–C(5) and H–C(1′), preventing a conformational analysis as described here for the dinucleosides **13** and **18**. The *C(6)*-substituted units (unit II of **13** and both units of **18**) adopt a *syn*-conformation, evidenced by δ(H–C(2′)) of 4.76 – 4.84 ppm. The upfield shift for H–C(2′/I) (0.45 ppm) relative to H–C(2′/II) of the *C(6/I)*-unsubstituted **13** evidences an *anti*-configured unit I. All ribofuranosyl moieties of **13** and **18** adopt a (*N*)-conformation (J(1′,2′)/J(3′,4′) in the range of 0.55 – 0.6). Larger J(4′,5′b) than J(4′,5′a) values suggest that the more strongly shielded H_b–C(5′) corresponds to the $H_{pro\text{-}R}$–C(5′), in agreement with [33]. *gg*/*gt*/*tg* Ratios of 45 : 50 : 5 and 30 : 65 : 5 were calculated for unit I of **13** and **18** from the coupling constants in *Table 8* (*Exper. Part*). These rotameric equilibria agree well with solvated monoplexes of **13**, and with a mixture of solvated monoplexes and a base(I)-stacked duplex of **18**.

Biological Testing. The employment of *Xenopus laevis* embryos and tadpoles as efficient and cost-effective vertebrate animal models for *in vivo* drug-discovery screens, and the estimation of drug toxicities was recently reviewed [38]. For example, *Xenopus* embryos were successfully used to identify novel anti-angiogenic compounds which had comparable bioactivities in a mouse model of neovascularization [39].

The unprotected, H_2O-soluble dinucleosides **13** and **18** were evaluated for toxicity and teratogenicity using the *Xenopus* embryos (*Fig. 7*). Compound testing covered embryonic development from the onset of blood circulation until embryos became tadpoles and reached feeding stages. The studies were conducted according to the protocols approved by the Veterinary Office of the Canton of Zurich, Switzerland (Permit No. 1997/2004 to *A. W. B.*). *Xenopus* embryos were obtained by *in vitro* fertilization, staged, and documented as described in [39]. The embryos (25 embryos per *Petri* dish in a final volume of 5 ml) were treated from stage 32 (1 d, 16 h post fertilization) to stage 48 (7 d, 12 h) with 0.1x MMR (0.1 M NaCl, 2 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM *HEPES*, pH 7.8) alone or 0.1x MMR supplemented with 5, 10, and 20 μM of **13**, or 5, 10, and 20 μM of **18**. The embryos were monitored daily for any evidence of abnormal embryonic development or altered morphology. No adverse

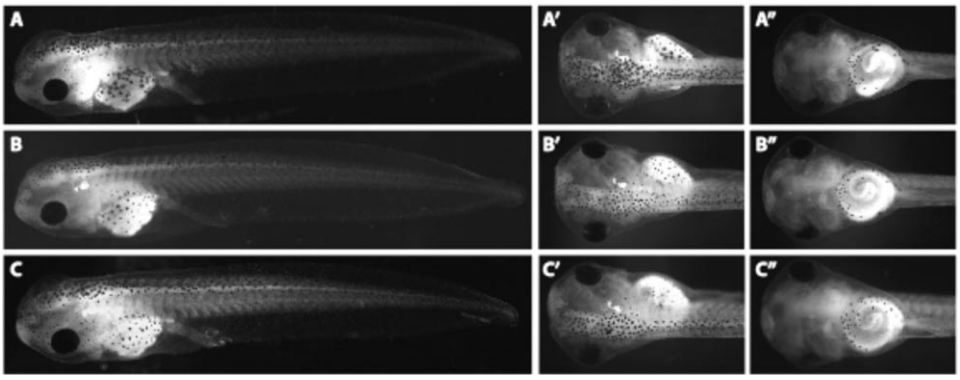

Fig. 7. *Exposure of* Xenopus laevis *embryos to* **13** *and* **18** *shows no developmental defects at the highest concentration. X. laevis* stage 45 tadpoles are shown in lateral (*A*, *B*, and *C*), dorsal (*A′*, *B′*, and *C′*), and ventral views (*A″*, *B″*, and *C″*). Embryos were immersed from stage 32 to stage 48 in salt water only (*A*), or in salt water containing either 20 μM of **13** (*B*) or 20 μM of **18** (*C*).

side effects of the treatments with **13** or **18** could be observed at all concentrations tested, and survival of the embryos was 100%.

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Experimental Part

General and Procedure for ^{1}H-NMR Studies. See [1].

N^4*-Benzoyl-5′*-O-*[dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylidenecytidine* (**3**). A soln. of **2** [11] (22.4 g, 57.8 mmol) and 1*H*-imidazole (12.6 g, 185 mmol) in DMF (350 ml) was treated dropwise with 'thexyl(dimethyl)chlorosilane' (= TDSCl = dimethyl(1,1,2-trimethyl)silyl chloride; 16.5 g, 92.5 mmol) and stirred for 12 h. The mixture was diluted with MeOH (50 ml) and evaporated. A soln. of the residue in AcOEt (500 ml) was washed with H_2O (2 ×) and brine, dried ($MgSO_4$), and evaporated. Crystallization from EtOH gave **3** (27.8 g, 91%). Colourless crystals. R_f (CH_2Cl_2/AcOEt 4 :1) 0.23. M.p. 153.0–154.5°. $[\alpha]_D^{25} = +10.7$ ($c = 0.75$, $CHCl_3$). UV ($CHCl_3$): 260 (22860), 310 (9480). IR (ATR): 3231*w*, 3075*w*, 2957*w*, 2865*w*, 1693*m*, 1665*s*, 1611*m*, 1555*m*, 1484*s*, 1433*w*, 1384*m*, 1376*m*, 1339*m*, 1301*m*, 1263*s*, 1250*s*, 1212*m*, 1186*w*, 1141*m*, 1102*s*, 1069*s*, 1042*w*, 1023*m*, 995*m*, 966*w*, 941*w*, 907*w*, 891*w*, 867*m*, 843*s*, 825*s*, 810*s*, 791*m*, 779*s*, 752*m*, 718*m*, 695*s*, 671*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 4*; additionally, 8.84 (br. *s*, BzN*H*); 7.91–7.87 (*m*, 2 arom. H); 7.61–7.46 (*m*, 3 arom. H, H–C(5)); 1.58 (*sept.*, $J = 6.9$, Me_2CH); 1.58, 1.34 (2*s*, Me_2CO_2); 0.85 (*d*, $J = 6.9$, Me_2CH); 0.82 (*s*, Me_2CSi); 0.11 (*s*, Me_2Si). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 5*; additionally, 166.48 (br. *s*, NHC=O); 133.19 (*d* and *s*); 129.04, 127.58 (4*d*); 113.87 (*s*, Me_2CO_2); 34.10 (*d*, Me_2CH); 27.40, 25.50 (2*q*, Me_2CO_2); 25.50 (*s*, Me_2CSi); 20.48, 20.45 (2*q*, Me_2CSi); 18.67, 18.63 (2*q*, Me_2CH); −3.04, −3.27 (2*q*, Me_2Si). MALDI-MS: 530.3 (100, $[M + H]^+$). Anal. calc. for $C_{27}H_{39}N_3O_6Si$ (529.71): C 61.22, H 7.42, N 7.93; found: C 61.10, H 7.32, N 7.91.

N^4*-Benzoyl-5′*-O-*[dimethyl(1,1,2-trimethylpropyl)silyl]-6-(hydroxymethyl)-2′,3′*-O-*isopropylidenecytidine* (**4**). A soln. of ^{i}Pr$_2$NH (17.4 g, 172 mmol) in THF (130 ml) was cooled to 0°, treated dropwise with 1.6M BuLi in hexane (108 ml, 172 mmol), and cooled after 90 min to −70°. The cooled soln. of LDA was transferred during 25 min *via* a *Teflon* canula to a −70° cold soln. of **3** (18.2 g, 34.4 mmol) in THF (350 ml). The soln. was stirred for 1 h at −70°, treated dropwise with DMF (27 ml, 344 mmol), stirred for 1 h at −60°, warmed to −20°, and treated with AcOH (11 ml). The mixture was poured into sat. NH_4Cl soln. and extracted with AcOEt (3 ×). The combined org. layers were washed with H_2O (2 ×) and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue in EtOH (200 ml) was treated dropwise

Table 4. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Cytidine Mononucleotides* **3**–**10** *in* $CDCl_3$[a])

	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
H–C(5)	[b])	7.61[c])	[b])	7.47[c])	[b])	[b])	7.67[c])	[b])
H–C(6)	8.15	–	–	–	–	–	–	7.73
CH_a–C(6)	–	4.72	4.21	4.26	4.57	4.22	5.27	–
CH_b–C(6)	–	4.72	4.21	4.16	4.49	4.19	5.20	–
H–C(1′)	5.98	5.92	5.84	5.76	5.98	5.86	5.71	5.65
H–C(2′)	4.76	5.30	5.30	5.36	5.33	5. 31	5.34	5.13
H–C(3′)	4.74	4.91	4.90	5.17	4.93	4.94	4.97	4.80
H–C(4′)	4.39	4.19	4.13	4.21	4.23	4.12	4.22	4.30
H_a–C(5′)	3.94	3.87	3.87	3.92–3.83	3.87	3.34	3.34	3.33
H_b–C(5′)	3.78	3.80	3.81	3.92–3.83	3.81	3.27	3.29	3.33
J(5,6)	7.5	–	–	–	–	–	–	7.4
J(H_a,H_b)	–	[d])	[d])	12.9	12.9	12.8	13.5	–
J(1′,2′)	1.8	1.2	1.0	2.5	1.1	0.9	1.0	1.6
J(2′,3′)	6.2	6.5	6.5	6.7	6.4	6.5	6.5	6.5
J(3′,4′)	2.7	4.1	4.1	4.0	4.0	3.8	3.6	3.9
J(4′,5′a)	2.5	5.4	5.5	[c])	5.5	6.9	7.0	6.7
J(4′,5′b)	3.6	7.1	7.2	[c])	7.3	7.1	7.4	6.7
J(5′a,5′b)	11.6	10.7	10.5	[c])	10.7	13.6	13.6	[c])

[a]) Assignments based on a HSQC spectrum (**9** and **10**). [b]) Hidden by aromatic signals at 7.65–7.45 ppm. [c]) Broad signal. [d]) Not assigned.

Table 5. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of the Cytidine Mononucleotides* **3**–**10** *in* $CDCl_3$

	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
C(2)[b])	154.66	155.55	157.69	155.77	154.8	155.4	152.16	154.54
C(4)	162.48	162.66	162.16	162.55	162.20	162.52[b])	162.50	163.14
C(5)[b])	96.29	96.64	97.15	97.82	98.84	97.52	98.68	96.98
C(6)	145.01	160.26	158.96	157.61	155.21	157.58[b])	154.67	147.16
CH_2–C(6)	–	61.33	62.80	62.83	41.17	62.94	64.42	–
C(1′)	94.15	92.50	93.11	93.33	90.66	93.40	93.26	97.17
C(2′)	86.33	84.31	84.23	83.38	84.19	85.00	84.74	85.06
C(3′)	80.31	82.66	83.02	80.70	82.78	85.08	84.87	83.70
C(4′)	88.02	90.20	90.29	88.42	90.66	88.83	89.17	87.53
C(5′)	63.26	64.03	62.80	62.99	64.12	31.72	31.61	31.36

[a]) Assignments based on HSQC spectra (**9** and **10**). [b]) Broad signal.

with a soln. of $NaBH_4$ (1.43 g, 37.8 mmol) in EtOH (200 ml), stirred for 30 min, and diluted with sat. NH_4Cl soln. After evaporation of the org. solvents, the mixture was extracted with AcOEt (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (Et_2O/pentane 4:1) gave **4** (16.6 g, 86%). Colourless solid. R_f (Et_2O/cyclohexane 4:1) 0.20. $[\alpha]_D^{25} = +7.2$ (c = 0.75, $CHCl_3$). UV ($CHCl_3$): 262 (22120), 312 (8600). IR (ATR): 3285*w* (br.), 2957*w*, 2867*w*, 1675*m*, 1649*m*, 1609*s*, 1567*s*, 1477*m*, 1414*m*, 1351*s*, 1249*s*, 1210*m*, 1158*m*, 1129*m*, 1082*s*, 1061*s*, 1000*w*, 971*w*, 901*w*, 873*m*, 828*s*, 777*s*, 702*m*, 640*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): see *Table 4*; additionally, 8.92 (br. *s*, BzN*H*); 7.85–7.83 (*m*, 2 arom. H); 7.56–7.42 (*m*, 3 arom. H); 1.59 (*sept.*, J = 6.9, Me_2CH); 1.52, 1.32 (2*s*, Me_2CO_2); 0.85 (*d*, J = 6.9, *Me*$_2$CH); 0.82 (*s*, *Me*$_2$CSi); 0.08, 0.06 (2*s*, Me_2Si); OH signal not visible.

^{13}C-NMR (100 MHz, $CDCl_3$); assignments based on a HMBC and a HSQC spectrum): see *Table 5*; additionally, 167.10 (br. *s*, NHC=O); 133.39 (*d*); 133.11 (*s*); 129.14 (2*d*); 127.83 (2*d*); 113.76 (*s*, Me_2CO_2); 34.26 (*d*, Me_2CH); 27.33, 25.45 (2*q*, Me_2CO_2); 25.45 (*s*, Me_2CSi); 20.52, 20.47 (2*q*, Me_2CSi); 18.65, 18.61 (2*q*, Me_2CH); −3.14, −3.16 (2*q*, Me_2Si). HR-MALDI-MS: 598.2351 (27, $[M + K]^+$, $C_{28}H_{41}KN_3O_7Si^+$; calc. 598.2345), 582.2794 (37, $[M + Na]^+$, $C_{28}H_{41}N_3NaO_7Si^+$; calc. 582.2602), 560.2794 (100, $[M + H]^+$, $C_{28}H_{42}N_3O_7Si^+$; calc. 560.2787). Anal. calc. for $C_{28}H_{41}N_3O_7Si$ (559.73): C 60.08, H 7.38, N 7.51; found: C 60.11, H 7.52, N 7.28.

N^4-*Benzoyl-5′*-O-*[dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylidene-6*-{[(4-*methoxyphenyl)(diphenyl)methoxy]methyl}cytidine* (**5**). A stirred soln. of **4** (857 mg, 1.53 mmol), DMAP (10 mg, 0.08 mmol) und EtN^iPr_2 in CH_2Cl_2 (50 ml) was cooled to 0° and treated in portions with MMTrCl (1.89 g, 6.12 mmol). After 4 h, the mixture was diluted with H_2O (20 ml), and the phases were separated. The aq. phase was extracted with CH_2Cl_2 (2×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (Et_2O/CH_2Cl_2 1:9) gave **5** (1.11 g, 87%). Colourless foam. R_f (Et_2O/CH_2Cl_2 1:9) 0.28. $[\alpha]_D^{25} = -11.8$ ($c = 1.0$, $CHCl_3$). IR (ATR): 3400–3100*w* (br.), 2956*w*, 2865*w*, 1681*m*, 1612*s*, 1567*s*, 1507*m*, 1474*m*, 1448*m*, 1424*m*, 1371*m*, 1351*s*, 1314*m*, 1301*m*, 1249*s*, 1210*m*, 1180*m*, 1155*m*, 1063*s*, 1034*s*, 1001*m*, 976*w*, 931*w*, 900*w*, 874*w*, 828*s*, 777*m*, 766*m*, 745*m*, 698*s*, 671*w*, 631*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 4*; additionally, 8.53 (br. *s*, BzN*H*); 7.85–7.83 (br. *d*, $J = 6.7$, 2 arom. H); 7.64–7.50 (*m*, 7 arom. H, H–C(5)); 7.40–7.22 (*m*, 8 arom. H); 6.87–6.84 (*m*, 2 arom. H); 3.78 (*s*, MeO); 1.59 (*sept.*, $J = 6.8$, Me_2CH); 1.46, 1.31 (2*s*, Me_2CO_2); 0.85 (*d*, $J = 7.0$, Me_2CH); 0.82 (*s*, Me_2CSi); 0.07, 0.04 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 5*; additionally, 158.96 (*s*, MeO*C*); 143.35 (*s*, 2 C); 134.33 (*s*); 133.18 (*s* and *d*); 130.58–127.37 (several *d*); 113.47 (*d*, 2 C); 113.22 (*s*, Me_2CO_2); 88.48 (*s*, Ph_2C); 55.37 (*q*, MeO); 34.28 (*d*, Me_2CH); 27.41, 25.67 (2*q*, Me_2CO_2); 25.48 (*s*, Me_2CSi); 20.62, 20.57 (2*q*, Me_2CSi); 18.73, 18.69 (2*q*, Me_2CH); −2.96, −3.03 (2*q*, Me_2Si); NHC=O signal hidden by the noise. HR-MALDI-MS: 854.3845 (31, $[M + Na]^+$, $C_{48}H_{57}N_3NaO_8Si^+$; calc. 854.3807), 832.4003 (42, $[M + H]^+$, $C_{48}H_{58}N_3O_8Si^+$; calc. 832.3988), 273.1287 (100, $MMTr^+$, $C_{20}H_{17}O^+$; calc. 273.1274). Anal. calc. for $C_{48}H_{57}N_3O_8Si$ (832.08): C 69.29, H 6.90, N 5.05; found: C 69.02, H 6.95, N 5.08.

N^4-*Benzoyl-2′,3′*-O-*isopropylidene-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}cytidine* (**6**). A soln. of **5** (6.32 g, 7.6 mmol) in THF (100 ml) was treated with $(HF)_3 \cdot NEt_3$ (19.3 g, 120 mmol), stirred for 20 h at 23°, poured into sat. $NaHCO_3$ soln., and extracted with CH_2Cl_2 (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (CH_2Cl_2/AcOEt 3:7) gave **6** (5.24 g, 93%). Colourless foam. R_f (CH_2Cl_2/AcOEt 3:7) 0.19. $[\alpha]_D^{25} = -23.1$ ($c = 1.0$, $CHCl_3$). UV ($CHCl_3$): 262 (26300), 310 (10550). IR (ATR): 3384*w*, 3059*w*, 2984*w*, 2934*w*, 1667*m*, 1611*s*, 1568*s*, 1508*m*, 1478*m*, 1447*m*, 1423*m*, 1371*s*, 1352*s*, 1301*s*, 1249*s*, 1211*m*, 1178*m*, 1156*m*, 1102*s*, 1062*s*, 1031*s*, 976*m*, 902*m*, 872*w*, 830*m*, 791*w*, 765*w*, 726*m*, 698*s*, 669*w*, 640*m*, 631*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 4*; additionally, 8.76 (br. *s*, BzN*H*); 7.90 (br. *d*, $J = 7.2$, 2 arom. H); 7.65–7.49 (*m*, 7 arom. H); 7.41–7.23 (*m*, 8 arom. H); 6.87–6.84 (*m*, 2 arom. H); 3.92–3.83 (*m*, 2 H–C(5′), OH); 3.78 (*s*, MeO); 1.41, 1.31 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 5*; additionally, 166.36 (br. *s*, NHC=O); 158.96 (*s*); 143.34 (*s*, 2 C); 134.20 (*s*); 133.27 (*d*); 132.87 (*s*); 130.54–127.39 (several *d*); 113.79 (*s*, Me_2C); 113.45 (*d*, 2 C); 88.56 (*s*, Ph_2C); 55.36 (*q*, MeO); 27.46, 25.50 (2*q*, Me_2C). HR-MALDI-MS: 712.2617 (32, $[M + Na]^+$, $C_{40}H_{39}N_3NaO_8^+$; calc. 712.2635), 273.1277 (100, $MMTr^+$, $C_{20}H_{17}O^+$; calc. 273.1274).

N^4-*Benzoyl-6-(chloromethyl)-5′*-O-*[dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylidenecytidine* (**7**). A soln. of **4** (3.94 g, 7.04 mmol) and EtN^iPr_2 (1.47 ml, 8.45 mmol) in CH_2Cl_2 (100 ml) was cooled to 0°, treated dropwise with a soln. of Ms_2O (1.35 g, 7.74 mmol) in CH_2Cl_2 (40 ml), and stirred for 90 min. The mixture was treated with a soln. of LiCl (4.49 g, 106 mmol) in DMF (60 ml) and stirred for 4 h at 23°. The mixture was poured into brine and extracted with AcOEt (3×). The combined org. layers were washed with H_2O (3×) and brine, dried ($MgSO_4$), and evaporated. FC (pentane/AcOEt 5:2) gave **7** (3.25 g, 80%). Colourless foam. R_f (Et_2O/CH_2Cl_2 1:11) 0.22. $[\alpha]_D^{25} = -12.5$ ($c = 1.0$, $CHCl_3$). IR (ATR): 3400–3100*w* (br.), 2957*w*, 2867*w*, 1680*s*, 1615*s*, 1564*s*, 1500*w*, 1475*m*, 1420*w*, 1406*w*, 1354*s*, 1248*s*, 1210*m*, 1155*m*, 1129*m*, 1080*s*, 1001*w*, 982*w*, 898*w*, 873*m*, 828*s*, 778*m*, 737*m*, 701*m*, 661*m*, 616*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 4*; additionally, 8.79 (br. *s*, BzN*H*); 7.88 (br. *d*, $J = 7.5$, 2 arom. H); 7.64–7.58 (*m*, 1 arom. H, H–C(5)); 7.56–7.47 (*m*, 2 arom. H); 1.59 (*sept.*, $J = 6.9$, Me_2CH); 1.56, 1.35 (2*s*, Me_2CO_2); 0.85 (*d*, $J = 6.9$, Me_2CH); 0.82, 0.81 (2*s*, Me_2CSi); 0.07, 0.05 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 5*; additionally, 166.5 (br. *s*, NHC=O); 134.33 (*d*); 132.77 (*s*); 129.09 (*d*, 2 C); 127.65 (*d*,

2 C); 113.57 (*s*, Me_2CO_2); 34.29 (*d*, Me_2CH); 27.41, 25.55 (2*q*, Me_2CO_2); 25.47 (*s*, Me_2CSi); 20.60, 20.54 (2*q*, Me_2CSi); 18.74, 18.69 (2*q*, Me_2CH); −3.01 (*q*, Me_2Si). HR-MALDI-MS: 600.2267 (29, $[M + Na]^+$, $C_{28}H_{40}ClN_3NaO_6Si^+$; calc. 600.2267), 578.2459 (68, $[M + H]^+$, $C_{28}H_{41}ClN_3O_6Si^+$; calc. 578.2448), 542.2667 (100, $[M - Cl]^+$, $C_{28}H_{40}N_3O_6Si^+$; calc. 542.2681). Anal. calc. for $C_{28}H_{40}ClN_3O_6Si$ (578.18): C 58.17, H 6.97, N 7.27; found: C 57.98, H 6.96, N 7.07.

5′-S-Acetyl-N⁴-benzoyl-2′,3′-O-isopropylidene-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-5′-thiocytidine (**8**). A soln. of PPh_3 (4.21 g, 16.1 mmol) in THF (30 ml) was cooled to 0°, treated dropwise with DIAD (3.25 g, 16.1 mmol), and stirred for 10 min. The mixture was treated with a soln. of **6** (7.38 g, 10.7 mmol) in THF (10 ml), stirred for 10 min, treated dropwise with AcSH (1.14 g, 15 mmol), and stirred for another 90 min at 0°. The mixture was diluted with H_2O and extracted with AcOEt (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (pentane/AcOEt 2:1 → 0:1) gave **8** (7.91 g, 99%). Yellow foam. R_f (AcOEt/pentane 1:1) 0.35. $[\alpha]_D^{25} = +3.9$ (c = 1.0, $CHCl_3$). UV ($CHCl_3$): 245 (12600), 273 (11800). IR (ATR): 3400–3200*w* (br.), 3054*w*, 2986*w*, 2931*w*, 1683*s*, 1610*s*, 2986*w*, 1508*m*, 1478*m*, 1447*m*, 1418*m*, 1371*m*, 1351*m*, 1301*m*, 1248*s*, 1210*m*, 1180*m*, 1155*m*, 1134*w*, 1091*s*, 1061*s*, 1031*s*, 1001*m*, 980*m*, 899*w*, 871*m*, 831*m*, 795*w*, 765*w*, 746*w*, 698*s*, 628*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 4*; additionally, 8.85–8.55 (br. *s*, BzN*H*); 7.90 (br. *d*, J = 7.2, 2 arom. H); 7.64–7.58 (*m*, 1 arom. H); 7.55–7.45 (*m*, 4 arom. H, H–C(5)); 7.42–7.23 (*m*, 10 arom. H); 6.88–6.83 (*m*, 2 arom. H); 3.78 (*s*, MeO); 2.32 (*s*, AcS); 1.44, 1.30 (2*s*, Me_2C). ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 5*; additionally, 195.13 (*s*, SC=O); 159.22 (*s*); 144.65, 143.48 (2*s*); 134.45 (*s*); 133.36 (*d* and *s*); 130.72–127.52 (several *d*); 113.59 (*d*, 2 C); 113.59 (*s*, Me_2C); 88.64 (*s*, Ph_2C); 55.38 (*q*, MeO); 30.72 (*q*, *Me*C=O); 27.23, 25.47 (2*q*, Me_2C); signal of NHC=O hidden by the noise. HR-MALDI-MS: 786.2234 (10, $[M + K]^+$, $C_{42}H_{41}KN_3O_8S^+$; calc. 786.2246), 770.2499 (10, $[M + Na]^+$, $C_{42}H_{41}N_3NaO_8S^+$; calc. 770.2507), 748.2683 (10, $[M + H]^+$, $C_{42}H_{42}N_3O_8S^+$; calc. 748.2687), 273.1281 (100, $MMTr^+$; calc. 273.1274). Anal. calc. for $C_{42}H_{41}N_3O_8S \cdot H_2O$ (765.87): C 65.87, H 5.66, N 5.49; found: C 66.00, H 5.66, N 5.49.

5′-S-Acetyl-N⁴-benzoyl-2′,3′-O-isopropylidene-6-{[(methylsulfonyl)oxy]methyl}-5′-thiocytidine (**9**). A soln. of **8** (3.78 g, 5.05 mmol) in CH_2Cl_2 (135 ml) was treated dropwise with Cl_2CHCO_2H (15 ml) and $^iPr_3SiH$ (2.41 g, 15.2 mmol), stirred for 45 min, and poured into sat. $NaHCO_3$ soln. The mixture was extracted with CH_2Cl_2 (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue in CH_2Cl_2 (100 ml) was cooled to 0° and treated with EtN^iPr_2 (1.06 ml, 6.06 mmol) and dropwise with Ms_2O (968 mg, 5.56 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred for 2 h at 0° and poured into H_2O. After separation of the layers, the aq. phase was extracted with CH_2Cl_2 (2×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/pentane 2:1) gave **9** (1.93 g, 69%). Colourless foam. R_f (AcOEt/pentane 3:2) 0.23. $[\alpha]_D^{25} = +11.5$ (c = 0.5, $CHCl_3$). IR (ATR): 3327*w*, 3129*w*, 2988*w*, 2935*w*, 1678*s*, 1617*s*, 1567*s*, 1498*w*, 1479*m*, 1422*w*, 1347*s*, 1242*s*, 1210*m*, 1175*s*, 1158*m*, 1091*s*, 1056*s*, 1002*m*, 967*m*, 948*m*, 899*w*, 870*m*, 833*m*, 800*m*, 785*m*, 761*w*, 701*m*, 664*w*, 625*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 4*; additionally, 8.73 (br. *s*, BzN*H*); 7.88 (br. *d*, J = 7.3, 2 arom. H); 7.66–7.49 (*m*, 3 arom. H); 3.20 (*s*, MsO); 2.34 (*s*, AcS); 1.53, 1.34 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 5*; additionally, 194.74 (*s*, SC=O); 166.54 (br. *s*, NHC=O); 133.46 (*d*); 132.65 (*s*); 129.06 (2*d*); 127.72 (2*d*); 113.84 (*s*, Me_2C); 38.78 (*q*, MsO); 30.78 (*q*, *Me*C=O); 27.21, 25.45 (2*q*, Me_2C). HR-MALDI-MS: 576.1090 (100, $[M + Na]^+$, $C_{23}H_{27}N_3NaO_9S_2^+$; calc. 576.1081). Anal. calc. for $C_{23}H_{27}N_3O_9S_2$ (553.61): C 49.90, H 4.92, N 7.59; found: C 49.82, H 5.03, N 7.34.

5′-S-Acetyl-N⁴-benzoyl-2′,3′-O-isopropylidene-5′-thiocytidine (**10**). A suspension of NaH (60% in oil; 340 mg, 8.52 mmol) in THF (15 ml) was cooled to 0°, treated dropwise with a soln. of **2** (1.65 g, 4.26 mmol) in THF (50 ml), and stirred for 20 min. The soln. was treated with 1-tosyl-1*H*-imidazole (1.04 g, 4.69 mmol) warmed to r.t., stirred for 4 h, and diluted with sat. NH_4Cl soln. After evaporation of the org. solvents, the aq. mixture was extracted with CH_2Cl_2 (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A stirred soln. of the residue and AcSK (9.73 g, 85.2 mmol) in DMF (25 ml) was heated under N_2 to 70° for 6 h. DMF was evaporated, and a suspension of the residue in CH_2Cl_2 was washed with H_2O (2×). The org. layer was dried ($MgSO_4$) and evaporated. Crystallisation from toluene gave **10** (1.29 g, 69%). Grey solid. R_f (Et_2O) 0.23. M.p. 170.0–171.1°. $[\alpha]_D^{25} = +40.6$ (c = 1.0, $CHCl_3$). UV ($CHCl_3$): 268 (21800), 308 (6640). IR (ATR): 3400–3200*w* (br.), 3144*w*, 3066*w*, 2988*w*, 2933*w*, 1666*s*, 1624*s*, 1551*m*, 1477*s*, 1400*w*, 1373*m*, 1351*w*, 1157*w*, 1132*w*, 1087*s*, 1057*s*,

1028*m*, 1012*w*, 994*w*, 968*w*, 898*w*, 873*w*, 850*w*, 785*m*, 704*m*, 624*m*. ^{1}H-NMR (300 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 4*; additionally, 8.65 (br. *s*, BzN*H*); 7.88 (*d*, *J* = 7.5, 2 arom. H); 7.66–7.59 (*m*, 1 arom. H); 7.56–7.49 (*m*, 2 arom. H, H–C(5)); 2.37 (*s*, AcS); 1.56, 1.36 (2*s*, Me_2C). ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 5*; additionally, 194.70 (*s*, SC=O); 166.95 (br. *s*, NHC=O); 133.28 (*d*); 133.03 (*s*); 129.02 (2*d*); 127.76 (2*d*); 114.25 (*s*, Me_2C); 30.66 (*q*, *Me*C=O); 27.09, 25.30 (2*q*, *Me*$_2$C). ESI-MS: 484.0 (10, $[M + K]^+$), 468.1 (100, $[M + Na]^+$), 446.1 (30, $[M + H]^+$). Anal. calc. for $C_{21}H_{23}N_3O_6S$ (445.50): C 56.62, H 5.20, N 9.43; found: C 56.33, H 5.30, N 9.39.

*5′-*O*-[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′-*O*-isopropylidenecytidine-6-methyl-(6^1 → 5′-*S*)-2′,3′-*O*-isopropylidene-5′-thiocytidine* (**11**). A soln. of **10** (374 mg, 0.84 mmol) and **7** (485 mg, 0.84 mmol) in MeOH (15 ml) was treated with 7M NH_3 in MeOH (16 ml) and stirred for 5 h. The precipitate was filtered off, and the filtrate was evaporated. FC (CH_2Cl_2/MeOH 9:1) of the combined solids gave **11** (557 mg, 93%). Colourless solid. R_f (CH_2Cl_2/MeOH 9:1) 0.20. M.p. 213.7–215.1°. $[\alpha]_D^{25} = -158.0$ (*c* = 1.0, $CHCl_3$). UV ($CHCl_3$): 245 (12600), 273 (11800). IR (ATR): 3328*w*, 3185*w*, 2957*w*, 2865*w*, 1638*s*, 1531*m*, 1486*m*, 1373*s*, 1289*w*, 1250*m*, 1208*m*, 1156*m*, 1131*w*, 1062*s*, 1000*m*, 978*w*, 936*w*, 875*m*, 829*s*, 783*s*, 732*m*, 677*w*, 610*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 6*; additionally, 1.57 (*sept.*, *J* = 6.9, Me_2CH); 1.55, 1.52, 1.33, 1.32 (4*s*, 2 Me_2CO_2); 0.83 (*d*, *J* = 6.9, *Me*$_2$CH); 0.80, 0.79 (2*s*, Me_2CSi); 0.03, 0.02 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 7*; additionally, 115.36, 112.86 (2*s*, 2 Me_2C); 34.23 (*d*, Me_2CH); 27.50, 27.37, 25.59, 25.40 (4*q*, 2 *Me*$_2$CO$_2$); 25.34 (*s*, Me_2CSi); 20.52, 20.45 (2*q*, *Me*$_2$CSi); 18.65, 18.60 (2*q*, *Me*$_2$CH); −3.04, −3.16 (2*q*, Me_2Si). HR-MALDI-MS: 759.3175 (47, $[M + Na]^+$, $C_{33}H_{52}N_6NaO_9SSi^+$; calc. 759.3178), 737.3346 (100, $[M + H]^+$, $C_{33}H_{53}N_6O_9SSi^+$; calc. 737.3359). Anal. calc. for $C_{33}H_{52}N_6O_9SSi \cdot H_2O$ (754.97): C 52.50, H 7.21, N 11.13; found: C 52.49, H 7.18, N 11.12.

X-Ray Analysis of **11** · *MeOH*. Crystals of **11** · MeOH suitable for X-ray analysis were obtained by slow evaporation of a MeOH soln. of **11**. Crystal data at 220 K for 2 $C_{33}H_{52}N_6O_9SSi \cdot CH_4O$ (1504.9); orthorhombic $P2_12_12_1$; *a* = 15.3705(3), *b* = 17.1904(4), *c* = 30.4388(6) Å. *V* = 8042.7(3) Å^3; *Z* = 4; D_{calc} = 1.243 Mg/m^3. *Bruker-Nonius Kappa-CCD* with MoK_α radiation (λ = 0.7107 Å). The structure was solved by direct methods [40] and refined by full-matrix least-squares analysis [41] including an isotropic extinction correction. All heavy atoms were refined anisotropically (H-atoms isotropic, whereby H-positions are based on stereochemical considerations). *R* = 0.0718, R_w = 0.1592 for 941 parameters and 8719 reflections with $I > 2\sigma(I)$ and $\tau < 23.53°$.

*2′,3′-*O*-Isopropylidenecytidine-6-methyl-(6^1 → 5′-*S*)-2′,3′-*O*-isopropylidene-5′-thiocytidine* (**12**). A soln. of **11** (231 mg, 314 μmol) in THF (2.5 ml) was treated with $(HF)_3 \cdot NEt_3$ (1.0 ml, 6.28 mmol), stirred for 14 h, treated with 25% NH_4OH (1 ml), and evaporated. The aq. residue was extracted with $CHCl_3$ (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC ($CHCl_3$/MeOH/NH_4OH 6:1:0.07) gave **12** (176 mg, 94%). Colourless solid. R_f (CH_2Cl_2/MeOH 4:1) 0.31. $[\alpha]_D^{25} = -116.1$ (*c* = 0.5, $CHCl_3$/MeOH 1:1). IR (ATR): 3317*w*, 3183*w*, 2986*w*, 2925*w*, 2852*w*, 1639*s*, 1530*m*, 1487*s*, 1374*s*, 1293*w*, 1265*m*, 1208*m*, 1156*m*, 1054*s*, 1024*s*, 1003*s*, 875*m*, 820*w*, 789*m*, 751*s*, 664*w*, 611*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 6*; additionally, 4.36 (br. *s*, OH); 1.55, 1.54, 1.35, 1.33 (4*s*, 2 Me_2C). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HSQC spectrum): see *Table 7*; additionally, 115.05, 113.69 (2*s*, 2 Me_2CO_2); 27.52, 27.50, 25.55, 25.47 (4*q*, 2 *Me*$_2$C). HR-MALDI-MS: 633.1722 (66, $[M + K]^+$, $C_{25}H_{34}KN_6O_9S^+$; calc. 633.1740), 617.1989 (100, $[M + Na]^+$, $C_{25}H_{34}N_6NaO_9S^+$; calc. 617.2000).

*Cytidine-6-methyl-(6^1 → 5′-*S*)-5′-thiocytidine* (**13**). A soln. of **11** (55 mg, 75 μmol) in CF_3CO_2H/H_2O 1:1 (0.8 ml) was stirred for 3 h and evaporated. FC (CH_2Cl_2/MeOH/NH_4OH 4:5:1) gave **13** (33 mg, 86%). Colourless solid. R_f (CH_2Cl_2/MeOH/NH_4OH 4:5:1) 0.29. $[\alpha]_D^{25} = +22.4$ (*c* = 0.2, H_2O). UV (H_2O): 273 (12250), 237 (10500). IR (ATR): 3329*m*, 3198*m*, 2928*w*, 1725*w*, 1637*s*, 1607*s*, 1527*m*, 1485*s*, 1385*m*, 1280*w*, 1210*w*, 1183*w*, 1093*s*, 1040*s*, 893*w*, 858*w*, 784*m*, 731*w*, 680*w*, 612*w*. ^{1}H-NMR (400 MHz, D_2O, 23°; assignments based on a DQF-COSY and a HSQC spectrum): see *Table 8*. ^{1}H-NMR (500 MHz, H_2O/D_2O 9:1, 23°): signals of NH: 7.3–7.0 (br. *s*, 0.25 H); 6.9–6.65 (br. *s*, 0.15 H). ^{13}C-NMR (400 MHz, D_2O; assignments based on a DQF-COSY and a HSQC spectrum): see *Table 9*. HR-MALDI-MS: 537.1375 (32, $[M + Na]^+$, $C_{19}H_{26}N_6NaO_9S^+$; calc. 537.1374), 383.1121 (100, $[M - C_5H_8O_4 + H]^+$, $C_{14}H_{19}N_6O_5S^+$; calc. 383.1132). Anal. calc. for $C_{19}H_{26}N_6O_9S \cdot 2\,H_2O$ (550.54): C 41.45, H 5.49, N 15.27; found: C 41.44, H 5.30, N 15.17.

Table 6. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Cytidine Dinucleotides* **11**, **12**, **14**, *and* **15** *in $CDCl_3$, and of* **16** *and* **17** *in (D_6)DMSO*[a])

	11	**12**	**14**	**15**	**16**	**17**
Cytidine unit (I)						
H_aN–C(4/I)	10.80	9.83	10.64	10.16	7.41	7.67–7.46
H_bN–C(4/I)	5.42	5.24	5.40	5.55	7.32	7.67–7.46
H–C(5/I)	5.64	5.73	5.42	5.44	5.84	5.89
CH_a–C(6/I)	7.23[b])	7.16[b])	4.10	4.10	4.37	4.38
CH_b–C(6/I)	–	–	4.00	4.00	4.30	4.32
H–C(1′/I)	5.16	5.18	5.62	5.65	5.69	5.689
H–C(2′/I)	5.30	5.26	5.22	5.22	5.17	5.18
H–C(3′/I)	4.64	4.68	4.59	4.62	4.81	4.805
H–C(4′/I)	4.09	4.12	3.98	4.04–3.98	4.11	4.12
H_a–C(5′/I)	2.95	2.96	2.93	2.95	2.88	2.89
H_b–C(5′/I)	2.70	2.70	2.67	2.69	2.82	2.84
J(H_a,H_b/I)	7.4[c])	7.4[c])	12.5	12.5	14.6	14.7
J(1′,2′/I)	2.1	1.7	2.3	2.0	< 1.0	< 1.0
J(2′,3′/I)	6.7	6.8	6.9	6.9	6.3	6.3
J(3′,4′/I)	6.6	6.1	6.6	*ca.* 6.6	4.0	4.0
J(4′,5′a/I)	11.1	11.1	11.1	10.9	6.3	6.7
J(4′,5′b/I)	2.1	1.9	1.6	1.5	7.7	7.9
J(5a′,5′b/I)	15.4	15.4	15.4	15.5	13.8	14.1
Cytidine unit (II)						
H_aN–C(4/II)	9.41	9.39	9.30	9.37	7.24	7.67–7.46
H_bN–C(4/II)	7.14	7.29	7.22	7.37–7.27	7.24	7.67–7.46
H–C(5/II)	5.81	5.83	5.89	5.93	5.61	5.685
CH_a–C(6/II)	3.84	3.84	3.81	3.86	3.71	3.75
CH_b–C(6/II)	3.48	3.43	3.39	3.39	3.62	3.65
H–C(1′/II)	5.77	5.70	5.77	5.69	5.80	5.79
H–C(2′/II)	5.35	5.37	5.36	5.36	5.18	5.18
H–C(3′/II)	4.88	5.09	4.88	5.16	4.78	4.815
H–C(4′/II)	4.13	4.21	4.14	4.20	3.97	3.98
H_a–C(5′/II)	3.76	3.82	3.77	3.77	3.78–3.62	3.58
H_b–C(5′/II)	3.72	3.75	3.73	3.73	3.78–3.62	3.49
J(H_a,H_b/II)	14.0	14.2	13.9	14.3	14.5	14.4
J(1′,2′/II)	< 1.0	1.6	< 1.0	1.6	< 1.0	< 1.0
J(2′,3′/II)	6.3	6.4	6.3	6.4	6.3	6.2
J(3′,4′/II)	3.7	4.0	3.8	3.8	3.6	4.2
J(4′,5′a/II)	5.5	2.7	5.5	3.1	6.4	5.6
J(4′,5′b/II)	7.5	3.8	7.6	3.0	7.2	6.2
J(5a′,5′b/II)	10.6	[d])	10.7	12.0	[d])	11.8

[a]) Assignments based on DQF-COSY (for **11**), HSQC (for **11**, **12**, **14**, **16**, and **17**), HMBC (for **11**, **14**, **16**, and **17**), and ROESY spectra (for **11**, **12**, **14**, and **15**). [b]) H–C(6/I). [c]) J(5,6/I). [d]) Not assigned.

5′-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′-O-isopropylidenecytidine-6-methyl-(6′ → 5′-S)-2′,3′-O-isopropylidene-6-[[(4-methoxyphenyl)(diphenyl)methoxy]methyl]-5′-thiocytidine (**14**). A soln. of **8** (50 mg, 67 μmol) in MeOH (1 ml) was treated with powdered K_2CO_3 (28 mg, 101 μmol), stirred for 10 min, treated with **7** (39 mg, 67 μmol), and stirred for 12 h. After evaporation, a suspension of the residue in CH_2Cl_2 was washed with half sat. NH_4Cl soln., dried ($MgSO_4$), and evaporated. FC (CH_2Cl_2/

Table 7. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of the Cytidine Dinucleotides* **11**, **12**, **14**, *and* **15** *in $CDCl_3$, and of* **16** *and* **17** *in (D_6)DMSO*[a])

	11	**12**	**14**	**15**	**16**	**17**
Cytidine unit (I)						
C(2/I)	156.01	155.97	157.15	157.88	155.70	154.99
C(4/I)	167.02	167.04	166.34	166.25	165.65	165.01
C(5/I)	96.04	96.10	96.86	96.90	93.36	93.29
C(6/I)	145.64	145.28	153.17	153.06	155.93	156.45
CH_2–C(6/I)	–	–	62.89	62.91	59.27	59.20
C(1′/I)	98.41	98.31	91.39	91.52	90.76	90.83
C(2′/I)	82.75	83.11	82.72	80.90	84.37	84.31[b])
C(3′/I)	83.84	83.99	84.02	84.13	84.28	84.16
C(4′/I)	89.27	90.22	89.23	88.77	88.93	88.88
C(5′/I)	30.31	30.94	30.38	30.64	32.80	32.96
Cytidine unit (II)						
C(2/II)	156.98	157.75	156.94	157.13	155.70	154.92
C(4/II)	165.63	165.71	165.68	165.73	164.84	164.15
C(5/II)	100.30	100.14	100.39	100.65	96.06	96.03
C(6/II)	149.21	149.83	149.19	149.23	151.85	152.55
CH_2–C(6/II)	31.64	31.72	31.41	31.25	31.75	31.86
C(1′/II)	91.00	91.46	90.97	91.38	90.88	90.75
C(2′/II)	84.45	83.84	84.58	84.39	84.37	84.23[b])
C(3′/II)	83.04	81.23	83.10	82.82	82.64	82.05
C(4′/II)	90.27	88.39	90.49	89.51	89.54	89.06
C(5′/II)	64.57	62.91	64.69	63.10	63.90	62.09

[a]) Assignments based on DQF-COSY (for **11**), HSQC (for **11**, **12**, **14**, **16**, and **17**), and HMBC spectra (for **11**, **14**, **16**, and **17**). [b]) Assignments may be interchanged.

MeOH 14:1) gave **14** (43 mg, 62%). Yellow foam. R_f (CH_2Cl_2/MeOH 9:1) 0.28. $[\alpha]_D^{25} = -170.6$ ($c = 0.9$, $CHCl_3$). UV ($CHCl_3$): 243 (21400), 276 (15100). IR (ATR): 3332*w*, 2957*w*, 2933*w*, 2868*w*, 1640*s*, 1537*s*, 1509*m*, 1479*m*, 1380*m*, 1301*w*, 1250*m*, 1209*m*, 1179*m*, 1156*m*, 1063*s*, 1034*s*, 1002*m*, 937*w*, 901*w*, 873*w*, 829*s*, 779*m*, 739*w*, 701*m*, 631*w*. ^{1}H-NMR (600 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 6*; additionally, 7.49–7.48 (*m*, 4 arom. H); 7.37–7.27 (*m*, 8 arom. H); 6.89–6.86 (*m*, 2 arom. H); 3.81 (*s*, MeO); 1.57 (*sept.*, $J = 6.9$, Me_2CH); 1.54, 1.44, 1.33, 1.28 (4*s*, 2 Me_2CO_2); 0.83 (*d*, $J = 6.9$, Me_2CH); 0.80, 0.79 (2*s*, Me_2CSi); 0.03, 0.02 (2*s*, Me_2Si). ^{13}C-NMR (150 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 7*; additionally, 159.22 (*s*, MeO*C*); 143.65, 143.46, 134.43 (3*s*); 130.56–127.62 (several *d*); 115.11 (*s*, Me_2CO_2); 113.59 (*d*, 2 C); 112.80 (*s*, Me_2CO_2); 88.43 (*s*, Ph_2C); 55.43 (*q*, MeO); 34.26 (*d*, Me_2CH); 27.56, 27.38, 25.61, 25.39 (4*q*, 2 Me_2CO_2); 25.36 (*s*, Me_2CSi); 20.55, 20.47 (2*q*, Me_2CSi); 18.67, 18.61 (2*q*, Me_2CH); −2.99, −3.13 (2*q*, Me_2Si). HR-MALDI-MS: 1077.4220 (10, $[M + K]^+$, $C_{54}H_{70}KN_6O_{11}SSi^+$; calc. 1077.4224), 1061.4485 (100, $[M + Na]^+$, $C_{54}H_{70}N_6NaO_{11}SSi^+$; calc. 1061.4496), 1039.4700 (16, $[M + H]^+$, $C_{54}H_{71}N_6O_{11}SSi^+$; calc. 1039.4665), 273 (77, $MMTr^+$). Anal. calc. for $C_{52}H_{70}N_6O_{11}SSi$ (1039.33): C 62.40, H 6.79, N 8.09; found: C 62.13, H 6.99, N 7.89.

*2′,3′-*O*-Isopropylidenecytidine-6-methyl-(6^1 → 5′-*S*)-2′,3′-*O*-isopropylidene-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-5′-thiocytidine* (**15**). A soln. of **14** (125 mg, 120 μmol) in THF (1 ml) was treated with $(HF)_3 \cdot NEt_3$ (0.39 ml, 2.4 mmol), stirred for 14 h, and neutralized with 25% aq. NH_4OH (0.2 ml). After evaporation, the aq. residue was extracted with $CHCl_3$ (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC ($CHCl_3$/MeOH/NH_4OH 7:1:0.08) gave **15** (95 mg, 88%). Pale yellow solid. R_f ($CHCl_3$/MeOH/NH_4OH 7:1:0.08) 0.34. $[\alpha]_D^{25} = -162.1$ ($c =$

Table 8. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Deprotected Cytidine Dinucleotides* **13** *and* **18** *in D_2O*[a])

	13	**18**[b])		**13**	**18**
Cytidine unit (I)			Cytidine unit (II)		
H–C(5/I)	6.07	6.15	H–C(5/II)	6.06	5.97
CH_a–C(6/I)	7.70[c])	4.63	CH_a–C(6/II)	3.91	3.86, *3.67*
CH_b–C(6/I)	–	4.59	CH_b–C(6/II)	3.87	3.80, *3.61*
H–C(1′/I)	5.88	5.47, *5.53*	H–C(1′/II)	5.79	5.76
H–C(2′/I)	4.39	4.76, *4.84*	H–C(2′/II)	4.84	4.80
H–C(3′/I)	4.20	4.42	H–C(3′/II)	4.47	4.45
H–C(4′/I)	4.25	4.02 – 3.97, *4.15*	H–C(4′/II)	4.00	4.02 – 3.97
H_a–C(5′/I)	3.15	3.07, *3.24*	H_a–C(5′/II)	3.89	3.89
H_b–C(5′/I)	2.99	2.91, *3.03*	H_b–C(5′/II)	3.79	3.77
$J(H_a,H_b/I)$	7.6[d])	15.1	$J(H_a,H_b/II)$	15.0	15.0, *15.0*
J(1′,2′/I)	3.6	2.7, *3.0*	J(1′,2′/II)	3.6	3.6
J(2′,3′/I)	5.4	6.5, *6.3*	J(2′,3′/II)	6.4	6.7
J(3′,4′/I)	6.5	7.8, *7.8*	J(3′,4′/II)	6.3	5.0
J(4′,5′a/I)	3.8	3.2, *3.8*	J(4′,5′a/II)	2.9	3.0
J(4′,5′b/I)	6.7	8.4, *8.4*	J(4′,5′b/II)	5.6	5.8
J(5a′′,5′b/I)	14.6	14.6, *14.1*	J(5a’,5′b/II)	12.3	12.3

[a]) Assignments based on DQF-COSY (for **13**), HSQC (for **13** and **18**), and HMBC spectra (for **18**). [b]) 9 : 1 Mixture of isomers. Data of the minor isomer in italics. [c]) H–C(6/I). [c]) J(5,6/I).

Table 9. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of the Deprotected Cytidine Dinucleotides* **13** *and* **18** *in D_2O*[a])

	13	**18**		**13**	**18**
Cytidine unit (I)			Cytidine unit (II)		
C(2/I)	156.67	156.36	C(2/II)	157.36	156.96
C(4/I)	165.65	165.05	C(4/II)	164.70	164.39
C(5/I)	95.98	94.67	C(5/II)	97.65	97.98
C(6/I)	141.80	157.24	C(6/II)	153.42	154.18
CH_2–C(6/I)	–	59.48	CH_2–C(6/II)	32.88	33.22
C(1′/I)	90.94	92.19	C(1′/II)	91.59	91.94
C(2′/I)	73.15	72.02	C(2′/II)	71.37	71.69
C(3′/I)	71.59	72.43	C(3′/II)	69.46	69.79
C(4′/I)	81.89	82.77	C(4′/II)	83.56	83.90
C(5′/I)	32.68	32.88	C(5′/II)	61.53	61.89

[a]) Assignments based on DQF-COSY (for **13**), HSQC (for **13** and **18**), and HMBC spectra (for **18**).

0.5, $CHCl_3$/MeOH 1 : 1). IR (ATR): 3331*w*, 3104*w*, 2988*w*, 2934*w*, 1635*s*, 1534*s*, 1509*m*, 1482*s*, 1382*s*, 1299*m*, 1251*m*, 1211*m*, 1180*m*, 1155*m*, 1062*s*, 1033*m*, 1001*m*, 871*m*, 833*w*, 790*w*, 749*s*, 703*m*, 666*w*, 631*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 6*; additionally, 7.49 – 7.44 (*m*, 4 arom. H); 7.37 – 7.27 (*m*, 8 arom. H, HN–C(4/II)); 6.88 – 6.85 (*m*, 2 arom. H); 3.80 (*s*, MeO); 1.57, 1.44, 1.35, 1.28 (4*s*, 2 Me_2C); HO–C(5′/II) hidden by the noise. ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 7*; additionally, 159.16 (*s*, MeO*C*); 143.65, 143.48, 134.46 (3*s*); 130.58 – 127.59 (several *d*); 114.98, 113.60 (2*s*, 2 Me_2C); 113.60 (*d*, 2 C); 88.38 (*s*, Ph_2C); 55.43 (*q*, MeO); 27.54, 27.47, 25.59, 25.35 (4*q*, 2 Me_2C). HR-MALDI-MS: 935.3048 (20, [*M* +

K]$^+$, $C_{46}H_{52}KN_6O_{11}S^+$; calc. 935.3046), 919.3319 (100, [*M* + Na]$^+$, $C_{46}H_{52}N_6NaO_{11}S^+$; calc. 919.3307), 273.1274 (50, MMTr$^+$, $C_{20}H_{17}O^+$; calc. 273.1274).

*5′-*O*-[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′-*O*-isopropylidenecytidine-6-methyl-(6^1 → 5′-*S*)-6-(hydroxymethyl)-2′,3′-*O*-isopropylidene-5′-thiocytidine* (**16**). A soln. of **14** (156 mg, 150 μmol) in CH_2Cl_2 (1.8 ml) was treated with Cl_2CHCO_2H (200 μl) und $^iPr_3SiH$ (92 μl, 450 μmol), stirred for 1 h, diluted with $CHCl_3$ (20 ml), and washed with $NaHCO_3$ soln. The aq. layer was extracted with $CHCl_3$ (2 ×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC ($CHCl_3$/MeOH/NH_4OH 7:1:0.08) gave **16** (96 mg, 83%). Pale yellow foam. R_f ($CHCl_3$/MeOH/NH_4OH 7:1:0.08) 0.25. $[\alpha]_D^{25} = -135.8$ ($c = 0.5$, $CHCl_3$/MeOH 1:1). IR (ATR): 3327*w*, 3186*w*, 2957*w*, 2865*w*, 1719*w*, 1640*s*, 1532*s*, 1473*m*, 1380*m*, 1309*w*, 1251*m*, 1209*m*, 1182*w*, 1157*m*, 1081*s*, 1060*s*, 998*m*, 983*m*, 873*m*, 828*s*, 784*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO; assignments based on a HSQC and a HMBC spectrum): see *Table 6*; additionally, 5.9–5.6 (br. *s*, OH); 1.57 (*sept.*, $J = 6.9$, Me_2CH); 1.46, 1.45, 1.27, 1.26 (4*s*, 2 Me_2CO_2); 0.83, 0.82 (2*d*, $J = 6.9$, Me_2CH); 0.78, 0.77 (2*s*, Me_2CSi); 0.01, 0.00 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, (D_6)DMSO; assignments based on a HSQC and a HMBC spectrum): see *Table 7*; additionally, 112.37, 111.90 (2*s*, 2 Me_2CO_2); 33.65 (*d*, Me_2CH); 27.02 (2 C), 25.10, 25.00 (3*q*, 2 Me_2CO_2); 24.69 (*s*, Me_2CSi); 20.21, 20.16 (2*q*, Me_2CSi); 18.33, 18.28 (2*q*, Me_2CH); −3.31, −3.44 (2*q*, Me_2Si). HR-MALDI-MS: 805.3035 (55, [*M* + K]$^+$, $C_{34}H_{54}KN_6O_{10}SSi^+$; calc. 805.3023), 789.3311 (80, [*M* + Na]$^+$, $C_{34}H_{54}N_6NaO_{10}SSi^+$; calc. 789.3284), 767.3450 (100, [*M* + H]$^+$, $C_{34}H_{55}N_6O_{10}SSi^+$; calc. 767.3464).

*2′,3′-*O*-Isopropylidenecytidine-6-methyl-(6^1 → 5′-*S*)-6-(hydroxymethyl)-2′,3′-*O*-isopropylidene-5′-thiocytidine* (**17**). A soln. of **16** (125 mg, 163 μmol) in THF (2 ml) was treated with $(HF)_3 \cdot NEt_3$ (0.53 ml, 3.26 mmol), stirred for 18 h, and neutralized with 7M NH_3 in MeOH (1.5 ml). The mixture was diluted with H_2O and brine, and extracted with $CHCl_3$/MeOH 9:1 (3 ×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC ($CHCl_3$/MeOH/NH_4OH 5:1:0.06) gave **17** (97 mg, 95%). Colourless solid. R_f ($CHCl_3$/MeOH/NH_4OH 5:1:0.06) 0.33. $[\alpha]_D^{25} = -189.1$ ($c = 0.5$, $CHCl_3$/MeOH 1:1). IR (ATR): 3323*w*, 3178*w*, 2986*w*, 2931*w*, 1724*w*, 1639*s*, 1529*s*, 1482*m*, 1381*m*, 1309*w*, 1263*w*, 1241*w*, 1208*m*, 1182*w*, 1157*m*, 1085*m*, 1049*s*, 1023*s*, 999*s*, 872*m*, 822*w*, 788*m*, 760*w*, 719*w*, 685*w*, 627*w*, 610*w*. ^{1}H-NMR (400 MHz, (D_6)DMSO; assignments based on a HSQC and a HMBC spectrum): see *Table 6*; additionally, 5.9–5.75 (*m*, 2 OH); 1.46, 1.27 (2*s*, 2 Me_2C). ^{13}C-NMR (100 MHz, (D_6)DMSO; assignments based on a HMBC and a HSQC spectrum): see *Table 7*; additionally, 112.44, 112.25 (2*s*, 2 Me_2C); 27.14, 27.02, 25.19, 25.01 (4*q*, 2 Me_2C). HR-MALDI-MS: 663.1820 (29, [*M* + K]$^+$, $C_{26}H_{36}KN_6O_{10}S^+$; calc. 663.1845), 647.2093 (100, [*M* + Na]$^+$, $C_{26}H_{36}N_6NaO_{10}S^+$; calc. 647.2106).

*Cytosine-6-methyl-(6^1 → 5′-*S*)-6-(hydroxymethyl)-5′-thiocytidine* (**18**). A soln. of **14** (140 mg, 135 μmol) in CF_3CO_2H/H_2O 1:1 (1 ml) was treated with $^iPr_3SiH$ (250 μl, 1.22 mmol), stirred for 3 h, and evaporated. FC (CH_2Cl_2/MeOH/NH_4OH 2:3:0.075) gave **18** (55 mg, 75%). Colourless solid. R_f (CH_2Cl_2/MeOH/NH_4OH 4:5:1) 0.30. $[\alpha]_D^{25} = -8.0$ ($c = 0.28$, H_2O). UV (H_2O): 240 (10200), 272 (11100). IR (ATR): 3327*m*, 3200*m*, 2927*w*, 2870*w*, 1730*w*, 1638*s*, 1531*s*, 1482*m*, 1385*m*, 1266*w*, 1208*w*, 1180*w*, 1093*s*, 1038*s*, 1000*m*, 894*w*, 838*w*, 786*w*, 730*w*, 698*w*, 678*w*. ^{1}H-NMR (600 MHz, D_2O, 23°; assignments based on a HSQC and a HMBC spectrum; 9:1 mixture of isomers): see *Table 8*. ^{1}H-NMR (500 MHz, H_2O/D_2O 9:1, 23°, excitation sculping; 9:1 mixture of isomers): signals of NH: 7.6–7.4 (br. *s*, 0.2 H); 7.25–6.8 (3 br. *s*, 0.15 H). ^{13}C-NMR (100 MHz, D_2O; assignments based on a HSQC and a HMBC spectrum): see *Table 9*. HR-MALDI-MS: 567.1482 (100, [*M* + Na]$^+$, $C_{20}H_{28}N_6NaO_{10}S^+$; calc. 567.1480). Anal. calc. for $C_{20}H_{28}N_6O_{10}S \cdot 2\,H_2O$ (580.57): C 41.38, H 5.56, N 14.48; found: C 41.46, H 5.28, N 14.56.

*N^4-Benzoyl-5′-*O*-[dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′-*O*-isopropylidenecytidine-6-methyl-(6^1 → 5′-*S*)-N^4-benzoyl-2′,3′-*O*-isopropylidene-6-[[(4-methoxyphenyl)(diphenyl)methoxy]methyl]-5′-thiocytidine* (**19**). A soln. of **8** (931 mg, 1.25 mmol) in degassed THF/MeOH 1:1 (15 ml) was cooled to −10° and treated dropwise with a 1M soln. of MeSNa in degassed MeOH (2.5 ml, 2.5 mmol). The mixture was stirred for 3 h at −10° and poured into 0.1M HCl (25 ml). The mixture was diluted with brine and extracted with AcOEt (3 ×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **7** (734 mg, 1.25 mmol) in degassed DMF (7 ml) was treated with LiBr (59 mg, 674 μmol) and Cs_2CO_3 (407 mg, 1.25 μmol), stirred for 4 h, and diluted with sat. NH_4Cl soln. The mixture was extracted with AcOEt. The combined org. layers were washed with H_2O (3 ×) and

brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/pentane/MeOH 1:1:0.004) gave **19** (932 mg, 60%). Yellow foam. R_f (AcOEt/pentane/MeOH 1:1:0.004) 0.20. $[\alpha]_D^{25} = -63.4$ ($c = 0.5$, $CHCl_3$). IR (ATR): 3430–3150*w* (br.), 2955*w*, 2865*w*, 1674*s*, 1608*s*, 1565*s*, 1504*w*, 1473*m*, 1447*m*, 1415*w*, 1353*s*, 1313*m*, 1300*w*, 1248*s*, 1210*m*, 1180*m*, 1155*m*, 1063*s*, 1033*s*, 1001*m*, 976*m*, 900*w*, 871*m*, 828*s*, 778*m*, 766*w*, 746*w*, 699*s*, 660*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 8.65 (br. *s*, 2 BzN*H*); 7.87–7.86 (*m*, 4 arom. H); 7.62–7.23 (*m*, 18 arom. H, H–C(5/I), H–C(5/II)); 6.87–6.83 (*m*, 2 arom. H); 6.01 (br. *s*, H–C(1′/II)); 5.94 (br. *s*, H–C(1′/I)); 5.35 (*dd*, $J = 6.4, 0.9$, H–C(2′/II)); 5.28 (*dd*, $J = 6.4, 0.8$, H–C(2′/I)); 5.02 (*dd*, $J = 6.4, 4.2$, H–C(3′/I)); 4.94 (*dd*, $J = 6.4, 4.0$, H–C(3′/II)); 4.27–4.18 (*m*, CH_2–C(6/I), H–C(4′/I), H–C(4′/II)); 3.86 (*dd*, $J = 10.6, 7.2$, H_a–C(5′/II)); 3.80 (*dd*, $J = 10.6, 5.7$, H_b–C(5′/II)); 3.79, 3.68 (2*d*, $J = 14.3$, CH_2–C(6/II)); 3.09 (*dd*, $J = 13.8, 7.6$, H_a–C(5′/I)); 3.01 (*dd*, $J = 13.8, 6.3$, H_b–C(5′/I)); 1.59 (*sept.*, $J = 6.9$, Me_2CH); 1.54, 1.48, 1.34, 1.31 (4*s*, 2 Me_2CO_2); 0.84 (*d*, $J = 6.9$, Me_2CH); 0.81 (*s*, Me_2CSi); 0.06, 0.04 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 166.13, 165.91 (2 br. *s*, 2 NHC=O); 162.45, 161.77 (2 br. *s*, C(4/I), C(4/II)); 159.07 (*s*, MeO*C*); 157.70 (br. *s*, C(6/II)); 157.25 (br. *s*, C(6/I)); 155.42 (br. *s*, C(2/II)); 155.30 (br. *s*, C(2/I)); 143.43, 143.31, 134.31 (3*s*); 133.30, 133.20 (2*d*); 130.72–127.38 (several *d*); 113.63 (*s*, Me_2CO_2); 113.46 (2*d*); 113.23 (*s*, Me_2CO_2); 98.07, 97.65 (2 br. *d*, C(5/I), C(5/II)); 93.13 (*d*, C(1′/I)); 92.63 (*d*, C(1′/II)); 90.62 (*d*, C(4′/II)); 89.56 (*d*, C(4′/I)); 88.58 (*s*, Ph_2C); 84.73 (*d*, C(3′/I)); 84.69 (*d*, C(2′/I)); 84.29 (*d*, C(2′/II)); 83.00 (*d*, C(3′/II)); 64.12 (*t*, C(5′/II)); 62.88 (*t*, CH_2–C(6/I)); 55.23 (*q*, MeO); 34.39 (*t*, C(5′/I)); 34.13 (*d*, Me_2CH); 33.80 (*t*, CH_2–C(6/II)); 27.24, 27.15, 25.41, 25.33 (4*q*, 2 Me_2CO_2); 25.27 (*s*, Me_2CSi); 20.40, 20.35 (2*q*, Me_2CSi); 18.52, 18.48 (2*q*, Me_2CH); −3.23, −3.28 (2*q*, Me_2Si); 2 signals of C(1) of Bz hidden by the *d*s at 133.30, 133.20. HR-MALDI-MS: 1269.5009 (62, $[M + Na]^+$, $C_{68}H_{78}N_6NaO_{13}SSi^+$; calc. 1269.4981), 273.1274 (100, $MMTr^+$, $C_{20}H_{17}O^+$; calc. 273.1274).

N^4-*Benzoyl-5′*-O-*[dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylidenecytidine-6-methyl-(6^1 → 5′*-S)-N^4-*benzoyl-2′,3′*-O-*isopropylidene-6-{[(methylsulfonyl)oxy]methyl}-5′-thiocytidine* (**20**). A soln. of **19** (630 mg, 505 μmol) in CH_2Cl_2 (4.5 ml) was treated sequentially with Cl_2CHCO_2H (0.5 ml) and $^iPr_3SiH$ (310 μl, 1.52 mmol), stirred for 75 min, and poured into sat. $NaHCO_3$ soln. The mixture was extracted with CH_2Cl_2 (3×). The combined org. layers 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 (106 μl, 606 μmol) and dropwise with a soln. of Ms_2O (97 mg, 556 μmol) in CH_2Cl_2 (2 ml). The mixture was stirred for 3 h at 0°, poured into ice/water, and extracted with CH_2Cl_2 (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/pentane/MeOH 2:1:0 → 2:1:0.003) gave **20** (420 mg, 79%). Yellow foam. R_f (CH_2Cl_2/MeOH 19:1) 0.35. $[\alpha]_D^{25} = -51.8$ ($c = 0.5$, $CHCl_3$). IR (ATR): 3430–3150*w* (br.), 3156*w*, 3129*w*, 3063*w*, 2953*w*, 2865*w*, 1673*s*, 1608*s*, 1564*s*, 1475*m*, 1417*w*, 1352*s*, 1247*s*, 1210*m*, 1176*m*, 1157*m*, 1066*s*, 1001*m*, 972*m*, 950*w*, 901*w*, 871*w*, 829*s*, 798*m*, 780*m*, 701*m*, 662*m*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 8.85–8.4 (br. *s*, 2 BzN*H*); 7.87–7.86 (*m*, 4 arom. H); 7.62–7.23 (*m*, 6 arom. H, H–C(5/I), H–C(5/II)); 6.04 (*d*, $J = 1.0$, H–C(1′/II)); 5.78 (*d*, $J = 1.0$, H–C(1′/I)); 5.36 (*dd*, $J = 6.4, 1.0$, H–C(2′/II)); 5.32 (*dd*, $J = 6.5, 1.0$, H–C(2′/I)); 5.32, 5.20 (2*d*, $J = 13.5$, CH_2–C(6/I)); 5.05 (*dd*, $J = 6.5, 3.9$, H–C(3′/I)); (*dd*, $J = 6.4, 4.0$, H–C(3′/II)); 4.38 (*ddd*, $J = 7.0, 6.3, 4.0$, H–C(4′/I)); 4.21 (*ddd*, $J = 7.2, 5.6, 4.0$, H–C(4′/II)); 3.87 (*dd*, $J = 10.6, 5.6$, H_a–C(5′/II)); 3.81 (*dd*, $J = 10.6, 7.3$, H_b–C(5′/II)); 3.84, 3.72 (2*d*, $J = 14.2$, CH_2–C(6/II)); 3.20 (*s*, MsO); 3.12 (*dd*, $J = 13.9, 7.5$, H_a–C(5′/I)); 3.03 (*dd*, $J = 13.9, 6.2$, H_b–C(5′/I)); 1.58 (*sept.*, $J = 6.9$, Me_2CH); 1.57, 1.55, 1.35 (6 H) (3*s*, 2 Me_2CO_2); 0.85 (*d*, $J = 6.9$, Me_2CH); 0.82 (*s*, Me_2CSi); 0.07, 0.05 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 166.40 (br. *s*, 2 NHC=O); 162.57, 161.80 (2 br. *s*, C(4/I), C(4/II)); 157.72 (br. *s*, C(6/II)); 155.52 (br. *s*, C(2/II)); 154.78 (br. *s*, C(6/I)); 152.02 (br. *s*, C(2/I)); 133.48, 133.20 (2*d*); 132.63 (br. *s*, 2 C); 129.09–127.68 (several *d*); 114.07, 113.29 (2*s*, 2 Me_2CO_2); 98.52, 98.22 (2 br. *d*, C(5/I), C(5/II)); 93.18 (*d*, C(1′/I)); 92.64 (*d*, C(1′/II)); 90.62 (*d*, C(4′/II)); 89.92 (*d*, C(4′/I)); 84.73 (*d*, C(3′/I)); 84.53 (*d*, C(2′/I)); 84.26 (*d*, C(2′/II)); 82.95 (*d*, C(3′/II)); 64.29 (*t*, CH_2–C(6/I)); 64.06 (*t*, C(5′/II)); 38.62 (*q*, MsO); 34.14 (*t*, C(5′/I)); 34.14 (*d*, Me_2CH); 33.69 (*t*, CH_2–C(6/II)); 27.25, 27.10, 25.38, 25.29 (4*q*, 2 Me_2CO_2); 25.29 (*s*, Me_2CSi); 20.41, 20.36 (2*q*, Me_2CSi); 18.52, 18.49 (2*q*, Me_2CH); −3.24, −3.27 (2*q*, Me_2Si). HR-MALDI-MS: 1075.3566 (44, $[M + Na]^+$, $C_{49}H_{64}N_6NaO_{14}S_2Si^+$; calc. 1075.3538), 1053.3759 (21, $[M + H]^+$, $C_{49}H_{65}N_6O_{14}S_2Si^+$; calc. 1053.3764), 730.3166 (100, $[M - C_{13}H_{12}N_3O_5S]^+$, $C_{36}H_{52}N_3O_9SSi^+$; calc. 730.3188). Anal. calc. for $C_{49}H_{64}N_6O_{14}S_2Si$ (1053.29): C 55.88, H 6.12, N 7.98; found: C 56.01, H 6.12, N 7.91.

5′-S-*Acetyl*-N^4-*benzoyl-2′,3′*-O-*isopropylidenecytidine-6-methyl-(6^1 → 5′*-S)-N^4-*benzoyl-2′,3′*-O-*isopropylidene-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-5′-thiocytidine* (**21**). A soln. of **8** (504 mg, 674 μmol) in degassed THF/MeOH 1:1 (7 ml) was cooled to −10° and treated dropwise with a 1M soln. of MeSNa in degassed MeOH (1.35 ml, 1.35 mmol). The mixture was stirred for 3 h at −10° and poured into 0.1M HCl (14 ml). The mixture was diluted with brine and extracted with AcOEt (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **9** (373 mg, 674 μmol) in degassed DMF (9 ml) was treated with LiBr (59 mg, 674 μmol) and Cs_2CO_3 (220 mg, 674 μmol), stirred for 4 h, and poured into sat. NH_4Cl soln. The mixture was extracted with AcOEt. The combined org. layers were washed with H_2O (3×) and brine, dried ($MgSO_4$) and evaporated. FC (AcOEt/pentane/MeOH 3:2:0.005) gave **21** (486 mg, 62%). Yellow foam. R_f (AcOEt/pentane 2:1) 0.25. $[\alpha]_D^{25} = -45.9$ (c = 0.5, $CHCl_3$). IR (ATR): 3430–3160*w* (br.), 3059*w*, 2986*w*, 2931*w*, 1674*s*, 1607*s*, 1564*s*, 1505*m*, 1476*m*, 1448*m*, 1416*m*, 1352*s*, 1314*m*, 1303*m*, 1244*s*, 1210*m*, 1180*m*, 1155*m*, 1090*s*, 1061*s*, 1001*m*, 982*m*, 900*w*, 870*m*, 831*m*, 789*w*, 765*w*, 746*w*, 698*s*, 662*w*, 630*w*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 8.9–8.6 (br. *s*, 2 BzN*H*); 7.91–7.84 (*m*, 4 arom. H); 7.62–7.23 (*m*, 18 arom. H, H–C(5/I), H–C(5/II)); 6.89–6.83 (*m*, 2 arom. H); 6.01 (br. *s*, H–C(1′/II)); 5.96 (br. *s*, H–C(1′/I)); 5.35 (*dd*, J = 6.4, 0.7, H–C(2′/II)); 5.28 (*dd*, J = 6.5, 0.7, H–C(2′/I)); 5.05 (*dd*, J = 6.5, 4.2, H–C(3′/I)); 4.98 (*dd*, J = 6.4, 3.5, H–C(3′/II)); 4.28–4.25 (*m*, CH_2–C(6/I), H–C(4′/I)); 4.17 (*td*, $J \approx$ 7.1, 3.8, H–C(4′/II)); 3.77, 3.68 (2*d*, J = 13.5, CH_2–C(6/II)); 3.76 (*s*, MeO); 3.33 (*dd*, J = 13.6, 7.2, H_a–C(5′/II)); 3.27 (*dd*, J = 13.6, 7.2, H_b–C(5′/II)); 3.10 (*dd*, J = 13.8, 7.8, H_a–C(5′/I)); 2.99 (*dd*, J = 13.8, 6.0, H_b–C(5′/I)); 2.29 (*s*, AcS); 1.53, 1.48, 1.35, 1.31 (4*s*, 2 Me_2C). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 194.89 (*s*, SC=O); 166.40, 165.73 (2 br. *s*, 2 NHC=O); 162.43, 161.91 (2 br. *s*, C(4/I), C(4/II)); 159.08 (*s*, MeO*C*); 157.56 (br. *s*, C(6/II)); 157.25 (br. *s*, C(6/I)); 155.48 (br. *s*, C(2/II)); 155.26 (br. *s*, C(2/I)); 143.46, 143.33, 134.31 (3*s*); 133.18, 133.13 (2*d*); 130.61–127.37 (several *d*); 113.64, 113.46 (2*s*, 2 Me_2C); 113.46 (*d*, 2 C); 98.55, 97.71 (2 br. *d*, C(5/I), C(5/II)); 93.17 (*d*, C(1′/I)); 92.81 (*d*, C(1′/II)); 89.77 (*d*, C(4′/I)); 89.26 (*d*, C(4′/II)); 88.61 (*s*, Ph_2C); 85.00 (*d*, C(3′/II)); 84.96 (*d*, C(2′/II)); 84.72 (*d*, C(3′/I)); 84.69 (*d*, C(2′/I)); 62.92 (*t*, CH_2–C(6/I)); 55.23 (*q*, MeO); 34.35 (*t*, C(5′/I)); 33.85 (*t*, CH_2–C(6/II)); 31.57 (*t*, C(5′/II)); 30.54 (*q*, *Me*C=O); 27.16, 27.10, 25.35, 25.24 (4*q*, 2 *Me*$_2$C); 2 signals of C(1) of Bz hidden by the *d*s at 133.18, 133.13. HR-MALDI-MS: 1185.3731 (49, $[M + Na]^+$, $C_{62}H_{62}N_6NaO_{13}S_2^+$; calc. 1185.3708), 273.1274 (100, $MMTr^+$, $C_{20}H_{17}O^+$; calc. 273.1274). Anal. calc. for $C_{62}H_{62}N_6O_{13}S_2 \cdot H_2O$ (1181.33): C 63.04, H 5.46, N 7.11; found: C 62.97, H 5.63, N 7.35.

N^4-*Benzoyl-5′*-O-*[dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylidenecytidine-6-methyl-[(6^1 → 5′*-S)-N^4-*benzoyl-2′,3′*-O-*isopropylidene-5′-thiocytidine-6-methyl]$_2$-(6^1 → 5′*-S)-N^4-*benzoyl-2′,3′*-O-*isopropylidene-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-5′-thiocytidine* (**22**). A soln. of **19** (172 mg, 155 μmol) in THF/MeOH 1:1 (3 ml) was cooled to −10° and treated dropwise with a 1M soln. of MeSNa in degassed MeOH (310 μl, 310 μmol). The mixture was stirred for 3 h at −10°, poured into 0.1M HCl (3 ml), and extracted with AcOEt (3×). The combined org. layers were washed with H_2O and brine, dried ($MgSO_4$), and evaporated. A soln. of the residue and **21** (163 mg, 155 μmol) in degassed DMF (5 ml) was treated with LiBr (14 mg, 155 μmol) and Cs_2CO_3 (51 mg, 155 μmol), stirred for 2 h, and diluted with sat. NH_4Cl soln. The mixture was extracted with AcOEt (3×). The combined org. layers were washed with H_2O (3×) and brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/^{i}PrOH 99:1 → 49:1) gave **22** (166 mg, 51%). Yellow foam. R_f (AcOEt) 0.56. $[\alpha]_D^{25} = -76.1$ (c = 0.75, $CHCl_3$). UV ($CHCl_3$): 262 (97200), 316 (40800). IR (ATR): 3400–3000*w* (br.), 2968*w*, 2931*w*, 1669*s*, 1607*s*, 1563*s*, 1506*m*, 1473*m*, 1447*m*, 1414*w*, 1352*s*, 1314*m*, 1245*s*, 1209*m*, 1180*m*, 1155*m*, 1088*s*, 1059*s*, 1000*m*, 981*m*, 900*w*, 870*m*, 831*m*, 787*w*, 765*w*, 746*w*, 699*s*, 660*w*, 630*w*, 595*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 9.1–8.5 (br. *s*, 4 BzN*H*); 7.88–7.81 (*m*, 8 arom. H); 7.60–7.23 (*m*, 24 arom. H, H–C(5/I–IV)); 6.85–6.83 (*m*, 2 arom. H); 6.05, 6.02 (2 br. *s*, H–C(1′/II), H–C(1′/III)); 6.02 (br. *s*, H–C(1′/IV)); 5.92 (br. *s*, H–C(1′/I)); 5.33 (br. *d*, $J \approx$ 7.2, H–C(2′/IV)); 5.32 (br. *d*, $J \approx$ 7.5), 5.30 (br. *d*, J = 6.5) (H–C(2′/II), H–C(2′/III)); 5.25 (br. *d*, J = 6.5, H–C(2′/I)); 5.07–5.01 (*m*, H–C(3′/II), H–C(3′/III)); 4.99 (br. *dd*, J = 6.0, 3.9, H–C(3′/I)); 4.92 (br. *dd*, J = 5.7, 3.7, H–C(3′/IV)); 4.322, 4.315, (2*td*, J = 7.0, 3.7, H–C(4′/II), H–C(4′/III)); 4.23 (*td*, J = 7.0, 4.0, H–C(4′/I)); 4.21 (*s*, CH_2–C(6/I)); 4.19 (*ddd*, J = 7.0, 6.0, 4.4, H–C(4′/IV)); 3.80 (*dd*, J = 10.6, 5.6, H_a–C(5′/IV)); 3.76 (*s*, MeO); 3.86–3.67 (*m*, CH_2–C(6/II–IV), H_b–C(5′/IV)); 3.08–3.01 (*m*, 2 H–C(5′/I–III)); 1.57 (*sept.*, J = 6.9, Me_2CH);

1.55, 1.54, 1.53, 1.46, 1.34 (6 H), 1.33, 1.29 (7*s*, 4 Me_2CO_2); 0.84 (*d*, *J* = 6.9, *Me*$_2$CH); 0.81 (*s*, Me_2CSi); 0.06, 0.04 (2*s*, Me_2Si). ^{13}C-NMR (125 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 167.74–166.10 (4 br. *s*, 4 NHC=O); 162.50, 162.30–161.68 (4 br. *s*, C(4/I–IV)); 159.07 (*s*, MeO*C*); 157.66–156.84 (4 br. *s*, C(6/I–IV)); 155.74–154.87 (4 br. *s*, C(2/I–IV)); 143.41, 143.30, 134.30 (3*s*); 133.15, 133.05, 133.01 (2 C) (3*d*); 130.58–127.38 (several *d*); 113.66, 113.59, 113.46, 113.23 (4*s*, 4 Me_2CO_2); 113.46 (*d*, 2 C); 99.14–97.67 (4 br. *d*, C(5/I–IV)); 93.16 (*d*, C(1′/I)); 92.61–92.57 (3*d*, C(1′/II–IV)); 90.55 (*d*, C(4′/IV)); 90.06, 89.86 (2*d*, C(4′/II), C(4′/III)); 89.44 (*d*, C(4′/I)); 88.56 (*s*, Ph_2C); 84.85, 84.83 (2*d*, C(2′/II), C(2′/III), C(3′/II), C(3′/III)); 84.68 (*d*, C(2′/I), C(3′/I)); 84.29 (*d*, C(2′/IV)); 83.00 (*d*, C(3′/IV)); 64.11 (*t*, C(5′/IV)); 62.88 (*t*, *C*H_2–C(6/I)); 55.24 (*q*, MeO); 34.19 (*d*, Me_2CH); 34.32, 34.23, 34.14 (3*t*, *C*H_2–C(6/II–IV)); 33.82, 33.81, 33.69 (3*t*, C(5′/I–III)); 27.27, 27.21, 27.18, 27.15, 25.42, 25.34, 25.29, 25.27 (8*q*, 4 Me_2CO_2); 25.23 (*s*, Me_2CSi); 20.41, 20.36 (2*q*, *Me*$_2$CSi); 18.53, 18.49 (2*q*, *Me*$_2$CH); −3.21, −3.27 (2*q*, Me_2Si); 4 signals of C(1) of Bz hidden by the *d*s at 133.15–133.01. HR-MALDI-MS: 2116.7098 (24, $[M + K]^+$, $C_{108}H_{120}KN_{12}O_{23}S_3Si^+$; calc. 2116.7191), 2100.7383 (100, $[M + Na]^+$, $C_{108}H_{120}N_{12}NaO_{23}S_3Si^+$; calc. 2100.7452). Anal. calc. for $C_{108}H_{120}N_{12}O_{23}S_3Si \cdot H_2O$ (2096.47): C 61.87, H 5.87, N 8.02; found: C 61.57, H 6.00, N 7.89.

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylidenecytidine-6-methyl-[(6*^{1} → *5′*-S*)-2′,3′*-O-*isopropylidene-5′-thiocytidine-6-methyl]*$_2$-(6^1 → *5′*-S*)-2′,3′*-O-*isopropylidene-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-5′-thiocytidine* (**23**). A soln. of **22** (199 mg, 96 μmol) in CH_2Cl_2/sat. NH_3 in MeOH 1:5 (6 ml) was stirred in a pressure tube for 7 h and evaporated. FC (CH_2Cl_2/MeOH/NH_4OH 90:9:1) gave **23** (122 mg, 77%). Pale yellow foam. R_f (CH_2Cl_2/MeOH/NH_4OH 90:9:1) 0.26. $[\alpha]_D^{25}$ = −186.1 (*c* = 0.75, $CHCl_3$). UV ($CHCl_3$): 241 (30700), 276 (28200). IR (ATR): 3463*w*, 3333*w*, 3186*w*, 3054*w*, 2985*w*, 2936*w*, 1638*s*, 1534*s*, 1511*m*, 1475*w*, 1380*m*, 1373*m*, 1304*w*, 1251*m*, 1209*m*, 1180*m*, 1156*m*, 1061*s*, 1000*m*, 871*m*, 830*m*, 786*m*, 757*w*, 733*m*, 701*m*, 591*w*, 575*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HMBC, HSQC, and a ROESY spectrum): 10.78/5.35 (2 br. *s*, H_2N–C(4/I)); 10.08/7.29 (2 br. *s*, H_2N–C(4/II)); 10.04/7.29 (2 br. *s*, H_2N–C(4/III)); 9.43/7.01 (2 br. *s*, H_2N–C(4/IV)); 7.50–7.48 (*m*, 4 arom. H); 7.38–7.29 (*m*, 8 arom. H); 6.91–6.88 (*m*, 2 arom. H); 6.04 (*s*, H–C(5/II)); 5.88 (*s*, H–C(5/III)); 5.72 (*s*, H–C(1′/IV)); 5.71 (*d*, *J* = 1.3, H–C(1′/II)); 5.67 (*s*, H–C(5/IV)); 5.66 (*d*, *J* = 1.4, H–C(1′/III)); 5.60 (br. *s*, H–C(1′/I)); 5.44 (*s*, H–C(5/I)); 5.301 (*d*, *J* ≈ 6.5, H–C(2′/IV)); 5.299 (*d*, *J* = 6.5, H–C(2′/II)); 5.25 (*dd*, *J* = 6.5, 1.3, H–C(2′/III)); 5.15 (*dd*, *J* = 6.5, 1.3, H–C(2′/I)); 4.83 (*dd*, *J* = 6.1, 4.0, H–C(3′/IV)); 4.67 (*t*, *J* = 6.5, H–C(3′/II)); 4.61 (br. *t*, *J* ≈ 7.2, H–C(3′/III)); 4.60 (*t*, *J* = 6.9, H–C(3′/I)); 4.15–4.03 (*m*, H–C(4′/I–IV)); 4.01, 3.94 (2*d*, *J* = 11.8, CH_2–C(6/I)); 4.00 (*d*, *J* ≈ 11.5, CH_a–C(6/II)); 3.92 (*d*, *J* = 13.8, CH_a–C(6/III)); 3.83 (*s*, MeO); 3.77 (*dd*, *J* = 10.2, 5.4, H_a–C(5′/IV)); 3.74–3.68 (*m*, H_b–C(5′/IV), CH_a–C(6/IV)); 3.36, 3.35 (2*d*, *J* = 13.5, CH_b–C(6/II–IV)); 3.00 (*dd*, *J* = 15.6, 11.1, H_a–C(5′/I)); 2.95 (*dd*, *J* = 15.4, 11.0, H_a–C(5′/II)); 2.87 (br. *t*, *J* = 13.2, H_a–C(5′/III)); 2.74 (br. *d*, *J* = 15.0, H_b–C(5′/I), H_b–C(5′/II)); 2.65 (br. *d*, *J* = 14.3, H_b–C(5′/III)); 1.57 (*sept.*, *J* = 6.8, Me_2CH); 1.57, 1.52, 1.51, 1.42, 1.35, 1.31, 1.29, 1.27 (8*s*, 4 Me_2CO_2); 0.79 (*d*, *J* = 6.9, *Me*$_2$CH); 0.76 (*s*, Me_2CSi); 0.04, 0.01 (2*s*, Me_2Si). ^{13}C-NMR (125 MHz, $CDCl_3$; assignments based on a HMBC and a HSQC spectrum): 166.16, 166.07, 166.03, 165.51 (4*s*, C(4/I–IV)); 159.02 (*s*, MeO*C*); 157.84 (*s*, C(2/II)); 157.69 (*s*, C(2/III)); 156.99 (*s*, C(2/I)); 156.52 (*s*, C(2/IV)); 152.81 (*s*, C(6/I)); 148.67 (*s*, C(6/IV)); 148.29 (*s*, C(6/II)); 148.20 (*s*, C(6/III)); 143.33, 143.17, 134.21 (3*s*); 130.34–127.51 (several *d*); 115.03, 114.85, 114.68, 112.64 (4*s*, 4 Me_2CO_2); 113.48 (*d*, 2 C); 101.13 (*d*, C(5/II)); 100.99 (*d*, C(5/III)); 100.20 (*d*, C(5/IV)); 96.89 (*d*, C(5/I)); 91.32 (*d*, C(1′/I)); 90.59 (*d*, C(1′/IV)); 89.57–89.44 (5*d*, C(1′/II), C(1′/III), C(4′/II–IV)); 89.29 (*d*, C(4′/I)); 88.15 (*s*, Ph_2C); 84.12 (*d*, C(2′/IV)); 83.88–83.84 (3*d*, C(3′/I–III)); 83.04–82.95 (3*d*, C(2′/II), C(2′/III), C(3′/IV)); 82.48 (*d*, C(2′/I)); 64.33 (*t*, C(5′/IV)); 62.79 (*t*, *C*H_2–C(6/I)); 55.32 (*q*, MeO); 34.06 (*d*, Me_2CH); 31.41 (*t*, *C*H_2–C(6/IV)); 31.18 (*t*, *C*H_2–C(6/III)); 30.89 (*t*, *C*H_2–C(6/II)); 30.53 (*t*, C(5′/I)); 30.32 (*t*, C(5′/II)); 30.06 (*t*, C(5′/III)); 27.63, 27.61, 27.38 (2 C), 25.64 (2 C), 25.43 (2 C) (5*q*, 4 *Me*$_2$$CO_2$); 25.15 (*s*, Me_2CSi); 20.34, 20.32 (2*q*, *Me*$_2$CSi); 18.52, 18.50 (2*q*, *Me*$_2$CH); −3.01, −3.16 (2*q*, Me_2Si). HR-MALDI-MS: 1684.6426 (100, $[M + Na]^+$, $C_{80}H_{104}N_{12}NaO_{19}S_3Si^+$; calc. 1684.6403), 1662.6611 (99, $[M + H]^+$, $C_{80}H_{105}N_{12}O_{19}S_3Si^+$; calc. 1662.6584).

Cytidine-6-methyl-[(6^1 → *5′-S)-5′-thiocytidine-6-methyl]*$_2$*-(6*^{1} → *5′*-S*)-6-(hydroxymethyl)-5′-thiocytidine* (**24**). A soln. of **23** (33 mg, 20 μmol) in HCO_2H/H_2O 4:1 (1 ml) was treated with ^{i}Pr_3SiH (25 μl, 122 μmol), stirred for 20 h and evaporated at 25°. The residue was treated with NH_4OH (0.1 ml) and H_2O (1 ml) and lyophilised. FC ($CHCl_3$/MeOH/H_2O 1:3:0.3, NH_2 phase) gave **24** (15 mg, 69%) as a colour-

less solid. R_f ($CHCl_3$/MeOH/H_2O 1:3:0.3, NH_2 phase) 0.22. $[\alpha]_D^{25} = -91.8$ ($c = 0.25$, DMSO). UV (H_2O): 243 (18500), 272 (19500). IR (ATR): 3333*m*, 3201*m*, 2923*w*, 2870*w*, 1637*s*, 1531*s*, 1481*m*, 1384*m*, 1268*w*, 1093*s*, 1034*s*, 999*s*, 898*w*, 839*w*, 787*w*, 695*w*, 611*w*. ^{1}H-NMR (600 MHz, (D_6)DMSO; assignments based on a DQF-COSY, HMBC, and HSQC spectrum): 8.00–7.10, 7.40, 7.27, 7.19, 7.14 (5 br. *s*, H_2N–C(4/I–IV)); 5.90 (*s*, H–C(5/I)); 5.72, 5.65 (2*s*, H–C(5/II, III)); 5.65 (*s*, H–C(5/IV)); 5.49 (br. *s*, H–C(1′/IV)); 5.45 (br. *s*, H–C(1′/II, III)); 5.25 (*d*, $J = 2.6$, H–C(1′/IV)); 5.22–4.69 (br. *s*, OH); 4.56 (*dd*, $J = 5.9$, 4.0, H–C(2′/IV)); 4.52–4.50 (*m*, H–C(2′/I–III)); 4.37 (br. *s*, CH_2–C(6/I)); 4.26–4.18 (*m*, H–C(3′/I–III)); 4.15 (*t*, $J = 5.9$, H–C(3′/IV)); 3.82–3.70 (*m*, H–C(4′/I–IV), CH_a–C(6/II–IV)); 3.65–3.58 (CH_b–C(6/II–IV), H_a–C(5′/IV)); 3.44 (*dd*, $J = 11.8$, 5.1, H_b–C(5′/IV)); 2.85–2.69 (*m*, 2 H–C(5′/I–III)). ^{1}H-NMR (300 MHz, 2 mg in D_2O, 23°): 6.06 (*s*, H–C(5/I)); 5.86 (*s*, 1 H), 5.85 (*s*, 2 H) (H–C(5/II–IV)); 5.70 (*d*, $J = 3.3$, H–C(1′/IV)); 5.65 (*d*, $J = 2.5$), 5.63 (*d*, $J = 2.5$) (H–C(1′/II–III)); 5.49 (*d*, $J \approx 2.5$, 0.1 H), 5.43 (*d*, $J \approx 2.5$, 0.9 H) (H–C(1′/I)); 4.86–4.65 (*m*, partially erased due to irradiation of the HDO signal, H–C(2′/I–IV), H–C(3′/I–IV)); 4.56 (br. *s*, CH_2–C(6/I)); 4.43–4.34 (*m*, H–C(4′/I–IV)); 3.97–3.66 (*m*, 2 H–C(5′/IV), CH_2–C(6/II–IV)); 3.05–2.80 (*m*, 2 H–C(5′/I–III)). ^{13}C-NMR (150 MHz, (D_6)DMSO; assignments based on a DQF-COSY, HMBC, and HSQC spectrum): 165.50 (*s*, C(4/I)); 164.80, 164.72, 164.68 (3*s*, C(4/II–IV)); 157.00 (*s*, C(6/I)); 156.07, 155.94 (2 C), 155.90 (3*s*, C(2/I–IV)); 152.05, 151.86, 151.76 (3*s*, C(6/II–IV)); 96.92 (2 C), 96.67 (2*d*, C(5/II–IV)); 92.60 (*d*, C(5/I)); 92.49, 92.37, 92.31 (3*d*, C(1′/I–III)); 91.54 (*d*, C(1′/IV)); 84.90 (*d*, C(4′/IV)); 84.51, 84.34, 83.76 (3*d*, C(4′/I–III)); 73.13, 73.02, 72.95 (3*d*, C(3′/I–III)); 71.33, 71.29, 71.13 (2 C) (3*d*, C(2′/I–IV)); 70.04 (*d*, C(3′/IV)); 62.18 (*t*, C(5′/IV)); 58.84 (*t*, CH_2–C(6/I)); 32.53, 32.29 (2 C) (2*t*, C(5′/I–III)); 32.08, 31.88 (2 C) (2*t*, CH_2–C(6/II–IV)). HR-MALDI-MS: 1109.2756 (100, $[M + Na]^+$, $C_{40}H_{54}N_{12}NaO_{18}S_3^+$; calc. 1109.2733).

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