Oligonucleotide Analogues with Integrated Bases and Backbone

Part 32[1])

Thiomethylene- and Aminomethylene-Linked GG Dinucleosides of the ONIB Type: Formation of Quadruplexes

by **Martina Schulze-Adams**, **Bruno Bernet***, **David Touboul**[2]), **Daniel Egli**, **Lorenz Herdeis**, and **Andrea Vasella***

Laboratorium für Organische Chemie, ETH Zürich, Vladimir-Prelog-Weg 3, CH-8093 Zürich (e-mail: vasella@org.chem.ethz.ch)

The G[s]G dinucleoside **6** and the G[s]G* dinucleoside **8** were prepared by alkylation of the guanosine thiols derived from **2** and **5**, respectively, by the *C(8)*-chloromethylated guanosine **4** that was obtained from alcohol **3**. Dinucleosides **6** and **8** were deacylated to **7** and **9**, and fully deprotected to **10** and **11**, respectively. The G[N]G dinucleoside **16** was obtained by reductive amination of aldehyde **13** with an iminophosphorane derived from azide **14** and deprotection of the resulting dimer **15**. In the solid state of **6**, and in a solution of **6** and **8** in $CDCl_3$, H–N(1/I) and H–N(1/II) are engaged in intramolecular H-bonds to the C=O of the isobutyryl protecting groups, and HN of the isobutyryl group of unit I forms an interresidue, intramolecular H-bond to N(7/II), leading to a *syn* orientation of the nucleobase at unit I, to a *tg* orientation of the sulfanyl moiety, and to an orthogonal orientation of the nucleobases, preventing any base pairing. The silylated and isopropylidenated dinucleosides **7** and **9** are present in DMSO solution as solvated monoplexes. Broad ^{1}H-NMR signals of the nucleosides **7** and **16** in $CHCl_3$ solution evidence equilibrating G-quadruplexes. The quadruplex formation of **7** and **16** was established by ^{1}H-NMR spectroscopy (only of **16**), vapour pressure osmometry, mass spectrometry, and CD spectroscopy. The *C(6(I))*-hydroxymethylated analogue **9** in $CDCl_3$ and the fully deprotected dinucleosides **10** and **11** in H_2O form only weakly π–π stacked associates, but no G-quadruplexes, as evidenced by CD spectroscopy.

Introduction. – The ability of monomeric guanines to form polymorphic associates[3]) has been appreciated since *Bang* reported that guanylic acid forms a gel and since *Gellert et al.* determined its structure [7]. Increasing attention has been directed at the ability of guanosine-rich sequencences of DNA and RNA to form *Hoogsteen* H-bonded quartets that stack *via* π–π interactions to form polymorphic G-quadruplexes. Guanosine-rich DNA regions are widely spread in the genome and prevalent especially in telomeres and in oncogene promoters, so that G-quadruplex DNA is considered a promising therapeutic target [2][3][5b][8][9]. There is considerable interest in small compounds such as modified guanosines that interact with G-quadruplexes and function as ligands [10][11] and much interest in the supramolecular chemistry of

1) Part 31: see [1].

2) Present address: Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex (e-mail: David.Touboul@icsn.cnrs-gif.fr)

3) For reviews, see, *e.g.*, [2–6].

modified guanines [5c][6a][6b][6e][12] and various applications of their associates [6d].

We have synthesised novel oligonucleoside analogues (ONIBs[4])) characterised by a variety of linkers between C(5′) of a mononucleoside and C(6) of a neighbouring pyrimidine or C(8) of a neigbouring purine, and wondered about the ability of thiomethylene- and aminomethylene-linked di- and oligoguanosines of this type to form G-quartets and G-quadruplexes[5]). It is known that small structural changes may have a strong impact on the ability of guanosines to self-assemble [18]. We did not expect – on the basis of *Maruzen* models – that substitution of C(8) of the guanosines would impair association. It has indeed been reported that an 8-aminoguanosine [12] and similarly an 8-methylguanosine unit [19] inserted at any one of the positions of oligodeoxynucleotides with a sequence of between three and five deoxyguanosines promotes association to form polymorphic G-quadruplexes. For the *Maruzen* modeling, we assumed a parallel orientation that appears to be favoured in RNA quadruplexes [20–22] due to the disfavoured *syn* conformation of the glycosidic bond [23]. However, as the structure of ONIBs implies substitution of C(8) of purines that may favour a *syn* conformation also in quartets [24][25], we could not assume a unique quadruplex structure. We thus intended, in a scouting study, to synthesize the G*[s]G$^{(*)}$[6])) and G*[N]G dinucleosides, and to evaluate their association. We intended to synthesise these dinucleosides by following the methodology we described for adenosine derivatives in previous papers of this series[4]). The G*[s]G$^{(*)}$ dinucleosides should be obtained by lithiation of C(8) of a protected 2,3-*O*-isopropylideneguanosine to allow introduction of an electrophilic *C(1)* substituent by formylation, reduction, and activation, followed by substitution with a *C(5′)*-thioguanosine derivative. The G*[N]G dinucleoside should be prepared by reductamination of a *C(8)*-formyl guanosine using a phosphinimine derived from a *C(5′)*-aminodeoxyguanosine [1][15]. Several of the required intermediates have recently been described [1][13].

Results and Discussion. – 1. *Synthesis of G*[s]G$^{(*)}$ Dinucleosides.* The desired *C(8/I)*-unsubstituted thiomethylene-linked dinucleoside **10** and the *C(8/I)*-hydroxymethylated analogue **11** were obtained by *S*-alkylation of the chloromethylated guanosine **4** with the 5′-thiols derived from thioacetate **2** and from the 4-(methoxy)trityl thioether **5** [13], respectively, followed by deprotection (*Scheme 1*).

Thioacetate **2** was synthesized from 2,3-*O*-isopropylideneguanosine (**1** [26]) by 5′-*O*-tosylation [27], substitution with excess thioacetate in DMF, and introduction of the isobutyryl group [28]. The chloromethylated guanosine **4** was obtained from the known alcohol **3** [13] by treatment with MsCl in the presence of collidine. Attempted purification of **4** led to partial decomposition, and the crude product was used for the *S*-

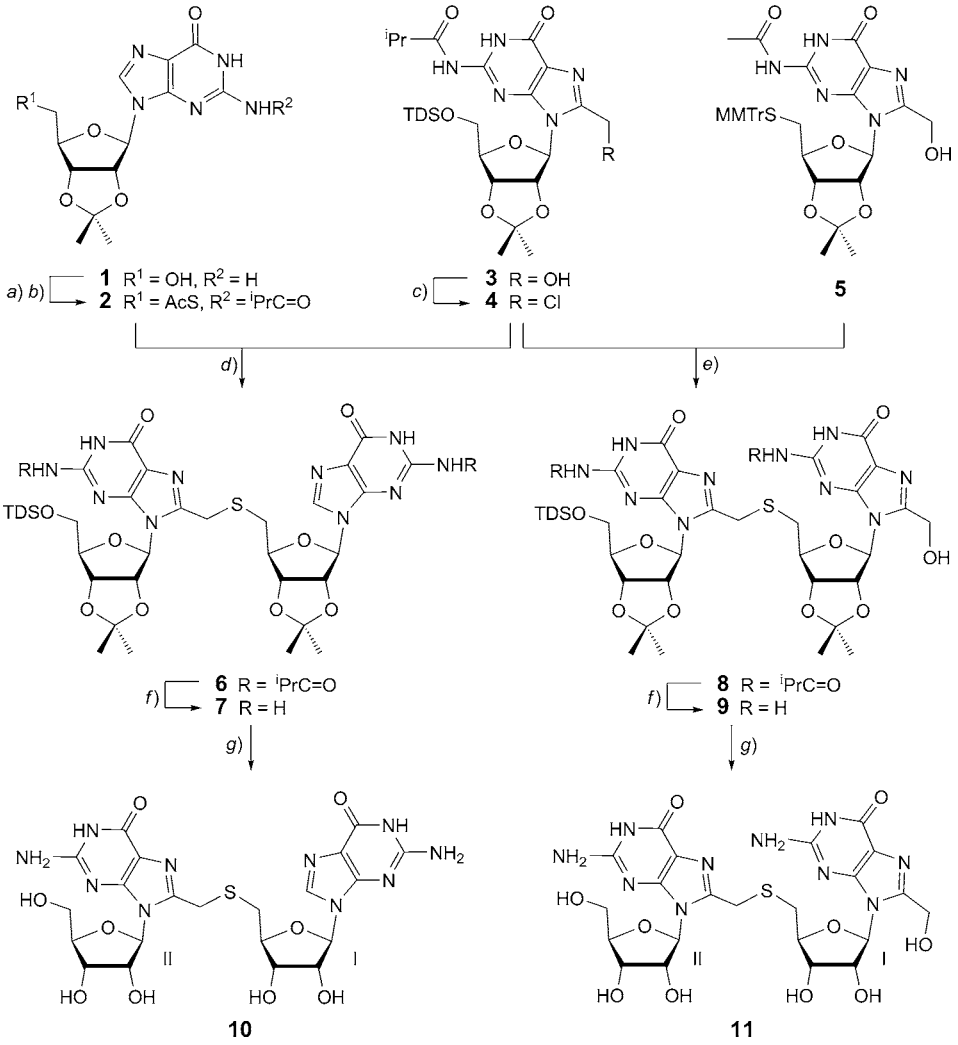
---

4) Abbreviation of the originally suggested term ‘OligoNucleotides Integrating Backbone and bases’.

5) Compare [1][13–17] and ref. cit. there.

6) *Conventions for abbreviated notation:* The substitution at C(6) of pyrimidines and C(8) of purines is denoted by an asterisk (*); for example, C* and G* for hydroxymethylated cytidine and guanosine derivatives, respectively. C$^{(*)}$ and G$^{(*)}$ 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 and [N] for an NH group.

Scheme 1



p-TsCl, pyridine/toluene; 51% of *p*-toluenesulfonate. *b*) 1. AcSK, DMF. 2. Isobutyryl chloride, pyridine; 91%. *c*) MsCl, 2,4,6-collidine, CH_2Cl_2; 86%. *d*) **2**, K_2CO_3, MeOH; then **4**, K_2CO_3, KCl, DMF; 89%. *e*) **5**, CF_3COOH (TFA), Me_3SiH, CH_2Cl_2; then **4**, K_2CO_3, KCl, DMF; 70%. *f*) NH_3/MeOH, CH_2Cl_2; 50% of **7**; 77% of **9**. *g*) HCO_2H/H_2O 4:1; 70% of **10** and an unassigned side product; 68% of **11**. TDS = thexyl(dimethyl)silyl = dimethyl(1,1,2-trimethylpropyl)silyl, MMTr = (monomethoxy)trityl = (4-methoxyphenyl)diphenylmethyl.

alkylations. However, crude **4** decomposed when we attempted to obtain the dinucleoside **6** by adding K_2CO_3 to a mixture of **2** and **4** in degassed MeOH. Thus, we deacetylated thioacetate **2** by treatment with K_2CO_3 in MeOH and deprotonated the resulting thiol by K_2CO_3 in DMF. Addition of KCl (*ca.* 50 equiv.) led to a thick

paste, ensuring that chloro derivative **4** did not decompose before it reacted with the thiolate anion to afford the thiomethylene-linked dinucleoside **6** in 89% yield. The use of aqueous 5% NaH_2PO_4 in the workup of **6** avoided the partial desilylation that occurred when using brine or saturated aqueous NH_4Cl solution. Dinucleoside **6** was deacylated with NH_3 in MeOH to yield 50% of silyl ether **7** upon chromatographic purification on a diol stationary phase. Attempts to purify dinucleosides **6** and **7** by liquid/liquid or liquid/solid extraction, crystallization, flash chromatography (FC, normal, reversed, amino, and cyano phase), or gel permeation chromatography (GPC) were not successful. The silyl and isopropylidene groups of **7** were removed with $HCOOH/H_2O$ 1:1 to yield 42% of an inseparable 3:2 mixture of the desired fully deprotected **10** and a major side-product that was not analyzed. The ^1H- and ^{13}C-NMR spectra of the crude showed signal doubling for the H- and C-atoms of the ribosyl unit I and for HC(8/I), whereas the mass spectrum of the side-product suggested a mass that is higher by two units than that of **10**. The formation of a xanthosine (replacement of ^{i}PrC(O)NH by OH) can be excluded on the basis of the absence of the corresponding characteristic signals in the ^{13}C-NMR spectrum.

For the synthesis of the *C(8/I)*-substituted dinucleoside **11**, we cleaved the 4-(methoxy)trityl thioether **5** [13] by CF_3COOH (TFA) in the presence of Me_3SiH in CH_2Cl_2 (*Scheme 1*). The resulting thiol was deprotonated (K_2CO_3 in DMF) and coupled with **4**, similarly to the thiol derived from **2**, to afford 70% of dinucleoside **8**. Deacylation of **8** with NH_3 in $MeOH/CH_2Cl_2$ furnished the silyl ether **9** (77%) that was fully deprotected to **11** (68%).

2. *H-Bonded Monoplexes of the* N^2*-Acylated G*[s]G$^{(*)}$ Dinucleosides* **6** *and* **8** *in the Solid State and in* $CHCl_3$ *Solution*. Crystallisation of **6** from $MeOH/CH_2Cl_2$ gave crystals free of solvent and suitable for X-ray analysis[7]). In the solid state, **6** does not show any base pairing (*Fig. 1*). Instead, an intramolecular H-bond from HN–C(2/I) to N(7/II) (H···N distance 2.018 Å; N–H···N angle 149.4°) is responsible for the orthogonal orientation of the guanine units (89.9°), for the *syn* orientation of the 8-unsubstituted guanine base of unit I ($\chi^I = 57.4°$), and for the *tg* orientation of the sulfanyl group (torsion angle O–C(4′/I)–C(5′/I)–S 162.6°). Unit II shows the expected *syn* orientation of the 8-substituted guanine unit ($\chi^{II} = 104.1°$; between *syn* and *high syn*) and a favourable *gt* orientation of the silyloxy group (torsion angle O–C(4′/II)–C(5′/II)–O 54.3°). The furanosyl rings of unit I and II adopt a $^3T_2$ and a ^1E conformation, respectively. The C=O groups of both isobutyryl substituents act as H-bond acceptors of H–N(1) (H···N(1/I) distance 1.867 Å; H···N(1/II) distance 1.806 Å) and thereby prevent base pairing of **6**. Intermolecular H-bonds from HN–C(2/II) to N(7/I) (H···N distance 1.807 Å; N–H···N angle 171°) are responsible for the stacking of **6** along the *b* axis.

The association of the N^2-acylated dinucleosides **6** and **8** in $CDCl_3$ was investigated by NMR spectroscopy. The HMBC spectrum of **6** allowed to unambiguously assign the H–N(1/I) signal (cross-peaks between C(5/I) and both H–N(1/I) and H–C(8/I)), the

---

7) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-977676. 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 CB2 1EZ (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

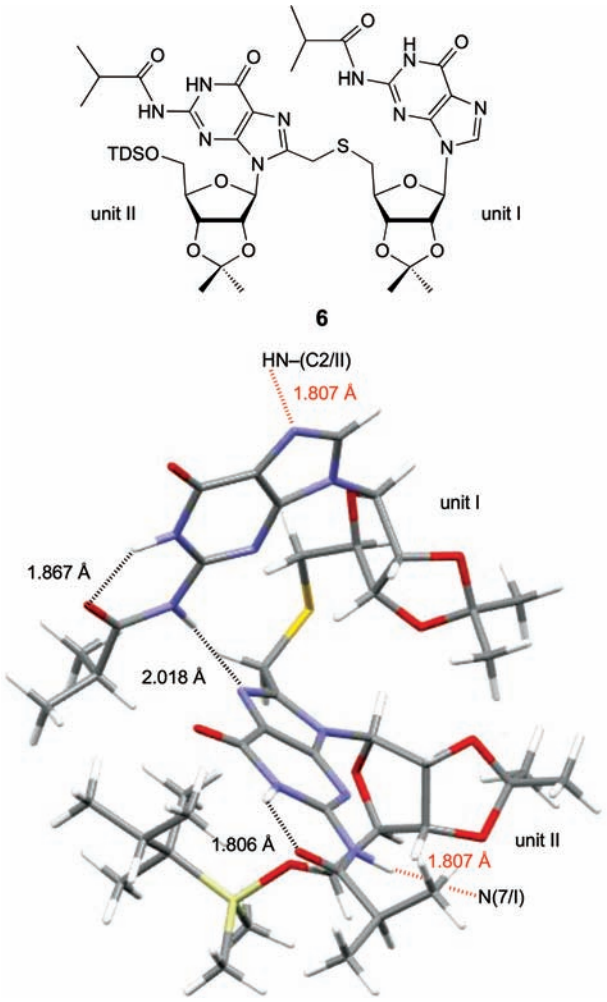


Fig. 1. *Crystal structure of the* N^2*-acylated dinucleoside* **6**. The H-Bonds are indicated by dashed lines (intramolecular H-bonds in black and intermolecular H-bonds in red).

H–N(1/II) signal (cross-peak between C(5/II) and H–N(1/II)), and the HN–C(2) signals (cross-peaks between HN–C(2) and C=O). The HMBC spectrum of **8** that is devoid of H–C(8/I) allows to differentiate only between the H–N(1) and HN–C(2) signals; the assignment to units I and II is based on the comparison with **6**. The strong downfield shift for H–N(1/I) (**6**: 12.63 ppm, **8**: 12.54 ppm) and H–N(1/II) (**6**: 12.38 ppm, **8**: 12.23 ppm) is similar to that of H–N(1) of guanosines in oligomeric guanine ribbons (*ca.* 12.0 ppm [29]) and in guanosine–cytosine duplexes (12.4–13.4 ppm [30]), and evidences strong H-bonding. Also one of the HN–C(2) groups (**6**: 12.26 ppm, **8**: 12.03 ppm) is involved in a strong intra- or intermolecular H-bond,

whereas the upfield shift for the other HN–C(2) (**6**: 10.25 ppm, **8**: 9.29 ppm) indicates an equilibrium between partially H-bonded species, considering that HN–C(2) of unassociated N^2-isobutyrylated and O^6-protected guanosines resonates in the range of 7.68 – 7.80 ppm [13]. These NH chemical shifts suggest a similar structure of **6** and **8** in apolar solvents as found in the solid state of **6**. H–N(1/I and 1/II) form a persistent H-bond to C=O of the isobutyryl groups, HN–C(2/I) forms a persistent intramolecular H-bond to N(7/II), and HN–C(2/II) a partly persistent intermolecular H-bond. The distal orientation of H–N(1) and HN–C(2) – a consequence of the N(1)–H ··· O=C H-bond – prevents to a large extent base pairing of N^2-acylated guanosines. Nevertheless, the broad H–N(1/II) signal of **8** suggests that intermolecular association may compete with intramolecular H-bonding.

The influence of the substituents at C(2), C(8), and C(5′) of isopropylidenated adenosine [17], and of a few guanosine mononucleosides [13] upon the orientation of the nucleobase and the O- or S-substituent at C(5′) have already been analysed. Most useful criteria for the characterisation oft *anti*/*syn* orientations in the adenosine (and pyrimidine) derivatives are the chemical shift for H–C(2′) in conjunction with the ribosyl ring conformation as affected by intramolecular H-bonds from HO–C(5′) to N(3), and the rotational equilibria for the C(4′)CH_2R group. These criteria can be applied, with some adjustments, to assign the *anti*/*syn* orientation of guanosines. The chemical shifts for H–C(2′) of **2** (5.15 ppm) and for **4** (5.43 ppm), adopting the (*N*) and predominantly an (*N*) conformation, evidence an *anti* conformation of **2** and a *syn* conformation of **4**, as expected from the substitution at C(8) of **4**. In the dinucleosides, the downfield shift for H–C(2′/II) (**6**: 5.62 ppm, **8**: 5.65 ppm; *Table 2* in the *Exper. Part*) evidences a *syn* orientation for unit II. The upfield shift for H–C(2′/I) (5.00 – 5.01 ppm) might evidence an *anti* orientation of the 8-unsubstituted unit I of **6** and, rather unexpectedly, also of the 8-hydroxymethylated guanosyl unit I of **8**. However, an anisotropy effect leads to a strong upfield shift for H–C(3′/I) relative to H–C(3′/II) ($\Delta\delta$ *ca.* 1.2 ppm) for **6** and **8**. Also H–C(2′/I) may show a clear and weaker anisotropy effect ($\Delta\delta$ relative to H–C(2′/II), *ca.* 0.6 ppm), casting some doubt on the above conformational assignment. It cannot be excluded, therefore, that unit I of **6** and **8** adopt a *syn* conformation, as required for the postulated intramolecularly H-bonded species. As expected, the sulfanyl substituent of unit I prefers exclusively a *tg* orientation (*gg*/*gt*/*tg* = – 5 : 4 : 101 (**6**) and – 3 : 3 : 100 (**8**)), as calculated from *J*(4′,5′a/I) 11.5 – 11.6 and *J*(4′,5′b/I) 3.4 – 3.5 Hz by the formulae given in [17], assuming that the more deshielded H–C(5′) is $H_{pro\text{-}S}$, whereas the silyloxy group of unit II prefers a *gt* orientation (*gg*/*gt*/*tg* 13 : 60 : 27 (**8**) and 14 : 51 : 35 (**10**)) that is also observed in the solid-state structure of **6**. Interestingly, also thioacetate **2** prefers completely the *tg* conformation, whereas the corresponding A, U, and C analogues adopt a *gt*/*tg* 1 : 1 equilibrium [14][17]. This suggests a stronger preference for the *tg* conformation of 5′-sulfanylated guanosines.

3. *Solvated Monoplexes of the G*[*s*]G*$^{(*)}$ *Dinucleosides* **7** *and* **9** – **11** *in DMSO*. The isopropylidenated and silylated dinucleosides **7** and **9** are well soluble in chlorinated solvents (up to 200 mM), but give rise to strong line broadening. Therefore, **7**, **9**, and the fully deprotected dinucleosides **10** and **11** were analysed by NMR spectroscopy of their solutions in (D_6)DMSO (*Table 2* and *3* in the *Exper. Part*). As expected for this solvent, these dinucleosides are present as completely solvated monoplexes. This is evidenced

by the upfield shift of H–N(1/I,II) (**7**: 9.7 – 10.1 ppm, **9**: 10.2 – 10.8 ppm)[8]) and of H_2N–C((2/I,II)) of **7**, **10**, and **11**, resonating at 6.2 – 6.73 ppm [29][31][32]. A fast H/D exchange prevented detecting the H–N(1/I,II) signals of **10** and **11**. The sulfanyl moiety of the isopropylidene acetals **7** and **9** still prefers a *tg* orientation (*gg*/*gt*/*tg ca.* 5 : 30 : 65), while that of the fully deprotected **10** and **11** adopts a *ca.* 1 : 1 *gt*/*tg* equilibrium. HO–C(5′/II) of **7** and **9** prefers a *tg* orientation (*gg*/*gt*/*tg ca.* 2 : 2 : 6), and of **10** and **11** a *gg* orientation (*gg*/*gt*/*tg ca.* 55 : 25 : 20). H–C(2′/I) of the completely deprotected **10** and **11** resonate at 4.60 and 5.01 ppm evidencing an *anti*- and a *syn*-oriented guanosyl unit, respectively. H–C(2′/II) of **10** and **11** resonate both at 4.72 ppm, showing a characteristic upfield shift ($\Delta\delta = 0.3$ ppm relative to H–C(2′/I) of **11**) for *syn*-oriented purine bases possessing a C(5′)OH ··· N(3) H-bond (*cf.* [17] and refs. cit. there). Both ribosyl units of **10** and **11** adopt an (*S*) conformation. H–C(2′/II) of the isopropylidenated **7** and **9** resonate at 5.47 – 5.48 ppm. This appears to be a typical shift for *syn*-configured isopropylidenated guanosines in DMSO. The rather small and similar upfield shifts for H–C(2′/I) (**7**: 5.21, **9**: 5.26 ppm) suggest a *ca.* 1 : 1 *syn*/*anti* orientation of the 8-unsubstituted and the 8-hydroxymethylated guanosyl moieties of unit I.

4. *Association of the G*[s]G(*) Dinucleosides* **7** *and* **9** *in Apolar Solvents, and of* **10** *and* **11** *in* H_2O. Guanosine-rich nucleotides may *a priori* form several isomers of G-quartets (G-tetrads) [33] by G · G *Hoogsteen* base pairing (*Fig. 2*, *a*). The possibility of the formation of quadruplexes comprising two G-quartets from G*[x]G(*) dinucleosides (x = s or n) was investigated by *Maruzen* modeling (*Fig. 2*, *b*). It is mandatory that the guanines of unit I form one G-quartet and the guanines of unit II the other one. In a quadruplex, the four monomers adopt the same conformation. The quartets may be arranged in a parallel (*Fig 2, b*, left-hand picture) or in an antiparallel fashion (right-hand picture), and the guanine moiety of unit I may be in an *anti* or a *syn* orientation. The interconversion of these four quadruplexes can only occur by separation of the base pairs, similar conformational changes of the individual dinucleosides, and reestablishing the base pairing. Concerted rotation about the C(4′/I)–C(5′/I) bonds of a given quadruplex leads from *gg* to *gt* and *tg* rotamers with an increasing distance between the quartets. The classical *anti* or *syn* orientation of the nucleobase is kept during the rotation with the exception of *tg* rotamers of the *anti*-configured quadruplex which adopt a conformation ($\chi = -55°$) with severe steric interaction between H–C(2′/I) and H/$HOCH_2$–C(8). π–π Stacking is only a stabilizing factor for distances < 4 Å (compare with an optimal distance of 3.4 Å in natural nucleosides). Considering that 5′-sulfanylated nucleosides avoid a *gg* conformation and 8-substituted guanosines strongly prefer a *syn* conformation, these *Maruzen* modelings predict for G*[s]G(*) dinucleosides a quadruplex characterized by an *anti* and *gt* conformation of unit I, and by parallel quartets with a short distance between the quartets (3.5 – 4.0 Å). This quadruplex is formed more easily from **7** and **10** than from **9** and **11**.

The ^{1}H-NMR spectra of 5 mM solutions of **7** and **9** at room temperature in $CDCl_3$ are characterized by broad signals in the absence or in the presence of KCl, potassium picrate, or NaCl. An analysis of the self-association by ^{1}H-NMR spectroscopy, similarly as in [17], was, therefore, not attempted. Line-broadening was also observed for

---

[8]) *Defrancq* and co-workers [31] postulated a value of 10.69 ppm as typical for free guanosines in DMSO.

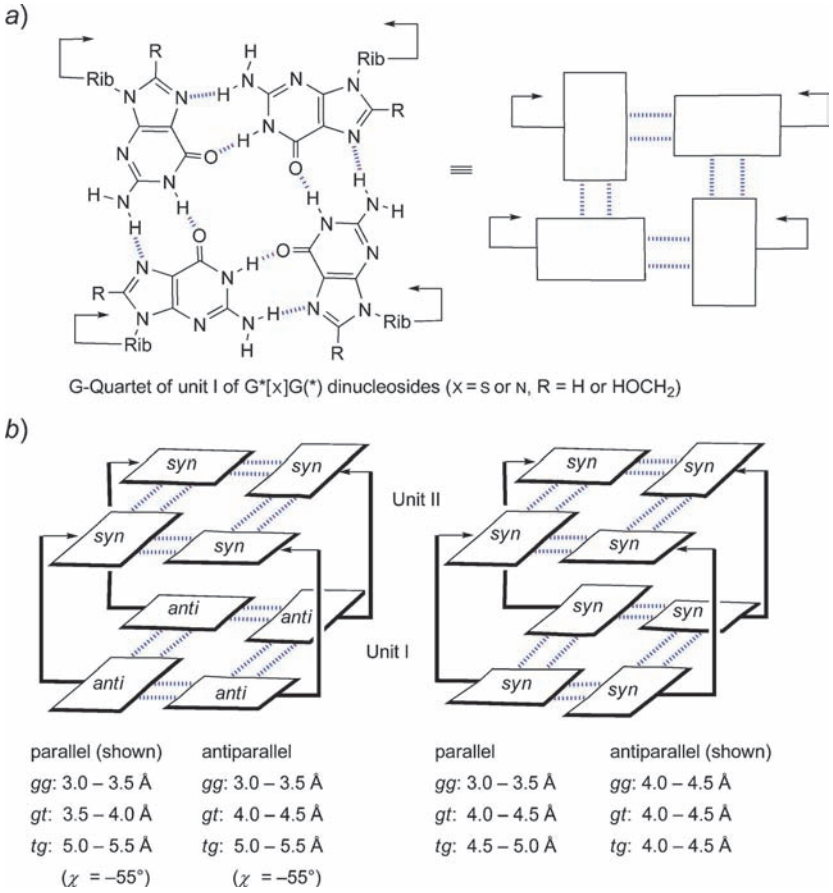


Fig. 2. a) Hoogsteen *base pairing for the formation of G quartets* (H-bonds indicated by blue hashed lines). The orientation of the guanine moieties along the *x*- and *y*-axis of the drawing is schematically represented by rectangles. b) *Schematic representation of the quadruplexes of G*[*X*]*G(*) dinucleosides* (X = S or N) *obtained by* Maruzen *modeling with estimated distances between the G quartets as depending on the conformation of unit I and the relative orientation of the quartets.* 'Parallel *vs.* antiperallel orientation' refers to the orientation of G moieties in the two quartet planes of the quadruplex along the *x*- and *y*-axis (the sense of rotation). The parallel orientation is shown in the left-hand representation of *b*), the antiparallel orientation in the right-hand one.

solutions of **7** in CD_2Cl_2 at $-70°$ and in CD_2ClCD_2Cl at $+100°$. This line-broadening evidences equilibria of the monoplex with one or several aggregates, but does not allow their characterisation. However, the molecular weight of *ca.* 3341 g/mol, as determind by vapour pressure osmometry (VPO) at 23° of a 5 mM solution of **7** in $CHCl_3$, suggested the formation of a quadruplex of **7**, characterized by a molecular mass of 3268 g/mol.

The aggregation of the dinucleosides **7** and **9**–**11** was further examined by mass spectrometry and circular dichroism (CD) spectroscopy. The ESI-TOF-MS (positive-

ion mode) of a 5 mM solution of **7**[9]) in $CHCl_3$ containing ammonium salts showed a single peak at *m/z* 3283, corresponding to $[4\,M+NH_4]^+$ or $(n\cdot[4\,M+NH_4])^{n+}$ (*Fig. 3,a*) (*cf.* [34]). No peak corrresponding to $[4\,M+2\,NH_4]^{2+}$ was observed. The peak at *m/z* 3283 was analyzed by MS/MS (*Fig. 3,b*). The by far most prominent peak in the resulting spectrum still corresponds to the quadruplex $[4\,M+NH_4]^+$, and confirms that a 5 mM solution of **7** (in contact with NH_3) in $CHCl_3$ is predominantly an NH_4^+-containing quadruplex. Minor peaks of decreasing intensity (20–2%) correspond to the monoplex M^+, duplex $[2\,M]^+$, triplex $[3\,M]^+$, and quadruplex $[4\,M]^+$. To evaluate the propensity of **7** to form the NH_4^+-containing quadruplexes in solution, we diluted the 5 mM solution of **7** in $CHCl_3$ to 2.5, 1, 0.5, 0.25, 0.1, and 0.05 mM (*Fig. 3,c*). The $[4\,M+NH_4]^+$ peak in the ESI-TOF mass spectra remained the most prominent one down to a concentration of 0.25 mM. It was still detected for the 0.05 mM solution. The monoplex peak $[M+H]^+$ (*m/z* 817.3; the charge carrier being a proton rather than an NH_4^+ ion) was observed upon dilution to 0.5 mM, its intensity increasing with decreasing concentration. The association constant for the formation of these quadruplexes was estimated[10]) as between 10^{13} and 10^{14} M^3, and $-\Delta G$ at 25° to *ca.* 19 kcal/mol, using the *van't Hoff* equation. Assuming two H-bonds per guanosine moiety and neglecting contributions from π–π stacking, this corresponds to 2.4 kcal/mol per H-bond, a distinctly smaller value than *ca.* 9 kcal/mol as obtained by calculation for the gas phase [35].

The influence of alkali and magnesium halides on the association of a 5 mM solution of **7** and ammonium salt in $CHCl_3$ was investigated by ESI-TOF-MS (*Fig. 4*), considering that the stability sequence of cationic complexes appears to depend on the structure [12][36] and on hydration, among other factors [37]. Addition of powdered NaI resulted in the sodium adduct $[4\,M+Na]^+$ (*m/z* 3288) as the most prominent peak; ammonium is thus easily replaced by a sodium cation. Addition of powdered KI gave only a very weak peak of the potassium adduct $[4\,M+K]^+$ (*m/z* 3304), the most abundant peak still being the one for $[4\,M+NH_4]^+$. There were two additional (unassigned) weak peaks at *m/z* 3291 and 3294 (dashed arrow). The mass spectrum was hardly affected by the addition of powdered MgI_2; the only detected peak corresponds to $[4\,M+NH_4]^+$. Hence, K^+ and Mg^{2+} complex too weakly to displace NH_4^+ as guest of the quadruplex. Addition of powdered LiI led to dissociation of the ammonium quadruplex $[4\,M+NH_4]^+$ and to the dominant formation of the lithium monoplex $[M+Li]^+$ (*m/z* 823.4).

The CD spectrum of such G-quartets is characterized by a degenerate exciton couplet centered at 258 nm, *i.e.*, at a UV absorption maximum that corresponds to a low or zero ellipticity [38]. A CD band at *ca.* 260 nm was associated with parallel sheets of G-quartets in G-quadruplexes, and a CD band at *ca.* 290 nm with heteropolar stacking

---

[9]) Obtained by deacylating **6** by NH_3 in MeOH, evaporation, dissolution of the crude product in $CHCl_3$, evaporation, and dissolution of the residue in the required amount of $CHCl_3$.

[10]) As evidenced by the VPO measurements, four monoplexes at the concentration M are in equilibrium with one quadruplex (at the concentration M_4). The association constant is then $K_{ass} = [M_4]/[M]^4$. The mass balance is $c_0 = M + 4\,M_4$. At a concentration between $2.5\cdot10^{-4}$ and 10^{-4} M, the integral of the monoplex peak is equal to the integral of quadruplex peak, thus $M = M_4$. At that concentration, the mass balance simplifies to $c_0 = 5\,M$, the association constant for the formation of quadruplexes cancels to $K_{ass} = [M]^{-3}$, thus between $8\cdot10^{12}$ and $1.25\cdot10^{14}$ M^3.

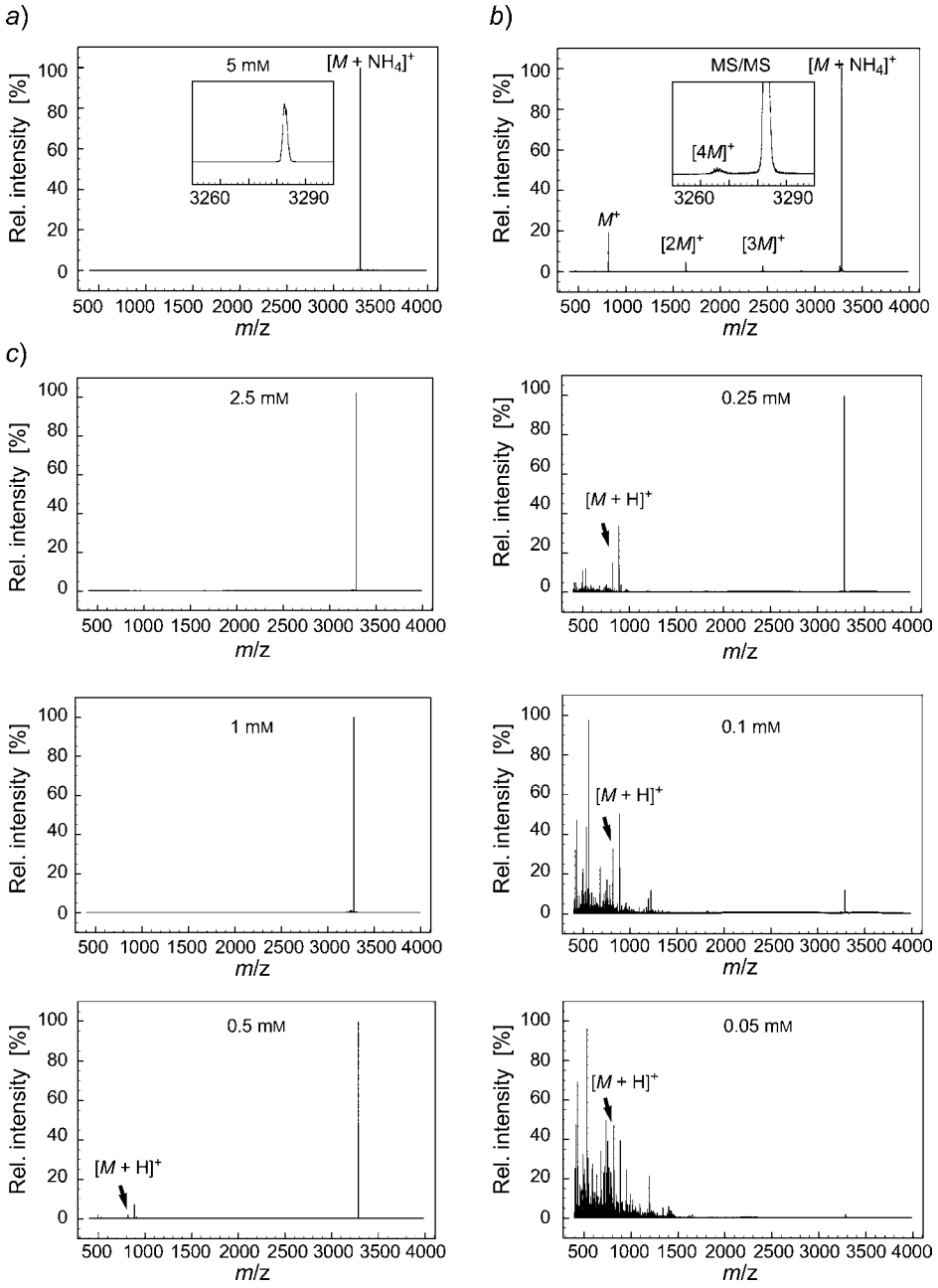


Fig. 3. *ESI-TOF-MS of the silylated and isopropylidenated dinucleoside* **7** *in* $CHCl_3$. *a*) $c = 5 \cdot 10^{-3}$ M. *b*) MS/MS of the peak at 3284 Da. *c*) $c = 2.5\ 10^{-3}$ to $5 \cdot 10^{-5}$ M.

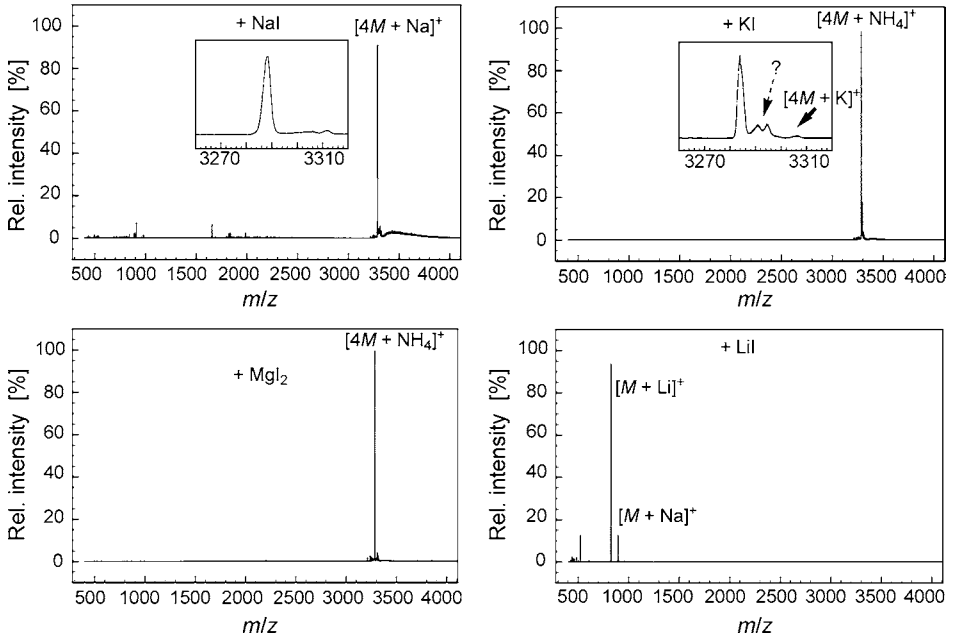


Fig. 4. *Influence of alkali and magnesium iodides, respectively, on the ESI-TOF-MS of a* $5 \cdot 10^{-3}$ M *solution of the silylated and isopropylidenated dinucleoside* **7** *in* $CHCl_3$

('head to head' or 'tail to tail') [39], alternating *syn* and *anti* conformations [38a], and tiltet/twisted sheets of G-quartets [38c]. Large ellipticities (*ca.* 80,000 degree cm^2 decimol^{-1}) hint at the formation of a quadruplex that may be completely formed, or partially disaggregated into quartets.

The CD spectrum of a 10^{-3} M solution in $CHCl_3$ of the silylated and isopropylidenated dinucleoside **7** shows a typical negative exciton couplet centered at 285 nm with extrema at 295 and 267 nm, and a large molar ellipticity ($[\theta] \approx 80{,}000$ degree cm^2 decimol^{-1}; *Fig. 5,a*). It evidences the formation of a quadruplex characterized by a *syn* conformation of unit II and an *anti* conformation of unit I, as suggested by *Maruzen* modeling. In agreement with this, the CD spectrum of the *C(8/I)*-hydroxymethylated analogue **9** shows only a positive *Cotton* effect with the maximum at 270 nm. The molar ellipticity ($[\theta] \approx 35{,}000$ degree cm^2 decimol^{-1}) evidences π–π stacking (compare with the poor π–π stacking of the mononucleoside **12**; $[\theta] < 10{,}000$ degree cm^2 decimol^{-1}), but the CD spectrum is not in keeping with a π–π stacked quadruplex structure. As suggested by *Maruzen* models (*Fig. 2*), a quadruplex possessing a *syn* and *gt* conformation of unit I, as favoured by the *C(8/I)*-hydroxmethyl substitution of **9**, should not show π–π-stacking due to the large distance between the quartets (>4 Å).

The propensity of **7** to form a quadruplex was further investigated. A very weak dependence on concentration of the CD spectrum of **7** in $CHCl_3$ evidences a high stability of the quadruplex in the concentration range from 10^{-3} to 10^{-5} M (*Fig. 5,b*). Addition of NH_4I or KI to a 10^{-3} M solution of **7** in $CHCl_3$ increased the amplitude of the trough at 295 nm, addition of NaI reduced it; both salts reduced the amplitude of

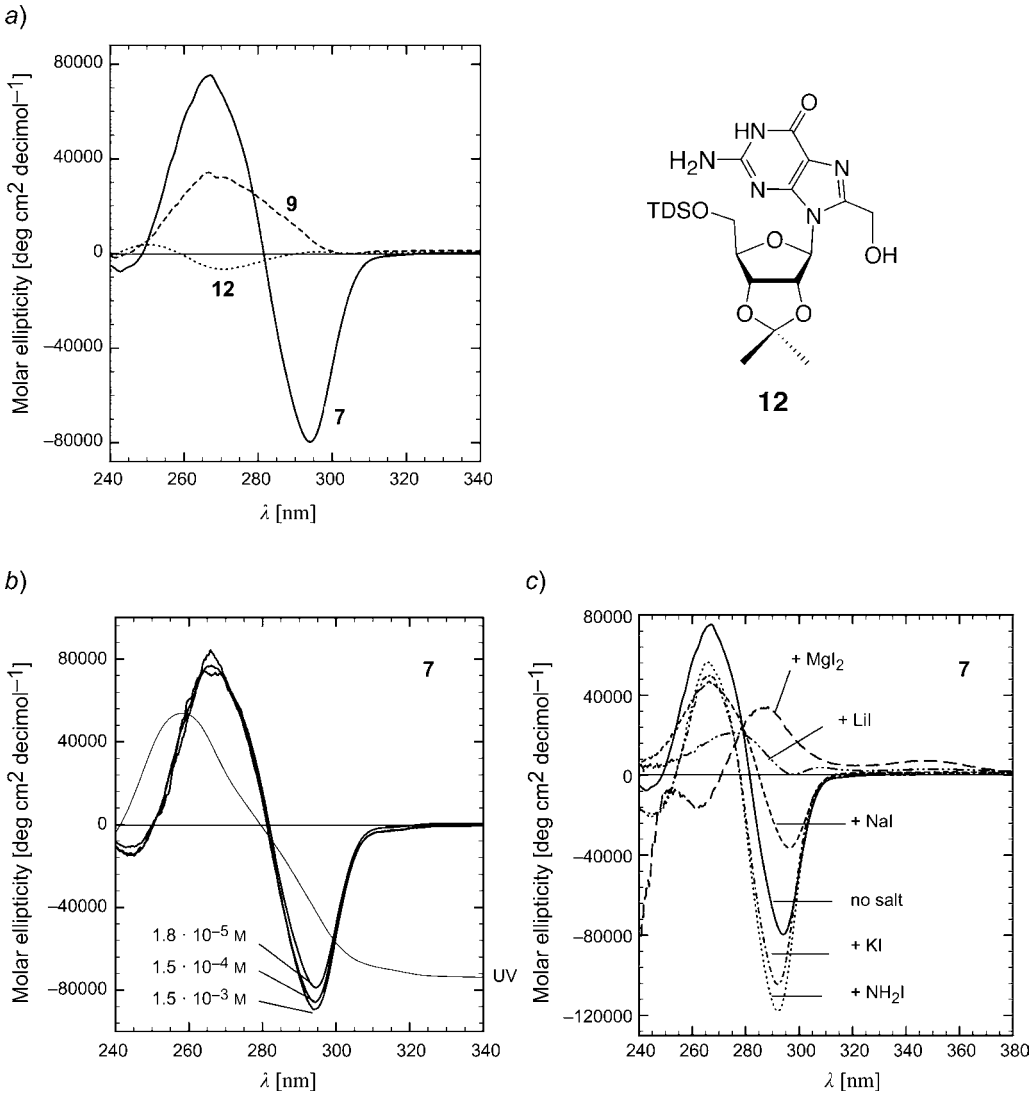


Fig. 5. a) *CD Spectra of dinucleosides* **7**, **9**, *and deisobutyrylated mononucleoside* **12** (derived from **3**) *in* $CHCl_3$. b) *Concentration dependence of the CD spectra of* **7** *and its UV spectrum.* c) *Influence of XI* (X = Li, Na, K, NH_4) *and* MgI_2 *on the CD spectrum of a* 10^{-3} M *solution of* **7** *in* $CHCl_3$.

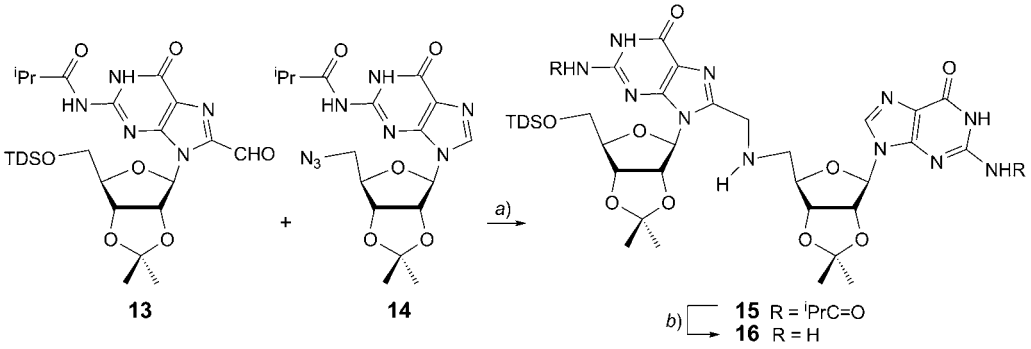
the peak at 265 nm (*Fig. 5, c*). This suggests that the dominating exciton couplet of the quadruplex is overlaid by CD absorptions of other π–π-stacked species. A positive *Cotton* band at 275 ($[\theta] \approx 20{,}000$ degree cm^2 decimol^{-1}) or 290 nm ($[\theta] \approx 35{,}000$ degree cm^2 decimol^{-1}) resulting from the addition of LiI and MgI_2, respectively, suggests the formation of other π–π-stacked aggregates. The CD results for the addition of these iodides to the empty quadruplex of **7** are essentially in agreement

with the ESI mass spectra denoting the formation of a quadruplex of **7** with ammonium as guest.

The CD spectra of aqueous solutions of the fully deprotected dinucleosides **10** (as a 3:2 mixture with an unassigned side-product) and **11** are characterized by considerably lower amplitudes ($[\theta] \leq 12{,}500$ degree cm^2 decimol^{-1}) of the bands, as compared to those of their silylated and isopropylidenated precursors **7** and **9** in $CHCl_3$. These weak molar ellipticities evidence the presence of several weakly π–π-stacked species, but the absence of substantial amounts of π–π-stacked quadruplexes.

5. *Synthesis, Conformation, and Association of the G*[*N*]G Dinucleosides* **15** *and* **16**. The G*[N]G dinucleoside **15** was obtained in a yield of 70% by reductamination of aldehyde **13** [1] with the phosphinimine derived from azido nucleoside **14** [1] and reduction of the resulting imine (*Scheme 2*). Deacylation of **15** with either NH_3 or MeONa in MeOH gave 83% of the partially protected dinucleoside **16**.

Scheme 2



13 14

a) 1. **14**, Me_3P, THF; then **13**. 2. $NaCNBH_3$, ^{i}PrOH/AcOH 1:1; 88%. *b*) MeONa, MeOH; 83%.

One would expect that **15** in $CDCl_3$ forms an inter-residue H-bond from HN–C(2/I) to N(7/II) as already found for the G*[s]G$^{(*)}$ dinucleosides **6** and **8** (see *Fig. 1*), albeit with a lower persistence, since the *tg* conformation of unit I of **15** is expected to be disfavoured, in contradistinction to **6** and **8**. An equilibrium between the free and the H-bonded species of **15** is evidenced by the downfield shifts of H–N(1/I) (12.8–12.6 ppm) and H–N(1/II) (12.03 ppm), the upfield shift of HN–C(2(II) (10.24 ppm), and the coalescence of HN–C(2/I) (detected by integration at 11.0–12.5 ppm). Weaker upfield shifts of H–C(2′/I) and H–C(3′/I) of **15** as compared to those of **6** (5.05 *vs.* 5.00 ppm and 4.18 *vs.* 3.69 ppm, resp.; *Tables 2* and *4* in the *Exper. Part*) corroborate this equilibrium. J(4′,5′a/I) of 7.2 and J(4′,5′b/I) of 4.0 Hz show a preference for the *tg* conformation and suggest a *ca.* 1:1 mixture of the free and the H-bonded species.

Addition of 1% of CD_3OD led to complete cleavage of the inter-residue H-bond of **15**. The ^{1}H-NMR spectra of **15** in $CDCl_3/CD_3OD$ 99:1 and of **16** in (D_6)DMSO evidence the presence of completely solvated monoplexes (*Table 4* in the *Exper. Part*). The chemical shifts for H–N(1) and HN–C=O of **15** (12.1–12.6 and 10.5–11.6 ppm, resp.) and for H–N(1) and NH_2 of **16** (10.71/10.77 and 6.60/6.64 ppm, resp.) agree well with their complete solvation. The downfield shift of H–C(2′/II) (**15**: 5.50 ppm, **16**:

5.52 ppm) and the upfield shift of H–C(2′/I) (**15**: 5.12 ppm, **16**: 5.15 ppm) reveal the expected *syn* conformation of unit II and a *syn*/*anti* equilibrium for unit I. This equilibrium is corroborated by strong cross-peaks between H–C(8/I) and both H–C(1′/I) and H–C(2′/I) of **15** and **16**. The TDSO group of unit II of **15** and **16** adopts a 1:1 *gt*/*tg* orientation, as evidenced by J(4′,5′) values of 5.6–7.0 Hz. Smaller J(4′,5′) values for unit I (5.1–6.0 Hz) reveal a *ca.* 1:1:1 *gg*/*gt*/*tg* equilibrium of the (guanosylmethyl)-amino substituent.

The ^{1}H-NMR spectrum of a 15 mM solution of **16** in $CDCl_3$ at room temperature shows broad signals for a 4:1 mixture of isomers, preventing the assignment of coupling constants. Nevertheless, an unambiguous assignment was possible for the major isomer with the help of DQF-COSY, HSQC, and ROESY spectra (*Table 4* in the *Exper. Part*). The downfield shifts of H–C(2′/II) (6.00 ppm) and H–C(2′/I) (5.95 ppm) suggest a *syn* conformation for both units. In the ROESY spectrum, a cross-peak between H–C(1′/II) and H_aC–C(8/II) confirms the *syn* conformation of unit II. However, equally strong cross-peaks between H–C(8/I) and both H–C(1′/I) and H–C(2′/I) reveal a 1:1 *syn*/*anti* equilibrium for unit I; the strong downfield shift for H–C(2′/I) must then be due to an anisotropy effect. Broad signals prevent any conclusions about the furanose ring conformation and the orientation of the substituents at C(5′/I) and C(5′/II). The minor species adopt completely an *anti* conformation of unit I as evidenced by a DQF-COSY cross-peak between H–C(1′/I) at 6.25 ppm and H–C(2′/I) resonating upfield at 5.04 ppm.

A quadruplex of **16** should lead *a priori* to six NH signals, four at low field (> 10 ppm) and two at high field (5.5–7.5 ppm). The ^{1}H-NMR spectrum of a 15 mM solution of **16** in $CDCl_3$ exhibits eleven signals in the range of 6 to 14 ppm, integrating for 0.1 to 0.7 H equivalents (see *Exper. Part*). Three of them appear between 7.6 and 9.3 ppm, and are hardly due to quartets. The quadruplex formation in pure $CDCl_3$ is slow, and different equilibria were found for other samples of **16**. Thus, in a sample more advanced in equilibration, five NH signals were observed at room temperature at 11.60, 11.05, 9.30, 7.75, and 6.20 ppm. Cooling to $-40°$ led to a doubling of the NH signals, suggesting a mixture of at least two H-bonded species. The ROESY spectrum of a third sample showed signals for two quartets (12.75/9.95/5.25 and 12.65/9.85/7.10 ppm) and signals for a free NH_2 group (7.95/6.05 ppm).

The quadruplexes of **16** were completely persistent in $CDCl_3$/(D_6)DMSO 9:1. A 85:15 mixture of two associated species was observed. H–N(I and II) appear as sharp *singlet*s at 11.88 and 11.85 ppm (major species), and at 12.07 and 12.02 ppm (minor species). The downfield shift agrees well with the formation of quadruplexes, unfortunately, the signals for H_2N–C(2/I and II) are hidden due to fast H/D exchange, probably catalyzed by traces of acid. The independence of δ(H–N(I and II) upon concentration (25 to 0.9 mM) and upon temperature (25 to $-50°$) evidences stable quadruplexes; there is no equilibration between the two associated species.

In $CDCl_3$/(D_6)DMSO 9:1, the signals of unit II of **16** are sharp, and those of unit I and of CH_2–C(8/II) are broad, but distinctly less so than in $CDCl_3$. This suggests that the guanines of unit II form a more stable quartet. The signals for corresponding H-atoms of the two species partially or completely overlap, only those of H–C(1′/I) and H–C(1′/II) are completely separated ($\Delta\delta$ of 0.03 and 0.09 ppm, resp.). This suggests a similar conformation, but a different H-bonding network for the two species. The

upfield shift of H–C(2′/I) (5.06 ppm; *Table 4* in the *Exper. Part*) and the downfield shift of H–C(2′/II) (5.84 ppm) evidence a largely predominant *anti* conformation of unit I (compare with *anti*/*syn* $\approx 1:1$ in $CDCl_3$), and confirm the expected *syn* conformation of unit II. The TDSO moiety of unit II adopts a 1:1 *gt*/*tg* orientation. Despite the broad signals, it was possible to assign coupling constants also for unit I. Small $J(4',5'a/I)$ and $J(4',5'b/I)$ couplings (< 1.5 Hz) reveal a *gg* orientation of the (guanylmethyl)amino group. Unit I prefers a northern conformation ($J(1',2')/J(3',4) < 0.35$), and unit II a 1:1 northern/southern equilibrium ($J(1',2')/J(3',4) \approx 1$).

Thus, ^{1}H-NMR spectroscopy of **16** in $CDCl_3/(D_6)DMSO$ 9:1 evidences a non-equilibrating 85:15 mixture of two quadruplexes possessing the *anti* and *gg* conformation of unit I. *Maruzen* modeling predicts a similar π–π-stacking of such quadruplexes with parallel and antiparallel orientation of the G-quartets (*Fig. 2*). The ^{1}H-NMR data indeed agree well with this prediction. An equilibration is improbable, since it would require a concerted cleavage of all H-bonds of a quartet, a concerted reorientation of the guanosyl units, and formation of the new H-bonds. However, both quadruplexes may be in equilibrium with other associated species, *e.g.*, with those possessing incompletely formed quartets; this is evidenced by the broad signals for the H-atoms of unit I. The formation of tetrameric complexes is corroborated by VPO molecular mass determination of a 10 mM solution of **16** in CH_2Cl_2, yielding a relative molecular mass of 3154.94 g/mol (3.94 times the molecular mass).

Stacking of the associated species of **16** was investigated by CD spectroscopy. The CD spectrum of a 1 mM solution of **16** in $CDCl_3$ shows minima at 305 and 268 nm, and maxima at 283 and 250 nm. The large molar ellipticities (up to 40,000 deg cm^2 decimol^{-1}) evidence π–π-stacking of the quartets. The maximum at 283 nm suggests a *syn* orientation, while the minimum at 268 and maximum at 250 nm suggest an *anti* orientation [40] (*Fig. 6*), in agreement with the ^{1}H-NMR analysis that evidenced two associated quadruplexes with both *anti* and *syn* orientation of unit I and a *syn* orientation at unit II. The slight decrease at 268 nm and the slight increase at 250 nm of the molar ellipticity upon increasing temperature may indicate a decrease of an unidentified π–π-stacked species in favour of the quadruplex with *anti* orientation of unit I, whereas the fraction of the other quadruplex with a *syn* orientation of unit I remained constant.

The concentration dependence of the association of **16** in $CHCl_3$ was further investigated by ESI mass spectrometry. The spectra of a 1, 0.1, and 0.05 mM solution of **16** in $CHCl_3$ show peaks for the monoplex (M), a quadruplex (Q), a duplex of quadruplexes (DQ), and an unassigned species (*Fig. 7* and *Table 1*). The peaks at *m*/*z* 1621.0 and 3219.6 (*Entries 6* and *13*) may be assigned to a mono- or a dication; a dication appears more probable. Hence, the monocation peaks *m*/*z* 800.2–838.2 (*Entries 1–3*) are assigned to the monoplex, the dication peaks at *m*/*z* 1611.4–1621.9 (*Entries 4–6*) to a quadruplex, and both the trication peaks at *m*/*z* 2146.3–2165.6 (*Entries 9–12*) and the dication peaks at *m*/*z* 3219.6–3239.6 (*Entries 13–16*) to a duplex of quadruples. The peaks at *m*/*z* 1942 and 1950 (*Entries 7* and *8*) show a mass difference of 8, suggesting the presence of dications containing sodium and potassium, respectively. This would hint at an incomplete quintuplex, but we can not assign an explicit structure. To obtain a clearer view of the concentration dependence, we summarized the percentages for the individual associated species and gave the relative

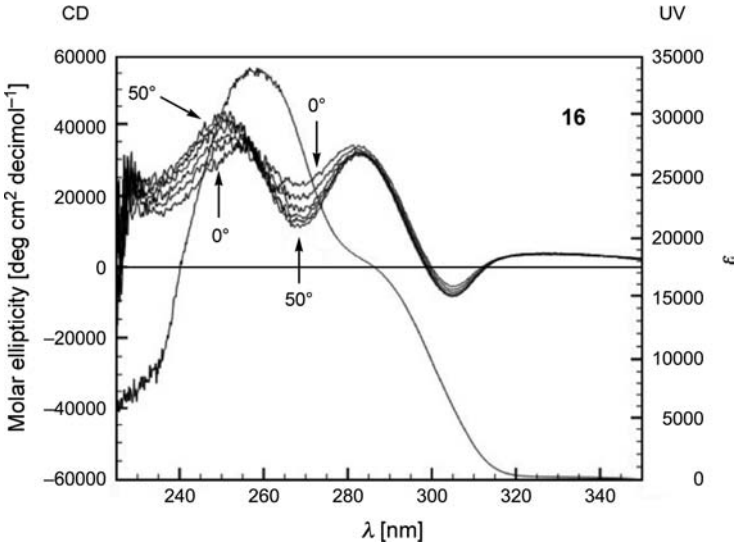


Fig. 6. *UV* and *temperature-dependent CD spectra in 10° steps from 0 to 50° of a 1 mM solution of* **16** *in* $CDCl_3$

intensities of these sums in parenthesis (*Table 1*). The duplex of quadruplexes is dominating at a 1 mM concentration (DQ/Q/M 92:2:6). Its intensity decreases with increasing dilution in favour of a quadruplex (0.1 mM: DQ/Q/M 58:26:14 and 0.05 mM: DQ/Q/M 33:41:14). Thus, these ESI mass spectra evidence a high stability of the quadruplex. Protonation leads to some clevage to the monoplex, and the presence of sodium or potassium cations favours the formation of a duplex of the quadruplexes.

Conclusions. – The propensity for the formation of G-quadruplexes is well evidenced for the silylated and isopropylidenated G*[s]G and G*[N]G dinucleosides **7** and **16** in $CHCl_3$ by VPO measurements, and ESI-MS and CD recordings. CD spectra suggest that the silylated and isopropylidenated G*[s]G* dinucleoside **9** in $CHCl_3$ and the fully deprotected G*[s]G(*) dinucleosides **10** and **11** do not form appreciable amounts of quadruplexes.

We thank Dr. *W. B. Schweizer* for the X-ray analysis, and the ETH Zürich and *Syngenta AG*, Basel, for generous support.

Experimental Part

General. Solvents were freshly distilled: THF from Na/benzophenone, CH_2Cl_2, MeOH, DMF, pyridine, $^iPr_2NH$, and EtN^iPr_2 from CaH_2. Reactions were run under N_2. Vapour Pressure Osmometry (VPO): *Corona 117* apparatus at the indicated concentration. Qual. TLC: Precoated silica-gel plates silica gel *60 F254* (SiO_2; *Merck*); detection by spraying with 'Mostain' and heating. Flash chromatography (FC): silica gel *Merck 60* (0.04–0.063 mm). Optical rotations: 1-dm cell at 25° and 589 nm. The temp. dependent CD (10° steps from 0° to 50°) and UV (20°) spectra: 1 mM soln. in $CDCl_3$ in a 1-mm *Suprasil* cell. FT-IR: ATR or 1% soln. in the indicated solvent; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR

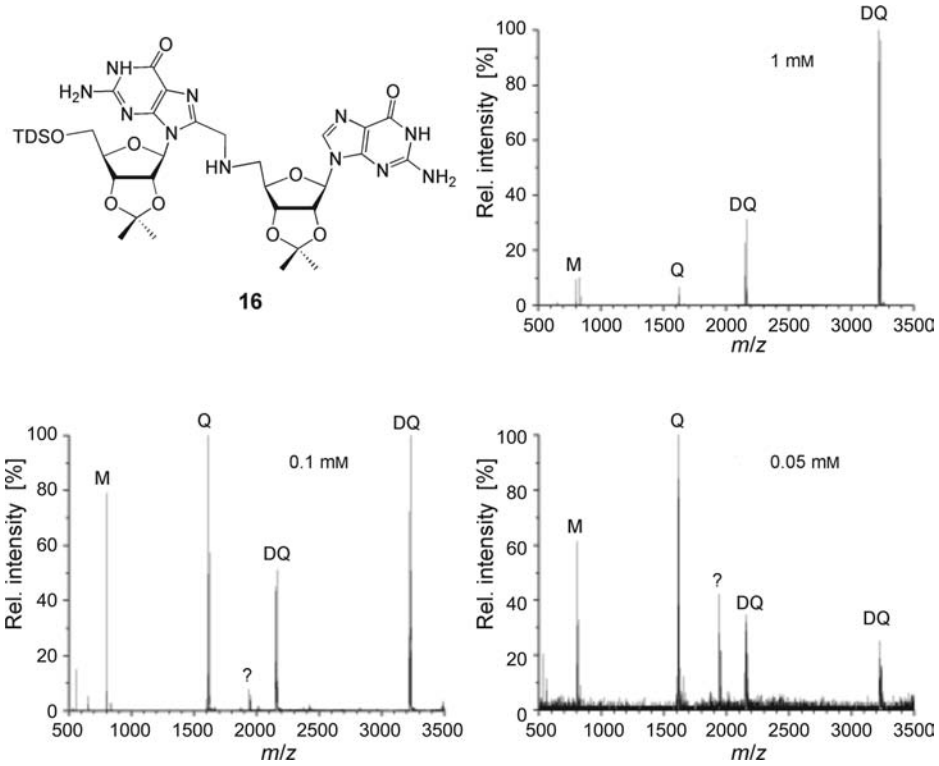


Fig. 7. *ESI Mass spectra of* **16** *for 1 mM, 0.1 mM, and 0.05 mM concentrations in* $CHCl_3$. DQ, peaks from a duplex of quadruplexes, *Q*, peaks from a quadruplex, *M*, peaks from the monoplex, *?*, unindentified peaks.

spectra: at 300 or 500 MHz, and 75 or 125 MHz, resp.; δ in ppm relative to the solved peaks ($CHCl_3$ = 7.28 and 77.0 ppm), *J* in Hz. MS: matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) with 0.05M indol-3-acrylic acid (IAA) in THF or with 0.05 α-cyano-4-hydroxycinnamic acid (CCA) in MeCN/EtOH/H_2O, and high-resolution (HR)-MALDI-MS with 0.05M 2,5-dihydrobenzoic acid (DHB) in THF or with 3-hydroxypicolinic acid (3-HPA) in THF.

*5′-*S*-Acetyl-*N^2*-isobutyryl-2′,3′-*O*-isopropylidene-5′-thioguanosine* (**2**). *2′,3′-*O*-Isopropylideneguanosine* (**1** [26]; 867 mg, 2.86 mmol) was transformed into *2′,3′-O*-isopropylidene-*5′-O*-tosylguanosine (655 mg, 51%) according to [27]. A soln. of this *p*-toluenesulfonate (3.268 g, 6.8 mmol) in DMF (15 ml) was treated with AcSK (7.7 g, 68 mmol), heated for 2 h to 60°, cooled to 24°, diluted with $CHCl_3$ (500 ml), and washed with H_2O (4 × 300 ml). After evaporation, a cold (0°) suspension of the orange residue in pyridine (70 ml) was treated dropwise with isobutyryl chloride (1.40 ml, 13.4 mmol), warmed to 24°, and washed with a 5% aq. NaH_2PO_4 soln. After evaporation, the residue was partitioned between AcOEt and H_2O. The org. layer was washed with H_2O and brine, dried ($MgSO_4$), filtered, and evaporated. FC (CH_2Cl_2/MeOH 1:33) gave **2** (2.8 g, 91%). White solid. R_f (AcOEt/cyclohexane 7:1) 0.84. $[\alpha]_D^{23} = +139.5$ (c = 1.0, $CHCl_3$). UV ($CHCl_3$): 285 (9580), 261 (12790), 255 (13230). IR ($CHCl_3$): 3310*w*, 3214*w*, 3019*m*, 2939*w*, 1698*s*, 1681*s*, 1607*s*, 1563*s*, 1537*w*, 1476*w*, 1419*m*, 1385*w*, 1375*w*, 1254*m*, 1188*w*, 1155*m*, 1095*m*, 1075*m*, 1021*m*, 948*w*, 877*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 2*; additionally, 12.13 (br. *s*, exchanged with D_2O, H–N(1)); 9.92 (br. *s*, exchanged with D_2O, HN–C(2)); 2.72 (*sept.*, *J* = 6.9, Me_2CH); 2.50 (*s*, AcS); 1.59, 1.30 (2*s*, Me_2CO_2); 1.31, 1.24 (2*d*, *J* = 6.9, Me_2CH). ^{13}C-NMR (75 MHz, $CDCl_3$): see *Table 3*; additionally, 197.75 (*s*, SC=O); 179.35 (*s*, NC=O); 114.08 (*s*, Me_2CO_2); 36.12 (*d*,

Table 1. *Relative Intensity* [%] *of the ESI-MS Peaks for Solutions of the G*[N]G Dinucleoside* **16** *in* $CHCl_3$

Entry	Spezies	Calculated *m/z*	Concentration [mM]		
			1 mM	0.1 mM	0.05 mM
1	$[\mathbf{16}+H]^+$	800.2	9	79	62
2	$[\mathbf{16}+Na]^+$	822.2	10	3	5
3	$[\mathbf{16}+K]^+$	838.2	3	3	9
Σ 1–3			22 (6%)	85 (14%)	76 (14%)
4	$[(\mathbf{16})_4+H+Na]^{2+}$	1611.4	1	100	100
5	$[(\mathbf{16})_4+H+K]^{2+}$	1619.4	1	57	84
6	$[(\mathbf{16})_4+2\ Na]^{2+}$ (or $[(\mathbf{16})_2+Na]^+$)	1621.9	7	9	30
Σ 4–6			9 (2%)	166 (26%)	214 (41%)
7	$?=[(\mathbf{16})_5-135+Na]^{2+}$	1942	0	8	42
8	$?=[(\mathbf{16})_5-135+K]^{2+}$	1950	0	6	22
Σ 7–8			0 (0%)	14 (2%)	66 (12%)
9	$[(\mathbf{16})_8+H+2\ Na]^{3+}$	2146.3	0	18	14
10	$[(\mathbf{16})_8+3\ Na]^{3+}$	2153.6	10	45	32
11	$[(\mathbf{16})_8+2\ Na+K]^{3+}$	2160.1	31	51	35
12	$[(\mathbf{16})_8+Na+2\ K]^{3+}$	2165.6	6	11	20
13	$[(\mathbf{16})_8+2\ Na]^{2+}$ (or $[(\mathbf{16})_4+Na]^+$)	3219.6	100	73	12
14	$[(\mathbf{16})_8+Na+K]^{2+}$	3227.3	96	36	19
15	$[(\mathbf{16})_8+3\ Na-H]^{2+}$	3230.3	55	100	25
16	$[(\mathbf{16})_8+2\ Na+K-H]^{2+}$	3239.6	39	30	16
Σ 9–16			337 (92%)	364 (58%)	173 (33%)

Me_2CH); 30.32 (*q*, *Me*C=O); 26.91, 25.19 (2*q*, Me_2CO_2); 18.91, 18.74 (2*q*, Me_2CH). HR-MALDI-MS (3-HPA): 474.1426 (38, $[M+Na]^+$, $C_{19}H_{25}N_5NaO_6S^+$; calc. 474.1418), 452.1599 (100, $[M+H]^+$, $C_{19}H_{26}N_5O_6S^+$; calc. 452.1598), 264.1085 (50), 222.0978 (42, $C_9H_{12}N_5O_2^+$; calc. 222.0986). Anal. calc. for $C_{19}H_{25}N_5O_6S$ (451.50): C 50.54, H 5.58, N 15.51; found: C 50.58, H 5.63, N 15.32.

*8-(Chloromethyl)-5′-*O*-[dimethyl(1,1,2-trimethylpropyl)silyl]-*N^2*-isobutyryl-2′,3′-*O*-isopropylideneguanosine* (**4**). A soln. of **3** [13] (1 g, 1.77 mmol) and freshly distilled 2,4,6-trimethylpyridine (286 µl, 3.54 mmol) in CH_2Cl_2 (5.9 ml) was treated dropwise with freshly distilled MsCl (288 µl, 3.7 mmol), stirred for 2 h at 24°, diluted with Et_2O (100 ml), and washed with 5% aq. KH_2PO_4 soln. The aq. layer was extracted with Et_2O. The combined org. layers were dried ($MgSO_4$), filtered, and evaporated. A soln. of the residue in 1,4-dioxane was evaporated in high vacuum to afford **4** (890 mg, 86%) that was directly used for the next step. White powder. ^{1}H-NMR (300 MHz, $CDCl_3$): see *Table 2*; additionally, 12.03 (br. *s*, exchanged with D_2O, H–N(1)); 8.08 (*s*, exchanged with D_2O, HN–C(2)); 2.61 (*sept.*, $J=6.9$, Me_2CHC=O); 1.61, 1.38 (2*s*, Me_2CO_2); 1.60 (*sept.*, $J\approx6.9$, $Me_2CHC(Me_2)$Si); 1.29 (*d*, $J=6.9$, Me_2CHC=O); 0.86 (*d*, $J=6.9$, $Me_2CHC(Me_2)$Si); 0.83 (*s*, Me_2CSi); 0.09, 0.07 (2*s*, Me_2Si).

*5′-*O*-[Dimethyl(1,1,2-trimethylpropyl)silyl]-*N^2*-isobutyryl-2′,3′-*O*-isopropylideneguanosine-8-methyl-(8^1 → 5′-*S*)-*N^2*-isobutyryl-2,3-*O*-isopropylidene-5′-thioguanosine* (**6**). A mixture of **2** (130 mg, 0.29 mmol) and K_2CO_3 (120 mg, 0.87 mmol) in MeOH (1.5 ml) was stirred at 23° for 15 min, diluted with AcOEt (30 ml), washed with sat. aq. NH_4Cl soln. (2 × 15 ml), dried ($MgSO_4$), filtered, and evaporated. A soln. of the residue in DMF (0.4 ml) was treated with K_2CO_3 (156 mg, 0.27 mmol), stirred for 5 min at 24°, and treated with KCl (100 mg, 1.36 mmol) and then with crude **4** (156 mg, *ca.* 0.26 mmol) in 7 portions over 1.5 h. The mixture was stirred for 9 h (→ yellow soln.), diluted with AcOEt (25 ml), washed with 5% aq. NaH_2PO_4 (3 × 15 ml), dried ($MgSO_4$), and evaporated. FC ($MeOH/CH_2Cl_2$ 1:49 →

Table 2. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Monomers* **2** *and* **4**, *and the Dimers* **6**–**11**.

Compound	**2**	**4**	**6**	**8**	**7**	**9**	**10**[a])	**11**
Solvent	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	(D_6)DMSO	(D_6)DMSO	(D_6)DMSO	(D_6)DMSO
Unit I								
H–C(8)	7.67	–	7.56	–	7.85	–	7.90 (7.91)	–
CH_a–C(8)	–	4.93	–	4.82	–	4.52[b])	–	4.53
CH_b–C(8)	–	4.76	–	4.78	–	4.45[b])	–	4.46
H–C(1′)	5.98	6.14	5.77	6.27	5.92	6.10	5.686 (5.678)	5.83
H–C(2′)	5.15	5.43	5.00	5.01	5.21	5.26	4.60	5.01
H–C(3′)	4.91	5.00	3.69	3.700	4.77	4.88	4.06 (4.047)	4.19
H–C(4′)	4.28	4.13	4.08	4.06–4.00	4.13	4.10–3.99	4.00 (3.96)	3.96
H_a–C(5′)	4.14	3.81	3.15	3.12	2.88	2.90	2.99 (2.96)	3.09
H_b–C(5′)	2.77	3.77	2.968	2.972	2.74	2.73	2.838 (2.831)	2.89
$J(H_a,H_b)$	–	12.4	–	13.2	–	13.4	–	13.1
J(1′,2′)	1.6	3.0	1.0	1.3	2.4	1.8	6.2 (6.2)	5.9
J(2′,3′)	6.5	6.7	6.1	6.4	6.3	6.3	5.6 (5.6)	5.6
J(3′,4′)	3.3	4.5	2.8	3.7	3.3	3.6	3.3 (3.3)	3.4
J(4′,5′a)	11.8	4.6	11.5	11.6	8.5	8.7	6.3 (6.3)	6.8
J(4′,5′b)	3.6	4.6	3.4	3.5	5.5	5.5	6.6 (6.6)	6.8
J(5′a,5′b)	13.3	11.3	13.9	14.1	13.8	13.8	13.8 (13.8)	13.7
Unit II								
CH_a–C(8)			4.02	4.02	3.92	3.93	3.99	4.01
CH_b–C(8)			3.94	3.93	3.89	3.83	3.87	3.87
H–C(1′)			6.35	6.36	6.11	6.10	5.76	5.76
H–C(2′)			5.62	5.65	5.48	5.47	4.72	4.72
H–C(3′)			4.99	5.06	5.13	5.13	4.12	4.12
H–C(4′)			4.19	4.26–4.20	4.04	4.10–3.99	3.88	3.87
H_a–C(5′)			3.68	3.705	3.68	3.68	3.66	3.66
H_b–C(5′)			3.62	3.64	3.64	3.62	3.55	3.55
$J(H_a,H_b)$			15.2	15.2	14.5	14.3	14.4	14.5
J(1′,2′)			0.9	1.3	1.2	1.3	6.6	6.4
J(2′,3′)			6.0	6.0	6.2	6.3	5.5	5.7
J(3′,4′)			4.3	3.9	4.0	3.9	3.1	3.5
J(4′,5′a)			4.3	5.0	7.0	6.9	3.7	3.9
J(4′,5′b)			7.2	6.7	5.1	5.0	4.2	4.2
J(5′a,5′b)			11.3	11.1	11.2	11.2	11.9	12.0

[a]) 3 : 2 mixture of **10** and an unassigned side product (values in parenthesis). [b]) *H*OCH_2–C(8/I) at 5.59 ppm, $J(CH_a,OH) = 6.0$ Hz, $J(CH_b,OH) = 5.2$ Hz.

1 : 19) gave **6** (222 mg, 89%). Colourless foam. R_f (MeOH/CH_2Cl_2/NH_4OH 9 : 1 : 0.1) 0.60. M.p. 290–293°. UV ($CHCl_3$): 287 (23830), 264 (27910). IR ($CHCl_3$, 10.5μ): 3419*w*, 3170*w*, 3018*m*, 2977*m*, 2936*w*, 2870*w*, 1691*s*, 1607*s*, 1561*s*, 1469*w*, 1419*w*, 1375*w*, 1316*w*, 1253*m*, 1191*w*, 1157*m*, 1080*m*, 948*w*, 875*w*, 830*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 2*; additionally, 12.63 (*s*, exchanged with D_2O, H–N(1/I)); 12.38 (*s*, exchanged with D_2O, H–N(1/II)); 12.27 (*s*, exchanged with D_2O, HN–C(2/I)); 10.25 (*s*, exchanged with D_2O, HN–C(2/II)); 2.973 (*sept.*, $J = 6.8$, Me_2CHC=O/I); 2.77 (*sept.*, $J = 6.8$, Me_2CHC=O/II); 1.54 (*sept.*, $J = 6.8$, Me_2C*H*C(Me_2)Si); 1.54, 1.44, 1.210, 0.94 (4*s*, 2 Me_2CO_2); 1.25, 1.207 (2*d*, $J = 6.8$, *Me*$_2$CHC=O); 1.240, 1.236 (2*d*, $J = 6.9$, *Me*$_2$CHC=O); 0.81 (*d*, $J = 6.9$, *Me*$_2$CHC(Me_2)Si); 0.77, 0.76 (2*s*, *Me*$_2$CSi); −0.01, −0.03 (2*s*, Me_2Si). ^{13}C-NMR

Table 3. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of the Monomer* **2**, *and the Dimers* **6**–**8**, **10**, *and* **11**.

Compound Solvent	**2** $CDCl_3$	**6** $CDCl_3$	**8** $CDCl_3$	**7** (D_6)DMSO	**10**[a]) (D_6)DMSO	**11** (D_6)DMSO
Unit I						
C(2)	147.98	149.00	148.80	153.66[b])	153.62[b])	153.25[b])
C(4)	147.19	147.64[b])	148.54[b])	150.39	151.32	153.02
C(5)	122.12	122.09	120.23	116.88	116.82	115.70[c])
C(6)	155.43	156.01	155.92	156.65	156.68	156.65[d])
C(8)	138.44	138.84	147.82	136.11	135.79 (135.82)	147.40
CH_2–C(8)	–	–	58.76	–	–	56.53
C(1′)	91.14	91.11	89.86	88.42	86.55 (br.)	88.39
C(2′)	85.17	84.60	84.74	83.42	72.53 (72.44)	71.00
C(3′)	83.10	82.93	83.09	82.98	72.41 (br.)	72.49
C(4′)	87.70	88.86	88.53	85.17	83.27 (83.32)	83.36
C(5′)	30.82	34.50	34.77	32.82	33.51 (33.54)	33.76
Unit II						
C(2)		149.45	149.23	155.34[b])	153.16[b])	153.14[b])
C(4)		148.30[b])	148.57[b])	151.39	151.75	151.79
C(5)		119.04	119.28	115.33	115.79	115.56[c])
C(6)		154.67	154.52	156.34	156.27	156.41[d])
C(8)		148.15[b])	148.47	143.86	144.68	145.03
CH_2–C(8)		30.40	30.58	27.26	27.82	28.03
C(1′)		89.62	89.79	88.22	88.05	88.10
C(2′)		83.36	83.32	83.31	71.82	71.87
C(3′)		81.21	81.47	81.13	70.28	70.30
C(4′)		88.72	88.33	88.04	85.66	85.69
C(5′)		63.62	63.41	63.40	61.97	61.76

[a]) 3 : 2 mixture of **10** and an unassigned side product (values in parenthesis). [b])[c])[d]) Assignments may be interchanged.

(125 MHz, $CDCl_3$; assignments based on DEPT, HSQC, and HMBC spectra): see *Table 3*; additionally, 180.92 (*s*, Me_2CHC=*O*/I); 179.92 (*s*, Me_2CHC=*O*/II); 113.59, 113.54 (2*s*, 2 Me_2CO_2); 36.12, 35.76 (2*d*, 2 Me_2CHC=O); 34.01 (*d*, $Me_2CHC(Me_2)Si$); 27.29, 26.79, 25.31, 23.99 (4*q*, 2 Me_2CO_2); 25.27 (*s*, Me_2CSi); 20.23, 20.22 (2*q*, $Me_2CHC(Me_2)Si$); 19.83, 19.06 (2*q*, Me_2CHC=O); 19.00, 18.47 (2*q*, Me_2CHC=O); 18.53, 18.39 (2*q*, Me_2CSi); −3.41, −3.55 (2*q*, Me_2Si). Anal. calc. for $C_{43}H_{64}N_{10}O_{11}SSi$ (957.19): C 53.96, H 6.74, N 14.63; found: C 53.87, H 6.70, N 14.43.

Crystal Structure of **6**. Colourless crystals of **6** were obtained from $MeOH/CH_2Cl_2$. Crystal data were were collected on a *Bruker-Nonius Kappa-CCD* instrument with MoK_α radiation ($\lambda = 0.7107$ Å) at 173.2 K. The structure was determined by direct methods [41] and refined by full-matrix least-squares analysis [42] including an isotropic extinction correction. All heavy atoms were refined anisotropically (H-atoms isotropic, H-positions based on stereochemical considerations). $R_{gt} = 0.0553$, w$R_{gt} = 0.1592$ for 595 parameters and 9339 reflections with $I > 2\sigma(I)$ and $\tau < 26.02°$, resulting in $C_{43}H_{64}N_{10}O_{11}SSi$ (957.18): orthorhombic $P2_12_12_1$; $a = 10.24190(10)$, $b = 17.4273(2)$, $c = 26.8566(5)$ Å, $\beta = 107.263$ (1). $V = 4793.60(11)$ Å^3; $Z = 4$; $D_x = 1.326$ Mg/m^3.

*5′-*O*-[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′-*O*-isopropylideneguanosine-8-methyl-(8′ → 5′-*S*)-2,3-*O*-isopropylidene-5′-thioguanosine* (**7**). A soln. of **6** (70 mg, 73 μmol) in CH_2Cl_2 (1.5 ml) was treated with sat. NH_3 soln. in MeOH (4.5 ml) and stirred in a sealed tube for 96 h. Evaporation and FC (diol phase; $CHCl_3$/MeOH 24 : 1) gave **7** (30 mg, 50%). R_f (diol phase; $CHCl_3$/MeOH 24 : 1) 0.19. $[\alpha]_D^{23} = -125.3$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3485*w*, 3292*w*, 3225*m*, 3019*s*, 2959*m*, 2865*w*, 1686*s*, 1633*m*,

1601*m*, 1536*w*, 1489*w*, 1427*w*, 1375*m*, 1254*w*, 1156*w*, 1087*m*, 865*w*, 832*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO; assignments based on HSQC and HMBC spectra): see *Table 2*; additionally, 10.1–9.7 (br. *s*, exchanged with D_2O, H–N(1/I) and H–N(1/II)); 6.65, 6.58 (2 br. *s*, exchanged with D_2O, H_2N–C(2/I) and H_2N–C(2/II)); 1.51, 1.47, 1.32, 1.24 (4*s*, 2 Me_2CO_2); 1.49 (*sept.*, $J = 6.9$, Me_2CH); 0.78, 0.77 (2*d*, $J = 6.8$, Me_2CH); 0.73, 0.72 (2*s*, Me_2CSi); −0.087, −0.091 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, (D_6)DMSO; assignments based on DEPT, HSQC, and HMBC spectra): see *Table 3*; additionally, 113.08, 112.72 (2*s*, 2 Me_2CO_2); 33.53 (*d*, Me_2CH); 26.91, 26.78, 25.24, 24.87 (4*q*, 2 Me_2CO_2); 24.66 (*s*, Me_2CSi); 20.10, 20.03 (2*q*, Me_2CH); 18.22, 18.14 (2*q*, Me_2CSi); −3.71 (*q*, Me_2Si).

Guanosine-8-methyl-(8^I → 5′-S)-5′-thioguanosine (**10**). A soln. of **7** (8 mg, 9 μmol) in HCO_2H/H_2O 4:1 (1 ml) was stirred for 18 h and evaporated. A soln. of the residue in aq. NH_4OH/H_2O 1:6 (3 ml) was lyophilized. FC (NH_2 phase; 1,4-dioxane/H_2O 3:2) gave a 3:2 mixture (5 mg, 70%). R_f (NH_2 phase; 1,4-dioxane/H_2O 3:2) 0.40. UV (H_2O): 257 (21800). IR (ATR): 3321*m*, 3146*m*, 2932*m*, 1681*s*, 1628*s*, 1594*s*, 1535*m*, 1504*w*, 1486*w*, 1411*m*, 1360*m*, 1229*w*, 1167*w*, 1113*m*, 1078*m*, 1043*s*, 943*w*, 915*w*, 872*w*. ^{1}H-NMR (400 MHz, (D_6)DMSO; 3:2 mixture of **10** and an unassigned side product; assignment based on DQF-COSY and HMBC spectra): see *Table 2*; additionally, 6.55 (br. *s*, exchanged with D_2O, NH_2); 6.50 (br. *s*, exchanged with D_2O, NH_2); 6.37 (br. *s*, exchanged with D_2O, NH_2); 6.25 (br. *s*, exchanged with D_2O, NH_2); 5.9–4.8 (br. *s*, exchanged with D_2O, several OH). ^{13}C-NMR (125 MHz, (D_6)DMSO; 3:2 mixture of **10** and a unassigned side-product; assignments based on DEPT, HSQC, and HMBC spectra): see *Table 3*. HR-MALDI-MS (3-HPA): 619.1128 (10), 618.1530 (8), 617.1490 (37, $[M + Na]^+$, $C_{21}H_{26}N_{10}NaO_9S^+$; calc. 617.1497), 597.1310 (19), 596.1709 (14), 595.1677 (54, $[M + H]^+$, $C_{21}H_{27}N_{10}O_9S^+$; calc. 595.1678), 479.1201 (19), 464.1287 (19), 463.1256 (100, $[M - C_5H_9O_4 + 2\,H]^+$, $C_{16}H_{19}N_{10}O_5S^+$; calc. 463.1256), 235.0713 (32).

5′-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-N^2-isobutyryl-2′,3′-O-isopropylideneguanosine-8-methyl-(8^I → 5′-S)-N^2-isobutyryl-8-(hydroxymethyl)-2,3-O-isopropylidene-5′-thioguanosine (**8**). A soln. of **5** [13] (50 mg, 0.07 mmol) in CH_2Cl_2 (0.8 ml) was treated with TFA (12 μl, 0.15 mmol) and Me_3SiH (23 μl, 0.15 mmol), stirred at 23° for 15 min, diluted with AcOEt (30 ml), washed with KOH/KH_2PO_4 buffer (pH 7; 2 × 15 ml), dried ($MgSO_4$), filtered, and evaporated. A soln. of the residue in DMF (0.1 ml) was treated with K_2CO_3 (19 mg, 0.14 mmol), stirred for 5 min at 24°, treated with KCl (100 mg, 1.35 mmol) and then with **4** (41 mg, 0.07 mmol) in six portions over 0.5 h. The mixture was stirred for 4.5 h (→ yellow soln.), diluted with AcOEt (25 ml), washed with aq. KOH/KH_2PO_4 buffer (pH 7; 3 × 15 ml), dried ($MgSO_4$), filtered, and evaporated to afford crude **8** (*ca.* 85% pure, 57 mg, 70%). UV ($CHCl_3$): 288 (23360), 263 (27050). ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a HSQC and a HMBC spectrum): see *Table 2*; additionally, 12.54 (*s*, H–N(1/I)); 12.23 (br. *s*, H–N(1/II)); 12.03 (*s*, HN–C(2/I)); 9.29 (*s*, HN–C(2/II)); 2.976 (*sept.*, $J = 6.9$, Me_2CHC=O/I); 2.72 (*sept.*, $J = 6.8$, Me_2CHC=O/II); 1.9–1.75 (br. *s*, OH); 1.59, 1.32 (2*s*, Me_2CO_2); 1.55 (*sept.*, $J = 6.8$, $Me_2CCH(Me)_2Si$); 1.41, 0.91 (2*s*, Me_2CO_2); 1.260, 1.243 (2*d*, $J = 6.6$, Me_2CHC=O); 1.238, 1.230 (2*d*, $J = 6.8$, Me_2CHC=O); 0.81 (*d*, $J = 6.8$, $Me_2CHC(Me_2)$-Si); 0.770, 0.766 (2*s*, Me_2CSi); −0.012, −0.016 (2*s*, Me_2Si). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on DEPT, HSQC, and HMBC spectra): see *Table 3*; additionally, 180.68 (*s*, Me_2CHC=O/I); 179.16 (*s*, Me_2CHC=O/II); 113.70, 113.11 (2*s*, 2 Me_2CO_2); 36.41 (*d*, Me_2CHC=O/II); 35.71 (*d*, Me_2CHC=O/I); 34.05 (*d*, $Me_2CHC(Me_2)Si$); 27.39, 25.62 (2*q*, Me_2CO_2); 26.86, 24.22 (2*q*, Me_2CO_2); 25.27 (*s*, Me_2CSi); 20.25, 20.22 (2*q*, $Me_2CHC(Me_2)Si$); 19.86, 18.51 (2*q*, Me_2CHC=O); 19.03 (*q*, Me_2CHC=O); 18.45, 18.41 (2*q*, Me_2CSi); −3.44, −3.52 (2*q*, Me_2Si). ESI-MS: 1009.5 (100, $[M + Na]^+$, $C_{44}H_{66}N_{10}NaO_{12}SSi^+$; calc. 1009.42), 987.5 (23, $[M + H]^+$, $C_{44}H_{67}N_{10}O_{12}SSi^+$; calc. 987.44), 588.0 (26), 413.3 (21), 304.2 (71).

5′-O-[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′-O-isopropylideneguanosine-8-methyl-(8^I → 5′-S)-2′,3′-O-isopropylidene-8-(hydroxymethyl)-5′-thioguanosine (**9**). A soln. of crude **8** (*ca.* 85% pure; 42 mg, 36 μmol) in CH_2Cl_2 (0.3 ml) was treated with sat. NH_3 soln. in MeOH (1.2 ml) and stirred in a sealed tube for 48 h. Evaporation and FC (diol phase; toluene/MeOH 7:1 → 4:1) gave **9** (23 mg, 77%). ^{1}H-NMR (300 MHz, (D_6)DMSO): see *Table 2*; additionally, 10.8–10.2 (br. *s*, H–N(1/I) and H–N(1/II)); 6.73, 6.60 (2 br. *s*, H_2N–C(2/I) and H_2N–C(2/II)); 1.51, 1.48, 1.35, 1.24 (4*s*, 2 Me_2CO_2); 1.49 (*sept.*, $J = 6.9$, Me_2CH); 0.78, 0.77 (*d*, $J = 6.8$, Me_2CH); 0.73 (*s*, Me_2SiC); −0.09 (*s*, Me_2Si). ESI-MS: 887.3 (50), 886.3 (76), 885.3 (100, $[M + K]^+$, $C_{36}H_{54}KN_{10}O_{10}SSi^+$; calc. 869.34), 870.3 (43), 869.2 (75, $[M + Na]^+$, $C_{36}H_{54}N_{10}NaO_{10}SSi^+$; calc. 869.34), 685.1 (26), 304.2 (37).

Guanosine-8-methyl-(8^{1} → 5′-S)-8-(hydroxymethyl)-5′-thioguanosine (**11**). A soln. of **9** (8 mg, 9 μmol) in HCO_2H/H_2O 4:1 (1 ml) was stirred for 18 h and evaporated. A soln. of the residue in NH_4OH/H_2O 1:6 (3.5 ml) was lyophilized. FC (NH_2 phase; $CHCl_3$/MeOH/NH_4OH 1:3:1) gave **11** (4 mg, 68%). R_f (NH_2 phase; $CHCl_3$/MeOH/NH_4OH 1:3:0.5) 0.23. $[\alpha]_D^{25} = -14.5$ ($c = 0.25$, DMSO). UV (H_2O): 260 (25100). IR (ATR): 3317*m*, 3210*m*, 3132*m*, 2936*w*, 1682*s*, 1633*s*, 1598*s*, 1506*w*, 1424*m*, 1364*m*, 1290*w*, 1228*w*, 1201*w*, 1115*w*, 1082*m*, 1037*m*, 945*w*, 915*w*. ^{1}H-NMR (400 MHz, (D_6)DMSO; assignments based on DQF-COSY and HMBC spectra): see *Table 2*; additionally, 6.6–6.2 (*m*, exchanged with D_2O). ^{13}C-NMR (100 MHz, (D_6)DMSO; assignment based on DEPT, HSQC, and HMBC spectra): see *Table 3*. HR-MALDI-MS (3-HPA): 663.1327 (32, $[M+K]^+$, $C_{22}H_{28}KN_{10}O_{10}S^+$; calc. 663.1342), 647.1602 (100, $[M+Na]^+$, $C_{22}H_{28}N_{10}NaO_{10}S^+$; calc. 647.1603), 625.1759 (20, $[M+H]^+$, $C_{22}H_{29}N_{10}O_{10}S^+$; calc. 625.1783), 493.1358 (63, $[M - C_5H_9O_4 + 2\,H]^+$, $C_{17}H_{21}N_{10}O_6S^+$; calc. 493.1361), 456.0348 (48), 398.0557 (26), 312.0759 (49), 282.0874 (28), 235.0557 (31).

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]*-N^2-*isobutyryl-2′,3′*-O-*isopropylideneguanosine-8-methyl-(8^{1} → 5′*-N)-*5′-amino-5′deoxy*-N^2-*isobutyryl-2′,3′*-O-*isopropylideneguanosine* (**15**). A soln. of **14** [1] (1.26 g, 3 mmol) in THF (10 ml) was treated with a 1M soln. of Me_3P in THF (3.3 ml), stirred for 2 h at 25°, treated with a soln. of **13** [13] (1.69 g, 3 mmol) in THF (10 ml), stirred for 4 d, and taken to dryness. A soln. of the residue in CH_2Cl_2 was washed with H_2O (3×) and brine, dried ($MgSO_4$), filtered, and evaporated to afford the crude imine (2.24 g, 80%). R_f (CH_2Cl_2/MeOH 95:5) 0.38. HR-MALDI-MS (3-HPA): 938.4567 (100, $[M+H]^+$, $C_{43}H_{63}N_{11}O_{11}Si^+$; calc. 938.4556).

A suspension of the crude imine (375 mg, 0.4 mmol) in ^{i}PrOH/MeOH 13:2 (15 ml) was added dropwise to a mixture of $NaCNBH_3$ (38 mg, 0.6 mmol) in ^{i}PrOH/AcOH 1:1 (5.0 ml). After 2 h, the soln. was poured into 1M aq. NaOH, and extracted with CH_2Cl_2. The org. layer was washed with sat. $NaHCO_3$ soln. (2 ×), H_2O, and brine, dried ($MgSO_4$), filtered, and evaporated. FC (CH_2Cl_2/AcOEt/MeOH 94:2:4) gave **15** (330 mg, 88%). White solid. R_f (CH_2Cl_2/MeOH 95:5) 0.33. M.p. 263° (dec.). $[\alpha]_D^{25} = -102.7$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 286 (24885), 257 (31500). IR (ATR): 3190*w*, 3018*w*, 2973*w*, 1682*m*, 1606*m*, 1559*m*, 1466*w*, 1419*w*, 1375*w*, 1250*m*, 1214*s*, 1193*m*, 1157*m*, 1073*m*, 1033*w*, 948*w*, 874*w*, 830*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): see *Table 4*; additionally, 12.80–12.60 (br. *s*, H–N(1/I)); 12.5–11.0 (br. *s*, only detectable by integration, HN–C(2/I)); 12.03 (br. *s*, H–N(1/II)); 10.24 (br. *s*, HN–C(2/II)); 3.15, 2.80 (2 *sept.*, $J = 6.8$, 2 Me_2CHC=O); 2.7–2.0 (br. *s*, H–N(5′/I)); 1.56 (*sept.*, $J = 6.8$, Me_2CHC(Me_2)Si); 1.53, 1.51, 1.20, 1.14 (4*s*, 2 Me_2CO_2); 1.268, 1.263, 1.235, 1.227 (4*d*, $J = 6.8$, 2 Me_2CHC=O); 0.85 (*d*, $J = 6.8$, Me_2CHC(Me_2)Si); 0.791, 0.784 (2*s*, Me_2CSi); 0.00, −0.01 (2*s*, Me_2Si). ^{1}H-NMR (600 MHz, $CDCl_3/CD_3OD$ 99:1; assignments based on DQF-COSY, HSQC, and ROESY spectra): see *Table 4*; additionally, 12.6–12.1 (br. *s*, 0.2 H, H–N(1/I and II); 11.6–11.3, 10.8–10.5 (2 br. *s*, 0.2 H, HN–C(2/I and II)); 2.87, 2.69 (2 *sept.*, $J = 6.8$, 2 Me_2CHC=O); 1.57, 1.56, 1.39, 1.26 (4*s*, 2 Me_2CO_2); 1.54 (*sept.*, $J = 6.8$, Me_2CHC(Me_2)Si); 1.26 (*d*, $J = 6.8$, 9 H), 1.22 (*d*, $J = 6.9$, 3 H) (2 Me_2CHC=O); 0.82 (*d*, $J = 6.8$, Me_2CHC(Me_2)Si); 0.778, 0.776 (2*s*, Me_2CSi); 0.04, 0.01 (2*s*, Me_2Si). ^{13}C-NMR (150 MHz, $CDCl_3/CD_3OD$ 99:1; assignments based on DQF-COSY, HSQC, and ROESY spectra): see *Table 5*; additionally, 180.46, 179.81 (2*s*, 2 NC=O); 114.43, 114.12 (2*s*, 2 Me_2CO_2); 36.10, 35.88 (2*d*, 2 Me_2CHC=O); 34.06 (*d*, Me_2CHC(Me_2)Si); 27.29, 27.15, 25.53, 25.10 (4*q*, 2 Me_2CO_2); 25.39 (*s*, Me_2CSi); 20.27, 20.21 (2*q*, Me_2CSi); 19.21, 19.05, 18.97, 18.88 (4*q*, 2 Me_2CHC=O); 18.45, 18.42 (2*q*, Me_2CHC(Me_2)Si); −3.30, −3.48 (2*q*, Me_2Si). HR-MALDI-MS (3-HPA): 980.4291 (16), 979.4284 (34), 978.4253 (57, $[M+K]^+$, $C_{43}H_{65}KN_{11}O_{11}Si^+$; calc. 978.4271), 964.4555 (19), 963.4544 (57), 962.4508 (100, $[M+Na]^+$, $C_{43}H_{65}N_{11}NaO_{11}Si^+$; calc. 962.4532), 941.4711 (13), 940.4742 (23, $[M+H]^+$, $C_{43}H_{66}N_{11}O_{11}Si^+$; calc. 940.4708). Anal. calc. for $C_{43}H_{65}N_{11}O_{11}Si$ (940.13): C 54.94, H 6.97, N 16.39; found: C 55.02, H 7.10, N 16.10.

5′-O-*[Dimethyl(1,1,2-trimethylpropyl)silyl]-2′,3′*-O-*isopropylideneguanosine-8-methyl-(8^{1} → 5′*-N)-*5′-amino-5′-deoxy-2′,3′*-O-*isopropylideneguanosine* (**16**). A soln. of **15** (112 mg, 0.12 mmol) and MeONa (67 mg, 1.2 mmol) in MeOH (2.5 ml) was kept for 14 h at 25° and taken to dryness. A soln. of the residue in CH_2Cl_2 was treated with pentane. The precipitate was filtered off, washed with pentane, and dried to give **16** (80 mg, 83%). An anal. sample was obtained by FC (CH_2Cl_2/AcOEt/MeOH 90:3:7). White solid. R_f (CH_2Cl_2/MeOH 9:1) 0.21. M.p. 209° (dec.). $[\alpha]_D^{25} = +107.3$ ($c = 0.5$, MeOH). UV ($CHCl_3$) 287 (sh, 10820), 257 (20870). IR (ATR): 3280*w*, 3140*w*, 2956*w*, 1677*s*, 1602*m*, 1532*w*, 1483*w*, 1372*m*, 1252*w*, 1212*m*, 1184*w*, 1156*w*, 1069*s*, 898*w*, 827*m*. ^{1}H-NMR (500 MHz, (D_6)DMSO; assignments based on DQF-

Table 4. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the G*[N]G Dinucleosides* **15** *and* **16**.

Compound	**15**		**15**[a])		**16**[a])		**16**[b])	
Solvent	$CDCl_3$		$CDCl_3/CD_3OD$ 99 : 1		$(D_6)DMSO$		$CDCl_3/(D_6)DMSO$ 9 : 1	
	Unit I	Unit II	Unit I	Unit II	Unit I	Unit II	Unit I	Unit II
H–C(8)	7.62	–	7.71	–	7.88	–	7.14 (7.27)	–
CH_a–C(8)	–	4.24	–	4.11	–	3.90 (br.)	–	3.91 (4.44)
CH_b–C(8)	–	3.81	–	3.94	–	3.79 (br.)	–	3.49 (3.63)
H–C(1′)	5.78	6.41	5.83	6.17	5.87	6.31	5.69 (5.71)	6.04 (6.22)
H–C(2′)	5.05	5.66	5.12	5.50	5.15	5.52	5.06 (5.96)	5.84 (6.00)
H–C(3′)	4.18	5.02	4.70	5.09	4.84	5.11	5.25 (5.50)	4.82 (4.89)
H–C(4′)	4.11	4.13	4.22	4.17	4.13 (br.)	4.04	4.16 (4.51)	4.09 (4.18)
H_a–C(5′)	3.02	3.66	3.01	3.68	2.85 (br.)	3.64	3.07 (3.34)	3.42 (3.25)
H_b–C(5′)	2.89	3.615	2.97	3.65	2.77 (br.)	3.61	2.96 (3.14)	3.36 (3.20)
$J(H_a,H_b)$	–	14.2	–	14.8	–	14.2	–	13.2 (14.8)
J(1′,2′)	2.0	1.2	2.4	2.0	3.2	1.1	< 1.5	2.4
J(2′,3′)	6.4	6.4	6.4	6.2	6.3	6.2	6.5	6.3
J(3′,4′)	4.0	3.6	3.7	3.6	3.1	3.6	5.5	2.3
J(4′,5′a)	7.2	5.2	6.0	6.2	5.4	7.0	< 1.5	6.5
J(4′,5′b)	4.0	6.8	5.5	5.6	5.1	5.6	< 1.5	6.5
J(5′a,5′b)	13.0	11.0	13.0	11.0	12.2	11.1	10.7	10.6

[a]) Assignments based on DQF-COSY, HSQC, and ROESY spectra. [b]) Data of the major species of a 85 : 15 mixture; signals for the ribosyl unit I and CH_2–C(8/II) are broad. In parentheses, data of the major species of a *ca.* 4 : 1 mixture in $CDCl_3$ at 298 K; very broad signals prevent the determination of coupling constants.

Table 5. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of the G*[N]G Dinucleosides* **15** *and* **16** (assignments based on DQF-COSY, HSQC, HMBC, and ROESY spectra).

Compound	**15**		**16**		**16**	
Solvent	$CDCl_3/CD_3OD$ 99 : 1		$(D_6)DMSO$		$CDCl_3$ (at 298 K)	
	Unit I	Unit II	Unit I	Unit II	Unit I	Unit II
C(2)	147.90	147.90	153.62	153.26	151.94[a])	153.68[a])
C(4)	149.02	149.45	150.53	151.27	152.45	153.14
C(5)	121.84	119.55	116.94	115.09	114.96	114.65
C(6)	155.41	155.87	156.30	156.54	160.13[b])	160.75[b])
C(8)	138.60	148.50	136.06	145.94 (br.)	138.10	150.38
CH_2–C(8)	–	46.71	–	45.83 (br.)	–	46.39
C(1′)	90.70	89.94	88.19	88.08	93.22	89.67
C(2′)	84.30	83.74	82.80	83.13	81.92	82.18
C(3′)	82.02	81.70	81.75	81.36	83.71	82.27
C(4′)	86.57	87.41	84.47	87.97	86.59	88.70
C(5′)	50.21	63.12	50.05	63.43	51.24	62.81

[a]) [b]) Assignments may be interchanged.

COSY, HSQC, and ROESY spectra): see *Table 4*; additionally, 10.77, 10.71 (2 br. *s*, 2 H–N(1)); 6.64, 6.60 (2 br. *s*, 2 NH_2); 2.7–2.3 (br. *s*, H–N(5′/I)); 1.50, 1.49, 1.30, 1.27 (4*s*, 2 Me_2CO_2); 1.49 (*sept.*, $J = 6.9$, Me_2CH); 0.773, 0.770 (2*d*, $J = 6.9$, Me_2CH); 0.72, 0.71 (2*s*, Me_2CSi); −0.09, −0.11 (2*s*, Me_2Si). ^{1}H-NMR

(300 MHz, $CDCl_3$/(D_6)DMSO 9:1, NH_2 exchanged with D_2O; 85:15 mixture of diastereoisomers): signals of the major diastereoisomer, see *Table 4*; additionally, 11.88, 11.85 (2*s*, 2 H–N(1)); 1.50, 1.44, 1.36, 1.19 (4*s*, 2 Me_2CO_2); 1.33 (*sept.*, $J = 6.8$, Me_2CH); 0.61 (*d*, $J = 6.8$, Me_2CH); 0.554, 0.547 (2*s*, Me_2CSi); −0.25, −0.27 (2*s*, Me_2Si); signals of the minor distereoisomer: 12.07, 12.02 (2*s*, 2 H–N(1)); 6.12 (*d*, $J =$ 2.5, H–C(1′/II)); 5.69 (br. *s*, H–C(1′/I)); other signals partly or completely overlapping with the corresponding signals of the major diastereoisomer. ^{1}H-NMR (600 MHz, $CDCl_3$, 298 K; assignments based on DQF-COSY, HSQC, and ROESY spectra): see *Table 4*; additionally, 14.00 (0.1 H), 11.72 (0.1 H), 11.62 (0.4 H), 11.58 (0.2 H), 11.02 (0.4 H), 10.34 (0.1 H), 9.29 (0.3 H), 7.94 (0.1 H), 7.65 (0.25 H), 6.46 (0.15 H), 6.10 (0.7 H) (several NH signals); 1.67, 1.60, 1.49, 1.42 (4*s*, 2 Me_2CO_2); 1.39 (*sept.*, $J = 6.6$, Me_2CH); 0.66 (*d*, $J = 6.8$, Me_2CH); 0.58 (*s*, Me_2CSi); −0.31 (*s*, Me_2Si). ^{13}C-NMR (126 MHz, DMSO; assignments based on DQF-COSY, HSQC, and ROESY spectra): see *Table 5*; additionally, 113.14, 112.53 (2*s*, 2 Me_2CO_2); 33.51 (*d*, Me_2CH); 26.98, 26.86, 25.20, 24.61 (4*q*, 2 Me_2CO_2); 25.06 (*s*, Me_2CSi); 20.07, 19.99 (2*q*, Me_2CSi); 18.20, 18.13 (2*q*, Me_2CH); −3.71, −3.73 (2*q*, Me_2Si). ^{13}C-NMR (150 MHz, $CDCl_3$, 298 K; assignments based on DQF-COSY, HSQC, and ROESY spectra): see *Table 5*; additionally, 113.43, 112.85 (2*s*, 2 Me_2CO_2); 34.00 (*d*, Me_2CH); 27.50, 26.82, 25.73, 25.01 (4*q*, 2 Me_2CO_2); 25.14 (*s*, Me_2CSi); 20.17, 20.11 (2*q*, Me_2CSi); 18.35, 18.30 (2*q*, Me_2CH); −3.81, −3.89 (2*q*, Me_2Si). HR-MALDI-MS (3-HPA): 823.3738 (13), 822.3698 (25, $[M + Na]^+$, $C_{35}H_{53}N_{11}NaO_9Si^+$; calc. 822.3690), 802.3931 (15), 801.3911 (46), 800.3884 (100, $[M + H]^+$, $C_{35}H_{54}N_{11}O_9Si^+$; calc. 800.3875), 650.3415 (14), 649.3370 (32, $[M$ – guaninyl$]^+$, $C_{30}H_{49}N_6O_8Si^+$; calc. 649.3376), 497.2597 (18), 496.2575 (61), 466.2467 (24).

REFERENCES

[1] M. Schulze-Adams, D. Touboul, B. Bernet, A. Vasella, *Helv. Chim. Acta* **2014**, *97*, 1037.
[2] M. Duechler, *J. Drug Targeting* **2012**, *20*, 389.
[3] T. M. Bryan, P. Baumann, *Mol. Biotechnol.* **2011**, *49*, 198.
[4] M. Franceschin, *Eur. J. Org. Chem.* **2009**, 2225.
[5] a) S. M. Haider, S. Neidle, G. N. Parkinson, *Biochimie* **2011**, *93*, 1239; b) S. Balasubramanian, S. Neidle, *Curr. Opin. Chem. Biol.* **2009**, *13*, 345; c) S. Neidle, S. Balasubramanian, Quadruplex Nucleic Acids, RSC Publishing, Cambridge, UK, 2006.
[6] a) S. Lena, S. Masiero, S. Pieraccini, G. P. Spada, *Chem. Eur. J.* **2009**, *15*, 7792; b) S. Lena, S. Masiero, S. Pieraccini, G. P. Spada, *Mini-Rev. Org. Chem.* **2008**, *5*, 262; c) J. T. Davis, G. P. Spada, *Chem. Soc. Rev.* **2007**, *36*, 296; d) J. T. Davis, *Angew. Chem., Int. Ed.* **2004**, *43*, 668; e) S. Pieraccini, T. Giorgi, G. Gottarelli, S. Masiero, G. P. Spada, *Mol. Cryst. Liq. Cryst.* **2003**, *398*, 57.
[7] I. Bang, *Biochem. Z.* **1910**, *26*, 293; M. Gellert, M. N. Lipsett, D. Davies, *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 2013.
[8] D. J. Patel, A. T. Phan, V. Kuryavi, *Nucleic Acids Res.* **2007**, *35*, 7429.
[9] S. Neidle, G. Parkinson, *Nat. Rev. Drug. Discovery* **2002**, *1*, 383.
[10] S. Müller, D. A. Sanders, M. Di Antonio, S. Matsis, J.-F. Riou, R. Rodriguez, S. Balasubramanian, *Org. Biomol. Chem.* **2012**, *10*, 6537.
[11] S. Loic, A. Guédin, S. Amrane, N. Smith, F. Denat, J.-L. Mergny, D. Monchaud, *Chem. Commun.* **2011**, *47*, 4992; D. Monchaud, M.-P. Teulade-Fichou, *Org. Biomol. Chem.* **2008**, *6*, 627.
[12] J. Gros, A. Avino, J. Lopez de la Osa, C. Gonzalez, L. Lacroix, A. Pérez, M. Orozco, R. Eritja, J.-L.-Mergny, *Chem. Commun.* **2008**, 2926.
[13] L. Herdeis, S. Thomas, B. Bernet, A. Vasella, *Helv. Chim. Acta* **2013**, *96*, 1235.
[14] L. Herdeis, B. Bernet, A. Augustine, R. E. Kälin, A. Brändli, A. Vasella, *Helv. Chim. Acta* **2011**, *94*, 545.
[15] K. Chiesa, B. Bernet, A. Vasella, *Helv. Chim. Acta* **2010**, *93*, 1822; K. Chiesa, A. Shvoryna, B. Bernet, A. Vasella, *Helv. Chim. Acta* **2010**, *93*, 668.
[16] B. Bernet, Z. Johar, A. Ritter, B. Jaun, A. Vasella, *Helv. Chim. Acta* **2009**, *92*, 2596.
[17] A. Ritter, D. Egli, B. Bernet, A. Vasella, *Helv. Chim. Acta* **2008**, *91*, 673.
[18] I. C. M. Kwan, R. J. Delley, D. Hodgson, G. Wu, *Chem. Commun.* **2011**, *47*, 3882.
[19] P. L. T. Tran, A. Virgilio, V. Esposito, G. Citarella, J.-L. Mergny, A. Galeone, *Biochimie* **2011**, *93*, 399.

[20] A. Joachimi, A. Benz, J. S. Hartig, *Bioorg. Med. Chem.* **2009**, *17*, 6811.
[21] H. Martadinata, A. T. Phan, *J. Am. Chem. Soc.* **2009**, *131*, 2570.
[22] Y. Xu, K. Kaminaga, M. Komiyama, *J. Am. Chem. Soc.* **2008**, *130*, 11179.
[23] C.-F. Tang, R. H. Shafer, *J. Am. Chem. Soc.* **2006**, *128*, 5966.
[24] J. Gros, F. Rosu, S. Amrane, A. De Cian, V. Gabelica, L. Lacroix, J.-L. Mergny, *Nucleic Acids Res.* **2007**, *35*, 3064.
[25] V. Gubala, J. E. Betancourt, J. M. Rivera, *Org. Lett.* **2004**, *6*, 4735.
[26] A. M. Michelson, A. R. Todd, *J. Chem. Soc.* **1949**, 2476.
[27] E. J. Reist, P. A. Hart, L. Goodman, B. R. Baker, *J. Org. Chem.* **1961**, *26*, 1557.
[28] D. Flockerzi, G. Silber, R. Charubala, W. Schlosser, R. S. Varma, F. Creegan, W. Pfleiderer, *Liebigs Ann. Chem.* **1981**, 1568.
[29] S. Lena, G. Brancolini, G. Gottarelli, P. Mariani, S. Masiero, A. Venturini, V. Palermo, O. Pandoli, S. Pieraccini, P. Samori, G. P. Spada, *Chem. Eur. J.* **2007**, *13*, 3757.
[30] M. L. Colgrave, H. E. L. Williams, M. S. Searle, *Chem. Commun.* **2001**, 315.
[31] P. Murat, B. Gennaro, J. Garcia, N. Spinelli, P. Dumy, E. Defrancq, *Chem. Eur. J.* **2011**, *17*, 5791.
[32] T. Giorgi, F. Grepioni, I. Manet, P. Mariani, S. Masiero, E. Mezzina, S. Pieraccini, L. Saturni, G. P. Spada, G. Gottarelli, *Chem. Eur. J.* **2002**, *8*, 2143.
[33] J. Šponer, A. Mládek, N. Špačková, X. Cang, T. E. Cheatham III, S. Grimme, *J. Am. Chem. Soc.* **2013**, *135*, 9785.
[34] N. V. Hud, P. Schultze, V. Sklenar, J. Feigon, *J. Mol. Biol.* **1999**, *285*, 233.
[35] S.-i. Kawahara, Y. Takagi, K. Taira, H. Kobayashi, *Nucleic Acids Symp. Ser.* **2004**, *48*, 133; M. Meyer, T. Steinke, M. Brandl, J. Sühnel, *J. Comput. Chem.* **2001**, *22*, 109; J. Gu, J. Leszczynski, M. Bansal, *Chem. Phys. Lett.* **1999**, *311*, 209.
[36] P. Podbevšek, P. Šket, J. Plavec, *Nucleosides, Nucleotides, Nucleic Acids* **2007**, *26*, 1547; S. Mezzache, S. Alves, J.-P. Paumard, C. Pepe, J.-C. Tabet, *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1075; T. Aggerholm, S. C. Nanita, K. J. Koch, R. G. Cooks, *J. Mass Spectrom.* **2003**, *38*, 87; A. Wong, G. Wu, *J. Am. Chem. Soc.* **2003**, *125*, 13895.
[37] J. D. Gu, J. Leszczynski, *J. Phys. Chem. A* **2000**, *104*, 6308.
[38] a) A. Randazzo, G. P. Spada, M. W. da Silva, *Top. Curr. Chem.* **2013**, *330*, 67; b) G. Gottarelli, G. P. Spada, in 'Circular Dichroism: Principles and Applications', Eds. N. Berova, K. Nakanishi, R. W. Woody, Wiley-VCH, New York, pp. 547–561; c) G. Gottarelli, S. Masiero, G. P. Spada, *Enantiomer* **1998**, *3*, 429.
[39] S. Masiero, R. Trotta, S. Pieraccini, S. De Tito, R. Perone, A. Randazzo, G. P. Spada, *Org. Biomol. Chem.* **2010**, *8*, 2683.
[40] D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inskeep, H. Eyring, *J. Am. Chem. Soc.* **1971**, *93*, 1600.
[41] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **1999**, *32*, 115.
[42] G. M. Sheldrick, SHELXL97, Program for Refinement of Crystal Structures, University of Göttingen, Göttingen, 1997.

Received May 19, 2014