A Practical Synthesis of 3′-Thioguanosine and of Its 3′-Phosphoramidothioite (a Thiophosphoramidite)

by **Jasenka Matulic-Adamic** and **Leonid Beigelman***

Department of Chemistry & Biochemistry, *Ribozyme Pharmaceuticals, Inc.*, 2950 Wilderness Place, Boulder, Colorado 80301, USA

Dedicated to Prof. Dr. *Frank Seela* on the occasion of the 60th birthday

Starting from guanosine, an efficient method for the synthesis of 3′-thioguanosine (see **13**) and of its 3′-phosphoramidothioite (see **23**), suitable for automated incorporation into oligonucleotides, was developed. Reaction of 5′-N^2-protected guanosine with 2-acetoxyisobutyryl bromide afforded stereoselectively the 2′-*O*-acetyl-3′-bromo-β-D-xylofuranosyl derivative **3**, which was converted to a 7:3 mixture of the *S*-acyl ribofuranosyl intermediates **5** or **6** and the 3′,4′-unsaturated by-product **4**. The *S*-acylated nucleosides **5** and **6** were then converted in three steps to 5′-*O*-(4,4′-dimethoxytrityl)-3′-*S*-(pyridin-2-ylthio)-3′-thioguanosine (**11**), which served as a common intermediate for the preparation of free 3′-thionucleoside **13** and 3′-thionucleoside 3′-phosphoramidothioite **23**.

Introduction. – Oligonucleotides containing 3′-*S*-phosphorothioate linkages have attracted increasing interest as probes for studying the interaction of nucleic acids and their processing enzymes. In particular, these analogs have been used as probes in the elucidation of the roles of metal ions in phosphoester transfer reactions catalyzed by RNA [1][2] and ribonucleoprotein enzymes [3].

As part of our studies of chemically modified hammerhead ribozymes, we recently demonstrated [4] that the previously developed '5-ribo' nuclease-stabilized hammerhead motif can be further refined by systematic incorporation of 1-(β-D-xylofuranosyl)adenine (xA) and 1-(β-D-xylofuranosyl)guanine (xG) in place of the conserved ribopurine residues of the catalytic core. Modified ribozymes substituted with xA at positions A15.1 and A6 demonstrated catalytic activity close to the activity of the parent stabilized ribozyme and an improved nuclease stability, effectively reducing the number of unstabilized residues from 5 to 3. Unfortunately, analogous guanosine substitutions at positions G5, G8, and G12 substantially lowered catalytic rates. Based on these results, we wanted to incorporate 3′-deoxy-3′-thioguanosine in place of these highly conserved [5][6] guanosine residues in the stabilized catalytic domain. Replacing the sugar 3′-O-atom by the larger, more electropositive S-atom should favor the 3′-*endo* sugar pucker, making these analogs very good mimics of RNA [7–9]. At the same time, this modification is expected to increase ribozyme resistance to nuclease degradation [2][10]. In addition, 3′-thio analogs, when incorporated into the ribozyme substrate cleavage site, can also serve for the study of the mechanism of cleavage in the presence of divalent metal ions [1][3].

The synthesis of 3′-*S*-phosphorothioate-linked deoxyribodinucleotides by solution chemistry [3][11–13] and solid-phase chemistry was reported [1][14].

The synthesis of ribonucleotide 3′-*S*-phosporothiolate analogs has been limited to the preparation of UspU [10] and IspU [2] dimers by solution chemistry. Recently, *Sun*

et al. [15] described the synthesis of U, C, G, and I 3′-*S*-phosphoramidothioites and their incorporation into RNA by standard phosphoramidite solid-phase synthesis. This work enabled direct incorporation of 3′-thioribonucleosides into oligonucleotides.

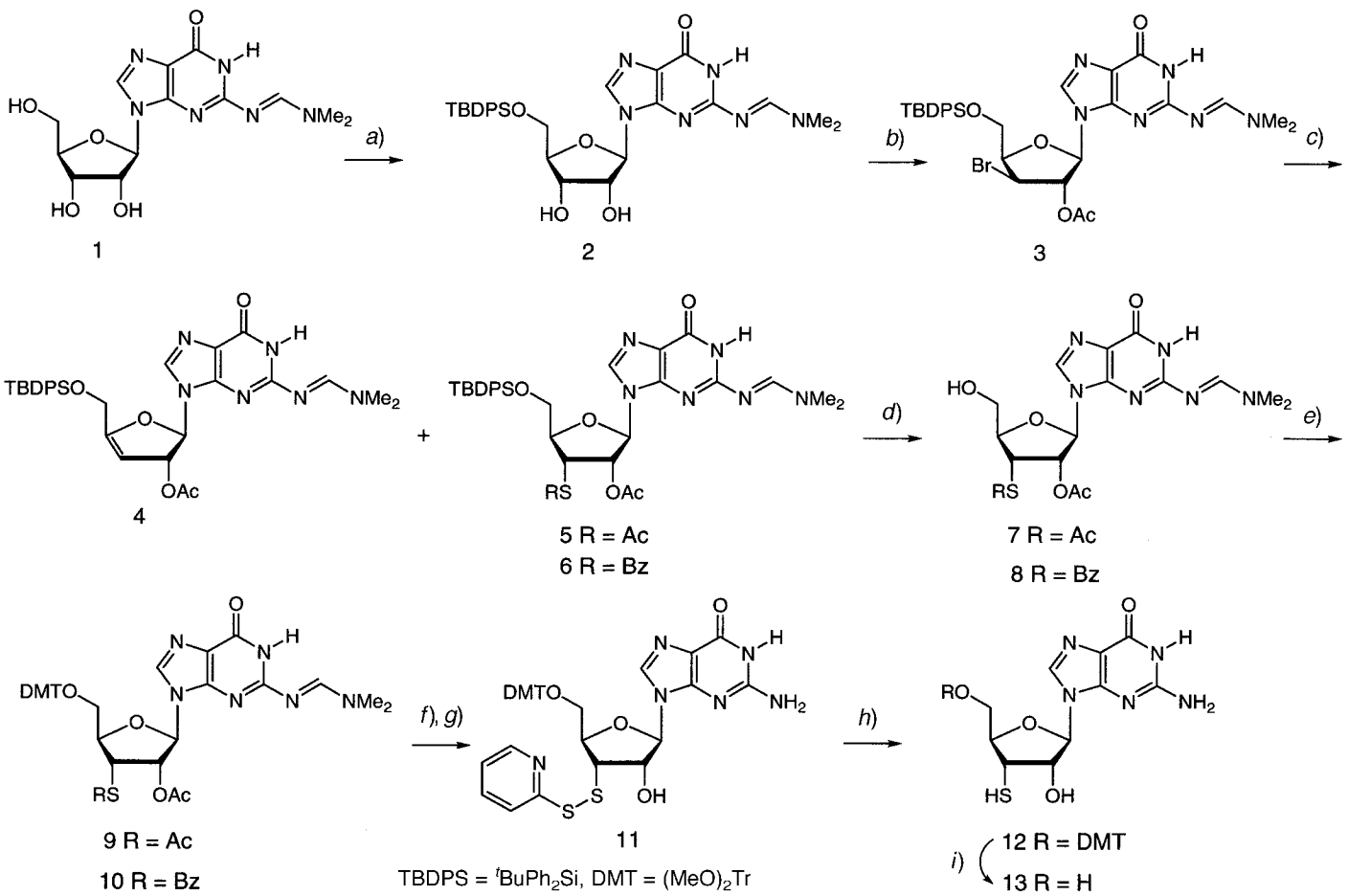
Two general approaches were investigated in the reported syntheses of 3′-thioribonucleosides. In the first approach, the target 3′-thioribonucleosides were prepared by condensation of an appropriately protected 3-thioribose derivative with the desired nucleoside base [16–18]. While glycosylation reactions using pyrimidines proceed in high yields and with N(1) regioselectivity [19], purine bases generally give more complex mixtures because both N(7) and N(9) of the purine base are reactive towards glycosylation [20]. *Sun et al.* [15] reported the first synthesis of 3′-thioguanosine derivatives by the above-described approach. Noticeably, peracylated 3-thioribose reacted with persilylated N^2-acetylguanine to provide the condensation product in *ca.* 40% yield. Subsequent synthetic steps proceeded with moderate yields resulting in a low overall yield of 3′-phosphoramidothioite.

The second general approach to the preparation of 3′-thioribonucleosides is based on the S_N2 displacement of an appropriate leaving group at C(3′) of the protected xylonucleoside. Synthesis of 3′-thioadenosine [21], 3′-thiouridine [10], and 3′-thioinosine [2][22] starting from preformed nucleosides has been reported. So far, this approach has not been applied to the synthesis of 3′-thioguanosine. Here we describe a novel and efficient synthesis of 3′-thioguanosine (**13**) and of its protected 3′-phosphoramidothioite **23** from guanosine as a starting material.

Results and Discussion. – We envisaged that (3′-bromo-3′-deoxy-β-D-xylofuranosyl)guanosine, which can be prepared in one step from guanosine and 2-acetoxyisobutyryl bromide (= 2-acetoxy-2-methylpropanoyl bromide; AcOibuBr; *Mattocks-Moffatt* reagent) [23], will provide an attractive starting material for the introduction of the 3′-thioribo functionality by nucleophilic displacement. Unfortunately, the reaction of guanosine with AcOibuBr results in a low yield of the mixture of bromo acetates of *xylo*- and *arabino*-configuration [24]. In general, reactions of base-unprotected purine nucleosides with this reagent result in the mixtures of *trans*-bromo acetates [23][24]. It was noted [25] that the reaction of N^2,5′-*O*-dibenzoylguanosine with AcOibuBr leads predominantly to the 2′-*O*-acetyl-3′-bromo-3′-deoxy-β-D-xylofuranosyl derivative. On the other side, it was recently reported [26] that the reaction of N^2-[(dimethylamino)methylene]guanosine (**1**), with AcOibuBr proceeded stereoselectively, yielding exclusively the 3′-bromo-3′-deoxy-β-D-xylofuranosyl derivative.

Encouraged by this last report [26], we decided to apply the same reaction conditions to the suitably 5′-protected N^2-[(dimethylamino)methylene]guanosine derivative **2** (*Scheme 1*). The 5′-protection in **2** should reduce the complexity of the product mixture by eliminating the possible formation of the mixture of 5′-OH, 5′-(2,5,5-trimethyl-4-oxo-1,3-dioxolanyl), and/or 5′-*O*-acylated derivatives in the reaction with AcOibuBr [24][26]. In this manner, identification of the reaction products becomes straightforward. We chose the 5′-*O*-(*tert*-butyl)diphenylsilyl (^{t}BuPh$_2$Si) protection because of its relatively high stability towards acidic conditions generated during the reaction with AcOibuBr in moist MeCN. This group is also expected to undergo selective cleavage in the presence of *S*-acyl groups. Thus, reaction of **1** [27] with ^{t}BuPh$_2$SiCl proceeded quantitatively to afford the 5′-*O*-silyl derivative **2**, which

Scheme 1



a) ^{t}BuPh$_2$SiCl, Py, r.t., 16 h. *b*) Me$_2$C(OAc)COBr, MeCN, H$_2$O, r.t., 3 h. *c*) KSAc or KSBz, DMF, 60°, 10 h. *d*) Bu$_4$NF·3 H$_2$O, AcOH, THF, r.t., 5 h. *e*) (MeO)$_2$TrCl, Py, r.t., 4 h. *f*) 40% aq. MeNH$_2$ soln., r.t., 16 h. *g*) 2,2′-Dithiobis[pyridine], DMF, 60°, 10 h. *h*) Dithiothreitol (DTT), CHCl$_3$, r.t., 3 h. *i*) 1N HCl in MeOH, DTT, r.t., 3 h.

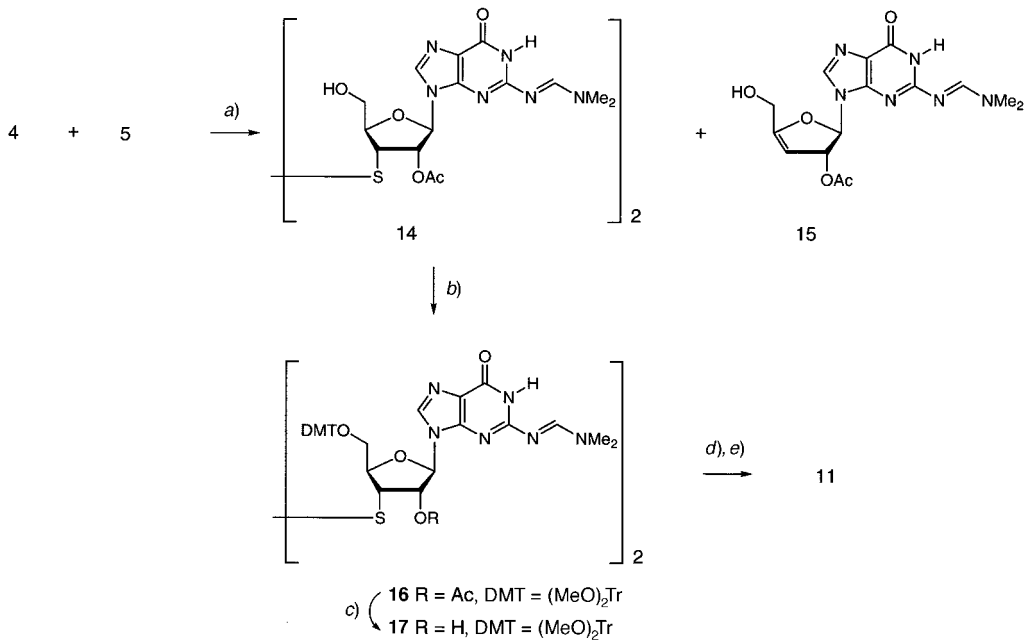
reacted smoothly with AcOibuBr to yield the desired 3′-bromo-3′-deoxy-β-D-xylofuranosyl derivative **3** in high yield. Only the *xylo*-isomer was obtained as judged by ^{1}H-NMR analysis. This result indicates that the nature of the guanosine protection at the NH_2 group directs the stereochemical outcome of this reaction. Reaction of **3** with potassium thioacetate or potassium thiobenzoate in DMF yielded the 3′-*S*-acetyl or 3′-*S*-benzoyl derivatives **5** and **6**, respectively, along with 3′,4′-unsaturated derivative **4**. The 3′,4′-unsaturated derivative **4** was easily identified by the characteristic absence of H–C(4′) and a down-field shift of H–C(3′) in the ^{1}H-NMR spectra. Compound **4** is formed by a competing elimination reaction analogous to the one observed with the carbohydrate analog [18]. The ratio was 7:3 in favor of the substitution products **5** or **6**, which could not be separated from the elimination product **4** at this stage. The mixture **4/5** or **4/6** was then treated with tetrabutylammonium fluoride (Bu_4NF) buffered with an excess of AcOH, and the product mixture was separated by chromatography to give the desired 5′-deprotected derivatives **7** and **8**, respectively, in good yield. The unsaturated derivative **4** was unstable under the acidic reaction conditions, and no attempt was made to isolate the products of its degradation. When triethylamine trihydrofluoride ($Et_3N \cdot 3\,HF$) was used for the deprotection, desilylation was incomplete.

According to our experience, it is highly desirable to keep guanosine derivatives protected with lipophylic groups during synthetic transformations because of the solubility problems and, therefore, low isolated yields when working with unprotected guanosines. Thus, **7** and **8** were 5′-reprotected in high yields with the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) group to give the fully protected derivatives **9** and **10**, respectively (*Scheme 1*). The $(MeO)_2Tr$ group provided a hydrophobic tag that simplified workup and purification of subsequent synthetic intermediates. Next, **9** and **10** were converted to 3′-(pyridin-2-yldithio) derivative **11** in 85 and 80% yield, respectively, in aqueous $MeNH_2$ solution for *S*-deacylation followed by the *in situ* SH protection by disulfide exchange with 2,2′-dithiobis[pyridine] in DMF. It was reported that the removal of a 2′-*O*-acyl protection in ribofuranosyl derivatives similar to **9** and **10** proceeds with difficulty [15][22]. We found that 40% aqueous $MeNH_2$ solution easily removed acyl and *N*-protecting groups from **9** and **10** and was the base of choice because, contrary to aqueous NH_3 solution, it completely solubilized the fully protected substrates. To synthesize the free nucleoside **13**, **11** was treated with dithiothreitol (DTT) in $CHCl_3$. When Et_3N was added to the reaction mixture, the reaction was faster than in its absence, but at the same time, **12** was converted to its Et_3NH^+ salt with the SH group. Final deprotection of the $(MeO)_2Tr$ group of **12** to give **13** was achieved with 1N HCl in MeOH in the presence of DTT which quenched the released $(MeO)_2Tr$ cation. In the absence of DTT, quantitative *S*-alkylation took place. To the best of our knowledge, this is the first report on the synthesis of 3′-thioguanosine (**13**).

It is worth noting that when unbuffered Bu_4NF in THF was used to desilylate the mixture **4/5**, the *S*-acetyl protecting group was also cleaved, leading to the formation of the disulfide **14** and of the 5′-desilylated 3′,4′-unsaturated derivative **15** (*Scheme 2*), which were separated by column chromatography (silica gel), **14** being, however, invariably contaminated with Bu_4NF. Attempted rechromatography of **15** led to its decomposition. Disulfide **14** was then 5′-$(MeO)_2$Tr-protected to afford **16**.

It appeared to us that, at this stage, the selective removal of the 2′-*O*-acetyl group of **16**, followed by the introduction of the 2′-*O*-[(*tert*-butyl)dimethylsilyl] (^{t}BuMe$_2$Si)

Scheme 2



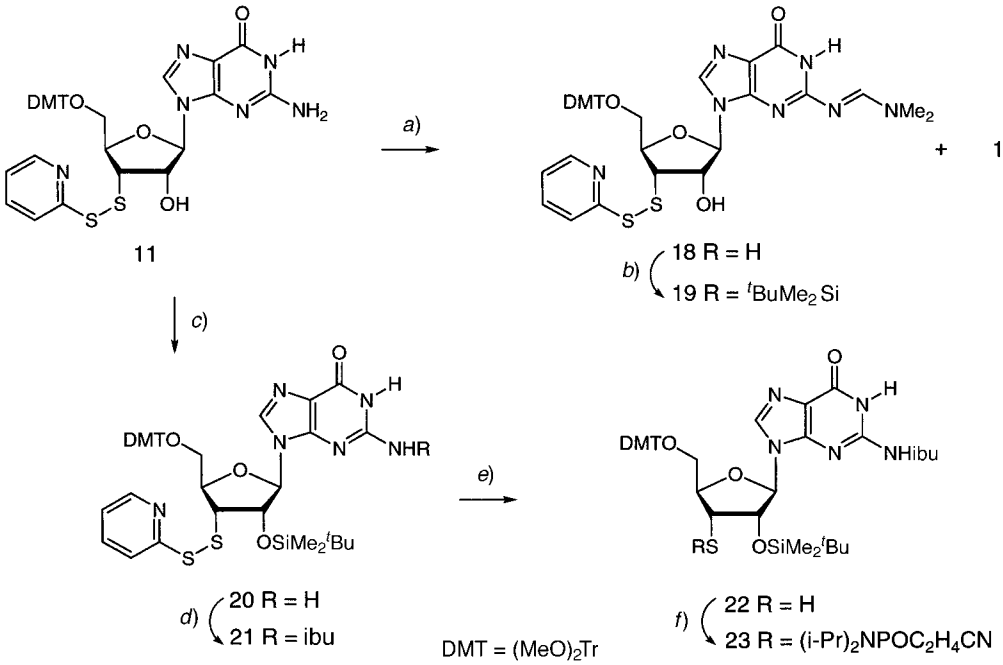
a) 1M Bu_4NF in THF, r.t., 3 h. *b*) $(MeO)_2TrCl$, Py, r.t., 4 h. *c*) Ion exchange with *AG1X8* (OH^-) or *Amberlyst A-26* (CN^-), MeOH, 55°, 16 h. *d*) 40% aq. $MeNH_2$ soln., r.t., 16 h. *e*) 2,2′-dithiobis[pyridine], DMF, 60°, 10 h.

protection, subsequent reduction of the 3′-disulfide and 3′-*S*-phosphitylation would be the shortest way to prepare the desired 3′-phosphoramidothioite building block. Reactions of **16** with mild deacylating agents like basic ion exchangers in OH^- or CN^- form [28] selectively removed the 2′-*O*-acetyl protection (→ **17**), but at the same time, the nucleoside was strongly adsorbed by the resin, resulting in low recoveries. We, therefore, abandoned this approach and instead applied the same strategy, *i.e.* base-catalyzed *S*-deacetylation followed by *S*-protection with the pyridylinylthio group, as used for the preparation of 3′-(pyridin-2-yldithio) derivative **11** from *S*-acylated derivatives **9** or **10**. In this manner, **11** was obtained from the disulfide **16** in 67% yield.

Our approach to the 3′-phosphoramidothioite synthesis is shown in *Scheme 3*. Reaction of **11** with dimethylformamide dimethyl acetal yielded the desired *N*-protected derivative **18** in 23% yield. Unfortunately, this reagent also effected the cleavage of the *S*-(pyridinylthio) protecting group, leading to formation of disulfide **17** in 33% yield. The 2′-OH function in the alcohol **18** was smoothly protected with the ^{t}BuMe$_2$Si group using (*tert*-butyl)dimethylsilyl trifluoromethanesulfonate (^{t}BuMe$_2$SiTf)(→ **19**). Alternatively, 5′-*O*-$(MeO)_2$Tr derivative **11** was first 2′-*O*-silylated with ^{t}BuMe$_2$SiCl[1]) to afford **20** and then *N*-protected using isobutyric anhydride (ibu_2O) in the presence of 4-(dimethylamino)pyridine (DMAP), yielding

[1]) The ^{t}BuMe$_2$SiTf reagent caused partial *N*-silylation of *N*-unprotected guanosine, making it unsuitable for the silylation of **11** (unpublished results).

Scheme 3



a) $Me_2NCH(OMe)_2$, Py, r.t., 16 h. *b*) ^{t}BuMe$_2$SiTf, Py, r.t. 5 h. *c*) ^{t}BuMe$_2$SiCl, Py, 1*H*-imidazole, r.t., 16 h. *d*) ibu_2O, Py, DMAP, r.t., 16 h, then 50°, 5 h. *e*) DTT, $CHCl_3$, Et_3N. *f*) $(^i Pr)_2NP(Cl)OC_2H_4CN$, $^i Pr_2EtN$, 1-methyl-1*H*-imidazole, r.t., 2 h.

the fully protected **21**. In the absence of DMAP, no reaction occurred. On the other hand, reaction of **20** with isobutyryl chloride led to *N*-bis-acylation. Reduction of **21** with DTT afforded the 3′-SH derivative **22**, which appeared as a mixture of two rotamers in the ^{1}H-NMR spectrum. Resonances of the major rotamer were in accordance with those reported by *Sun et al.* [15]. Phosphitylation of **22** under standard conditions afforded 3′-phosphoramidothioite **23**.

Conclusion. – An efficient synthesis of 3′-thioguanosine and its 3′-*S*-phosphoramidothioite from guanosine was devised. Transformation of the easily accessible key intermediate **3** to 3′-(pyridin-2-yldithio) synthon **11**, followed by protection of the 2-NH_2 and 2′-OH group, reduction, and phosphitylation provided the target 3′-*S*-phosphoramidothioite **23** in 13% overall yield from guanosine. Keeping all synthetic intermediates protected with lipophylic groups enabled their chromatographic purification and, consequently, a good recovery of the products. Incorporation of 3′-*S*-phosphoramidothioite **23** into ribozymes and subsequent mechanistic studies are in progress and will be published in due course.

Experimental Part

General. All reactions were carried out under a positive pressure of Ar in anh. solvents. Commercially available reagents and anh. solvents were used without further purification. Anal. TLC: *Merck* silica gel *60* F_{254}

plates (Art. 5554). Flash column chromatography (FC): *Merck* 0.040-0.063 mm silica gel *60*. ^{1}H- and ^{31}P-NMR Spectra: at 400.075 and 161.947 Hz, resp. in $CDCl_3$, unless stated otherwise; chemical shifts δ in ppm rel. to $SiMe_4$ and H_3PO_4, resp. Mass spectra: fast-atom bombardment (FAB) method.

5′-O-*[(*tert*-Butyl)diphenylsilyl]*-N^2-*[(dimethylamino)methylene]guanosine* (**2**). To a stirred soln. of *N*^{2}-[(dimethylamino)methylene]guanosine (**1**) [24] (5.5 g, 16.3 mmol) in pyridine (100 ml), (*tert*-butyl)diphenylsilyl chloride (6.2 ml, 23.8 mmol) was added under Ar. The mixture was stirred at r.t. for 16 h, then quenched with MeOH (20 ml), and evaporated to a syrup. The residue was precipitated from EtOH/Et_2O: **2** (9 g, 96%). ^{1}H-NMR ((D_6)DMSO + D_2O): 8.46 (*s*, CH=N); 7.89 (*s*, H–C(8)); 7.58–7.31 (*m*, 2 Ph); 5.81 (*d*, *J*(1′,2′) = 4.8, H–C(1′)); 4.46 (‘*t*’, *J*(2′,1′) = 4.8, H–C(2′)); 4.23 (‘*t*’, *J*(3′,2′) = 5.0, H–C(3′)); 3.97 (*m*, H–C(4′)); 3.84 (*dd*, *J*(5′,4′) = 2.8, *J*(5′,5″) = 12.0, H–C(5′)); 3.74 (*dd*, *J*(5″,4′) = 4.4, *J*(5″,5′) = 12.0, H′–C(5″)); 3.05 (*s*, H′–C(5′)Me); 2.97 (*s*, Me); 0.94 (*s*, *t*-Bu), HR-MS (FAB$^+$): 577.26095 ($C_{29}H_{36}N_6O_5Si^+$, $[M+H]^+$; calc. 577.2595).

1-[2-O-*Acetyl-3-bromo-5*-O-*[(*tert*-butyl)diphenylsilyl]-3-deoxy-β*-D-*xylofuranosyl]*-N^2-*[(dimethylamino)-methylene]guanine* (**3**). To a soln. of **2** (5.8 g, 10 mmol) and H_2O (0.12 ml) in MeCN (130 ml) at 0°, 2-acetoxyisobutyryl bromide (5.56 ml, 38 mmol) was added, and the mixture was stirred at r.t. for 3 h. The soln. was poured into sat. aq. $NaHCO_3$ soln. (100 ml) and extracted with CH_2Cl_2 (3 × 200 ml). The combined org. layers were dried (Na_2SO_4) and evaporated: chromatographically pure (6 g, 87%). White foam **3**. ^{1}H-NMR: 8.97 (br. *s*, NH); 8.62 (*s*, CH=N); 7.82 (*s*, H–C(8)); 7.73–7.31 (*m*, 2 Ph); 6.09 (*s*, H–C(2′)); 5.92 (*d*, *J*(1′,2′) = 1.6, H–C(1′)); 4.42 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 4.06 (*dd*, *J*(5′,4′) = 5.6, *J*(5′,5″) = 10.4, H–C(5′)); 3.97 (*dd*, *J*(5″,4′) = 6.4, *J*(5″,5′) = 10.4, H′–C(5′)); 3.17 (*s*, 1 MeN); 3.07 (*s*, 1 MeN); 2.19 (*s*, Ac); 1.07 (*s*, *t*Bu). HR-MS (FAB$^+$): 681.1850 ($C_{31}H_{37}BrN_6O_5Si^+$, $[M+H]^+$; calc. 681.1856).

1-[2-O-*Acetyl-5*-O-*[(*tert*-butyl)diphenylsilyl]-3-deoxy-β*-D-glycero-*pent-3-enofuranosyl]*-N^2-*[(dimethylamino)methylene]guanine* (**4**) *and 2′*-O,*3′*-S-*Diacetyl-5′*-O-*[(*tert*-*butyl)diphenylsilyl]*-N^2-*[(dimethylamino)methylene]-3′-thioguanosine* (**5**). To a soln. of **3** (5.4 g, 7.9 mmol) in dry DMF (50 ml), potassium thioacetate (2.7 g, 23.6 mmol) was added, and the mixture was stirred at 60° for 16 h and then evaporated to a syrup. The residue was partitioned between an aq. $NaHCO_3$ soln./brine 1:1 and CH_2Cl_2, the org. layer dried (Na_2SO_4) and evaporated, and the residue chromatographed (silica gel, gradient 2–10% MeOH/CH_2Cl_2): **4/5** (4.8 g). Yellowish foam. ^{1}H-NMR: **4/5** 3:7; 8.65 (*s*, CH=N, **5**); 8.61 (*s*, CH=N, **4**); 7.73 (*s*, H–C(8), **5**); 7.52 (*s*, H–C(8), **4**); 7.66–7.27 (*m*, Ph); 6.39 (*d*, *J*(1′,2′) = 1.4, H–C(1′), **4**); 6.03 (*s*, H–C(1′), **5**); 6.02 (*d*, *J*(1′,2′) = 1.4, H–C(2′), **4**); 5.83 (*s*, H–C(2′), **5**); 5.44 (*s*, H–C(3′), **4**); 4.75 (*dd*, *J*(3′,2′) = 5.8, *J*(3′,4′) = 10.2, H–C(3′), **5**); 4.28 (*m*, 2 H–C(5′), **4**); 4.18 (*ddd*, *J*(4′,5′) = 2.2, *J*(4′,5″) = 4.8, *J*(4′,3′) = 10.2, H–C(4′), **5**); 3.90 (*dd*, *J*(5′,5″) = 11.5, *J*(5′,4′) = 2.2, H–C(5′), **5**); 3.82 (*dd*, *J*(5″,5′) = 11.5, *J*(5″,4′) = 4.8, H′–C(5″), **5**); 3.14 (*s*, 1 MeN, **4**); 3.09 (*s*, 1 MeN, **5**); 3.08 (*s*, 1 MeN, **4**); 3.04 (*s*, 1 MeN, **5**); 2.31 (*s*, AcS, **5**); 2.16 (*s*, AcO, **5**); 2.13 (*s*, AcO, **4**); 1.07 (*s*, *t*Bu); 0.99 (*s*, *t*Bu).

When potassium thiobenzoate was used instead of potassium thioacetate, an inseparable mixture of **4** and *2′*-O-*acetyl-3′*-S-*benzoyl-5′*-O-*[(*tert*-*butyl)diphenylsilyl]*-N^2-*[(dimethylamino)methylene]-3′-thioguanosine* (**6**) was obtained in a similar yield and ratio to the unsaturated derivative **4** as above.

2′-O,*3′*-S-*Diacetyl*-N^2-*[(dimethylamino)methylene]-3′-thioguanosine* (**7**). To the above mixture **4/5** (0.9 g) in THF (15 ml), AcOH (0.37 ml, 6.5 mmol) was added, followed by $Bu_4NF \cdot 3\,H_2O$ (0.82 g, 2.6 mmol). The mixture was stirred at r.t. for 5 h, then diluted with CH_2Cl_2, and washed with H_2O and 10% aq. $NaHCO_3$ soln. The aq. layers were back-washed with CH_2Cl_2 and the combined org. layers dried (Na_2SO_4) and evaporated. FC (silica gel, gradient 2–10% MeOH/CH_2Cl_2) afforded **7** (300 mg, 46% from **3**). Yellowish foam. ^{1}H-NMR: 8.87 (br. *s*, NH); 8.78 (*s*, CH=N); 7.73 (*s*, H–C(8)); 5.80 (*d*, *J*(1′,2′) = 2.0, H–C(1′)); 5.76 (*dd*, *J*(2′,1′) = 2.0, *J*(2′,3′) = 6.4, H–C(2′)); 4.94 (*dd*, *J*(3′,2′) = 6.4, *J*(3′,4′) = 9.6, H–C(3′)); 4.22 (*d*, *J*(4′,3′) = 9.6, H–C(4′)); 4.04 (br. *s*, OH–C(5′)); 3.99 (*d*, *J*(5′,5″) = 12.0, H–C(5′)); 3.71 (*d*, *J*(5″,5′) = 12.0, H′–C(5′)); 3.19 (*s*, 1 MeN); 3.04 (*s*, 1 MeN); 2.34 (*s*, AcS); 2.13 (*s*, AcO). HR-MS (FAB$^+$); 439.1405 ($C_{17}H_{22}N_6O_6S^+$, $[M+H]^+$); calc. 439.1355).

2′-O-*Acetyl-3′*-S-*benzoyl*-N^2-*[(dimethylamino)methylene]-3′-thioguanosine* (**8**). According to the same procedure as above, **8** was synthesized from **4/6** in *ca.* 70% yield. ^{1}H-NMR: 8.90 (br. *s*, NH); 8.57 (br. *s*, NH); 7.69 (*s*, H–C(8)); 5.88 (*m*, H–C(1′), H–C(2′)); 5.30 (*m*, H–C(3′)); 4.34 (*d*, *J*(4′,3′) = 9.2, H–C(4′)); 4.04 (*d*, *J*(5′,5″) = 12.8, H–C(5′)); 3.80 (*dd*, *J*(5″,OH) = 9.6, *J*(5′,5″) = 12.8, H′–C(5′)); 3.69 (br. *s*, OH–C(5′)); 3.25 (*s*, MeN); 3.10 (*s*, 1 MeN); 2.16 (*s*, AcO). HR-MS (FAB$^+$): 501.1561 ($C_{17}H_{22}N_6O_6S^+$, $[M+H]^+$; calc. 501.1556).

2′-O,*3′*-S-*Diacetyl-3′-deoxy-5′*-O-*(4,4′-dimethoxytrityl)*-N^2-*[(dimethylamino)-methylene-3′-thioguanosine* (**9**). To a soln. of **7** (720 mg, 1.64 mmol) in dry pyridine (15 ml), $(MeO)_2$TrCl (1.1 g, 3.3 mmol) was added. The mixture was stirred at r.t. for 4 h, quenched with MeOH, and evaporated to a syrup which was partitioned

between 5% aq. $NaHCO_3$ soln. and CH_2Cl_2. The org. layer was washed with brine, dried (Na_2SO_4), and evaporated, and the residue purified by FC (silica gel, gradient 1–5% MeOH/CH_2Cl_2) **9** (0.85 g, 70%). Colorless foam. ^{1}H-NMR: 8.69 (*s*, CH=N); 8.58 (br. *s*, NH); 7.69 (*s*, H–C(8)); 7.38–6.74 (*m*, H–C(8), 12 arom. H); 6.06 (*dd*, *J*(2′,3′) = 6.4, *J*(2′,1′) = 1.2, H–C(2′)); 5.82 (*d*, *J*(1′,2′) = 1.2, H–C(1′)); 4.73 (*dd*, *J*(3′,4′) = 10.6, *J*(3′,2′) = 6.4, H–C(3′)); 4.21 (*dq*, *J*(4′,3′) = 10.6, *J*(4′,5′) = 3.0, *J*(4′,5″) = 4.4, H–C(4′)); 3.78 (*s*, 2 MeO); 3.36 (*m*, 2 H–C(5′)); 3.07 (*s*, 1 MeN); 3.05 (*s*, 1 MeN); 2.26 (*s*, AcS); 2.15 (*s*, AcO). HR-MS (FAB$^+$); 741.2692 ($C_{38}H_{40}N_6O_8S^+$, $[M+H]^+$; calc. 741.2707).

*2′-*O*-Acetyl-3′-benzoyl-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-*N^2*-[(dimethylamino)methylene]-3′-thioguanosine* (**10**). As described for **9**, **8** was converted to **10** in 69% yield. ^{1}H-NMR: 8.80 (*s*, CH=N); 8.65 (br. *s*, NH); 7.70 (*s*, H–C(8)); 7.88–6.66 (*m*, 19 arom. H); 6.17 (*d*, *J*(2′,3′) = 5.8, H–C(2′)); 5.86 (*d*, *J*(1′,2′) = 1.2, H–C(1′)); 5.08 (*dd*, *J*(3′,4′) = 10.4, *J*(3′,2′) = 5.8, H–C(3′)); 4.31 (*m*, H–C(4′)); 3.67 (*s*, 2 MeO); 3.45 (*m*, 2 H–C(5′)); 3.06 (*s*, 2 MeN); 2.15 (*s*, AcO). HR-MS (FAB$^+$); 803.2855 ($C_{43}H_{42}N_6O_8S^+$, $[M+H]^+$; calc. 803.2863).

*5′-*O*-(4,4′-Dimethoxytrityl)-3′-(pyridin-2-ylthio)-3′-thioguanosine* (**11**). *a*) A soln. of **9** (530 mg, 0.38 mmol) in 40% aq. $MeNH_2$ (50 ml) was kept at r.t. for 16 h. The mixture was evaporated and the residual syrup dissolved in Ar-purged DMF (30 ml) containing 2,2′-thiobis[pyridine] (340 mg, 1.54 mmol). The mixture was heated at 60° for 10 h and then evaporated to a syrup. FC (silica gel, gradient 1–12% MeOH/CH_2Cl_2) afforded **11** (460 mg, 85%). Colorless solid. ^{1}H-NMR: 10.64 (br. *s*, NH); 8.39 (*m*, 1 H, Py); 7.83 (*s*, H–C(8)); 7.73–6.72 (*m*, 16 arom. H); 6.50 (*d*, *J*(OH,2′) = 4.80, OH–C(2′)); 6.45 (br. *s*, NH_2); 5.81 (*d*, *J*(1′,2′) = 2.4, H–C(1′)); 4.83 (*m*, H–C(2′)); 4.34 (*m*, H–C(4′)); 4.09 (*dd*, *J*(3′,2′) = 6.00, *J*(3′,4′) = 7.8, H–C(3′)); 3.70 (*s*, 2 MeO); 3.11 (*dd*, *J*(5′,5″) = 11.2, *J*(5″,4′) = 4.8, H′–C(5′)). HR-MS (FAB$^+$); 711.2076 ($C_{36}H_{34}N_6O_6S_2^+$, $[M+H]^+$; calc. 711.2060).

b) By the same procedure as above, but starting from *S*-benzoyl derivative **10**, target **11** was prepared in 80% yield.

c) Starting from **16** (830 mg, 1.12 mmol) and under the above conditions, derivative **11** (570 mg, 67%) was obtained.

*5′-*O*-(4,4′-Dimethoxytrityl)-3′-thioguanosine* (**12**). To the soln. of **11** (240 mg, 0.34 mmol) in $CHCl_3$ (14 ml), dithiothreitol (DTT; 125 mg, 0.81 mmol) was added, and the mixture was stirred at r.t. for 3 h. It was then evaporated to a syrup, and the product was precipitated by addition of peroxide-free Et_2O. The precipitate was filtered off, washed with Et_2O, and dried: 230 mg of crude **12**. ^{1}H-NMR ((D$_6$)DMSO): 10.63 (br. *s*, NH); 7.86 (*s*, H–C(8)); 7.32–6.80 (*m*, 13 arom. H); 6.49 (br. *s*, NH_2); 5.81 (*s*, H–C(1′)); 4.43 (*d*, *J*(2′,3′) = 4.8, H–C(2′)); 3.93 (*m*, H–C(4′)); 3.79 (*dd*, *J*(3′,2′) = 4.8, *J*(3′,4′) = 9.6, H–C(3′)); 3.71 (*s*, 2 MeO); 3.16 (*dd*, *J*(5″,5′) = 10.4, *J*(5″,4′) = 4.8, H′–C(5′)).

3′-Thioguanosine (**13**). The mixture of crude **12** (230 mg, 0.33 mmol) and DTT (150 mg) was dissolved in 1N HCl/MeOH (12 ml), and the mixture was kept at r.t. for 3 h. It was then evaporated and the residue co-evaporated with toluene two times. Addition of AcOEt afforded a precipitate which was filtered off, washed well with AcOEt and dried: **13** (90 mg, 79%). The product was reprecipitated from H_2O. ^{1}H-NMR (CD_3OD): 8.10 (*s*, H–C(8)); 5.91 (*s*, H–C(1′)); 4.37 (*d*, *J*(2′,3′) = 5.2, H–C(2′)); 3.97 (*m*, H–C(4′), H–C(5′)); 3.82 (*dd*, *J*(5″,5′) = 13.0, *J*(5″,4′) = 3.4, H′–C(5′)); 3.64 (*dd*, *J*(3′,2′) = 5.2, *J*(3′,4′) = 9.6, H–C(3′)). HR-MS (FAB$^+$): 300.0767 ($C_{10}H_{13}N_5O_4S^+$; $[M+H]^+$); calc. 300.0767).

*3′,3‴-Dithiobis[2′-*O*-acetyl-3′-deoxy-*N^2*-[(dimethylamino)methylene]guanosine]* (**14**) *and 1-(2-*O*-Acetyl-3-deoxy-β-*D*-glycero-pent-3-enofuranosyl)-*N^2*-[(dimethylamino)methylene]guanine* (**15**). To **4**/**5** (4.8 g) in THF (100 ml), 1M Bu_4NF in THF (10 ml) was added. The mixture was stirred for 3 h at r.t. and then evaporated to a syrup. FC (silica gel, gradient 2–10% MeOH/CH_2Cl_2) yielded the faster eluting **15** (1 g, 35% from **3**). Colorless foam. ^{1}H-NMR: 8.96 (br. *s*, NH); 8.57 (*s*, CH=N); 7.65 (*s*, H–C(8)); 6.41 (*s*, H–C(2′)); 6.04 (*s*, H–C(1′)); 5.42 (*m*, H–C(3′)); 4.32 (*m*, 2 H–C(5′)); 3.19 (*s*, MeN); 3.06 (*s*, MeN); 2.11 (*s*, Ac).

The slower eluting **14** was obtained as a yellowish solid (0.9 g, 29% from **3**). ^{1}H-NMR ((D$_6$)DMSO): 11.34 (br. *s*, NH); 8.53 (*s*, CH=N); 8.00 (*s*, H–C(8)); 5.97 (*d*, *J*(1′,2′) = 2.4, H–C(1′)); 5.89 (*dd*, *J*(2′,1′) = 2.4, *J*(2′,3′) = 6.0, H–C(2′)); 5.23 (*t*, *J*(OH,5′) = 5.6, OH–C(5′)); 4.11 (*m*, H–C(4′)); 4.02 (*dd*, *J*(3′,2′) = 6.0, *J*(3′,4′) = 8.4, H–C(3′)); 3.78 (*m*, H–C(5′)); 3.60 (*m*, H′–C(5′)); 3.10 (*s*, MeN); 3.00 (*s*, MeN); 2.06 (*s*, AcO). HR-MS (FAB$^+$): 791.2355 ($C_{30}H_{38}N_{12}O_{10}S_2^+$, $[M+H]^+$; calc. 791.2354).

*3′,3‴-Dithiobis[2′-*O*-acetyl-3′-deoxy-5′-*O*-(4,4′dimethoxytrityl)-*N^2*-[(dimethylamino)methylene]guanosine]* (**16**). To the soln. of **14** (400 mg, 0.5 mmol) in dry pyridine (10 ml), $(MeO)_2TrCl$ (508 mg, 1.5 mmol) was added and the mixture stirred 4 h at r.t. MeOH (10 ml) was added and the soln. evaporated to dryness. The residue was partitioned between sat. $NaHCO_3$ soln. and CH_2Cl_2, and the org. layer washed with brine, dried (Na_2SO_4), and evaporated to a syrup. FC (silica gel, gradient 2–10% MeOH/CH_2Cl_2) yielded **16** (620 mg, 71% yield). Yellowish foam. ^{1}H-NMR: 8.72 (br. *s*, NH); 8.01 (*s*, CH=N); 7.48–7.21 (*m*, H–C(8), 13 arom. H); 6.25

(*d*, *J*(2′,3′) = 4.8, H–C(2′)); 5.78 (*s*, H–C(1′)); 4.00 (*m*, H–C(3′), H–C(4′)); 3.78 (*s*, 2 MeO); 3.50 (br. *s*, 2 H–C(5′)); 3.14 (*s*, 1 MeN); 3.13 (*s*, 1 MeN); 1.82 (*s*, AcO). HR-MS (FAB^+): 1395.4943 ($C_{72}H_{74}N_{12}O_{14}S_2^+$, $[M+H]^+$; calc. 1395.4967).

*3′,3‴-Dithiobis[3′-deoxy-5′-*O*-(4,4′dimethoxytrityl)-*N^2*-[(dimethylamino)methylene]guanosine]* (**17**). *a*) To a soln. of **16** (60 mg, 0.04 mmol) in dry MeOH, ion exchange resin *AG1X8* (OH^-) (1 g) was added. The mixture was stirred at 55° for 16 h, the resin filtered off and washed well with hot MeOH, and the filtrate evaporated: pure **17** (16 mg, 28%). Colorless solid. ^{1}H-NMR ((D_6)DMSO): 11.34 (br. *s*, NH); 8.48 (*s*, CH=N); 7.92 (*s*, H–C(8)); 7.31–6.73 (*m*, 13 arom. H); 6.27 (*d*, *J*(OH,2′) = 5.2, OH–C(2′)); 5.88 (*d*, *J*(1′,2′) = 1.2, H–C(1′)); 4.60 (*m*, H–C(2′)); 4.16 (*m*, H–C(4′)); 4.08 (*m*, H–C(3′)); 3.65 (*s*, 2 MeO); 3.65 (*m*, 2 H–C(5′)); 3.02 (*s*, 1 MeN); 2.97 (*s*, 1 MeN). HR-MS (FAB^+): 1311.4746 ($C_{68}H_{70}N_{12}O_{12}S_2^+$, $[M+H]^+$; calc. 1311.4756).

b) With *Amberlyst A-26* (CN^-) under the above conditions, **17** was obtained from **16** in 21% yield.

*5′-*O*-(4,4′-Dimethoxytrityl)-*N^2*-[(dimethylamino)methylene]3′-*S*-(pyridin-2-ylthio)-3′-thioguanosine* (**18**) *and* **17**. To the soln. of **11** (400 mg, 0.56 mmol) in dry pyridine (5 ml), dimethylformamide dimethyl acetal (1.2 ml, 9 mmol) was added and the mixture stirred at r.t. for 16 h. Solvents were removed *in vacuo* and the residue chromatographed (silica gel, gradient 1–50% MeOH/CH_2Cl_2). Fractions containing the faster-running material gave **18** (110 mg, 23%). ^{1}H-NMR: 8.73 (br. *s*, NH); 8.53 (*s*, CH=N); 8.49 (*m*, 1 H, Py); 7.71 (*s*, H–C(8)); 7.62 (*m*, 1 H, Py); 7.44–6.79 (*m*, 15 arom. H); 6.09 (*s*, H–C(1′)); 4.55 (*d*, *J*(2′,3′) = 4.8, H–C(2′)); 4.23 (*dq*, *J*(4′,3′) = 10.5, *J*(4′,5′) = 2.8, *J*(4′,5′) = 3.4, H–C(4′)); 4.14 (*dd*, *J*(3′,2′) = 4.8, *J*(3′,4′) = 10.5, H–C(3′)); 3.78 (*s*, 2 MeO); 3.57 (*dd*, *J*(5″,5′) = 10.6, *J*(5′,4′) = 2.8, H–C(5′)); 3.41 (*dd*, *J*(5′,5″) = 10.6, *J*(5″,4′) = 3.4, H–C(5′)); 3.08 (*s*, 1 MeN); 3.05 (*s*, 1 MeN).

Fractions containing the slower running compound gave **17** (120 mg, 33%). Colorless foam. ^{1}H-NMR: identical to that of **17** obtained by the above procedures.

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(4,4′-dimethoxytrityl)-*N^2*-[(dimethylamino)methylene]-3′-*S*-(pyridin-3-ylthio)-3′-thioguanosine* (**19**). To a soln. of **18** (110 mg, 0.14 mmol) in dry pyridine (1 ml), ^{t}BuMe$_2$SiTf (0.103 ml, 0.45 mmol) was added. The mixture was stirred at r.t. for 5 h, then quenched with MeOH, and evaporated. The residue was dissolved in CH_2Cl_2, the org. phase washed with 5% aq. $NaHCO_3$ soln. and brine, dried (Na_2SO_4), and evaporated, and the syrup submitted to FC (silica gel, gradient 1–10% MeOH/AcOEt): **19** (90 mg, 71%). Colorless solid. ^{1}H-NMR: (br. *s*, NH); 8.50 (*s*, CH=N); 8.37 (*m*, 1 H, Py); 7.81 (*s*, H–C(8)); 7.50–6.74 (*m*, 16 arom. H); 5.98 (*d*, *J*(1′,2′) = 2.4, H–C(1′)); 4.75 (*dd*, *J*(2′,3′) = 5.0, *J*(2′,1′) = 2.4, H–C(2′)); 4.50 (*m*, H–C(4′)); 3.99 (*dd*, *J*(3′,2′) = 5.0, *J*(3′,4′) = 8.4, H–C(3′)); 3.76 (*s*, 2 MeO); 3.62 (*dd*, *J*(5′,5″) = 10.9. *J*(5′,4′) = 2.2. H–C(5′)); 3.38 (*dd*, *J*(5′,5″) = 10.9, *J*(5″,4′) = 4.4, H–C(5′)); 3.06 (*s*, 1 MeN); 3.04 (*s*, 1 MeN); 0.93 (*s*, *t*Bu); 0.17 (*s*, Me); 0.10 (*s*, Me). HR-MS (FAB^+): 880.3357 ($C_{45}H_{53}N_7O_6S_2Si^+$, $[M+H]^+$; calc. 880.3346).

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(4,4′-dimethoxytrityl)-3′-*S*-(pyridin-2-ylthio)-3′-thioguanosine* (**20**). To a soln. of **11** (410 mg, 0.58 mmol) in dry pyridine (36 ml), 1*H*-imidazole (2.36 g, 35 mmol) and ^{t}BuMe$_2$SiCl (4.29 g, 28 mmol) were added. The mixture was stirred at r.t. for 16 h and then evaporated to a syrup. The residue was partitioned between CH_2Cl_2 and sat. aq. $NaHCO_3$ soln., the org. layer washed with H_2O, dried (Na_2SO_4), and evaporated, and the syrup submitted to FC (silica gel, gradient 1–10% MeOH/CH_2Cl_2): **20** (430 mg, 85%). White foam. ^{1}H-NMR ((D_6)DMSO): 11.99 (br. *s*, NH); 8.39 (*m*, 1 H, Py); 7.84 (*s*, H–C(8)); 7.71 (*m*, 1 H, Py); 7.62–6.72 (*m*, 15 arom. H); 6.41 (br. *s*, NH_2); 5.80 (*d*, *J*(1′,2′) = 4.4, H–C(1′)); 5.04 ('*t*', *J*(2′,3′) = 4.4, H–C(2′)); 4.39 (*m*, H–C(4′)); 4.01 ('*t*', *J*(3′,4′) = 6.4, H–C(3′)); 3.69 (*s*, 2 MeO); 3.13 (*dd*, *J*(5″,5′) = 11.0, *J*(5″,4′) = 4.6, H′–C(5′)); 0.82 (*s*, *t*Bu); 0.08 (*s*, 1 Me); 0.06 (*s*, 1 Me). HR-MS (FAB^+): 825.2977 ($C_{42}H_{48}N_6O_6S_2Si^+$ $[M+H]^+$; calc. 825.2924).

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-O-(4,4′-dimethoxytrityl)-*N^2*-isobutyryl-3′-*S*-(pyridin-2-ylthio)-3′-thioguanosine* (**21**). To the soln. of **20** (310 mg, 0.38 mmol) in dry pyridine (5 ml), isobutyric anhydride (0.19 ml, 1.14 mmol) and 4-(dimethylamino)pyridine (46 mg, 0.38 mm) were added. The mixture was stirred at r.t. for 16 h and at 50° for 5 h, then quenched with MeOH (2 ml), and evaporated. The syrup was partitioned between CH_2Cl_2 and 5% aq. $NaHCO_3$ soln., the org. layer washed with brine, dried (Na_2SO_4), and evaporated and the syrup submitted to FC (silica gel, gradient 1–5% MeOH/CH_2Cl_2): **21** (320 mg, 95%). Colorless foam. ^{1}H-NMR: 11.94 (br. *s*, NH); 8.36 (*m*, 1 H, Py); 7.85 (*m*, 1 H, Py); 7.80 (*s*, H–C(8)); 7.58–6.71 (*m*, NH, 15 arom. H); 5.83 (*d*, *J*(1′,2′) = 5.2, H–C(1′)); 5.22 ('*t*', *J*(1′,2′) = 5.2, H–C(2′)); 4.50 (*m*, H–C(4′)); 4.27 (*t*, *J*(3′,4′) = 6.4, H–C(3′)); 3.76 (*s*, MeO); 3.75 (*s*, MeO); 3.56 (*dd*, *J*(5′,5″) = 11.0, *J*(5′,4′) = 1.8, H–C(5′)); 2.98 (*dd*, *J*(5″,5′) = 11.0, *J*(5″,4′) = 3.0, H′–C(5′)); 1.69 (*m*, Me_2CH); 0.94 (*d*, *J* = 7.2, Me); 0.76 (*d*, *J* = 7.2, Me); 0.88 (*s*, *t*Bu); 0.11 (*s*, Me); 0.06 (*s*, Me). HR-MS (FAB^+): 895.3380 ($C_{46}H_{54}N_6O_7S_2Si^+$, $[M+H]^+$, calc. 895.3343).

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(4,4′-dimethoxytrityl)-*N^2*-isobutyryl-3′-thioguanosine* (**22**). To the soln. of **21** (340 mg, 0.38 mmol) in $CHCl_3$ (20 ml), Et_3N (0.4 ml) and dithiotreitol DTT (140 mg, 0.91 mmol) were added, and the mixture was stirred for 1 h at r.t. The mixture was then washed with sat. aq. $NaHCO_3$ soln.

and H_2O, dried (Na_2SO_4) and evaporated. FC (silica gel, gradient 0.5–2% MeOH/CH_2Cl_2) afforded **22** (270 mg, 90%). ^{1}H-NMR of the major rotamer: 11.92 (br. *s*, NH); 7.93 (*s*, H–C(8)); 7.63–6.80 (*m*, NH, 15 arom. H); 5.83 (*d*, *J*(1′,2′) = 2.8, H–C(1′)); 4.74 (*dd*, *J*(2′,1′) = 2.8, *J*(2′,3′) = 5.4, H–C(2′)); 4.13 (br. *d*, *J*(4′,3′) = 7.6, H–C(4′)); 3.78 (*s*, MeO); 3.73 (*s*, MeO); 3.74 (*m*, H–C(3′)); 3.63 (*dd*, *J*(5′,5″) = 11.0, *J*(5′,4′) = 1.2, H–C(5′)); 3.27 (*dd*, *J*(5″,5′) = 11.0, *J*(5″,4′) = 3.0, H′–C(5′)); 1.63 (*d*, *J*(SH,3′) = 8.4, SH); 2.08 (*m*, Me_2CH); 1.09 (*d*, *J* = 6.8, Me); 0.98 (*d*, *J* = 6.8, Me); 0.91 (*s*, *t*Bu); 0.14 (*s*, Me); 0.08 (*s*, Me). HR-MS (FAB$^+$): 786.3354 ($C_{41}H_{51}N_5O_7SSi^+$, $[M+H]^+$; calc. 786.3357).

*2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(4,4′-dimethoxytrityl)-*N^2*-isobutyryl-3′-thioguanosine 3′-(2-Cyanoethyl Diisopropylphoramidothioite)* (**23**). Phosphitylation of **22** as described by *Sun et al.* [15] afforded a product which was purified by FC (0.5% EtOH/CH_2Cl_2 containing 1% Et_3N). The final product was obtained as a white powder by precipitation from toluene/pentane at 0°: 76% yield. ^{31}P-NMR: 163.5 (*s*); 159.6 (*s*). HR-MS (FAB$^+$): 986.4406 ($C_{50}H_{68}N_7O_8PSSi^+$, $[M+H]^+$; calc. 986.4435).

REFERENCES

[1] J. A. Piccirilli, J. S. Vyle, M. H. Caruthers, T. R. Cech, *Nature (London)* **1987**, *361*, 85.
[2] L. B. Weinstein, D. I. Earnshaw, R. Cosstick, T. R. Cech, *J. Am. Chem. Soc.* **1996**, *118*, 10341.
[3] E. J. Sontheimer, S. Sun, J. A. Piccirili, *Nature (London)* **1997**, *308*, 801.
[4] J. Matulic-Adamic, A. T. Daniher, C. Gonzalez, L. Beigelman, *Bioorg. Med. Chem. Lett.* **1998**, *9*, 157.
[5] L. Beigelman, J. A. McSwiggen, K. G. Draper, C. Gonzalez, K. Jensen, A. M. Karpeisky, A. S. Modak, J. Matulic-Adamic, A. B. DiRenzo, P. Haeberli, D. Sweedler, D. Tracz, S. Grimm, F. E. Wincott, V. G. Thackray, N. Usman, *J. Biol. Chem.* **1995**, *270*, 25702.
[6] D. B. McKay, *RNA* **1996**, *2*, 395.
[7] W. Guschlbauer, K. Jankowski, *Nucleic Acids Res.* **1980**, *8*, 1420.
[8] C. Thibaudeau, I. Plavec, N. Garg, A. Papchikhin, J. Chattopadhyaya, *J. Am. Chem. Soc.* **1994**, *116*, 4038.
[9] I. Plavec, C. Thibaudeau, J. Chattopadhyaya, *J. Am. Chem. Soc.* **1994**, *116*, 6558.
[10] X. Liu, C. B. Reese, *Tetrahedron Lett.* **1996**, *37*, 925.
[11] R. Cosstick, J. S. Vyle, *Nucleic Acids. Res.* **1990**, *18*, 829.
[12] X. Li, D. M. Andrews, R. Cosstick, *Tetrahedron* **1992**, *48*, 2729.
[13] X. Li, G. K. Scott, A. D. Baxter, R. J. Taylor, J. S. Vyle, R. Cosstick, *J. Chem. Soc., Perkin Trans. 1* **1994**, 2123.
[14] J. S. Vyle, B. A. Connolly, D. Kemp, R. Cosstick, *Biochemistry* **1992**, *31,* 3012.
[15] S. Sun, A. Yoshida, J. A. Piccirilli, *RNA* **1997**, *3*, 1352.
[16] K. J. Ryan, E. M. Acton, L. Goodman, *J. Org. Chem.* **1968**, *33*, 1783.
[17] X. Cao, M. D. Matteucci, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 807.
[18] M. Elzagheid, A. Azhayev, M. Oivanen, H. Lonnberg, *Collect. Czech. Chem. Commun., Special Issue* **1996**, *61*, S42.
[19] H. Vorbruggen, B. Bennua, *Chem. Ber.* **1981**, *114*, 1279.
[20] M. J. Robins, R. Zou, Z. Guo, S. F. Wnuk, *J. Org. Chem.* **1996**, *61*, 9207.
[21] R. Mengel, H. Griesser, *Tetrahedron Lett.* **1977**, 1177.
[22] A. P. Higson, G. K. Scott, D. J. Earnshaw, A. D. Baxter, R. A. Taylor, R. Cosstick, *Tetrahedron* **1996**, *52*, 1027.
[23] A. F. Russell, S. Greenberg, J. G. Moffatt, *J. Am. Chem. Soc.* **1973**, *95*, 4025.
[24] M. J. Robins, J. S. Wilson, D. Madej, N. H. Low, F. Hansske, S. F. Wnuk, *J. Org. Chem.* **1995**, *60*, 7902.
[25] T. C. Jain, J. G. Moffat, 'Abstracts, 165 National Meeting of the American Chemical Society', Dallas, Texas, April, 1973, CARB 15.
[26] G.-X. He, N. Bishofberger, *Tetrahedron Lett.* **1995**, *39*, 6991.
[27] T. L. Sheppard, A. T. Rosenblatt, R. Breslow, *J. Org. Chem.* **1994**, *59*, 7243.
[28] J. Herzig, A. Nudelman, H. E. Gottlieb, B. Fisher, *J. Org. Chem.* **1986**, *51*, 727.

Received August 26, 1999