Photolabile *N*-hydroxypyrid-2(1*H*)-one derivatives of guanine nucleosides: a new method for independent guanine radical generation†

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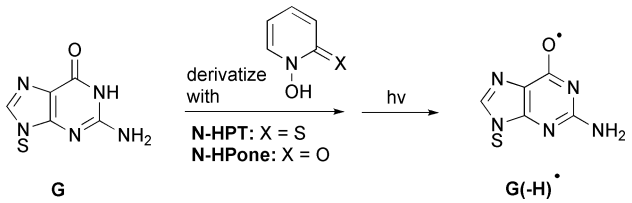
One-electron oxidized guanine is an important reactive intermediate in the formation of oxidatively generated damage in DNA and a variety of methods have been utilized for the abstraction of a single electron from the guanine moiety. In this study, an alternative approach for the site specific, independent generation of the guanine radical, utilizing *N*-hydroxypyrid-2(1*H*)-one as a photolabile modifier of guanine, is proposed. Novel photolabile 6-[(1-oxido-2-pyridinyl)oxo]-6-deoxy- and 2′,6-dideoxy-guanosine derivatives capable of generating the neutral guanine radical (G(-H)•) upon photolysis were synthesized and characterized. The generation of G(-H)• proceeds through homolysis of the N–O bond and was confirmed through continuous photolysis product analysis and trapping studies, as well as laser flash photolysis experiments.

Introduction

Extensive research over the past years on oxidatively produced DNA damage has uncovered a wealth of chemistry involving the sugar[1] and base[2] components of nucleosides, nucleotides, synthetic oligonucleotides and natural DNA. Of the four DNA bases, guanine (G) is the most easily oxidized, its reduction potential being the lowest among the nucleobases, and presents the richest chemistry.[3] Numerous studies on the long-distance hole-migration process through a DNA double strand have been based on trapping an electron hole by a guanine base. These studies have led to a detailed insight into the distance and sequence dependence of charge movement processes.[4-7] One electron oxidation of the G moiety results in the formation of a wide variety of oxidatively generated damage.[3,8-10] A variety of oxidants are known to abstract a single electron from G. These oxidants include $Br_2^{\bullet-}$ and $SO_4^{\bullet-}$,[11] $CO_3^{\bullet-}$,[12,13] light (photoionization) or type I photosensitization.[14-16] One electron reduction of 8-bromoguanine has recently been reported as an efficient method for producing the guanine radical cation ($G^{\bullet+}$).[17,18] The produced $G^{\bullet+}$ deprotonates at physiological pH with a p*K*a of 3.9 at the nucleoside level,[11] to produce the neutral guanine radical [G(-H)•]. The deprotonation of $G^{\bullet+}$ at the nucleoside level and in DNA occurs with a rate constant in the order of 10^7 s^{-1}, at neutral pH.[19] A recent report[20] suggests that hydroxyl radicals (OH•) react with guanine mainly by hydrogen atom abstraction from the 2-amino group, not by addition to the C-4 as previously believed,[21] resulting in the formation of a 2-aminyl radical which tautomerizes to G(-H)•. The lifetime of this radical depends on the presence of reactive species, but in the absence of these species, the lifetime can reach ~5 s.[22] Recent experiments showed that $^3O_2$ reacts with G(-H)• ($k < 10^6$ M^{-1} s^{-1}) at least 3 orders of magnitude more slowly than $O_2^{\bullet-}$, thereby kinetically favoring the reaction with $O_2^{\bullet-}$.[21] The reaction of G(-H)• with $O_2^{\bullet-}$ leads to the formation of imidazolone derivative (dIz), as the major one-electron oxidation product, which is hydrolytically unstable and is consequently transformed to oxazolone derivative (dZ).[14] The greater stability and decreased reactivity of $G^{\bullet+}$ in double-stranded DNA leads to hydration at the 8-position and, after a second one-electron oxidation, to the formation of 8-oxo-G. The neutral radical G(-H)• does not give rise to 8-oxo-G.[21]

Selective generation of carbon-centered sugar and base radicals has been accomplished by photo-reactive precursors using nucleosides or oligonucleotides (ODNs). Generation of a single radical species on duplex ODNs provides a powerful tool for elucidating the role of reactive intermediates in the formation of nucleic acid lesions. For example, radicals generated at C1′ and C4′ and, more recently, C5′ positions of the sugar moiety have been studied in detail by photolysis of the corresponding *tert*-butyl ketones.[23-28] Selective generation of pyrimidine base radicals has been also accomplished by photolysis of phenylthio-[29] or isopropylketones.[30] Photolabile precursors for the site selective generation of G(-H)• are missing.

Towards this effort, we proposed the use of either *N*-hydroxypyridine-2(1*H*)-thione (N-HPT) or *N*-hydroxypyrid-2(1*H*)-one (N-HPone) as photolabile modifiers at the 6-position of 2′-deoxyguanosine or guanosine (Scheme 1). Photolysis of the



Scheme 1 Proposed generation of G(-H)•.

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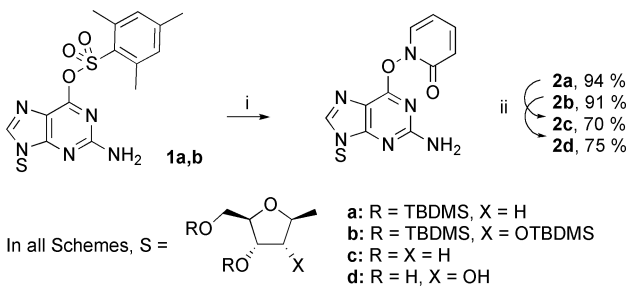
derivatized guanine moiety was expected to homolytically cleave the N–O bond and afford the guanine radical. We have recently evaluated the use of *N*-hydroxypyridine-2(1*H*)-thione (N-HPT) as a photolabile modifier and we did observe products arising from G(-H)$^{\bullet}$ formation.[31] However, product studies, as well as laser flash photolysis experiments indicated that both the chemistry and photochemistry of this functional group are rather complex. It has been reported that the photochemistry of N-HPone is much simpler compared to its sulfur analogue,[32,33] and we decided to utilize N-HPone, with the expectation that homolysis of the N–O bond will lead to the generation of the guanine radical. We present herein our results from synthetic and stability studies, accompanied by continuous photolysis product studies, trapping experiments and observation of the transient spectra of G(-H)$^{\bullet}$ by laser flash photolysis (LFP).

Results

Synthesis

The synthesis of O^6-[2-oxopyridin-1(2*H*)-yl]-guanosine and 2′-deoxyguanosine derivatives **2** is presented in Scheme 2. The known O^6-(2-mesitylsulfonyl) derivatives **1** were synthesized following reported procedures.[31,34] Introduction of the 2-oxopyridin-1(2*H*)-yl group, through DABCO-induced activation,[31] in dioxane, led to the formation of compounds **2a** and **2b** in excellent yields (94 and 91%, respectively). Products **2a** and **2b** were readily deprotected in the presence of TBAF in THF and the water soluble derivatives **2c** and **2d** were isolated in satisfactory yields (70 and 75%, respectively). Although the methodology was initially developed on the ribo-derivative **2d** for economy, it was then extended to **2c** and only the 2′-deoxy analogue **2c** was utilized in the photochemical studies described herein.

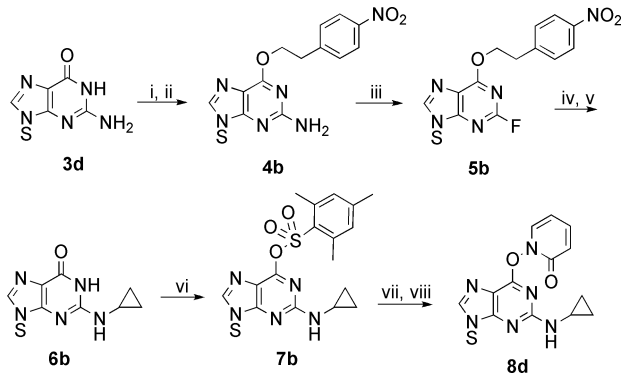


Scheme 2 Synthesis of O^6-[2-oxopyridin-1(2*H*)-yl]-guanosine and 2′-deoxyguanosine derivatives. (i) DABCO, dioxane; then N-HPone, DBU. (ii) TBAF, THF.

Synthesis of a G(-H)$^{\bullet}$ chemical trap

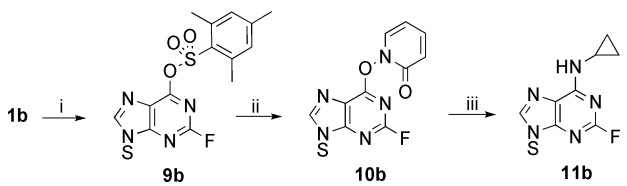
In an effort to provide an efficient trap for the guanine radical to be produced photolytically, a derivative of **2d** bearing a cyclopropyl group at N^2 was also synthesized, in accordance with the work of Saito and coworkers.[35] They proposed that the dG radical cation induces homolytic cyclopropane ring opening, as was evidenced by the formation of N^2-(3-hydroxypropanoyl)dG. Therefore, product analysis of the photolyzed mixture could provide chemical evidence for G(-H)$^{\bullet}$ generation in our system as well.

The synthesis of **8d** is presented in Scheme 3. The 2-(4-nitrophenyl)ethyl group was utilized for the protection of the O^6 position through a Mitsunobu reaction.[36] Conversion of **4b** to **5b** was accomplished by treatment with *tert*-butylnitrite in 70% anhydrous HF/pyridine at −20 °C using a modification of the procedure by Robins & Uznanski.[37] Unfortunately, the lability of the TBDMS protecting groups of the sugar moiety in the presence of the fluoride anion also resulted in partial deprotection together with the fluorination. Strict adherence to the experimental protocol is essential to achieve the optimal yield (71%). Otherwise, reapplication of the reaction mixture to the silylation conditions was necessary to improve the yield of **5b**. Substitution of the fluoro group by cyclopropylamine, followed by removal of the 2-(4-nitrophenyl)ethyl protecting group[38] afforded derivative **6b**. The photolabile adduct **8d** was obtained by following the previously applied experimental procedure for products **2c** and **2d** (*vide supra*) and **8d** was obtained in good yield.



Scheme 3 Synthesis of O^6-[2-oxopyridin-1(2*H*)-yl]-N^2-cyclopropyl-guanosine (**8d**). (i) TBDMSCl, imidazole, DMF, 87% (ii) NPE, DIAD, PPh_3, dioxane, 84% (iii) HF/pyridine 70/30, *t*-BuONO, pyridine, THF, 71% (iv) cyclopropylamine, Et_3N, DMF (v) DBU, pyridine, 53% (two steps) (vi) mesitylsulfonyl chloride, DMAP, DCM, 66% (vii) *N*-hydroxypyrid-2(1*H*)-one, DABCO, DBU, dioxane, 94% (viii) TBAF in THF, 75%.

Protection of the O^6 position of the base moiety is compulsory in order for the fluorination to take place. Utilization of the 2-(4-nitrophenyl)ethyl protecting group was successful, but it nevertheless introduced two additional synthetic steps. An interesting alternative approach would be to introduce the fluoro group at derivative **1b**, thus eliminating the extra protection–deprotection steps. Indeed, fluorination of **1b** followed by substitution by *N*-hydroxypyrid-2(1*H*)-one were successful, but when the substitution by cyclopropylamine was attempted, only formation of **11b** was observed (Scheme 4).



Scheme 4 Synthesis of 9-(2′,3′,5′-tri-*O*-TBDMS-β-D-ribofuranosyl)-*N*-cyclopropyl-2-fluoro-purin-6-amine (**11b**). (i) HF/pyridine 70/30, *t*-BuONO, pyridine, THF, 44% (ii) *N*-hydroxypyrid-2(1*H*)-one, DABCO, DBU, dioxane, 96% (iii) cyclopropylamine, Et_3N, DMF, 73%.

Photolabile adduct stability

Insertion of the photolabile adduct **2c** in synthetic oligonucleotides would require that suitable protection is applied and that it remains stable under the standard or modified conditions utilized in automated oligonucleotide synthesis, on solid support. Compound **2c** was indefinitely stable in D_2O at natural pD and its solutions showed no appreciable change after at least 2 months' storage at room temperature as indicated by NMR spectroscopy. Moreover, it was stable for at least 24 h when heated in an aqueous solution, at 80 °C, in the dark and exhibited the same stability in 80% aqueous acetic acid at room temperature as shown by HPLC experiments. The compound was relatively stable in 0.1 M I_2 in THF (1 h, at r.t., for >90% recovery) and in 25% aq. NH_4OH (2.5 h, at r.t., for >90% recovery).

Therefore, the modification can be inserted in synthetic oligonucleotides, but modified solid phase oligonucleotide synthesis with more base labile protection (*e.g.*, PAC protection[39]) would have to be used.

Photolysis of 2c in aqueous solutions

The photolysis of **2c** (blue peaks in Fig. 1) with pyrex-filtered (>320 nm) UVA light in H_2O, under argon, at natural pH, was followed by RP-HPLC. The reaction is practically completed after 30 min (>95% conversion of **2c**) yielding two main products that were characterized by LC/MS and by comparison with authentic samples. The peak at 16.5 min (Fig. 1) corresponds to 2′-deoxyguanosine generated in 38% yield, with respect to the consumed **2c**, whereas the peak at 14 min corresponds to 2-hydroxypyridine (50% yield). Yields were determined by calibration curves, obtained from known concentrations of authentic samples. The apparent size of the peaks corresponding to 2-hydroxypyridine (Fig. 1) is a result of the poor absorbance of this compound at 254 nm[40] and detection at 230 nm provides a more appropriate picture for its formation (Inset b in Fig. 1).

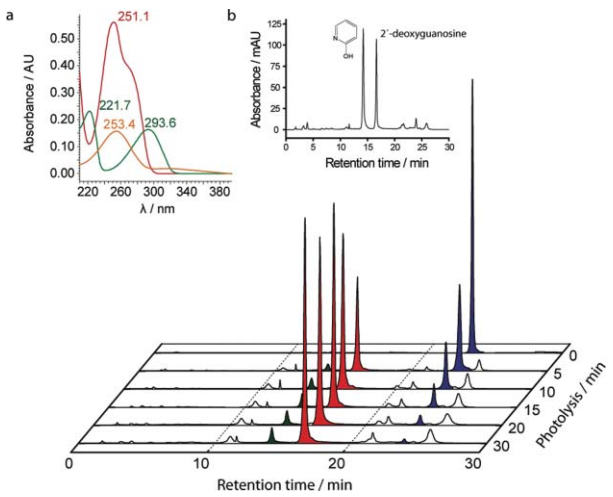


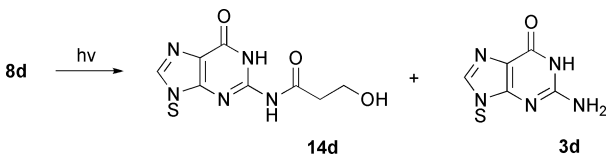
Fig. 1 HPLC profile of the photolysis of **2c** in H_2O, under argon, detection at 260 nm. Insets: (a) UV spectra for 2′-deoxyguanosine (λ_{max} at 251 nm), 2-hydroxypyridine (λ_{max} at 222 & 294 nm) and imidazolone (dIz, λ_{max} at 253 nm). (b) HPLC for the photolysis of **2c** (Rt 24 min) after 30 min reaction; detection at 230 nm.

When the photolysis was run in $H_2{}^{18}O$ and was monitored by LC-MS, no isotopic labeling in either 2′-deoxyguanosine or 2-hydroxypyridine was detected, indicating that homolysis of the N–O bond, rather than solvolysis of **2c**, had occurred (see also discussion below).

We did not proceed with a systematic characterization of the minor products at this stage. In the case of photolysis under air, however, a new nucleosidic product was identified as 2-amino-5-[(2-deoxy-β-D-ribosyl)amino]-4*H*-imidazol-4-one (imidazolone, dIz), from its characteristic UV (Fig. 1, inset a, λ_{max} at 253 nm) and MS spectra. Upon incubation of the reaction mixture in the dark, for 24 h, at 37 °C, the imidazolone peak gave its place to the corresponding oxazolone (Fig. S1, see ESI†). This guanine oxidation product was quantified after 30 min reaction, to ~5% yield, assuming an ε_{260} of 1.99×10^4 $M^{-1}cm^{-1}$.[41,42]

Photolysis of 8d in aqueous solutions

The analogue **8d** was photolyzed in H_2O for 25 min, under oxygen, and the photolyzed mixture was incubated overnight in the dark at ambient temperature. Analysis by LC-MS revealed that the major peak (at 17.9 min, Fig. S2, S3, see ESI†) corresponded to N^2-(3-hydroxypropanoyl)guanosine, suggesting that homolytic cyclopropane ring opening, followed by reaction with molecular oxygen and subsequent rearrangement to **14d** (Scheme 5), has occurred. Guanosine (**3d**) is also one of the photolysis products (peak at 14 min) in accord with the literature report.[35]



Scheme 5 Major products observed in the photolysis of **8d**.

Transient spectroscopy

Direct evidence of G(-H)• generation was obtained through time-resolved experiments. Laser flash photolysis of **2c** was performed by excitation at 308 nm, in acetonitrile:water (4:1) mixture, under an anaerobic atmosphere. *N*-Hydroxypyrid-2(1*H*)-one was also photolyzed, as a reference. After 2 microseconds, two contributions were distinguishable in the spectrum of **2c**. One of them corresponded to the 2-pyridyloxyl radical,[43,44] as assessed by comparison with the reference, and the other one displayed the typical features of the neutral guanine radical. The spectrum of this radical was obtained by subtraction of the 2-pyridyloxyl radical from the signal, but it was also obtained directly after longer delay times (Fig. 2); its assignment to the guanine radical was based on the characteristic transient absorption bands at *ca.* 310 nm, 390 nm and 520 nm.[11] The quantum yield of this radical was roughly estimated at 0.2, and its decay was biphasic. A relatively fast component with $\tau = 25$ μs was assigned to the caged G(-H)•/2-pyridyloxyl radical pair, whereas the longer lived (>1 ms) residual absorption was attributed to free G(-H)• radical. Quenching of the obtained guanine radical by oxygen, isopropanol or thiols[45] was not observed; thus the rate constant for these processes must be lower than 10^6 M^{-1} s^{-1} as has been reported.[21]

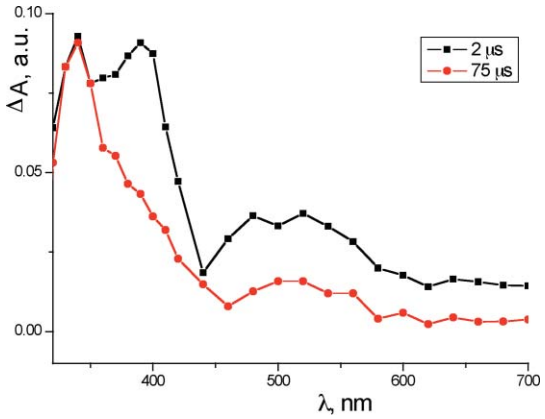


Fig. 2 Transient absorption spectra obtained 2 μs (squares) and 75 μs (circles) after excitation with 308 nm excimer laser pulse, generated upon photolysis of **2c**, in a deaerated acetonitrile:water 4:1 solution.

Discussion

The main products from the photolysis of **2c**, namely 2′-deoxyguanosine and 2-hydroxypyridine could have been produced by one of two possible routes. The first possible route would involve simple hydrolysis of either **2c** or more likely its corresponding excited state, since **2c** was proven, from the stability studies, to be stable in natural-pH aqueous solutions. This route was disproven since the photolysis of **2c** in $H_2{}^{18}O$ did not lead to any ^{18}O incorporation in either 2′deoxyguanosine or 2-hydroxypyridine as shown by LC-MS studies on the photolysis products. The lack of ^{18}O incorporation in any of the two major products also rules out the possibility that they are formed from a recombination reaction between the two radicals followed by hydrolysis of the adducts formed. The second possible route would involve homolysis of the N–O bond in **2c**, as expected, followed by one electron reduction of the resulting radical species. This could be effected through disproportionation reactions, and this possibility would require further oxidation of one of the two components, resulting in yields of <50% for the reduced species. The yields observed (38% for dGuo and 50% for 2-hydroxypyridine) point in this direction, and this issue deserves further study. Finally, hydrogen abstraction from the 2-deoxyribose moiety of the nucleosidic products of the photolysis mixture by a delocalized radical, such as G(-H)$^{\bullet}$ or 2-pyridyloxyl, is expected to be endothermic and therefore could be ruled out. The presence of dIz, a known dGuo oxidation product, in the oxygenated photolysis mixture, albeit in low yield, is a first indication of the intermediacy of G(-H)$^{\bullet}$.

Nevertheless, more direct and positive means of observing G(-H)$^{\bullet}$ were necessary in order to confirm the suitability of our system for the independent generation of G(-H)$^{\bullet}$. Therefore, we utilized the radical trap **8d**, following the work by Saito and coworkers.[35] According to this work, a G$^{+\bullet}$ containing a cyclopropylamino group at the 2-position leads, in the presence of oxygen and through the intermediacy of an *N*-centered radical cation, to the ring opened product **14d**, thus trapping the electron hole centered on this specific guanine. Photolysis of the suitably modified guanosine **8d**, in our system, did lead to the same product **14d**, thus confirming the intermediacy of G(-H)$^{\bullet}$. If a neutral *N*-centered radical is involved in this case, ring opening is expected to proceed with a rate of around 10^6 s^{-1} as has been estimated for a secondary cyclopropylamine analogue,[46] although a lower estimate for *N*-cyclopropylaniline was recently reported.[47]

The final direct evidence for the independent generation of G(-H)$^{\bullet}$ came from time-resolved experiments, where the characteristic transient absorption bands of G(-H)$^{\bullet}$ were observed. This radical, generated in a significant yield, recombines with the 2-pyridyloxyl radical within the cage (fast decay component) or escapes to give a longer lived free radical, which is able to react further leading to products. The guanine radical is a well characterized species that has been previously observed by transient spectroscopy,[11,12,48-50] but has not been directly generated by homolysis of an excited nucleoside derivative.

Conclusions

We have synthesized and characterized novel photolabile 6-[(1-oxido-2-pyridinyl)oxo]-6-deoxy- and 2′,6-dideoxy-guanosine derivatives capable of generating the neutral guanine radical upon photolysis. The generation of G(-H)$^{\bullet}$ was confirmed for **2c** through continuous photolysis product analysis and trapping studies, as well as laser flash photolysis experiments, and involves the homolysis of the N–O bond and the formation of the neutral guanine radical. In comparison to the previously studied N-HPT derivative,[31] the present system has exhibited, as expected, simpler photochemistry generating the desired G(-H)$^{\bullet}$. It should be emphasized that this system does not require molecular oxygen and, therefore, no other reactive oxygen species is formed. Nevertheless, the possible role of the 2-pyridyloxyl radical in the fate of G(-H)$^{\bullet}$ deserves further study. A systematic characterization of all minor products in the photolysis of **2c** and utilization of this system for the site-specific formation of G(-H)$^{\bullet}$ in synthetic oligonucleotides under very mild photolytic conditions is currently under study and will be reported in due course.

Experimental

Synthetic protocols

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-O^6-(2-oxopyridin-1(2*H*)-yl)-2′-deoxyguanosine (2a). A solution of **1a** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 1.87 g, 2.76 mmol) and DABCO (619 mg, 5.52 mmol) in anhydrous dioxane (25 mL) was stirred for 1 h under Ar in room temperature. Then, 2-oxopyridine *N*-oxide (1.53 g, 13.79 mmol) was added and after 30 min DBU (624 μL, 4.14 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with ethyl acetate (20 mL) and extracted first with water (15 mL), then with a sat. $NaHCO_3$ solution (15 mL) and finally with brine (15 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 96:4 CH_2Cl_2–EtOH to give 1.56 g of the product (94%). R_f: 0.35 in CH_2Cl_2–EtOH 96:4. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.07 [s, 12H, $Si(CH_3)_2$] 0.88 [s, 18H, $SiC(CH_3)_3$] 2.29–2.59 (m, 2H, H-2β, H-2α) 3.72 (dd, 1H, $J_{H4'} = 3.4$, $J_{H5'} = 11.6$ Hz, H-5″) 3.80 (dd, 1H, $J_{H4'} = 3.4$, $J_{H5''} = 11.6$ Hz, H-5′) 3.95 (dd, 1H, $J_{H3'} = 6.2$, $J_{H5'} = 3.4$ Hz, H-4′) 4.55 (dd, 1H, $J_{H4'} = 6.2$, $J_{H2\alpha} = 3.4$ Hz, H-3′) 4.96 (bs, 2H, H-2) 6.18 (dt, 1H, $J = 6.9$, 6.8, 1.5 Hz, OPyH-5) 6.28 (dd, 1H, $J_{H2\beta} = 6.2$, $J_{H2\alpha} = 6.2$ Hz, H-1′) 6.71 (dd, 1H, $J = 9.2$,

1.5 Hz, OPyH-3) 7.36 (dt, 1H, J = 9.2, 6.8, 2.0 Hz, OPyH-4) 7.51 (dd, 1H, J = 6.8, 2.0 Hz, OPyH-6) 8.02 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ −5.56, −5.47, −4.89, −4.74 [$Si(CH_3)_2$] 17.9, 18.3 [$SiC(CH_3)_3$] 25.7, 25.9 [$SiC(CH_3)_3$] 41.0 (C-2′) 62.6 (C-5′) 71.5 (C-3′) 83.8 (C-1′) 87.6 (C-4′) 104.8 (OPyC-5) 113.4 (C-5) 123.0 (OPyC-3) 136.0 (OPyC-6) 139.0 (C-8) 139.3 (OPyC-4) 155.1 (C-6) 157.4 (C-2) 158.5 (C-4) 158.9 ppm (OPyC-O). $[\alpha]_D^{25}$ = −7.2 (C = 1 M, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) 3427, 2958, 2934, 2890, 1674(C=O), 1632, 1600, 1578, 1463, 835. UV ($CHCl_3$, nm) 288 (ε = 13842 $M^{-1}cm^{-1}$).

***O*^{6}-(2-Oxopyridin-1(2*H*)-yl)-2′-deoxyguanosine (2c).** To a solution of **2a** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 100 mg, 0.17 mmol) in anhydrous THF (2 mL) were added activated molecular sieves (50 mg) and a 1 M solution of TBAF in THF (556 µL, 0.556 mmol). The reaction mixture was stirred for 1 h at room temperature under Ar, filtrated and washed with MeOH (5 mL). The filtrate was evaporated to dryness and the residue was purified by column chromatography using 95:5 CH_2Cl_2–EtOH to give 43 mg (70%) of the product. R_f: 0.13 in CH_2Cl_2–EtOH 96:4. ^{1}H-NMR (400 MHz, DMSO-d6) δ 2.22–2.28 (m, 1H, H-2β) 2.57–2.64 (m, 1H, H-2α) 3.48–3.60 (m, 2H, H-5′, H-5″) 3.83 (dd, 1H, $J_{H3'}$=7.4, $J_{H5'}$=4.5 Hz, H-4′) 4.37 (dd, 1H, $J_{H4'}$=7.4, $J_{H2\alpha}$=3.3 Hz, H-3′) 6.24 (dd, 1H, $J_{H2\beta}$=7.2, $J_{H2\alpha}$=7.2 Hz, H-1′) 6.32 (dt, 1H, J = 6.8, 6.8, 1.6 Hz, OPyH-5) 6.63 (dd, 1H, J = 9.3, 1.6 Hz, OPyH-3) 6.65 (s, 2H, H-2) 7.53 (dt, 1H, J = 9.3, 6.8, 1.9 Hz, OPyH-4) 8.11 (dd, 1H, J = 6.8, 1.9 Hz, OPyH-6) 8.25 ppm (s, 1H, H-8). ^{13}C-NMR (100 MHz, DMSO-d6) δ 40.5 (C-2′) 61.5 (C-5′) 70.6 (C-3′) 82.8 (C-1′) 87.6 (C-4′) 104.7 (OPyC-5) 111.5 (C-5) 121.7 (OPyC-3) 137.6 (OPyC-6) 139.5 (C-8) 140.0 (OPyC-4) 155.4 (C-6) 156.5 (C-2) 158.6 (C-4) 159.2 ppm (OPyC-O). UV (H_2O, nm) 214 (ε = 19924 $M^{-1}cm^{-1}$), 246 (ε = 7098 $M^{-1}cm^{-1}$), 291 (ε = 10810 $M^{-1}cm^{-1}$). HR-ESI-MS for $C_{15}H_{17}N_6O_5^+$ $[M + H]^+$, calcd 361.1255; found 361.1245.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*O*^{6}-(2-oxopyridin-1(2*H*)-yl)guanosine (2b). A solution of **1b** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 100 mg, 0.12 mmol) and DABCO (28 mg, 0.25 mmol) in anhydrous dioxane (1.5 mL) was stirred for 1 h under Ar at room temperature. Then, 2-oxopyridine *N*-oxide (69 mg, 0.62 mmol) was added and after 30 min DBU (28 µL, 0.19 mmol) was added and the reaction mixture was stirred in room temperature for 3 h. The reaction mixture was diluted with ethyl acetate (10 mL) and extracted first with water (5 mL), then with a sat. $NaHCO_3$ solution (5 mL) and finally with brine (5 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 7:3 ethyl acetate–hexane to give 89 mg (91%) of the product. R_f: 0.42 in ethyl acetate–hexane 7:3. ^{1}H-NMR (200 MHz, $CDCl_3$) δ −0.05, 0.00, 0.07, 0.09, 0.12, 0.13 [s, 3H, $Si(CH_3)_2$] 0.84, 0.90, 0.94 [s, 9H, $SiC(CH_3)_3$] 3.77 (dd, 1H, $J_{H4'}$ = 2.2, $J_{H5'}$ = 11.4 Hz, H-5″) 4.00 (dd, 1H, $J_{H4'}$ = 2.2, $J_{H5''}$ = 11.4 Hz, H-5′) 4.05–4.12 (m, 1H, H-4′) 4.29 (dd, 1H, $J_{H2'}$ = 4.2, $J_{H4'}$ = 4.2 Hz, H-3′) 4.39 (dd, 1H, $J_{H1'}$ = 4.2, $J_{H3'}$ = 4.2 Hz, H-2′) 4.93 (bs, 2H, NH_2) 5.89 (d, 1H, $J_{H2'}$ = 4.2 Hz, H-1′) 6.19 (dt, 1H, J = 7.0, 6.6, 1.5 Hz, OPyH-5) 6.73 (dd, 1H, J = 9.2, 1.5 Hz, OPyH-3) 7.38 (dt, 1H, J = 9.2, 6.6, 2.0 Hz, OPyH-4) 7.53(dd, 1H, J = 7.0, 2.0 Hz, OPyH-6) 8.16 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ −5.43, −5.38, −4.86, −4.76, −4.26 [$Si(CH_3)_2$] 17.9, 18.0, 18.5 [$SiC(CH_3)_3$] 25.7, 25.8, 26.1 [$SiC(CH_3)_3$] 61.9 (C-5′) 71.0 (C-3′) 76.2 (C-2′) 84.4 (C-4′) 88.4 (C-1′) 104.8 (OPyC-5) 113.5 (C-5) 123.1 (OPyC-3) 136.1 (OPyC-6) 139.0 (C-8) 139.6 (OPyC-4) 155.3 (C-6) 157.5 (C-2) 158.6 (C-4) 158.9 ppm (OPyC-O). $[\alpha]_D^{25}$ = −5.6 (C = 1 M, $CHCl_3$). IR ($CHCl_3$, cm^{-1}) 3429, 2959, 2934, 2860, 1674 (C=O), 1632, 1600, 1578, 1509, 1202, 836, 716. UV ($CHCl_3$, nm) 246 (ε = 9543 $M^{-1}cm^{-1}$), 289 (ε = 13898 $M^{-1}cm^{-1}$).

***O*^{6}-(2-Oxopyridin-1(2*H*)-yl)guanosine (2d).** To a solution of **2b** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 380 mg, 0.52 mmol) in anhydrous THF (8 mL) were added activated molecular sieves (150 mg) and a 1 M solution of TBAF in THF (1.74 mL, 1.74 mmol). The reaction mixture was stirred for 1 h at room temperature under Ar, filtrated and washed with MeOH (15 mL). The filtrate was evaporated to dryness and the residue was purified by column chromatography using 93:7 CH_2Cl_2–EtOH to give 149 mg (75%) of the product. R_f: 0.30 in CH_2Cl–EtOH 9:1. ^{1}H-NMR (200 MHz, D_2O) δ 3.75 (dd, 1H, $J_{H4'}$ = 3.4, $J_{H5'}$ = 13.0 Hz, H-5″) 3.85 (dd, 1H, $J_{H4'}$ = 3.4, $J_{H5''}$=13.0 Hz, H-5′) 4.17 (dd, 1H, $J_{H3'}$ = 5.4, $J_{H5'}$ = 3.4 Hz, H-4′) 4.36 (dd, 1H, $J_{H4'}$ = 5.4, $J_{H2'}$ = 5.4 Hz, H-3′) 4.69 (dd, 1H, $J_{H1'}$ = 5.4, $J_{H3'}$ = 5.4 Hz, H-2′) 5.90 (d, 1H, $J_{H2'}$ = 5.4 Hz, H-1′) 6.56 (dt, 1H, OPyH-5) 6.77 (dd, 1H, OPyH-3) 7.67 (dt, 1H, OPyH-4) 7.89 (dd, 1H, OPyH-6) 8.14 ppm (s, 1H, H-8). ^{13}C-NMR (100 MHz, DMSO-d6) δ 61.3 (C-5′) 70.4 (C-3′) 75.7 (C-2′) 84.5 (C-4′) 87.9 (C-1′) 104.8 (OPyC-5) 112.5 (C-5) 122.5 (OPyC-3) 137.1 (OPyC-6) 139.2 (C-8) 139.8 (OPyC-4) 155.2 (C-6) 156.8 (C-2) 158.6 (C-4) 159.0 ppm (OPyC-O). UV (H_2O, nm) 245 (ε = 8532 $M^{-1}cm^{-1}$), 292 (ε = 12749 $M^{-1}cm^{-1}$). HR-ESI-MS for $C_{15}H_{17}N_6O_6^+$ $[M + H]^+$, calcd 377.1204; found 377.1188.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*O*^{6}-[2-(4-nitrophenyl)-ethyl]-guanosine (4b). To a solution of 2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)-guanosine (**3b**) (1.00 g, 1.60 mmol) and Ph_3P (462 mg, 1.76 mmol) in anhydrous dioxane (4 mL) were added at 0 °C and under Ar, DIAD (347 µL, 1.76 mmol) and 4-nitrophenyl-ethanol (294 mg, 1.76 mmol). The reaction mixture was stirred at room temperature for 18 h, diluted with CH_2Cl_2 (15 mL) and extracted first with water (15 mL), then with a sat. $NaHCO_3$ solution (15 mL) and finally with brine (15 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 7:3 ethyl acetate–hexane to afford 1.04 g (84%) of the product as a yellowish powder. R_f: 0.65 in ethyl acetate–hexane 7:3. ^{1}H-NMR (200 MHz, $CDCl_3$) δ −0.22, −0.06 [s, 3H, $Si(CH_3)_2$] 0.10, 0.12 [s, 6H, $Si(CH_3)_2$] 0.77, 0.91, 0.94 [s, 9H, $SiC(CH_3)_3$] 3.27 (t, 2H, J_{CH2O} = 7.0 Hz, CH_2Ph) 3.75 (dd, 1H, $J_{H4'}$ = 2.2, $J_{H5'}$ = 11.2 Hz, H-5″) 3.94 (dd, 1H, $J_{H4'}$ = 2.2, $J_{H5''}$ = 11.2 Hz, H-5′) 4.07 (dd, 1H, $J_{H3'}$ = 5.6, $J_{H5'}$ = 2.2 Hz, H-4′) 4.25 (dd, 1H, $J_{H2'}$ = 5.6, $J_{H4'}$ = 5.6 Hz, H-3′) 4.47 (dd, 1H, $J_{H1'}$ = 5.6, $J_{H3'}$ = 5.6 Hz H-2′) 4.71 (t, 2H, J_{CH2Ph} = 7.0 Hz, CH_2O) 5.21 (bs, 2H, H-2) 5.91 (d, 1H, $J_{H2'}$ = 5.6 Hz, H-1′) 7.46 (d, J_{CH} = 8.4 Hz, 2H, Ph) 7.95 (s, 1H, H-8) 8.14 ppm (d, J_{CH} = 8.4 Hz, 2H, Ph). ^{13}C-NMR (50 MHz, $CDCl_3$) δ −5.45, −5.40, −5.13, −4.76, −4.73, −4.34 [$Si(CH_3)_2$] 17.9, 18.0, 18.5 [$SiC(CH_3)_3$] 25.6, 25.8, 26.0 [$SiC(CH_3)_3$] 35.2 (CH_2Ph) 62.7 (C-5′) 66.0 (CH_2O) 72.2 (C-3′) 76.4 (C-2′) 85.5 (C-4′) 87.3 (C-1′) 115.3 (C-5) 123.6 (2 × Ph) 129.9 (2 × Ph) 137.8 (C-8) 146.0 ($C-NO_2$) 146.8 (Ph) 154.0 (C-4) 159.1 (C-2) 160.6 ppm (C-6). *m/z* (ESI) 797.5 $(M + Na)^+$.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*O*^{6}-[2-(4-nitrophenyl)-ethyl]-2-fluoro-inosine (5b). A solution of **4b** (775 mg, 1.00 mmol) in anhydrous THF (2 mL) was added to a solution of HF/pyridine 70/30 (2.5 mL), pyridine (1 mL) and THF (1 mL) at –25 °C under Ar. Immediately, *t*-Bu-ONO (714 µL, 6.00 mmol) was added and the reaction mixture was stirred at –25 °C for 7 min exactly. The reaction mixture was then poured into ice-water (25 mL) and extracted with CH_2Cl_2 (5 × 15 mL). The combined organic layers were extracted with water (25 mL), then with a sat. $NaHCO_3$ solution (25 mL) and finally with brine (25 mL). The organic layer was dried over sodium sulfate, evaporated to dryness and the residue was purified by column chromatography using 5:5 ethyl acetate–hexane to afford 550 mg (71%) of the product. R_f: 0.75 in ethyl acetate–hexane 7:3. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.25, –0.05 [s, 3H, $Si(CH_3)_2$] 0.08, 0.12 [s, 6H, $Si(CH_3)_2$] 0.78, 0.91, 0.93 [s, 9H, $SiC(CH_3)_3$] 3.31 (t, 2H, J_{CH2O} = 6.8 Hz, CH_2Ph) 3.77 (dd, 1H, $J_{H4'}$ = 2.6, $J_{H5'}$ = 11.4 Hz, H-5″) 4.00 (dd, 1H, $J_{H4'}$ = 2.6, $J_{H5''}$ = 11.4 Hz, H-5′) 4.11 (dd, 1H, $J_{H3'}$ = 5.0, $J_{H5'}$ = 2.6 Hz, H-4′) 4.28 (dd, 1H, $J_{H2'}$ = 5.0, $J_{H4'}$ = 5.0 Hz, H-3′) 4.56 (dd, 1H, $J_{H1'}$ = 5.0, $J_{H3'}$ = 5.0 Hz, H-2′) 4.82 (t, 2H, J_{CH2Ph} = 6.8 Hz, CH_2O) 5.96 (d, 1H, $J_{H2'}$ = 5.0 Hz, H-1′) 7.47 (d, J_{CH} = 8.6 Hz, 2H, Ph) 8.14 (d, J_{CH} = 8.6 Hz, 2H, Ph) 8.26 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ –5.21, –4.46, –4.21 [$Si(CH_3)_2$] 18.0, 18.3, 18.7 [$SiC(CH_3)_3$] 25.8, 25.0, 26.3 [$SiC(CH_3)_3$] 35.2 (CH_2Ph) 62.7 (C-5′) 67.6 (CH_2O) 72.1 (C-3′) 76.2 (C-2′) 85.9 (C-4′) 88.7 (C-1′) 118.3 (C-5) 124.0 (2 × Ph) 130.1 (2 × Ph) 142.0 (C-8) 145.5 (C-NO_2) 147.1 (Ph) 155.9 (C-4) 160.1 (C-2) 162.0 ppm (C-6). ^{19}F-NMR (188 MHz, $CDCl_3$) δ –50.23 ppm.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-cyclopropyl-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine. To a solution of **5b** (550 mg, 0.70 mmol) and Et_3N (90 µL, 0.85 mmol) in anhydrous DMF (3.5 mL) was added cyclopropylamine (58 µL, 0.85 mmol) under Ar and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with $CHCl_3$ (35 mL) and extracted first with water (20 mL), then with a sat. $NaHCO_3$ solution (20 mL) and finally with brine (20 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was used without further purification for the next step. R_f: 0.72 in ethyl acetate–hexane 7:3. *m/z* (ESI) 813.5 [100, $(M - H^+)^-$].

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-cyclopropyl-guanosine (6b). To a solution of 2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-cyclopropyl-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanosine (we consider that the previous reaction was quantitative, 570 mg, 0.70 mmol) in anhydrous pyridine (8 mL) was added DBU 0.5 M (600 µL, 4.00 mmol) under Ar and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with $CHCl_3$ (20 mL) and extracted first with water (20 mL), then with a sat. $NaHCO_3$ solution (20 mL) and finally with brine (20 mL). The organic layer was dried over sodium sulfate, evaporated to dryness and the residue was purified by column chromatography using 97:3 $CHCl_3$–MeOH to afford 250 mg of the product as a white solid (overall yield 53%). R_f: 0.29 in $CHCl_3$–MeOH 97:3. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.12, –0.01 [s, 3H, $Si(CH_3)_2$] 0.11 [s, 12H, $Si(CH_3)_2$] 0.76–0.86 (m, 4H, 2 × CH_2) 0.83 [s, 9H, $SiC(CH_3)_3$] 0.93 [s, 18H, $SiC(CH_3)_3$] 2.75 (m, 1H, CH) 3.80 (dd, 1H, $J_{H4'}$ = 2.6, $J_{H5'}$ = 11.4 Hz, H-5″) 3.98 (dd, 1H, $J_{H4'}$ = 2.6, $J_{H5''}$ = 11.4 Hz, H-5′) 4.06 (dd, 1H, $J_{H3'}$ = 4.6, $J_{H5'}$ = 2.6 Hz, H-4′) 4.30 (dd, 1H, $J_{H2'}$ = 4.6, $J_{H4'}$ = 4.6 Hz, H-3′) 4.62 (dd, 1H, $J_{H1'}$ = 4.6, $J_{H3'}$ = 4.6 Hz, H-2′) 5.90 (d, 1H, $J_{H2'}$ = 4.6 Hz, H-1′) 7.97 (s, 1H, H-8) 11.8 ppm (bs, 1H, H-2). ^{13}C-NMR (50 MHz, $CDCl_3$) δ –5.38, –5.32, –4.78, –4.68, –4.42 [$Si(CH_3)_2$] 6.89, 7.23 (2 × CH_2) 17.9, 18.1, 18.5 [$SiC(CH_3)_3$] 23.8 (CH) 25.7, 25.8, 26.0 [$SiC(CH_3)_3$] 62.4 (C-5′) 71.7 (C-3′) 75.3 (C-2′) 84.9 (C-4′) 87.9 (C-1′) 116.9 (C-5) 135.5 (C-8) 156.7 (C-4) 157.4 (C-2) 158.7 ppm (C-6).

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-cyclopropyl-*O*^{6}-(mesitylsulfonyl)guanosine (7b). To a solution of **6b** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 250 mg, 0.38 mmol) and Et_3N (210 µl, 1.50 mmol) in 2 mL of anhydrous CH_2Cl_2 was added DMAP (12 mg, 0.09 mmol) and mesitylenechloride (180 mg, 0.83 mmol). The reaction mixture was stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the residue was dissolved in 15 mL of $CHCl_3$. The $CHCl_3$ solution was extracted first with water (15 mL), then with a sat. $NaHCO_3$ solution (15 mL) and finally with brine (15 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 15:85 ethyl acetate–hexane to give 210 mg of the product (66%).

R_f: 0.29 in ethyl acetate–hexane 15:85. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.22, –0.05 [s, 3H, $Si(CH_3)_2$] 0.09 [s, 12H, $Si(CH_3)_2$] 0.44–0.47 (m, 2H, CH_2) 0.68–0.71 (m, 2H, CH_2) 0.77 [s, 9H, $SiC(CH_3)_3$] 0.91 [s, 18H, $SiC(CH_3)_3$] 2.28 (s, 3H, CH_3) 2.54–2.62 (m, 1H, CH) 2.71 (s, 6H, 2 × CH_3) 3.77 (dd, 1H, $J_{H4'}$ = 2.4, $J_{H5'}$ = 11.0 Hz, H-5″) 3.98 (dd, 1H, $J_{H4'}$ = 2.4, $J_{H5''}$ = 11.0 Hz, H-5′) 4.07 (dd, 1H, $J_{H3'}$ = 5.0, $J_{H5'}$ = 2.4 Hz, H-4′) 4.28 (dd, 1H, $J_{H2'}$ = 5.0, $J_{H4'}$=5.0 Hz, H-3′) 4.60 (dd, 1H, $J_{H1'}$ = 5.0, $J_{H3'}$ = 5.0 Hz, H-2′) 5.12 (bs, 1H, H-2) 5.92 (d, 1H, $J_{H2'}$ = 5.0 Hz, H-1′) 6.94 (s, 2H, Ph) 8.01 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ –5.46, –4.99, –4.76, –4.50 [$Si(CH_3)_2$] 7.17, 7.35 (2 × CH_2) 17.7, 18.0, 18.4 [$SiC(CH_3)_3$] 21.0 (CH_3) 22.7 (2 × CH_3) 24.2 (CH) 25.6, 25.7, 26.0 [$SiC(CH_3)_3$] 62.4 (C-5′) 71.8 (C-3′) 75.2 (C-2′) 85.0 (C-4′) 87.8 (C-1′) 116.5 (C-5) 129.7 (C-8) 131.5 (2 × m-C) 139.6 (C-S) 140.1, 140.3 (o-C) 143.6 (p-C) 154.6 (C-4) 155.8 (C-2) 159.0 ppm (C-6).

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-cyclopropyl-*O*^{6}-(2-oxopyridin-1(2*H*)-yl)guanosine (8b). A solution of **7b** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 95 mg, 0.11 mmol) and DABCO (25 mg, 0.22 mmol) in anhydrous dioxane (2 mL) was stirred for 1 h under Ar at room temperature. Then, 2-oxopyridine *N*-oxide (62 mg, 0.56 mmol) was added and after 30 min DBU (25 µL, 0.17 mmol) was added and the reaction mixture was stirred in room temperature for 3 h. The reaction mixture was diluted with $CHCl_3$ (15 mL) and extracted with a sat. $NaHCO_3$ solution (2 × 15 mL) and brine (15 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 95:5 $CHCl_3$–MeOH to afford 80 mg of the product (94%) as a yellowish solid. R_f: 0.35 in $CHCl_3$–MeOH 95:5. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.03, 0.01, 0.08, 0.09, 0.11, 0.12, 0.13 [s, 3H, $Si(CH_3)_2$] 0.39–0.47 (m, 2H, CH_2) 0.61–0.64 (m, 2H, CH_2) 0.84, 0.91, 0.93 [s, 9H, $SiC(CH_3)_3$] 2.53–2.56 (m, 1H, CH) 3.78 (dd, 1H, $J_{H4'}$ = 2.2, $J_{H5'}$ = 11.4 Hz, H-5″) 4.04 (dd, 1H, $J_{H4'}$ = 2.2, $J_{H5''}$ = 11.4 Hz, H-5′) 4.08–4.10 (m, 1H, H-4′) 4.33 (dd, 1H, $J_{H2'}$ = 4.4, $J_{H4'}$ = 4.4 Hz, H-3′) 4.48 (dd, 1H, $J_{H1'}$ = 4.4, $J_{H3'}$ = 4.4 Hz, H-2′) 5.14 (bs, 2H, NH_2) 5.93 (d, 1H, $J_{H2'}$ = 4.4 Hz, H-1′) 6.14 (dt,

1H, OPyH-5) 6.73 (dd, 1H, OPyH-3) 7.35 (dt, 1H, OPyH-4) 7.51 (dd, 1H, OPyH-6) 8.14 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ –5.46, –5.37, –4.81, –4.55, –4.36 [$Si(CH_3)_2$] 7.09, 7.24 (2 × CH_2) 17.9, 18.0, 18.5 [$SiC(CH_3)_3$] 24.1 (CH) 25.7, 25.8, 26.1 [$SiC(CH_3)_3$] 61.8 (C-5′) 70.8 (C-3′) 75.6 (C-2′) 84.0 (C-4′) 88.4 (C-1′) 104.6 (OPyC-5) 112.1 (C-5) 123.1 (OPyC-3) 136.2 (OPyC-6) 138.9 (OPyC-4) 139.6 (C-8) 155.3 (C-2) 157.5 (C-6) 158.4 (OPyC-O) 159.1 ppm (C-4).

N^2-Cyclopropyl-O^6-(2-oxopyridin-1(2*H*)-yl)guanosine (8d). To a solution of **8b** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 38 mg, 0.05 mmol) in anhydrous THF (1 mL) were added activated molecular sieves (25 mg) and a 1 M solution of TBAF in THF (250 μL, 0.25 mmol). The reaction mixture was stirred for 1 h at room temperature under Ar, filtrated and washed with MeOH (15 mL). The filtrate was evaporated to dryness and the residue was purified by column chromatography using 9:1 CH_2Cl_2–EtOH to give 15 mg of the product (75%). R_f: 0.19 in $CHCl_3$–MeOH 93:7. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.34–0.38 (m, 2H, CH_2) 0.46–0.52 (m, 2H, CH_2) 2.37–2.39 (m, 1H, CH) 3.65 (dd, 1H, $J_{H4'}$ = 2.6, $J_{H5'}$ = 11.2 Hz, H-5″) 4.04 (dd, 1H, $J_{H4'}$ = 2.6, $J_{H5''}$ = 11.2 Hz, H-5′) 4.17 (m, 1H, H-4′) 4.28–4.30 (m, 1H, H-3′) 4.73 (m, 1H, H-2′) 5.71 (d, 1H, $J_{H2'}$ = 6.8 Hz, H-1′) 6.27 (dt, 1H, OPyH-5) 6.70 (dd, 1H, OPyH-3) 7.42 (dt, 1H, OPyH-4) 7.57 (dd, 1H, OPyH-6) 7.78 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 6.92, 7.26 (2 × CH_2) 24.2 (CH) 61.5 (C-5′) 70.9 (C-3′) 75.9 (C-2′) 84.6 (C-4′) 88.0 (C-1′) 104.6 (OPyC-5) 112.3 (C-5) 122.7 (OPyC-3) 137.4 (OPyC-6) 139.0 (C-8) 139.7 (OPyC-4) 155.5 (C-6) 157.0 (C-2) 158.9 (C-4) 159.3 ppm (OPyC-O). UV (H_2O, nm) 247 (ε = 7956 $M^{-1}cm^{-1}$), 292 (ε = 13412 $M^{-1}cm^{-1}$). HR-ESI-MS for $C_{18}H_{21}N_6O_6^+$ $[M + H]^+$, calcd 417.1517; found 417.1534.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-O^6-(mesitylsulfonyl)-2-fluoro-inosine (9b). A solution of **1b** (400 mg, 0.50 mmol) in anhydrous THF (2.4 mL) was added to a solution of HF/pyridine 70/30 (4 mL), pyridine (1.2 mL) and THF (1.2 mL) at –25 °C under Ar. Immediately, *t*-Bu-ONO (354 μL, 3.00 mmol) was added and the reaction mixture was stirred at –25 °C for 7 min exactly. The reaction mixture was then poured into ice-water (25 mL) and extracted with CH_2Cl_2 (5 × 15 mL). The combined organic layers were extracted with water (25 mL), then with a sat. $NaHCO_3$ solution (25 mL) and finally with brine (25 mL). The organic layer was dried over sodium sulfate, evaporated to dryness and the residue was purified by column chromatography using 2% EtOH in CH_2Cl_2 to afford 176 mg (44%) of the product. R_f: 0.75 in CH_2Cl_2–EtOH 98:2. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.25, –0.04 [s, 3H, $Si(CH_3)_2$] 0.09, 0.13 [s, 6H, $Si(CH_3)_2$] 0.78 [s, 9H, $SiC(CH_3)_3$] 0.92, 0.94 [s, 18H, $SiC(CH_3)_3$] 2.31 (s, 3H, CH_3) 2.77 (s, 6H, 2 × CH_3) 3.77 (dd, 1H, $J_{H4'}$ = 3.0, $J_{H5'}$ = 12.0 Hz, H-5″) 3.99 (dd, 1H, $J_{H4'}$ = 3.0, $J_{H5''}$ = 12.0 Hz, H-5′) 4.12 (dd, 1H, $J_{H3'}$ = 5.0, $J_{H5'}$ = 3.0 Hz, H-4′) 4.28 (dd, 1H, $J_{H2'}$ = 5.0, $J_{H4'}$ = 5.0 Hz, H-3′) 4.52 (dd, 1H, $J_{H1'}$ = 5.0, $J_{H3'}$ = 5.0 Hz, H-2′) 5.96 (d, 1H, $J_{H2'}$ = 5.0 Hz, H-1′) 6.99 (s, 2H, Ph) 8.40 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ –5.56, –5.52, –5.28, –4.88, –4.81, –4.53 [$Si(CH_3)_2$] 17.7, 17.9, 18.4 [$SiC(CH_3)_3$] 21.0 (CH_3) 22.6 (2 × CH_3) 25.4, 25.7, 25.9 [$SiC(CH_3)_3$] 62.2 (C-5′) 71.6 (C-3′) 76.0 (C-2′) 85.7 (C-4′) 88.6 (C-1′) 105.9 (C-5) 131.7 (2 × m-C) 140.8 (C-8) 143.8, 143.9 (o-C) 144.4 (p-C) 151.1 (C-S) 154.4 (C-4) 158.7 (C-6) 160.7 ppm(C-2). ^{19}F-NMR: (188 MHz, $CDCl_3$) δ –49.74 ppm. *m/z* (ESI) 811.3 [100, $(M + H)^+$].

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-2-fluoro-O^6-(2-oxopyridin-1(2*H*)-yl)inosine (10b). A solution of **9b** (dried by heating over phosphorus pentoxide *in vacuo* at 56 °C for 2 h before use, 120 mg, 0.15 mmol) and DABCO (34 mg, 0.30 mmol) in anhydrous dioxane (2 mL) was stirred for 1 h under Ar at room temperature. Then, 2-oxopyridine *N*-oxide (83 mg, 0.75 mmol) was added and after 30 min DBU (34 μL, 0.23 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with $CHCl_3$ (15 mL) and extracted with a sat. $NaHCO_3$ solution (15 mL) and brine (2 × 15 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 6:4 ethyl acetate–hexane to afford 102 mg of the product (96%). R_f: 0.43 in ethyl acetate–hexane 6:4. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.05, 0.03, 0.06, 0.09, 0.14, 0.16 [s, 3H, $Si(CH_3)_2$] 0.85, 0.92, 0.96 [s, 9H, $SiC(CH_3)_3$] 3.80 (dd, 1H, $J_{H4'}$ = 1.8, $J_{H5'}$ = 11.6 Hz, H-5″) 4.06 (dd, 1H, $J_{H4'}$ = 1.8, $J_{H5''}$ = 11.6 Hz, H-5′) 4.13–4.18 (m, 1H, H-4′) 4.32 (dd, 1H, $J_{H2'}$ = 5.0, $J_{H4'}$ = 5.0 Hz, H-3′) 4.46 (dd, 1H, $J_{H1'}$ = 5.0, $J_{H3'}$ = 5.0 Hz, H-2′) 5.99 (d, 1H, $J_{H2'}$ = 5.0 Hz, H-1′) 6.26 (dt, 1H, *J* = 8.4, 7.4, 1.6 Hz, OPyH-5) 6.78 (dd, 1H, *J* = 9.4, 1.6 Hz, OPyH-3) 7.44 (dt, 1H, *J* = 9.4, 7.4, 1.8 Hz, OPyH-4) 7.58 (dd, 1H, *J* = 8.4, 1.8 Hz, OPyH-6) 8.54 ppm (s, 1H, H-8). ^{19}F-NMR: (188 MHz, $CDCl_3$) δ –49.64 ppm.

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-2-fluoro-6-cyclopropylaminopurine riboside (11b). To a solution of **10b** (13 mg, 0.018 mmol) and Et_3N (38 μL, 0.27 mmol) in anhydrous DMF (1 mL) was added cyclopropylamine (1.9 μL, 0.027 mmol) under Ar and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture is diluted with $CHCl_3$ (5 mL) and extracted first with water (5 mL), then with a sat. $NaHCO_3$ solution (5 mL) and finally with brine (5 mL). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography using 2:8 ethyl acetate–hexane to afford 10 mg (73%) of the product. R_f: 0.52 in ethyl acetate–hexane 35:65. ^{1}H-NMR (200 MHz, $CDCl_3$) δ –0.16, –0.01 [s, 3H, $Si(CH_3)_2$] 0.09, 0.14 [s, 6H, $Si(CH_3)_2$] 0.63–0.71 (m, 2H, CH_2) 0.82 [s, 9H, $SiC(CH_3)_3$] 0.84–0.87 (m, 2H, CH_2) 0.92, 0.95 [s, 9H, $SiC(CH_3)_3$] 3.03 (m, 1H, CH) 3.73 (dd, 1H, $J_{H4'}$ = 2.8, $J_{H5'}$ = 11.4 Hz, H-5″) 4.02 (dd, 1H, $J_{H4'}$ = 2.8, $J_{H5''}$ = 11.4 Hz, H-5′) 4.12 (dd, 1H, $J_{H3'}$ = 4.6, $J_{H5'}$ = 2.8 Hz, H-4′) 4.29 (dd, 1H, $J_{H2'}$ = 4.6, $J_{H4'}$ = 4.6 Hz, H-3′) 4.60 (dd, 1H, $J_{H1'}$ = 4.6, $J_{H3'}$ = 4.6 Hz, H-2′) 5.91 (d, 1H, $J_{H2'}$ = 4.6 Hz, H-1′) 8.16 ppm (s, 1H, H-8). ^{13}C-NMR (50 MHz, $CDCl_3$) δ –5.21, –4.84, –4.54, –4.49, –4.19 [$Si(CH_3)_2$] 7.50, 7.64 (2 × CH_2) 18.0, 18.3, 18.7 [$SiC(CH_3)_3$] 24.0 (CH) 25.9, 26.0, 26.3 [$SiC(CH_3)_3$] 62.6 (C-5′) 72.0 (C-3′) 75.7 (C-2′) 85.6 (C-4′) 88.8 (C-1′) 131.8 (C-5) 139.5 (C-8) 157.3 (C-4) 157.7 (C-6) 161.6 ppm (C-2); ^{19}F-NMR: (188 MHz, $CDCl_3$) δ –49.45 ppm. *m/z* (ESI) 668.3 $(M + H)^+$.

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