A New Synthetic Approach for the Synthesis of N^2-Modified Guanosines

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Abstract: A new universal route for generating N^2-modified guanosines is developed and the synthesis of two examples is shown.

Key words: alkylations, alkyl halides, DNA , nucleosides, synthesis

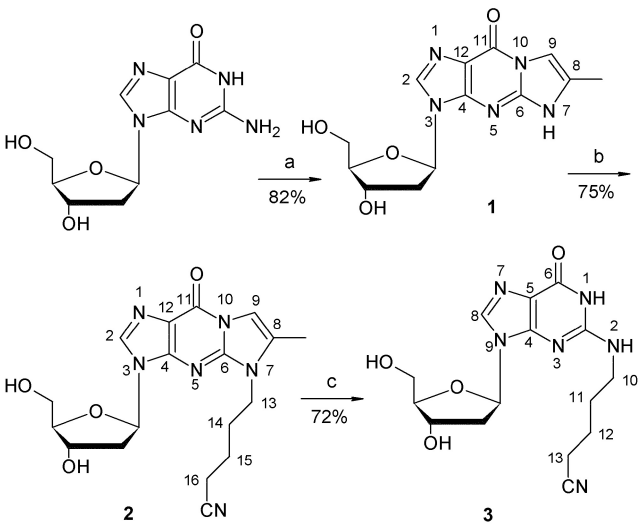
The insertion of appropriate reporter groups into specific regions of DNA is of great importance for understanding its structural motives and biological functions.[1] These markers usually possess physical properties, which allow detection and/or quantification. For example the marker can be a fluorophoric or paramagnetic group, so that they can be measured by fluorescence or EPR (electron paramagnetic resonance) spectroscopy.[2,3] Modified oligodeoxynucleotides are also of great interest for therapeutic use.[4]

Reporter groups for DNA are mainly either tethered to the 5-position of pyrimidines or the 7-position of deazapurines. This results in derivatization of the major groove of the B-helix. For labeling nucleosides in the minor groove especially, guanosine is an interesting candidate, because of its exocyclic amino function; this group points to the minor groove in a B-helix. Monoalkylation of this amino group of guanosine does not seem to impair its Watson–Crick base pairing capacity. When we tested the 2′,3′-dideoxy derivative of **3** in a T7-DNA-polymerase assay, we found adequate termination.[5]

The N^2-function of 2′-deoxyguanosine cannot be directly alkylated. Indeed the reaction at the N^7-position predominates and in some cases significant amounts of the O^6-alkylation products can be obtained.[6] Even in the presence of a weak base like potassium carbonate and with methyl iodide as alkylating agent, the reaction preferentially occurs at the N^1-position.[7]

For that reason a special strategy is needed for the synthesis of N^2-modified guanosines. The synthesis of 2-fluoroguanosine is possible; the fluorine can be replaced by an amine afterwards. This nucleophilic substitution works best with fluorine derivatives, in contrast to bromine or iodine derivatives.[8] Several attempts were necessary to optimize the fluorination step.[9,10] However, fluorination of O^6-unprotected guanosines is not convenient for the synthesis of N^2-modified guanosines, because of the low yields obtained.[11] By using protecting groups like benzyl or nitrophenylethyl (NPE) at the O^6-position better yields are achieved, especially with polyvinylpyridinium polyhydrogenfluoride (PVPHF) as the fluorination reagent.[12] Therefore this route has been used, even for biological applications.[13]

A simpler and shorter approach for the synthesis of N^2-modified guanosines uses wyosine as the protected intermediate. This motive has been known for 30 years.[14,15] Several derivatives have been synthesized because of their analogy to the tricyclic acyclovir and were proven to be active against herpes infections.[16] The wyosine motive can be also used as a protecting group for guanosine, as the tricycle (**1**) generated protects the N^1-position as well as the N^2-position. Therefore the possibility of double alkylations in the next step is prevented. Scheme 1 shows the route for the synthesis of N^2-modified guanosines. In connection with this guanosine reacts with freshly synthesized bromoacetone,[17] which is a teargas. Wyosine (**1**) is generated easily and in good yield. The following alkylation is universally applicable; aliphatic or arylalkyl bromides can be used as electrophilic agents. The alkylated wyosine **2** can be deprotected using mild conditions and then the modified N^2-guanosine **3** is readily obtained in a good yield (44% over three steps).



Scheme 1 Reagents and conditions: a) NaH, $CH_3C(O)CH_2Br$, NH_4OH, r.t.; b) K_2CO_3, $Br(CH_2)_4CN$, 2 d, r.t.; c) CH_3CN–H_2O, NBS, NH_4OH, r.t., 30 min.

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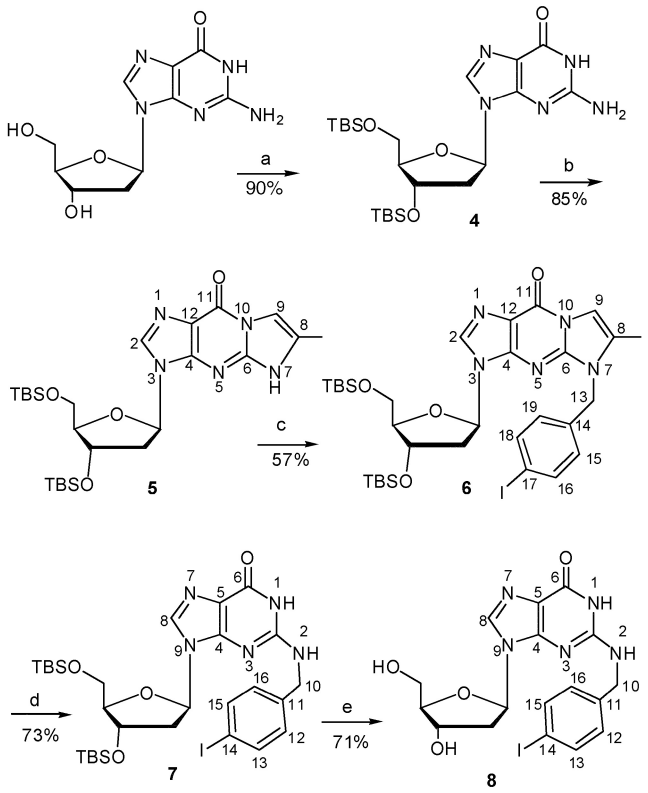
Due to the high polarity of guanosine, protecting the hydroxyl groups is advisable. Unfortunately, conversion to acetyl was not successful, because of the alkaline work-up required. To show the general applicability of this method, a second linker and nucleophilic agent was used (Scheme 2). In generating the N^2-modified guanosine **8** (23% over five steps), TBS groups were used as protecting groups for the hydroxyl groups of the sugar and *p*-iodobenzylbromide was used as an alkylating agent. The cleavage of the wyosine was performed analogously to Scheme 1.

The difference between the yields obtained can be explained by the different reactivities and the size of the electrophilic reagents used. This new synthetic approach for the synthesis of N^2-modified guanosines can be used for N^2-monoalkylation of guanosine, giving rise to minor groove labeling, which is yet almost unexplored. This might be especially helpful for monitoring DNA protein interactions whether they take place in the minor or major groove.



Scheme 2 Reagents and conditions: a) TBSCl, imidazole, DMF, r.t., 4 h; b) NaH, $CH_3C(O)CH_2Br$, NH_4OH, r.t.; c) K_2CO_3, *p*-iodobenzylbromide, r.t.; d) THF–H_2O, NBS, NH_4OH, r.t., 30 min; e) THF, TBAF, r.t., 20 min.

All ^1H and ^{13}C NMR spectra were measured on a Bruker AM 250 (250 MHz) spectrometer or on a Bruker Avance 400 (400 MHz) spectrometer. *J* values are given in Hz. MALDI-mass spectra were recorded on a Fisons VG Tofspec spectrometer and ESI-mass spectra on a Fisons VG Plattform II spectrometer. Column chromatography was carried out on silica gel 60 (Merck 9385, 34–62 μm).

2′-Deoxy-4-desmethylwyosine (1)

The reaction was carried out in a dry flask under an argon atmosphere. 2′-Deoxyguanosine (5 g; 18.71 mmol) was dissolved in anhyd DMSO (60 mL) and NaH (60% suspension; 940 mg, 19.64 mmol) was added. After stirring the solution for 1 h, bromoacetone (2.69 g; 19.64 mmol) was added and the mixture was stirred for 1 h. Then the reaction was completed by adding NH_4OH (30 mL). After 2 h the solution was reduced to a volume of 30 mL. The residue was dissolved in acetone (30 mL) and added to a stirred mixture of acetone (400 mL) and anhyd Et_2O (100 mL). After stirring at 0 °C for 3 h the resulting oil was isolated by decanting the solvent. The oil was washed with Et_2O (2 × 250 mL) and dried under reduced pressure. The powder obtained was taken up in H_2O (200 mL) and absorbed onto 15 g of silica gel by several co-evaporations with EtOH; column chromatography (CH_2Cl_2 → CH_2Cl_2–MeOH, 9:1) gave 4.7 g (82%) of **1**.

^1H NMR (250 MHz, DMSO-d_6): δ = 2.28 (d, 3 H, C8-CH_3), 2.32–2.68 (m, 2 H, H-2′), 3.51–3.63 (m, 2 H, 2 H-5′), 3.87–3.88 (m, 1 H, H-4′), 4.41 (m, 1 H, H-3′), 5.00–5.16 (t, 1 H, 5′-OH), 5.34–5.35 (d, 1 H, 3′-OH), 6.23–6.29 (t, 1 H, *J* = 6.8 Hz, H-1′), 7.37 (d, 1 H, H-9), 8.14 (s, 1 H, H-2), 12.38 (s, 1 H, N-H).

^{13}C NMR (62.8 MHz, DMSO-d_6): δ = 10.5 (C8-CH_3), 38.5–40.7 (C-2′ and C-3′ covered by DMSO), 70.76 (C-5′), 83.0 (C-4′), 87.6 (C-1′), 103.3 (C-9), 115.5 (C-12), 126.1 (C-8), 136.9 (C-2), 145.5 (C-6), 149.5 (C-4), 151.2 (C-11).

MS-ESI: *m*/*z* = 304.3 $[M - H]^-$.

Anal. Calcd for $C_{13}H_{15}N_5O_4$: C, 51.15; H, 4.95; N, 22.94. Found: C, 51.32; H, 4.95; N, 22.69.

7-Valerianacidnitrile-2′-deoxy-7-desmethylwyosine (2)

The reaction was carried out in a dry flask under an argon atmosphere. K_2CO_3 (0.48 g; 3.5 mmol) was added to a solution of **1** (1.00 g; 3.3 mmol) in anhyd DMF (25 mL). The suspension was stirred for 2 d. The mixture was filtered through celite and washed with warm DMF until UV-activity disappeared. The solvent was removed by using a nitrogen rotary evaporator and recrystallization of the residue from MeOH gave 0.96 g (75%) of **2**.

^1H NMR (250 MHz, DMSO-d_6): δ = 1.54–1.65 (m, 2 H, H-15), 1.77–1.89 (m, 2 H, H-14), 2.34 (d, 3 H, C8-CH_3), 2.49–2.59 (m, 3 H, H-2′, H-16), 2.67–2.77 (m, 1 H, H-2′), 3.49–3.66 (m, 2 H, H-5′), 3.84–3.89 (m, 1 H, H-4′), 4.07–4.11 (t, 2 H, *J* = 7.1 Hz, H-13), 4.41–4.43 (m, 1 H, H-3′), 4.92–4.96 (t, 1 H, *J* = 5.3 Hz, 5′-OH), 5.32–5.34 (d, 1 H, 3′-OH), 6.23–6.29 (t, 1 H, *J* = 7.0 Hz, H-1′), 7.48 (d, 1 H, H-9), 8.12 (s, 1 H, H-2).

^{13}C NMR (62.8 MHz, DMSO-d_6): δ = 6.2 (C8-CH_3), 9.6 (C-16), 21.9 (C-15), 27.5 (C-14), 38.4–40.6 (C-2′ and C-3′ covered by DMSO), 41.1 (C-13), 61.8 (C-5′), 83.6 (C-4′), 87.7 (C-1′), 103.1 (C-9), 115.9 (C-12), 120.4 (CN), 127.3 (C-8), 137.3 (C-2), 144.9 (C-6), 149.1 (C-4), 150.9 (C-11).

MS-ESI: *m*/*z* = 385.3 $[M - H]^-$.

Anal. Calcd for $C_{18}H_{21}N_6O_3$: C, 59.48; H, 7.49; N, 17.34. Found: C, 59.28; H, 7.55; N, 17.36.

2-Valerianacidnitrile-2′-deoxyguanosine (3)

To a solution of **2** (0.25 g; 0.65 mmol) in MeCN (5 mL) and H_2O (5 mL), NBS (0.14 g; 0.78 mmol) was added and the solution was stirred for 30 min. Afterwards a concd aq solution of NH_4OH was added and the mixture was stirred for another 30 min. The solvent was evaporated under reduced pressure and the residue was absorbed onto silica gel by several co-evaporations with EtOH; column chromatography (CH_2Cl_2–MeOH, 9:1) gave 0.16 g (72%) of **3**.

^1H NMR (250 MHz, DMSO-d_6): δ = 1.61–1.62 (m, 4 H, H-14 and H-15), 2.11–2.17 (m, 1 H, H-2′), 2.50–2.56 (m, 3 H, H-2′, H-16), 3.22-3.31 (m, 2 H, H-13), 3.42–3.63 (m, 2 H, H-5′), 3.84–3.89 (m,

1 H, H-4′), 4.42–4.46 (m, 1 H, H-3′), 4.70–4.73 (t, 1 H, *J* = 5.8 Hz, 5′-OH), 5.27–5.28 (d, 1 H, 3′-OH), 6.12–6.16 (t, 1 H, *J* = 7.1 Hz, H-1′), 6.59–6.62 (t, 1 H, N2-H), 7.89 (s, 1 H, H-8), 10.72 (s, 1 H, N1-H).

^{13}C NMR (62.8 MHz, DMSO-d_6): δ = 15.9 (C-16), 22.2 (C-15), 27.8 (C-14), 36.1 (C-2′), 38.6–40.4 (C-13 covered by DMSO), 61.9 (C-5′), 70.8 (C-3′), 85.1 (C-1′), 87.5 (C-4′), 117.6 (C-5), 120.5 (CN), 151.5 (C-4), 152.2 (C-2), 155.4 (C-6).

MS-ESI: *m*/*z* = 348.9 $[M - H]^+$.

Anal. Calcd for $C_{15}H_{20}N_6O_3$: C, 54.21; H, 6.07; N, 25.29. Found: C, 54.40; H, 6.31; N, 25.18.

3′,5′-*O*-Di-*tert*-butyldimethylsilyl-2′-deoxyguanosine (4)

The reaction was carried out in a dry flask under an argon atmosphere. 2′-Deoxyguanosine (5 g, 18.7 mmol) was suspended in anhyd DMF (23 mL). A TBS-solution (1 mol/L solution in THF; 75 mL, 4 equiv) and imidazole (8.9 g, 131 mmol, 7 equiv) was added to the suspension. After 10 min the solution began to clear and the product began to crystallize. The suspension was stirred overnight,

EtOH (30 mL) was added, and after stirring for 30 min the suspension was concentrated under reduced pressure. The crystals were filtered off under vacuum and washed with cold EtOH. The residue was dried under vacuum overnight and gave 8.36 g (90%) of **4**.

^{1}H NMR (250 MHz, DMSO-d_6): δ = –0.06 (s, 6 H, $SiCH_3$), 0.00 (s, 6 H, $SiCH_3$), 0.77 (6 s, 18 H, Si-*t*-Bu), 2.05–2.20 (m, 1 H, H-2′), 2.50–2.65 (m, 1 H, H-2′), 3.50–3.62 (m, 2 H, H-5′), 3.69–3.77 (m, 1 H, H-4′), 4.38–4.40 (m, 1 H, H-3′), 5.98–6.01 (t, 1 H, *J* = 6.0 Hz, H-1′), 6.43 (s, 2 H, NH_2), 7.8 (s, 1 H, H-8), 10.57 (s, 1 H, NH).

^{13}C NMR (62.8 MHz, DMSO-d_6): δ = –5.78 (2 s, $SiCH_3$), –5.2 (2 s, $SiCH_3$), 17.3 (SiC, quaternary), 17.6 (s, SiC, quaternary), 25.4 (Si-*t*-Bu), 39.2 (C-2′), 62.6 (C-5′), 71.9 (C-3′), 82.1 (C-1′), 86.8 (C-4′), 116.6 (C-5), 134.5 (C-8), 150.7 (C-4), 153.5 (C-2), 156.3 (C-6).

MS-ESI: *m*/*z* = 496.4 $[M - H]^+$

HRMS (ESI): *m*/*z* calcd for $C_{30}H_{41}N_5O_4Si_2$ $[M - H]^+$: 496.27698; found: 496.27763.

3′,5′-*O*-Di-*tert*-butyldimethylsilyl-2′-deoxy-4-desmethylwyosine (5)

The reaction was carried out in a dry flask under an argon atmosphere. By using a water bath (60 °C) **4** (4 g, 8.1 mmol) were dissolved in anhyd DMSO (80 mL). Subsequently NaH (95%; 224 mg; 8.9 mmol, 1.1 equiv) was added, which was accompanied by a vehement reaction; the resulting solution was stirred for 1 h. Then bromoacetone (1.1 g, 8.1 mmol) was added dropwise, whereby the solution changed color. The red solution was stirred overnight at r.t. protected from light. The solvent was removed under reduced pressure and column chromatography (CH_2Cl_2–MeOH, 40:1 → 20:1) of the residue gave 3.7 g (85%) of **5**.

^{1}H NMR (250 MHz, DMSO-d_6): δ = –0.08 (s, 6 H, $SiCH_3$), 0.00 (s, 6 H, Si-CH_3), 0.75 (4 s, 18 H, Si-*t*-Bu), 2.14 (s, 3 H, 8-CH_3), 2.15–2.22 (m, 1 H, H-2′), 2.55′2.8 (m, 1 H, H-2′), 3.50–3.65 (m, 2 H, H-5′), 3.70–3.80 (m, 1 H, H-4′), 4.41–4.44 (m, 1 H, H-3′), 6.11–6.17 (t, 1 H, *J* = 6.5 Hz, H-1′), 7.24 (s, 1 H, H-9), 7.96 (s, 1 H, H-2), 12.20 (s, 1 H, NH).

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = –5.4 (2 s, $SiCH_3$), –4.9 (2 s, $SiCH_3$), 10.5 (8-CH_3), 17.7 (s, SiC, quaternary), 17.9 (s, SiC, quaternary), 25.7 (Si-*t*-Bu), 39.7 (C-2′), 62.7 (C-5′), 71.9 (C-3′), 82.4 (C-1′), 86.9 (C-4′), 103.3 (C-9), 115.5 (C-12), 125.9 (C-8), 136.4 (C-2), 145.6 (C-6), 149.6 (C-4), 151.1 (C-11).

MS-ESI: *m*/*z* = 534.3 $[M - H]^+$

HRMS (ESI): *m*/*z* calcd for $C_{32}H_{42}N_5O_4Si_2$ $[M - H]^+$: 534.292634; found: 534.29285.

3′,5′-*O*-Di-*tert*-butyldimethylsilyl-2′-deoxy-7-(*p*-iodobenzyl)-4-desmethylwyosine (6)

The reaction was carried out in a dry flask under an argon atmosphere. To a solution of **5** (3.65 g; 6.84 mmol) in anhyd DMF (40 mL) were added K_2CO_3 (1.23 g; 8.88 mmol) and *p*-iodobenzylbromide (2.23 g; 7.52 mmol). After 30 min a precipitate formed; after stirring for a further 2 h, the solvent was removed under reduced pressure and column chromatography (CH_2Cl_2–*i*-PrOH, 40:1) of the residue gave 1.93 g (57%) of **6**.

^{1}H NMR (250 MHz, $CDCl_3$): δ = –0.02 (s, 6 H, $SiCH_3$), 0.00 (s, 6 H, $SiCH_3$), 0.81 (s, 18 H, Si-*t*-Bu), 2.12 (s, 3 H, 8-CH_3), 2.15–2.30 (m, 1 H, H-2′), 2.35–2.50 (m, 1 H, H-2′), 3.68–3.69 (m, 2 H, H-5′), 3.87–3.89 (m, 1 H, H-4′), 4.47–4.48 (m, 1 H, H-3′), 5.12 (s, 2 H, 13-CH_2), 6.20–6.30 (t, 1 H, *J* = 6.5 Hz, H-1′), 6.87 (d, 2 H, *J* = 8.3 Hz, ArH-15/19), 7.32 (s, 1 H, H-9), 7.56 (d, 2 H, *J* = 8.3 Hz, Ar-H-16/18), 7.87 (s, 1 H, H-2).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = –5.5 (2 s, $SiCH_3$), –4.8 (2 s, $SiCH_3$), 10.3 (8-CH_3), 17.9 (SiC, quaternary), 18.4 (SiC, quaternary), 25.9 (2 s, Si-*t*-Bu), 41.1 (C-2′), 45.3 (C-13), 62.9 (C-5′), 71.9 (C-3′), 83.6 (C-1′), 87.6 (C-4′), 93.6 (C-17), 104.0 (C-9), 116.9 (C-12), 126.2 (C-8), 128.9 (C-15/19), 135.4 (C-14), 136.4 (C-2), 138.0 (C-16/18), 145.7 (C-6), 149.3 (C-4), 151.7 (C-11).

MS-ESI: *m*/*z* = 750.3 $[M - H]^+$.

Anal.Calcd for $C_{32}H_{48}IN_5O_4Si_2$: C, 51.20; H, 6.41; N, 9.04. Found: C, 51.18; H, 6.34; N, 8.77.

3′,5′-*O*-Di-*tert*-butyldimethylsilyl-2-*N*-(*p*-iodobenzyl)-2′-deoxyguanosine (7)

To a solution of **6** (1.53 g; 2.04 mmol) in THF (50 mL), H_2O (20 mL) and NBS (0.45 g; 2.45 mmol) were added. After 40 min the reaction was stopped by the addition of an aq solution of NH_4OH (32%; 10 mL). After stirring for 30 min the two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were dried with Na_2SO_4, filtered, and evaporated. Column chromatography (CH_2Cl_2–*i*-PrOH, 30:1) of the residue gave 1.06 g (73%) of **7**.

^{1}H NMR (250 MHz, $CDCl_3$): δ = –0.03 (s, 6 H, $SiCH_3$), 0.00 (s, 6 H, $SiCH_3$), 0.77 (2 s, 18 H, Si-*t*-Bu), 2.15–2.20 (m, 1 H, H-2′), 2.21–2.35 (m, 1 H, H-2′), 3.64–3.66 (m, 2 H, H-5′), 3.88–3.89 (m, 1 H, H-4′), 4.38–4.39 (m, 1 H, H-3′), 4.46 (s, 2 H, N2-CH_2), 6.07 (t, 1 H, H-1′), 7.03 (d, 2 H, *J* = 8.3 Hz, *o*-ArH), 7.39 (s, 1 H, H-8), 7.47 (d, 2 H, *J* = 8.3 Hz, *m*-Ar-H), 8.1 (s, 1 H, N2-H), 12.05 (s, 1 H, N1-H).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = –5.5 (2 s, $SiCH_3$), –4.8 (2 s, $SiCH_3$), 18.3 (SiC, quaternary), 18.4 (SiC, quaternary), 25.8 (s, Si-*t*-Bu), 40.6 (C-2′), 44.3 (C-10), 62.9 (C-5′), 72.3 (C-3′), 84.1 (C-1′), 87.7 (C-4′), 91.9 (C-14), 117.2 (C-5), 128.6 (C-12/16), 135.3 (C-8), 137.2 (C-13/15), 139.3 (C-2), 150.9 (C-4), 152.6 (C-11), 159.1 (C-9).

MS-ESI: *m*/*z* = 712.5 $[M - H]^+$.

HRMS (ESI): *m*/*z* calcd for $C_{36}H_{45}IN_5O_2$ $[M - H]^+$: 712.220578; found: 712.22148

2-*N*-(*p*-Iodobenzyl)-2′deoxyguanosine (8)

The reaction was carried out in a dry flask and under an argon atmosphere. To a solution of **7** (1.05 g, 1.5 mmol) in anhyd THF (20 mL), TBAF (1 M in THF; 3.1 mL, 3.1 mmol, 2.1 equiv) was added dropwise. After 20 min the reaction was complete. The solution was evaporated and column chromatography (CH_2Cl_2–*i*-PrOH, 15:1) of the residue gave 503 mg (71%) of **8**.

^{1}H NMR (250 MHz, DMSO-d_6): δ = 2.15–2.20 (m, 1 H, H-2′), 2.50–2.65 (m, 1 H, H-2′), 3.45–3.55 (m, 1 H, H-5′), 3.75–3.85 (m, 1 H, H-5′), 4,3 (s, 1 H, 5′-OH), 4.44 (d, 2 H, *J* = 5.8 Hz, 10-CH_2), 4.85 (s, 1 H, 3′-OH), 5.26–5.27 (m, 1 H, H-4′), 6.08–6.14 (m, 1 H,

H-3′), 6.99 (t, 1 H, H-1′), 7.16 (d, 2 H, J = 8.3 Hz, *o*-ArH), 7.67 (d, 2 H, J = 8.3 Hz, *m*-Ar-H), 7.89 (s, 1 H, H-8), 10.74 (s, 1 H, N1-H).

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 43.4 (C-2′), 61.7 (C-10), 70.8 (C-5′), 82.9 (C-3′), 87.6 (C-1′), 92.7 (C-4′), 109.0 (C-14), 113.0 (C-5), 129.7 (C-12/16), 137.0 (C-13/15), 139.0 (C-2, C-8), 150.0 (C-4), 152.4 (C-11), 157.1 (C-6).

MS-ESI: m/z = 482.1 $[M - H]^-$.

HRMS (ESI): m/z calcd for $C_{16}H_{16}IN_5O_4$ $[M - H]^+$: 484.047624; found: 484.04569.

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