Regioselective Functionalization of Guanine: Simple and Practical Synthesis of 7- and 9-Alkylated Guanines Starting from Guanosine

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Abstract: Reaction of N2-acetyl-9- and/or -7-benzylated guanines 8 and 12 with selected alkylation agents in 1-methyl-2-pyrrolidone at 120 °C yielded the guaninium salts 9 and 13. The salts were consequently transformed by phase transfer hydrogenation into N7- and N9-isomers 10 and 14, respectively, in a highly regioselective manner. A convenient deoxygenation of both derivatives, achieved via the corresponding O6-arenesulfonates, into 2-aminopurine potential prodrugs was also established.

Key words: N2-acetyl-7- and 9-benzylguanine, benzylation, alkylations, hydrogenations, regioselectivity

Guanine is one of the five important bases of life and due to its polyfunctionality it is the most problematic chemistry occurs. The molecule is very difficult to functionalize selectively. Direct alkylation of guanine or guanosine produce several derivatives depending on pH used, implicating its amphoteric nature, whereas glycosylations of N1- or N7, N9-protected guanines affords N9/N7-isomeric mixtures.

A solution to the problem of the regioselective targeting (coupling) of the incoming respective alkyl or glycosyl substituent came of age when it turned out that this is the primary and practical route to manufacture clinically effective antiviral drugs. Examples include acyclovir (ACV), ganciclovir (GCV), penciclovir (PCV) which are active against herpes simplex virus 1 (HSV-1), varicella zoster virus (VZV), or human cytomegalovirus (HCMV). Extensive studies oriented towards achieving high regioselectivity revealed that a completely different approach is required for the use of alkylation agents that are not strongly activated by the β-oxygen function. If a solution for glycosylations has been apparently determined and explained by the role of the ‘6-enolate’ of diacetyl guanine (DACG) giving higher N9-regioselectivity, there is no such solution for the aimed introduction of respective alkyl side chains.

In this study, we wish to report on the solution of this problem with practical implications for the synthesis of penciclovir and famciclovir and on the novel regioselective synthesis of the N7-isomer of penciclovir. This strategy introduces a new approach for the synthesis of a potential new class of biologically active N2-alkyl substituted guanines.

Any rational strategy for the synthesis of alkyl substituted guanines requires mainly two steps: first the preparation of the adequately protected or constructed pure derivative that undergoes regioselective alkylation on either of two selected nitrogen atoms N9 or N7 and secondly, the introduction of the appropriate side chain, whereas further transformations into the target compounds e.g. alkylated purines were anticipated by design.

Searching for a practical and economic synthesis, it seemed reasonable to select guanosine (1) as the first choice for tentatively directing the reaction to position N7. However, none of the earlier reports deals with effective and high yielding alkylations of guanosine. Subsequent acid hydrolysis however afforded, e.g. 7-methyl and 7-ethylguanines in low yields. Complete alkylation of guanosine was achieved only with ethylene oxide. Furthermore, a thorough kinetic study of the benzylolation of guanosine, including the report on the properties and stability of the respective isomeric benzyl derivatives revealed the potential site of benzylolation (N7 >90%). It was suggested (noticed) that 7-benzylguanosine is converted into 7-benzylguanine (2) much faster than the respective 7-methyl substituted guanines. Excellent yield as well as easily performed regioselective protection of position N7 introduced 2 as a favorable candidate for subsequent alkylations to furnish N9-alkylated derivatives, and it seems competitive in all respects versus customary used starting materials, especially for a large scale synthesis.

For sure, 2 is the first available compound prepared from the keto form (guanosine and alike) that completely masks position N7 and leaves the N9 site open for attempted alkylations. Besides, the benzyl group could be readily deblocked via catalytic hydrogenation and has been widely used for that purpose. With compound 2 in our hands, the respective 7,9-dialkyl-guaninium salts are generally available. Further benzylolation of 2 with excess benzyl halide affords a bis-benzylated product 3 readily suited for making the desired N9-protected guanine 4. Thus, 2 was readily acetylated and treated with 2 equiv of benzyl bro-
mide in 1-methyl-2-pyrrolidone at 120 °C to provide 7,9-dibenzyl-N²-acetylguaninium bromide (3) (Scheme 1).

An attempt at chemoselective monodebenzylation of 3 was carried out in the presence of Pd/C (10%) and ammonium formate in MeOH. A remarkably selective monodebenzylation of 3 was achieved, much in favor of the anticipated 9-benzylguanine (4) (4/2 = 7:1 as determined by 'H NMR spectroscopy) in 71% yield. With this temporary benzyl protection on the way to access either N⁷- or N⁹-benzyl derivatives of guanine, we have established a general synthetic route to any kind of 7,9-dialkyl-guaninium salts designed to impose upon a regioselective manner synthesis of penciclovir and related drugs.

In fact, an almost identical approach has been reported, although no experimental details were given, neither was this route disclosed as general by providing N⁷- and N⁹-alkylated guanines and focused on the use of 3.19

Until recently,¹⁰,¹¹ it was a tenet that analogous N⁷ regioisomers of well-established antiviral drugs would not display antiviral activity. However, no comparisons have been made between N⁷-6-deoxyganciclovir,⁰¹ and N⁷-famciclovir-like isomers possessing all-carbon backbones of the side chain. Mostly, N²-isomeric products, obtained in minor quantities, were either discarded or were never processed further, although Bzowska et al.¹² reported that some derivatives pointed toward a new class of purine nucleoside phosphorylase inhibitors. To assess the ultimate objective, regioselective alkylations of N⁷ site devoid of any side products, extensive investigations were conducted on the already mentioned guanine derivatives 2, 4–7⁻¹⁴,¹⁶,¹⁷ (Figure 1).

One can observe that in a guanine system, a more thermodynamically stable product, namely the N⁹-isomer, is formed as a major component with transformed ‘6-enolate’ derivatives 5 and 6 in 1:9 and 2:8 ratio, respectively, whereas the introduction of ‘keto-forms’ 2 and 7 produced kinetic products in an equal level or in favor of N²-derivatives, depending on the properties of the base used in the reaction.⁵

The fixation of a ‘keto-form’ with appropriate derivatization facilitates a regioselectivity in favor of N² kinetic products (e.g. in 1-methylguanine). Glyoxal protection, especially, yielded an exceptional ratio (18:1) in favor of kinetic product, though in low yield.¹⁴

With all these data in mind, we now report on the regioselective kinetic formation of 7-alkylated guanine derivatives by alkylation of readily available 9-benzylguanine (4) starting from a novel compound 3. We also disclose a practical parallel regioselective route competing in all respects to already available synthetic procedures for manufacturing penciclovir.

The synthetic strategy adapted for 4 and 2 is shown in Scheme 2. A key reaction is the deprotection, e.g. catalytic dehydrogenation of temporarily protected guaninium salt 9 or 13 with simultaneous regioselective formation of the targeted products 10, 11, and 14, 15. Initially, the N²-acetyl-9-benzylguanine (8) was treated with 4-bromobut-1-yl acetate in 1-methyl-2-pyrrolidone for two hours at 120 °C. The resulting N²-acetyl-9-benzyl-7-(4-acetoxybut-1-yl)guaninium bromide (9a) was precipitated in EtOAc and the solid residue (88%) dried and used without further purification. At this point a critical step was introduced which was earlier already adopted for a very convenient and practical synthesis of the main synthon used in this work, namely 4. Compound 4 was obtained adequately from respective N²-acetyl-7,9-dibenzylguaninium bromide (3) which was subsequently treated with excessive
ammonium formate in refluxing MeOH and Pd/C (10%) as catalyst. After four hours, aqueous ammonia solution was added, the reaction mixture refluxed for one hour and the solvent removed to furnish a mixture of N\textsubscript{9}- and N\textsubscript{7}-benzylguanines in a N\textsubscript{9}/N\textsubscript{7} ratio of 7:1 in 80% overall yield. Pure 4 (71% yield) was easily separated after filtration of the resulting mixture through Celite and by thorough washings with MeOH. It is worthy to mention that by this procedure, a regioselectivity ratio in favor of the thermodynamically N\textsubscript{9}-substituted guanine isomer was obtained after use of temporary protection of the imidazole part of the purine ring.

When the same procedure was applied to mixed 7-alkyl-9-benzyl intermediate compound 9\textsubscript{a}, a smooth and clean reaction readily afforded 10\textsubscript{a} in 71% yield (no migration of either group to the accessible binding sites N\textsubscript{1}, N\textsubscript{3} and N\textsubscript{9} was observed by TLC during the reaction). A slight modification of the workup by using 2 N NaOH instead of ammonia furnished a partially protected 7-(4-acetoxybut-1-yl)guanine (10\textsubscript{a}), which was further acetylated with Ac\textsubscript{2}O in 1-methyl-2-pyrrolidone to provide N\textsubscript{2}-acetylated 11\textsubscript{a} (R\textsubscript{1} = CH\textsubscript{2}CO). Accordingly, the synthesis of the target compound 10\textsubscript{b} (N\textsubscript{7}-isomer of acetylated penciclovir) was achieved using 4-acetoxy-3-acetoxymethylbut-1-yl tosylate for the respective alkylation. The intermediate
guaninium salt 9b was precipitated out of Et₂O, the residue dissolved in MeOH and the reaction mixture processed like before to afford 7-(4-hydroxy-3-hydroxymethyl)but-1-ylguanine (10d) in 29% overall yield with spectroscopic data (see experimental) differing in all respects with relation to the N⁹-substituted isomer 16b, a well known drug penciclovir readily used in clinic. Corresponding analytical data were collected on the product prepared from 7-benzylguanine (2) via a similar strategy depicted in Scheme 2 in 47% overall yield. Since the alkylations were performed at elevated temperature (120 °C) it seemed reasonable that considerable extent of parallel alkylations may be expected with respect to two additional coupling sites, namely N¹ and N³. However, no migrations of either alkyl groups was observed in the reaction mixtures anticipating that 7,9-dialkyl salts were built as kinetic products affecting the most reactive N⁷ position, while the N⁹ nucleophilic site was temporarily masked with a benzyl group. It is obvious that the protected N⁷ position should direct alkylations to the thermodynamic N⁹-substituted product. Unlike with other useful and often used starting materials, no special tricks were needed in either case to achieve a regioselective alkylation for 2 and 4.

To the best of our knowledge, a new general and practical route directed to any respective N⁷-alkylated biologically important guanine analogues was thus developed. In this paper we want to back up this tenet with an example that offers significant advantage over previously reported preparations that mostly provided N⁷ isomers in minor quantities accompanying more or less successful attempts of regioselective synthesis of N⁷-substituted guanines. In addition, we wish to report on further transformations of these new, now readily available compounds that are rather scarce due to insignificant interest on this new class of compounds. However, the importance of a recently discovered 6-deoxy-N⁷-ganciclovir analogue certainly justifies the introduction of the independent procedure starting from N⁷-substituted guanine product 10c that mimics 2-amino-7-(1,3-dihydroxy-2-propanoxy)methyl)purine.

In Scheme 3 we disclose a new procedure avoiding the tedious chlorination which has been applied in N⁷-series for the synthesis of N²-acetyl-6-deoxycyclovir, an important prodrug. 7-(4-Acetoxybut-1-yl)-2-acetylguanine (11a) reacted with 2,4,6-trisopropylbenzenesulfonyl chloride (ArSO₂Cl), in anhydrous CH₂Cl₂ in the presence of triethylamine (2 equiv) and 4-(dimethylamino)pyridine (cat.) at room temperature to give intermediate 16 in 96% yield as a yellow foam, which was dissolved in EtOH. Hydrogenation in the presence of Pd/C (10%) as catalyst and triethylamine in a Parr apparatus at 85 °C and 3 bar pressure provided 17 in 50% yield. This experiment confirmed that our strategy can be potentially and generally adopted to a straightforward synthesis of any targeted N⁷-functionalized purins employing starting materials from natural sources (e.g. guanosine).

Finally, in order to avoid the use of halides or tosylates as side chain precursors we also anticipated the use of convenient cyclopropane reagent (6,6-dimethyl-5,7-dioxaspiro[2.5]octane-4,8-dione) which was used earlier although exhibiting poor selectivity with 5. We have found its effective use recently communicated on the alkylation of 2. Since the reactivity of cyclopropane synthons proved to be appropriate, the respective betaine was isolated after the application of the respective homocoupling addition reaction. Again, the resulting 9c was treated likewise with ammonium formate to furnish 10c in 63% yield. Compound 10c can be easily processed as reported by Choundary et al. for the N⁹-purine series (Scheme 4).
alkylations resulting in the formation of intermediates 3, 9, 15 which furnished penciclovir and its N7-isomeric analogue. They can also be further processed to famciclovir and its corresponding N7-analogue without any ambiguity of the alkylation site. This work may further find applicability for the synthesis of any purine acyclic nucleosides in satisfactory yields.

All reagents were of commercial grade. Reagent grade solvents were used without further purification. Melting points were determined on a Kofler apparatus and are uncorrected. Mass spectra were measured on an AutoSpecQ mass spectrometer of EBEqQ configuration (Micromass, Manchester, UK). All 1H NMR spectra are taken on a Varian Unit Plus spectrometer at 300 MHz at the National NMR Centre of Slovenia, in DMSO-d6 with DMSO as a standard at 2.50 ppm. Flash column chromatography was carried out on silica gel 60 (Merck, 40–63 nm) and TLC on silica gel 60 F254 (Merck) with detection by UV light. Evaporations were conducted in vacuo with a rotary evaporator.

7-Benzylguanine (2)
Guanosine (1; 56.6 g, 0.2 mol) was suspended in DMSO (150 mL) with stirring. The suspension was stirred for 20 min and then benzyl bromide (26 mL, 0.22 mol) was added. The reaction mixture was stirred at r.t. for 24 h, and the resulting solution was then transferred to a beaker. Aq 10% HCl (250 mL) was then added and the mixture was heated at 70 °C for 2 h, then cooled and filtered. The crystalline solid was washed with cold H2O, suspended in H2O and neutralized by addition of aq 6 M NaOH. The precipitate was filtered, washed with H2O and dried in vacuo at 120 °C to yield 41 g (85%) of 2 in a powder form identical in all respects with literature data.

1H NMR (DMSO-d6): δ = 7.43 (s, 1 H, NH), 8.07 (s, 1 H, H-8), 7.27–7.36 (m, 5 H, Ar), 6.13 (br s, 2 H, NH2), 5.41 (s, 2 H, NCH3).

MS (FAB): m/z = 242 (MH+, 100%).

N7-Acetyl-7-benzylguanine (12)
7-Benzylguanine (2; 9.65 g, 0.04 mol) was suspended in 1-methyl-2-pyrrolidone (94 mL) and then Ac2O (5.7 mL, 0.06 mol) was added. The resulting suspension was heated with stirring at 150 °C for 1 h. The solvent was removed and the residue suspended in EtOAc (50 mL) and filtered. The resulting solid was washed with acetone (10 mL) to afford 12 as a white powder (10.5 g, 93%) identical in all respects with the literature data; mp 236–238 °C (Lit. mp 211 °C).

1H NMR (DMSO-d6): δ = 12.11 (br s, 1 H, AcNH), 11.58 (s, 1 H, NH), 8.35 (s, 1 H, H-8), 7.31–7.35 (m, 5 H, Ar), 5.51 (s, 2 H, NCH3), 2.15 (s, 3 H, CH3CO).

MS (FAB): m/z = 284 (MH+, 100%).

HRMS: calcd for C21H20BrN5O2·HBr: 373.154; found: 373.155.

UV (H2O, pH 6.4): λmax = 266 nm (ε = 14,500).

N7-Acetyl-7,9-dibenzylguaninium Bromide (3)
Method A: Benzyl bromide (3.3 mL, 0.03 mol) was added to a suspension of N7-acetyl-7-benzylguanine (12; 5.6 g, 0.020 mol) in 1-methyl-2-pyrrolidone (50 mL). The reaction mixture was heated with stirring at 120 °C for 2 h, then poured while hot into EtOAc (150 mL) and stirred for 1 h. The resulting solid was filtered, washed with acetone (10 mL) and dried to give 3 (8.1 g, 90%).

1H NMR (DMSO-d6): δ = 12.61 (br s, 1 H, AcNH), 12.14 (s, 1 H, NH), 9.83 (s, 1 H, H-8), 7.38–7.52 (m, 10 H, Ar), 5.71 (s, 2 H, NCH3), 5.50 (s, 2 H, NCH3), 2.22 (s, 3 H, CH3CO).

13C NMR (DMSO-d6): δ = 174.3 (NHCOCH3), 157.58 (C-6), 150.90 (C-4), 147.71 (C-2), 140.09 (C-8), 110.64 (C-5), 134.38, 134.11, 129.14, 129.10, 129.01, 128.84, 128.35, 128.18 (CH2CH3), 51.90, 48.65 (CH3Ph).

MS (EI): m/z = 237 (M – HBr, 60%).

HRMS: calcd for C31H28BrN2O2·HBr: 373.154; found: 373.155.

Method B: Guanosine (4.53 g, 0.03 mol) was suspended in 1-methyl-2-pyrrolidone (50 mL). Ac2O (7.3 mL, 0.08 mol) was added and the reaction mixture stirred for 4 h at 150 °C. The solvent was removed, and fresh 1-methyl-2-pyrrolidone (25 mL) and benzyl bromide (7.2 mL, 0.06 mol) were added subsequently. The mixture was then heated for 2 additional h at 120 °C. The dark colored solution was poured into hot EtOAc (250 mL) with stirring. After sufficient cooling of the mixture, the salt 3 began to separate, which was collected after 1 h period on a filter and washed with acetone (2 × 25 mL). Slightly colored salt 3 was obtained after drying in vacuo (12.6 g, 92%). This material was used without further purification.
9-Benzylguanine (4)
The catalyst 10% Pd/C (1.2 g) was added to a solution containing 3 (11.5 g, 0.025 mol) and ammonium formate (4.4 g, 0.07 mol) in MeOH (230 mL). The mixture was heated with stirring at reflux for 4 h, filtered hot and the catalyst was washed with hot MeOH. Aq ammonia (20 mL) was added to the filtrate and the solution was reduced for 1 h. The solvent was removed and the residue crystallized from DMF to give a mixture of 7- and 9-benzylguanine in a ratio 1:7 (determined by 1H NMR spectroscopy (4.85 g, 80%)). A small quantity of the mixture (0.5 g) was transferred onto a filter funnel filled with Celite and washed with MeOH (500–700 mL). The resulting filtrate was concentrated to provide 7 (4.85 g, 80%); HRMS: m/z calcd for C_{12}H_{11}N_{5}O: 241.096; found: 241.096.

HRMS: m/z calcd for C_{14}H_{13}N_{5}O_{2}: 283.108; found: 283.108.

UV (H_2O, pH 6.2): 0.50 (1 H, CH, 2 CH, 2 CH3CO).

HRMS: m/z calcd for C_{16}H_{22}N_{5}O_{6}: 380.157; found: 380.157.

9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]guanine (10d)

9-Benzylguanine (4) was treated with 4-Acetoxy-3-acetoxymethylbut-1-yl tosylate (0.76 g, 2.1 mmol) at 120 °C continued for 18 h. The reaction mixture was refluxed for 4 h, then filtered and the remaining catalyst was washed with MeOH (10 mL). The solvent was removed under reduced pressure and the residue was dissolved in aq 1 M NaOH solution (10 mL) and heated on a steam bath for 30 min. The mixture was cooled, neutralized with conc HCl and allowed to stand for 3 h until white crystals began to separate. The solid was collected on a filter, washed with cold H_2O and finally dried in vacuo at 60 °C. White crystals were collected (0.6 g, 47%), identical in all respects with the literature data; mp >300 °C.

HRMS: m/z calcd for C_{12}H_{11}N_{5}O: 241.096; found: 241.096.

UV (H_2O, pH 6.2): 0.50 (1 H, CH, 2 CH, 2 CH3CO).

HRMS: m/z calcd for C_{14}H_{13}N_{5}O_{2}: 283.108; found: 283.108.

UV (H_2O, pH 6.5): C_{12}H_{11}N_{5}O; 253.118; found: 253.118.

UV (H_2O, pH 6.5): C_{12}H_{11}N_{5}O; 253.118; found: 253.118.

HRMS: m/z calcd for C_{17}H_{22}N_{5}O_{4}: 373.159; found: 373.159.

HRMS: m/z calcd for C_{19}H_{22}N_{5}O_{4}: 389.179; found: 389.179.

UV (H_2O, pH 6.5): C_{12}H_{11}N_{5}O; 253.118; found: 253.118.

UV (H_2O, pH 6.5): C_{12}H_{11}N_{5}O; 253.118; found: 253.118.

HRMS: m/z calcd for C_{17}H_{22}N_{5}O_{4}: 373.159; found: 373.159.

HRMS: m/z calcd for C_{19}H_{22}N_{5}O_{4}: 389.179; found: 389.179.

UV (H_2O, pH 6.5): C_{12}H_{11}N_{5}O; 253.118; found: 253.118.

UV (H_2O, pH 6.5): C_{12}H_{11}N_{5}O; 253.118; found: 253.118.

HRMS: m/z calcd for C_{17}H_{22}N_{5}O_{4}: 373.159; found: 373.159.

HRMS: m/z calcd for C_{19}H_{22}N_{5}O_{4}: 389.179; found: 389.179.
r.t. until a clear solution was obtained (2.5 h). The solvent was removed and the product was isolated after purification by flash chromatography (column prepared in CH₂Cl₂ and eluted with EtOAc).

Product 18, obtained as a yellow foam (2.21 g, 99%) was used in subsequent reactions.

1H NMR (CDCl₃): δ = 8.13 (br s, 1 H, AcNH), 8.02 (s, 1 H, H-8), 7.25 (s, 2 H, Ar), 4.25–4.11 (m, 6 H, NCH₂, 2 OCH₂), 3.72 (t, 4 H, 2 OCH₂), 1.99 (s, 6 H, 2 CH₃CO), 1.93–1.77 (m, 3 H, CH₂-3), 1.28 [d, J = 7.2 Hz, 2 H, Ar], 1.25 (s, 6 H, 2 CH₃). The resulting solution was stirred for 15 min at r.t. and evaporated and coevaporated with H₂O upon cooling of the solution. It was dissolved (2.65 g) in H₂O (20 mL) and in aq 40% MeNH₂ (9 mL). The combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. White powder was isolated after trituration of the residue with Et₂O. After drying product 19 was obtained and can be used in subsequent steps without any purification.

2-Amino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]purine (19) To a solution of 18 (8.5 g, 13.2 mmol) in EtOH (140 mL) were added the catalyst 10% Pd/C (1.4 g) and Et₃N (19.8 mL, 0.14 mol). The mixture subjected for hydrogenation in a Parr hydrogenator at 80–85 °C (3 bar) for 8 h. The catalyst was filtered off and washed with hot EtOH. The acetylated intermediate (31 g, 60%) separated out upon cooling of the solution. It was dissolved (2.65 g) in H₂O (20 mL) and in aq 40% MeNH₂ (9 mL). The resulting solution was stirred for 15 min at r.t. and evaporated and coevaporated with H₂O (10 mL) several times. The residue was recrystallized from EtOH to give 19 (1.7 g, 99%) identical in all respects with the literature data; mp 152–154 °C (Lit. 154–156 °C).

1H NMR (CDCl₃): δ = 8.55 (s, 1 H, H-6), 8.07 (s, 1 H, H-8), 6.49 (s, 2 H, NH), 4.44 (exchangeable with D₂O, 2 H, 2 OH), 4.11 (t, J = 7.2 Hz, 2 H, NCH₂), 3.1–3.5 (m, 4 H, 2 OCH₂), 1.76–1.45 (m, 3 H, CH₂-2, CH-3).

9-[4-Acetoxy-3-(acetoxyethyl)but-1-yl]-2-aminopurine (20) A mixture of 19 (1.75 g, 7.4 mmol), Ac₂O (1.7 mL) in pyridine (1.8 mL), and DMAP (90 mg, 0.74 mmol) in anhyd THF (30 mL) was stirred for 3 h at r.t. The solvent was evaporated and the residue was dissolved in H₂O. This mixture was extracted with CH₂Cl₂ (3 × 40 mL). The combined organic layers were dried (Na₂SO₄) and the solvent was evaporated. White powder was isolated after trituration of the mixture with Et₂O and with subsequent filtration to provide famciclovir (2.3 g, 97%) identical in all respects with the literature data; mp 100–102 °C (Lit. 102–103 °C).

1H NMR (CDCl₃): δ = 8.56 (s, 1 H, H-6), 8.09 (s, 1 H, H-8), 6.49 (s, 2 H, NH), 4.13 (t, J = 6.8 Hz, 2 H, NCH₂), 4.02 (d, J = 5.5 Hz, 4 H, 2 OCH₂), 1.99 (s, 6 H, 2 CH₃CO), 1.93–1.77 (m, 3 H, CH₂-2, CH-3).

N²-Acetyl-7-benzyl-9-[4-acetoxybut-1-yl]guaninium Bromide (21a) 4-Bromobut-1-yl acetate (10.7 g, 0.055 mol) was added to a suspension of 12 (14.1 g, 0.05 mol) in 1-methyl-2-pyrrolidinone (35 mL). The reaction mixture was heated for 2 h at 120 °C. The mixture was then cooled down and EtOAc (500 mL) was added. The precipitate was collected by filtration after 1 h, and the white salt obtained was washed with acetone and dried to give 13a (21 g, 88%), which was used without further purification.

1H NMR (CDCl₃): δ = 12.60 (br s, 1 H, AcNH), 12.17 (s, 1 H, NH), 9.83 (s, 1 H, H-8), 7.4–7.7 (m, 5 H, Ar), 5.72 (s, 2 H, CH₂Ph), 4.29 (t, J = 7.0 Hz, 2 H, NCH₂), 4.04 (t, J = 6.8 Hz, 2 H, OCH₂), 2.23 (s, 3 H, CH₂CON), 2.01 (s, 3 H, CH₂CO), 1.85–1.67 (m, 4 H, CH₂CH₂).

1C NMR (DMSO-d₆): δ = 174.01 (NHCOCH₂), 170.40 (CH₂CO), 151.42 (C-6), 150.49 (C-4), 147.52 (C-2), 139.93 (C-8), 134.21, 128.91, 128.40, 128.00 (CH₂CH₂), 110.32 (C-5), 63.00 (C-4), 45.30 (C-1), 24.91 (C-2‘), 25.11 (C-3‘), 23.91 (CH₂CON), 20.71 (CH₂CO).

HRMS: m/z calc for C₁₅H₁₆N₄O₄· Br⁺: 398.1928; found: 398.193.

Anal. Calc'd for C₁₅H₁₆N₄O₄· Br⁺: C, 58.16; H, 5.52; N, 22.94. Found: C, 58.05; H, 5.43; N, 22.94.

N²-Acetyl-9-(4-acetoxybut-1-yl)guaninium Bromide (13a) 4-Bromobut-1-yl acetate (2.9 g, 0.015 mol) was added to N²-acetyl-9-benzylguaninium (8: 2.83 g, 0.01 mol) in 1-methyl-2-pyrrolidinone (20 mL). The suspension was heated at 120 °C for 2 h. Et₂O (200 mL) was added to the cooled solution. After 1 h, the solvent mixture was decanted from the dark colored gummy residue, which was triturated with EtOAc. The solid obtained was filtered and washed with EtOAc. After drying product 9a (4.7 g, 99%) was obtained and was used in following experiments without further purification.

1H NMR (DMSO-d₆): δ = 12.00 (s, 1 H, AcNH), 11.70 (s, 1 H, NH), 9.44 (s, 1 H, H-8), 7.41 (m, 5 H, Ar), 5.38 (s, 2 H, CH₂Ph), 4.40 (t, J = 7.2 Hz, 2 H, NCH₂), 4.02 (t, 6.4 Hz, 2 H, OCH₂), 2.20 (s, 3 H, CH₂CON), 2.01 (s, 3 H, CH₂CO), 1.90–1.62 (m, 4 H, CH₂CH₂).
A mixture of 9a (4.7 g, 0.01 mol), ammonium formate (1.9 g, 0.03 mol), and 10% Pd/C (0.4 g) in MeOH (80 mL) was refluxed with stirring for 4 h, then filtered hot, and the filtrate was evaporated to dryness under reduced pressure. The catalyst was washed with hot water and neutralized with concd HCl. After 1 h, crystals formed were collected and washed with H2O and thoroughly dried to yield 10a (1.88 g, 71%); mp 220–223 °C.


HRMS: m/z calcd for C13H18N5O4: 266.125; found: 266.126.

Analysis of 2-Acetyl-7-(4-acetoxybut-1-yl)guanine (11a)

7-(4-Acetoxybutyl-1-yl)guanine (10a) (1 g, 4 mmol) was acetylated in a suspension in 1-methyl-2-pyrrolidone (10 mL) after addition of H2O (30 mL) and this solution was added to the residue. The mixture was then heated on a steam bath for 20 min and neutralized with concd HCl. After 1 h, crystals formed were collected and washed with H2O and thoroughly dried to yield 10a (1.88 g, 71%); mp 220–223 °C.

Analysis of 2-Acetyl-7-(4-acetoxybut-1-yl)-2-acetylamino-7H-purine (17)

To a solution of 16 (0.55 g, 1 mmol) in EtOH (25 mL) were added 10% Pd/C (0.2 g) and Et3N (0.15 mL, 1.1 mmol). Hydrogenation was performed in a Parr apparatus at 75 °C and 3 bar of pressure. After 6 h, the catalyst was removed by filtration and washed with hot EtOH. A mixture of N2-acetyl-7-(4-acetoxybutyl-1-yl)-guanine and 7-(4-acetoxybutyl-1-yl)-2-acetylamino-7H-purine (17) was obtained. The products were separated by flash chromatography using CH3OH:MeOH (2:1, 400 mL and 10:1, 400 mL) to obtain pure 17 (0.14 g, 50%) as a white solid; mp 131–135 °C.

Analysis of 7-(4-Acetoxybutyl-1-yl)-2-acetylaminomethyl-7H-purine (18)

To a solution of 16 (0.55 g, 1 mmol) in EtOH (25 mL) were added 10% Pd/C (0.2 g) and Et3N (0.15 mL, 1.1 mmol). Hydrogenation was performed in a Parr apparatus at 75 °C and 3 bar of pressure. After 6 h, the catalyst was removed by filtration and washed with hot EtOH. A mixture of N2-acetyl-7-(4-acetoxybutyl-1-yl)-guanine and 7-(4-acetoxybutyl-1-yl)-2-acetylamino-7H-purine (17) was obtained. The products were separated by flash chromatography using CH3OH:MeOH (2:1, 400 mL and 10:1, 400 mL) to obtain pure 17 (0.14 g, 50%) as a white solid; mp 131–135 °C.

Analysis of 7-(4-Acetoxybutyl-1-yl)nucleoside (19)

A mixture of 11a (0.5 g, 1.6 mmol), Et3N (0.9 mL, 6.4 mmol) and DMAP (10 mg, 0.1 mmol) in anhyd CH3Cl (20 mL) and 2,4,6-trisopropylbenzenesulfonfonyl chloride (1 g, 3.3 mmol) was stirred at r.t. until the solution became clear. The solution was washed with H2O (2×15 mL). The H2O phase was then washed with CHCl3 (20 mL). The joint organic phases were washed with brine (2×25 mL) and dried (Na2SO4). The solvent was removed on rotary evaporator and the product applied on a 'flash' chromatographic column and processed [CH3Cl (300 mL)] to obtain 16 (0.9 g, 96%) as a yellow foam.

Analysis of 7-(4-Acetoxybutyl-1-yl)nucleoside (19)

A mixture of 11a (0.5 g, 1.6 mmol), Et3N (0.9 mL, 6.4 mmol) and DMAP (10 mg, 0.1 mmol) in anhyd CH3Cl (20 mL) and 2,4,6-trisopropylbenzenesulfonfonyl chloride (1 g, 3.3 mmol) was stirred at r.t. until the solution became clear. The solution was washed with H2O (2×15 mL). The H2O phase was then washed with CHCl3 (20 mL). The joint organic phases were washed with brine (2×25 mL) and dried (Na2SO4). The solvent was removed on rotary evaporator and the product applied on a 'flash' chromatographic column and processed [CH3Cl (300 mL)] to obtain 16 (0.9 g, 96%) as a yellow foam.
Dimethyl 2-[(Guanin-7-yl)ethyl]malonate (21)

Method 1: Compound 9c (500 mg, 1.1 mmol), ammonium formate (174 mg, 2.76 mmol, 2.5 equiv) and 10% Pd/C (58 mg) were suspended in MeOH (30 mL) and the resulting mixture was kept at reflux with stirring for 2 d. Pd catalyst was filtered off and washed with hot MeOH to afford the crude product 11c after evaporation (250 mg, 63%).

Method 2: To a suspension of 9c (300 mg, 0.66 mmol) inaq MeOH (20 mL, 50% v/v) were added NaHCO₃ (27 mg, 0.33 mmol) and the catalyst (Pd/C 10%, 35 mg). The reaction mixture was subjected to hydrogenation at 9 bar and at 60 °C for 20 h. The 23 catalyst was filtered off and washed with hot MeOH (3 × 5 mL). The filtrate was dried to afford 11c (175 mg, 73%).

The N²-alkylated purine 11c (40 mg, 0.11 mmol) was dissolved in MeOH (0.2 mL) and stirred at r.t. for 16 h. The solvent was evaporated, and the residue was dissolved in H₂O. The resulting solution was neutralized with aq 1 M NaOH solution (pH 7). The precipitate formed was filtered off and dried to afford 21 (27 mg, 80%); mp 247–250 °C.


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