A Convenient Method for the Modification of 8-Bromoguanine via Its N^9-Tetrahydrofuranyl Derivative

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Abstract: A simple and straightforward method for the introduction of some N- and O-protecting groups into 8-bromoguanine has been developed using 2-acetylamino-8-bromo-6-oxo-9-(tetrahydrofuran-2-yl)-purine as a key intermediate.

Key words: alkylation, nucleobases, protecting groups, synthesis, tetrahydrofuranyl substituent

Purines bearing diverse substituents at various positions of the heterocycle are synthons in the preparation of novel nucleosides, nucleotides and oligonucleotides. They can function as antiviral, antibacterial and anticancer agents. Besides these uses, many possess antagonistic activity on adenosine receptors and influence numerous other enzymatic systems.[1]

Our research has been focused on the synthesis of new polyfunctional guanine derivatives. Guanine is the most problematic nucleobase owing to its poor solubility in most common organic solvents as well as its tendency towards unselective chemical transformations caused by the presence of many reaction centers in the molecule.

One of the methods we have extensively used for guanine modification is alkylation, which is one of the principal procedures of purine chemistry. However, in the case of guanine-type bases, it often suffers from serious drawbacks such as the formation of poorly separable isomeric mixtures.[2] Our previous research into the alkylation of 2-acetylamino-8-bromo-6-oxo-9(7)*H*-purine was motivated by the assumption that the electron-withdrawing bromine at position 8 of the purine cycle would not only improve the yield and regioselectivity of the guanine alkylation but it could also serve as a good leaving group in further chemical transformations of the alkylation products.[3] However, the use of 2-acetylamino-8-bromo-6-oxo-9(7)*H*-purine as the alkylating substrate did not solve the problem of process selectivity. Usually, a mixture of N^7/N^9-substituted products was formed and, in some cases, a significant amount of bis-alkylated derivatives was isolated.[3]

It is well documented that the regioselectivity of alkylation and glycosylation of nucleobases can be controlled by appropriate protection of the hydroxyl and amino groups present in their molecules. To our knowledge, the synthesis of the N- or O-protected derivatives of 2-acetylamino-8-bromo-6-oxo-9(7)*H*-purine has not been described in the literature. Therefore, the objective of this research was to develop methods for the introduction of protecting groups into 8-bromoguanine in order to transform it into a useful synthetic intermediate.

The first protecting group we examined was the *N*,*N*-diphenylcarbamoyl (DPC) functionality, which is widely used for the O^6-protection of guanine and guanosine.[4] The presence of the bulky and lipophilic DPC group in the guanine molecule may strongly favour its regioselective N^9-alkylation or glycosylation, help to avoid side reactions and make the nucleobase more soluble and, consequently, much easier to manipulate.[4] Furthermore, the O-DPC moiety at position 6 of the purine could be replaced by other functionalities, such as alkoxy or phenylthio.[5]

There is one general method for the carbamoylation of 6-oxopurines – their treatment with *N*,*N*-diphenylcarbamoyl chloride (DPC-Cl) and *N*,*N*-diisopropylethylamine in pyridine at ambient temperature.[4] N^2,$O^{2'}$,$O^{3'}$,$O^{5'}$-Tetraacetylated 2-amino-6-oxo-9-(β-D-ribofuranosyl)purine and some of its analogues as well as 2-acetylamino- and 2-isobutyrylamino-9-acetyl-6-oxopurine have been used as substrates for this reaction. In the latter case, the 9-acetyl group served as the protecting group, which was removed after carbamoylation by solvolysis.[4] Unfortunately, the existing methodology was unsuitable for the carbamoylation of 2-acetylamino-8-bromo-6-oxo-9(7)*H*-purine (**1**). We failed to convert compound **1** into its 9-acetylated derivative and the reaction of the 9-unprotected form of purine **1** with DPC-Cl afforded a disappointing mixture. There was therefore a need to find some other derivative of 8-bromopurine **1**, suitable for the introduction of the O^6-DPC function. To this end, we decided to study 2-acetylamino-8-bromo-6-oxo-9-(tetrahydrofuran-2-yl)purine (**2**), prepared previously through the reaction of **1** with 2,3-dihydrofuran (Scheme 1).[6] Treatment of intermediate **2** with DPC-Cl, under standard conditions,[4] afforded 2-acetylamino-8-bromo-6-(*N*,*N*-diphenyl-carbamoyloxy)-9-(tetrahydrofuran-2-yl)purine (**3**) in 78% yield, after purification by column chromatography. An attempt to recrystallize product **3** from hot ethanol revealed that the tetrahydrofuranyl moiety was removed under these conditions and the corresponding 2-acetylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9(7)*H*-purine (**4**) precipitated from the ethanolic solution in quantitative yield. Further experiments showed that product **4** could

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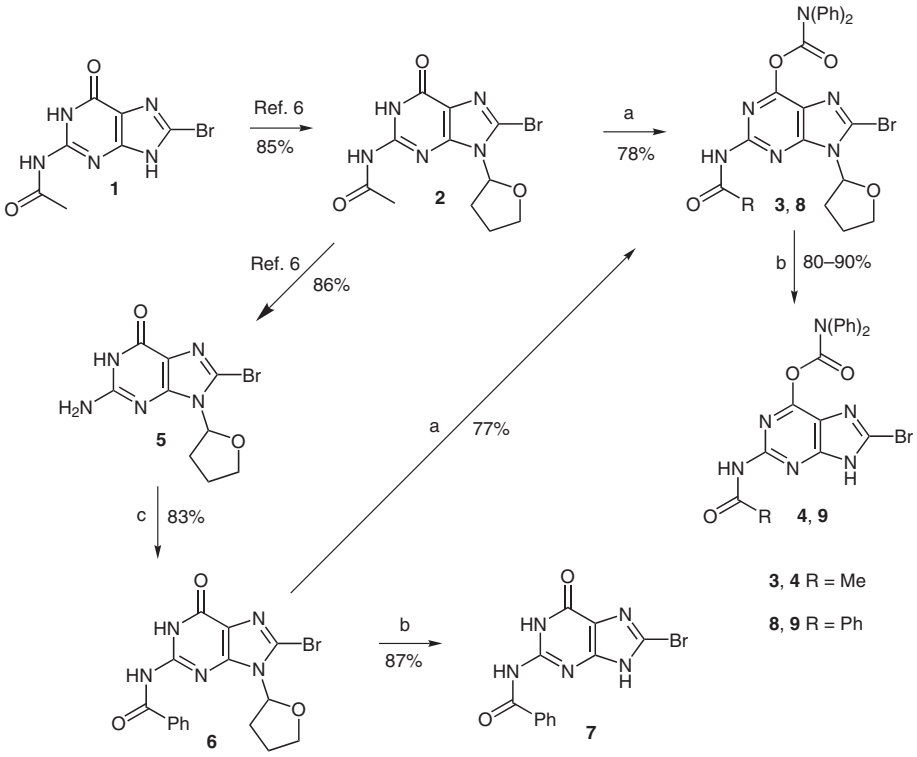


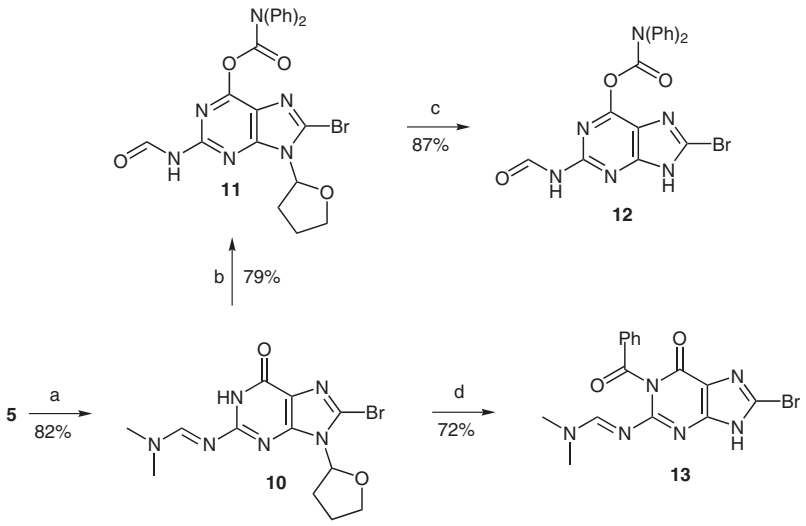
Scheme 1 *Reagents and conditions*: (a) DPC-Cl, DIPEA, py, r.t., 2.5–4 h; (b) EtOH, reflux, 20 min; (c) TMSCl, py, CH_2Cl_2, 0 °C→r.t., 30 min, then BzCl, 0 °C→r.t., 4 h.

also be obtained from crude **3**, although in lower yield. Therefore, 9-tetrahydrofuranyl derivative **2** turned out to be a convenient intermediate for the O^6-carbamoylation of purine **1**.

Taking into account the fact that in some reactions the N^2-benzoylated guanine derivatives are preferred over those that are N^2-acetylated, the next objective of our research was to synthesize 2-benzoylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9(7)*H*-purine (**9**), using the same intermediate **2** (Scheme 1). For benzoylation of the exocyclic amino group of compound **5**, obtained from deprotection of **2**,[6] instead of the conventional benzoyl chloride–pyridine method,[7] we decided to use the transient silylation approach developed recently for the preparation of N^2-acetyl- and N^2-phenacetyl derivatives of guanosine.[8] The approach worked well in our case and allowed us to synthesize 8-bromo-2-benzoylamino-6-oxo-9-(tetrahydrofuran-2-yl)purine (**6**) from nucleobase **5** in a three-step one-pot reaction (i.e. silylation with trimethylsilyl chloride, acylation with benzoyl chloride, de-silylation with aqueous sodium bicarbonate). Product **6** could be treated with hot ethanol, affording 2-benzoylamino-8-bromo-6-oxo-9(7)*H*-purine (**7**), or it could be carbamoylated to obtain 2-benzoylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9-(tetrahydrofuran-2-yl)purine (**8**) which, in turn, was easily transformed into 2-benzoylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9(7)*H*-purine (**9**). The purine **8** was less stable than the corresponding 2-acetylamino analogue **3** and partial loss of its tetrahydrofuranyl moiety occurred during the isolation process. On the other hand, the 2-benzoylamino nucleobase **9** was more soluble than derivative **4** and, furthermore, it could be recrystallized from ethanol.

Apart from acyl groups, dialkyl formamidines (especially *N*,*N*-dimethylformamidine) are widely used as protecting groups for the amino function of nucleosides. Taking into account the possible influence of *N*-amidine-type protection on the further alkylation of purine **1**,[9] the next object of our synthesis was 8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9(7)*H*-purine, with amidine protection at the amino group. The preparation of an analogous guanosine derivative has been described in literature[10] and, using this approach, 8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9-(tetrahydrofuran-2-yl)purine (**10**) was generated from compound **5** using *N*,*N*-dimethylformamide dimethylacetal in DMF (Scheme 2).

The carbamoylation of purine **10** afforded 8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-2-formylamino-9-(tetrahydro-2-furanyl)purine (**11**) as the sole reaction product instead of the expected 2-(*N*,*N*-dimethyliminoformamido) derivative. It is likely that the formamide obtained resulted from a partial hydrolysis of the formamidine function via the intermediate hemiorthoamide.[11] A similar transformation was also observed by us during the O^6-tosylation of 9-(2-acetoxyethoxymetyl)-2-(*N*,*N*-dimethyliminoformamido)-6-oxopurine.[12] The reasons behind this hydrolysis are not immediately obvious as the carbamoylation of **10** was carried out in anhydrous solvents under anhydrous conditions. Deprotection of **11** (ethanolic) and crystallization afforded the previously unknown 8-bromo-

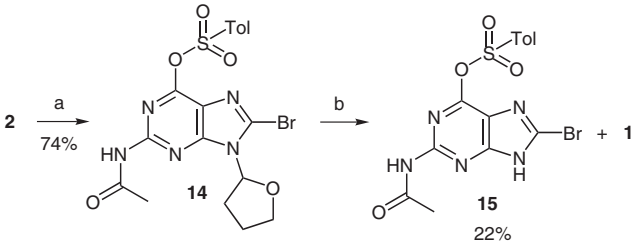


Scheme 2 *Reagents and conditions*: (a) *N*,*N*-dimethylformamide dimethylacetal, DMF, r.t., 4 h; (b) DPC-Cl, DIPEA, py, r.t., 6 h; (c) EtOH, reflux, 20 min; (d) BzCl, py, MeCN, r.t., 72 h.

6-(*N*,*N*-diphenylcarbamoyloxy)-2-formylamino-9(7)*H*-purine (**12**).

The next step of our research was to evaluate the possibility of protecting position 6 of purine **1** with a benzoyl group. Although guanine residues usually react with carboxylic acid chlorides and anhydrides at N^2, it has been reported that guanosine and its N^2-acyl derivatives can undergo acylation at O^6 when treated with an excess of substituted benzoyl chlorides in pyridine solution.[13] Unfortunately, our attempts at treating nucleobases **1** or **5** with benzoyl chloride in pyridine, led to a dark mixture of compounds. On the other hand, benzoylation of purine **5** in an acetonitrile/pyridine system[14] gave a single reaction product in reasonable yield. The product turned out to be 1-benzoyl-8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9(7)*H*-purine (**13**; Scheme 2). Therefore, it was evident that, alongside the benzoylation, cleavage of the tetrahydrofuranyl moiety occurred in the course of this reaction. The benzoylation of the guanine molecule at position 1 has not been previously described in literature. Recently, a similar pterin derivative, 2-(*N*,*N*-dimethyliminoformamido)-6-formyl-3-(*o*-toluoyl)pterin, was synthesized from 6-formylpterin in a three-component reaction [*o*-TolCl, DIPEA, DMF].[15] When we attempted to apply these reaction conditions to substrate **5** the only isolated product was derivative **10**. Evidently, in this case the reaction failed to go to completion and stopped at the second step, i.e. the interaction of the Vilsmeyer-type activated complex with the amino group.[15] This was possibly brought about by the extremely poor solubility of intermediate **10** in the reaction mixture. The same reason may also be responsible for the slow benzoylation of **5** to **13**; about 72 hours were necessary to complete this conversion.

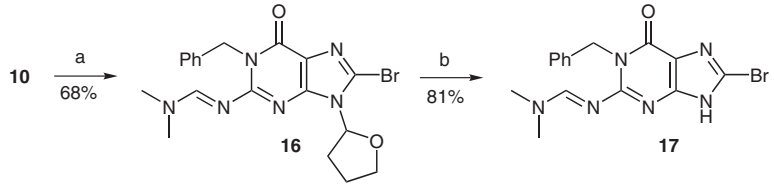
It is well documented that O^6-sulfonates can be useful synthetic intermediates for the transformation of guanosine derivatives. These compounds, being particularly susceptible to nucleophilic substitution at C-6, have found application in various substitution and hydrogenation reactions and also appear to be superior substrates in some cross-coupling reactions.[16] The sulfonylated products can be prepared easily and rapidly by treatment of the suitably protected guanosine derivatives with either arenesulfonyl chlorides in pyridine or with the triethylamine/4-(*N*,*N*-dimethylamino)pyridine (DMAP)/dichloromethane system.[16] The latter conditions have also been applied for the arenesulfonylation of 9-(2-acetoxyethoxymethyl)-2-acetylamino-6-oxopurine.[17] Since, to the best of our knowledge, there are no literature references to O^6-sulfonylated 8-bromoguanine or its derivatives, we therefore decided to apply our methodology to the synthesis of this compound. The reaction of purine **2** with 4-toluenesulfonyl chloride, under standard conditions, afforded 2-acetylamino-8-bromo-6-[(4-methylphenyl)sulfonyloxy]-9-(tetrahydrofuran-2-yl)purine (**14**) in 74% yield (Scheme 3).



Scheme 3 *Reagents and conditions*: (a) TsCl, Et_3N, DMAP, CH_2Cl_2, r.t., 24–48 h; (b) EtOH, reflux, 20 min.

However, when intermediate **14** was treated with boiling ethanol, in order to prepare the target 2-acetylamino-8-bromo-6-[(4-methylphenyl)sulfonyloxy]-9(7)*H*-purine (**15**), mostly starting material **1** was recovered, with derivative **15** only produced as a side product of the process.

Finally, to broaden the scope of our methodology, we applied it to the synthesis of purine derivative **1**, benzylated



Scheme 4 *Reagents and conditions*: (a) BnBr, K_2CO_3, DMF, r.t., 36 h; (b) EtOH, PTSA, reflux, 20 min.

in the pyrimidine part of the molecule. The selection of the benzyl group was motivated by its possible use for site-modification of the purine cycle, as well as for temporary protection of the 1,6-lactam function. Furthermore, it was of interest to evaluate the influence of the benzyl group on the N^9–C bond stability in compound **2**.

Initial attempts at treating nucleobase **2** with benzyl bromide, in the presence of potassium carbonate, resulted in a complex mixture of compounds. The only isolated products were 2-acetylamino-7-benzyl- and 2-acetylamino-9-benzyl-8-bromo-6-oxopurine,[3] which indicated that during this reaction the benzylation may be accompanied by the loss of the tetrahydrofuranyl residue. On the other hand, the interaction of 2-(*N*,*N*-dimethyliminoformamido)purine **10** with benzyl bromide proceeded smoothly with the formation of a single product to which the structure of 1-benzyl-8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9-(tetrahydrofuran-2-yl)purine (**16**) was assigned (Scheme 4).

The result obtained was in accordance with the literature data on the formation of an analogous 1-substituted derivative in the benzylation of 2′-deoxyguanosine bearing amidine-type protection at N^2.[18] Intermediate **16** turned out to be stable in boiling ethanol and, in order to remove the tetrahydrofuranyl residue and prepare the target 1-benzyl-8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9(7)*H*-purine (**17**), a catalytic amount of 4-toluenesulphonic acid had to be added to the ethanolic solution.

The structures of all the synthesized products were verified by their ^{1}H NMR spectra. The presence of the DPC function in compounds **3**, **4**, **8**, **9**, **11** and **12**, and of the benzoyl function in compounds **6**–**9** were confirmed by the presence of the corresponding aromatic protons signals. The chemical shift of the exocyclic NH-group proton singlet (δ = ~10 ppm) in **3**, **4**, **8** and **9** supported their structures as O^6,N^2-disubstituted purine derivatives rather than N^1-substituted.[19] The spectra of compounds **11** and **12** contained doublets (δ = 9.3 and 11.1 ppm) characteristic of the formamidine group. It is known that some monosubstituted formamides can exist in solution as *cis* and *trans* isomers, due to the restricted rotation of the single C–N bond. In such cases, two sets of signals for the protons of the amine group and of the formyl group are observed in the ^{1}H NMR spectra, with minor coupling constant (~0–3 Hz) for the *cis* NH–CH protons and major coupling constant (~10–13 Hz) for the corresponding *trans* protons.[20] Since only one set of signals, with coupling constants of ~9.5 Hz, were present in the spectra of derivatives **11** and **12**, a restricted rotation of the amide bond could therefore be excluded. The ^{1}H NMR spectra of **11** and **12** were also very similar to that of the previously described 9-(2-acetoxyethoxymethyl)-2-formylamino-6-[(4-methylphenyl)sulfonyloxy]purine, for which the structure was unambiguously assigned using X-ray crystallographic analysis.[12] The multiplet at δ = 7.5–7.8 ppm in the spectrum of **13**, corresponded to the protons of the benzoyl group introduced into the molecule. Furthermore, a notable upfield shift of the singlet signal from one NCH_3 group of **13**, in comparison with the corresponding singlet for the starting material **10**, was also a distinguishing feature of the ^{1}H NMR spectrum. As the proton spectrum of **13** gave little information about the benzoylation site, X-ray crystallographic analysis was used for this purpose (Figure 1).

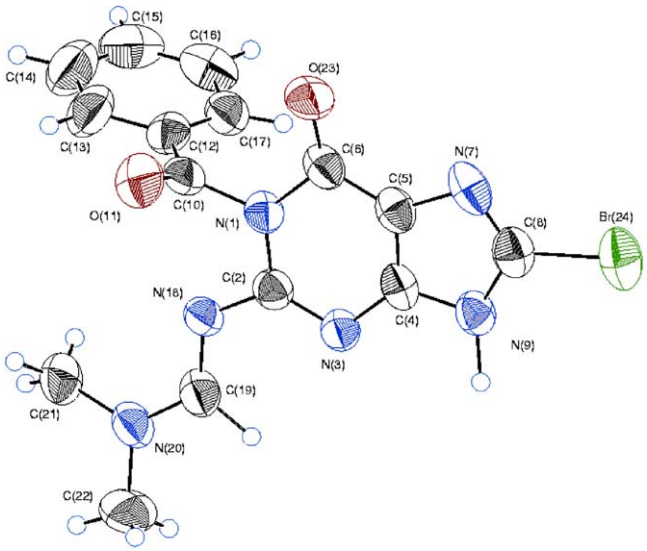


Figure 1 X-ray crystal structure of compound **13**.

Position 1 was excluded as the tosylation site in product **14** on the basis of the established fact that the presence of a substituent at position 1 in 2-acetylamino-6-oxopurines causes significant shift of the signal arising from the protons of the acetylamino function.[19] Such changes were not observed in the spectrum of **14**. The benzyl group in compound **16** was characterized by a multiplet at δ = ~7.2 ppm and by a two-proton singlet at δ = 5.37 ppm. The benzylation site in product **16** was proven by NOE measurements; irradiation of the protons at C-10 showed a strong nuclear Overhauser effect with the proton at C-12 as well with the protons of the NCH_3 group (Figure 2).

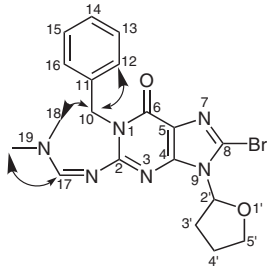


Figure 2 NOE interactions in compound **16**.

The loss of the tetrahydrofuranyl residue in products **4**, **7**, **9**, **12**, **13**, **15** and **17** was confirmed by the disappearance of all the corresponding proton signals and the appearance of the N^9-H and/or N^7-H proton singlets at δ = 13–14 ppm in their ^{1}H NMR spectra. ^{13}C NMR spectra of nucleobases **4**, **7**, **9**, **12**, **13**, **15** and **17** turned out to be unsuitable for the characterization of these compounds. The aromatic purine carbon atom signals were very broad, evidently due to the equilibrium between the N(7)H and N(9)H tautomeric forms in solution. The purity of all the compounds synthesized, except for the rather unstable intermediates **3**, **6**, **8**, **11** and **14**, were confirmed by elemental analysis.

In conclusion, we have developed an efficient method for the modification of 8-bromoguanine, using its 9-(tetrahydrofuran-2-yl) derivative as the key intermediate in the process. The method enables the introduction of several protecting groups into the guanine molecule and, therefore, allows access to a series of new nucleobases suitable for further chemical transformations. Furthermore, we believe that the elaborated procedure is general enough to be successfully used for the preparation of more novel purine derivatives.

All reagents were purchased from Acros Organics and used from freshly opened containers without any further purification. Reactions involving air- or moisture-sensitive reagents were carried out in anhydrous solvents under anhydrous argon. Merck TLC silica gel 60 F254 plates were used for TLC analyses and the products were visualized by UV irradiation. Column chromatography was carried out with Merck silica gel 60, particle size 0.040–0.063 mm. Melting points were determined on a Boetius table and are uncorrected. Elemental analyses were carried out on a Carlo Erba Elemental Analyzer. NMR spectra were taken on Varian Mercury 200 and Varian Mercury 400 spectrometers with HMDS as internal standard (δ = 0.055 ppm). The X-ray structure determination was performed on a Bruker-Nonius Kappa CCD automated diffractometer. Crystals of compound **13** were obtained by crystallization from ethanol.

2-Acetylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9-(tetrahydrofuran-2-yl)purine (3)

To a suspension of **2** (1.20 g, 3.5 mmol) in pyridine (18.0 mL) were added DIPEA (1.3 mL, 7.5 mmol) and DPC-Cl (0.93 g, 4.00 mmol). The reaction mixture was stirred for 2.5 h at r.t. whereupon a dark red solution was formed. H_2O (1.5 mL) was added and the mixture was stirred for another 20 min. All volatiles were removed under reduced pressure and the oily residue obtained was co-evaporated several times with toluene to remove the traces of pyridine and then taken up in EtOAc (100 mL). The solution was washed with 5% aq $NaHCO_3$ (30 mL), H_2O (3 × 30 mL) and brine (30 mL), dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (CH_2Cl_2–EtOH, 100:1.5) to afford chromatographically and spectrally pure **3** (1.47 g, 78.2%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.85–2.10 (s, 1 H, H-4′), 2.15 (s, 3 H, CH_3), 2.30–2.54 (m, 1 H, H-3′), 2.55–2.73 (m, 1 H, H-4′), 2.74–2.93 (m, 1 H, H-3′), 3.84–3.98 (m, 1 H, H-5′), 4.21–4.34 (m, 1 H, H-5′), 6.18–6.28 (m, 1 H, H-2′), 7.20–7.78 (m, 10 H, ArH), 10.79 (br s, 1 H, NH).

2-Acetylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9(7)*H*-purine (4)

Compound **3** (1.45 g, 2.7 mmol) was dissolved in warm EtOH (35 mL) and heated at reflux temperature. Within 5 min, a precipitate began to separate. After heating for an additional 15 min, the reaction mixture was allowed to cool to r.t. The precipitated solid was collected by filtration, washed with EtOH and dried in air to afford **4**.

Yield: 1.19 g (93.7%); mp 249–251 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.13 (s, 3 H, CH_3), 7.25–7.53 (m, 10 H, ArH), 10.69 (s, 1 H, NH), 14.39 (br s, 1 H, NH).

Anal. Calcd for $C_{20}H_{15}BrN_6O_3$: C, 51.41; H, 3.24; N, 17.98. Found: C, 51.47; H, 3.10; N, 17.65.

2-Benzoylamino-8-bromo-6-oxo-9-(tetrahydrofuran-2-yl)purine (6)

A suspension of **5** (0.6 g, 2.0 mmol) in a mixture of CH_2Cl_2 (40 mL) and pyridine (8 mL) was cooled to 0 °C in an ice-bath and TMSCl (1.5 mL, 11.8 mmol) was added, dropwise, over 2 min with stirring. The reaction was allowed to come to r.t. over 30 min, during which a clear solution was obtained. The flask was cooled again to 0–5 °C and BzCl (0.26 mL, 2.2 mmol) was added. The reaction mixture was stirred for 4 h at r.t., diluted with CH_2Cl_2 (20 mL) and poured into 5% aq $NaHCO_3$ (40 mL). The organic layer was separated, washed with 5% aq $NaHCO_3$ (20 mL), H_2O (2 × 20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a thick oil that was purified by flash chromatography on silica gel (CH_2Cl_2–EtOH, 100:2) to afford chromatographically and spectrally pure **6** (0.67 g, 82.7%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.85–2.07 (m, 1 H, H-4′), 2.31–2.51 (m, 1 H, H-3′), 2.53–2.71 (m, 1 H, H-4′), 2.72–2.90 (m, 1 H, H-3′), 3.82–3.96 (m, 1 H, H-5′), 4.18–4.32 (m, 1 H, H-5′), 6.12–6.22 (m, 1 H, H-2′), 7.43–7.72 (m, 3 H, ArH), 7.90–8.00 (m, 2 H, ArH).

2-Benzoylamino-8-bromo-6-oxo-9(7)*H*-purine (7)

Compound **6** (0.40 g, 1.0 mmol) was dissolved in warm EtOH (10 mL) and heated at reflux temperature for 15 min. The reaction mixture was cooled in a refrigerator and the precipitated solid was collected by filtration, washed with EtOH and dried in air to afford **7**.

Yield: 0.29 g (86.8%); mp >280 °C (decomp.).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 7.46–7.70 (m, 3 H, ArH), 7.97–8.06 (m, 2 H, ArH), 11.97 (s, 1 H, NH), 12.34 (s, 1 H, NH), 13.92 (br s, 1 H, NH).

Anal. Calcd for $C_{12}H_8BrN_5O_2$: C, 43.14; H, 2.41; N, 20.96. Found: C, 43.28; H, 2.30; N, 20.76.

2-Benzoylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9-(tetrahydrofuran-2-yl)-purine (8)

To a suspension of **6** (0.34 g, 0.8 mmol) in pyridine (4 mL) were added DIPEA (0.3 mL; 1.7 mmol) and DPC-Cl (0.21 g; 0.9 mmol). The reaction mixture was stirred for 4 h at r.t. whereupon a dark red solution formed. H_2O (0.5 mL) was added and the mixture was stirred for another 20 min. All volatiles were removed under reduced pressure and, after several co-evaporations with toluene to re-

move the traces of pyridine, an oily residue was obtained. The residue was purified by flash chromatography on silica gel (CH_2Cl_2–EtOH, 100:1.5) to afford chromatographically and spectrally pure **8** (0.37 g, 77.1%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.87–2.07 (m, 1 H, H-4′), 2.31–2.51 (m, 1 H, H-3′), 2.53–2.71 (m, 1 H, H-4′), 2.72–2.90 (m, 1 H, H-3′), 3.84–3.98 (m, 1 H, H-5′), 4.20–4.34 (m, 1 H, H-5′), 6.20–6.29 (m, 1 H, H-2′), 7.25–7.65 (m, 13 H, ArH), 7.89–8.00 (m, 2 H, ArH), 11.17 (s, 1 H, NH).

2-Benzoylamino-8-bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-9(7)*H*-purine (9)

Compound **8** (0.28 g, 0.47 mmol) was dissolved in a minimal amount of boiling EtOH. The solution was cooled in a refrigerator and the precipitated solid was collected by filtration, recrystallized from EtOH and dried in air to afford **9**.

Yield: 0.19 g (79.2%); mp 215–217 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 7.26–7.64 (m, 13 H, ArH), 7.91–7.99 (m, 2 H, ArH), 11.09 (s, 1 H, NH), 14.46 (br s, 1 H, NH).

Anal. Calcd for $C_{25}H_{17}BrN_6O_3$: C, 56.73; H, 3.24; N, 15.88. Found: C, 56.45; H, 3.06; N, 15.51.

8-Bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9-(tetrahydrofuran-2-yl)purine (10)

Compound **5** (1.20 g, 4.0 mmol) was dissolved in DMF (20 mL) with slight heating, then the solution was allowed to cool to r.t. and *N*,*N*-dimethylformamide dimethylacetal (2.38 g, 20.0 mmol) was added in one portion. The reaction mixture was stirred at r.t. and, within 10 min, a white solid began to separate. Stirring was continued for 4 h then the separated solid was collected by filtration, washed with EtOH and dried in air to afford **10** (1.16 g, 81.7%). The product was used for the next step without further purification. An analytical sample was recrystallized (EtOH–H_2O, 3:1) for analysis.

Mp 220–223 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.92–2.13 (m, 1 H, H-4′), 2.27–2.48 (m, 2 H, H-3′ and H-4′), 2.36 (s, 3 H, CH_3), 2.63–2.84 (m, 1 H, H-3′), 3.04 (s, 3 H, CH_3), 3.15 (s, 3 H, CH_3), 3.84–3.97 (m, 1 H, H-5′), 4.09–4.24 (m, 1 H, H-5′), 6.09–6.20 (m, 1 H, H-2′), 8.51 (s, 1 H, CH), 11.49 (s, 1 H, NH).

Anal. Calcd for $C_{12}H_{15}BrN_6O_2$: C, 40.58; H, 4.26; N, 23.66. Found: C, 40.39; H, 4.12; N, 23.61.

8-Bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-2-formylamino-9-(tetrahydrofuran-2-yl)purine (11)

To a suspension of **10** (0.36 g, 1.0 mmol) in pyridine (6 mL) were added DIPEA (0.40 mL, 2.3 mmol) and DPC-Cl (0.25 g, 1.1 mmol). The reaction mixture was stirred for 6 h at r.t. to obtain a dark red mixture. All volatiles were removed under reduced pressure and the oily residue was co-evaporated several times with toluene to remove the traces of pyridine and taken up in CH_2Cl_2 (50 mL). The CH_2Cl_2 solution was washed with 5% aq. $NaHCO_3$ (15 mL), H_2O (2 × 15 mL) and brine (15 mL), dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (CH_2Cl_2–EtOH, 50:1) to afford chromatographically and spectrally pure **11** (0.28 g, 78.9%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.90–2.12 (m, 1 H, CH-4′), 2.31–2.48 (m, 2 H, CH-4′ and CH-3′), 2.69–2.88 (m, 1 H, CH-3′), 3.85–3.99 (m, 1 H, CH-5′), 4.08–4.23 (m, 1 H, CH-5′), 6.19–6.28 (m, 1 H, NCH-2′), 7.03–7.53 (m, 10 H, ArH), 9.29 (d, *J* = 9.7 Hz, 1 H, CH), 11.20 (d, *J* = 9.7 Hz, 1 H, NH).

8-Bromo-6-(*N*,*N*-diphenylcarbamoyloxy)-2-formylamino-9(7)*H*-purine (12)

Compound **11** (0.24 g, 0.53 mol) was dissolved in EtOH (10 mL) and heated at reflux temperature for 20 min. The solution was cooled in a refrigerator and the precipitated solid was collected by filtration, recrystallized from EtOH and dried in air to afford **12**.

Yield: 0.21 g (87.5%); mp 264–265 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 7.23–7.64 (m, 10 H, ArH), 9.27 (d, *J* = 9.4 Hz, 1 H, CH), 11.08 (d, *J* = 9.4 Hz, 1 H, NH), 14.37 (br s, 1 H, NH).

Anal. Calcd for $C_{19}H_{13}BrN_6O_3 \cdot 0.5H_2O$: C, 49.37; H, 3.05; N, 18.18. Found: C, 49.39; H, 3.03; N, 17.95.

1-Benzoyl-8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9(7)*H*-purine (13)

To a suspension of **10** (1.24 g, 3.5 mmol) in MeCN (30 mL) were added pyridine (3.0 mL, 3.9 mmol), DMAP (0.043 g, 0.035 mmol) and BzCl (1.23 g, 1.0 mL, 8.75 mmol) in a dropwise manner. The reaction mixture was stirred at r.t. until a clear solution was obtained and TLC indicated the disappearance of the starting material (~72 h). The reaction mixture was diluted with EtOH (20 mL) and stirring was continued for another 30 min. All volatiles were removed under reduced pressure and, after several co-evaporations with toluene, a thick yellow oil was obtained. The oil was dissolved in $CHCl_3$ (100 mL) and any precipitate was removed by filtration. The filtrate was concentrated and the residue was purified by flash chromatography on silica gel ($CHCl_3$–EtOH, 40:1) to afford chromatographically and spectrally pure **9** (0.98 g, 72.1%). An analytical sample was recrystallized from EtOH and a crystal was submitted for X-ray crystallographic analysis (Figure 1 and Table 1).

Mp >165 °C (decomp.).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.59 (s, 3 H, CH_3), 3.05 (s, 3 H, CH_3), 7.51–7.83 (m, 5 H, ArH), 8.49 (s, 1 H, CH), 13.57 (s, 1 H, NH), 14.04 (s, 1 H, NH).

Anal. Calcd for $C_{15}H_{13}BrN_6O_2 \cdot 0.5C_2H_5OH$: C, 45.73; H, 3.84; N, 20.00. Found: C, 46.00; H, 3.67; N, 19.92.

2-Acetylamino-8-bromo-6-[(4-methylphenyl)sulfonyloxy]-9-(tetrahydrofuran-2-yl)purine (14)

To a suspension of **2** (0.34 g, 1.0 mmol) in CH_2Cl_2 (20 mL) were added Et_3N (0.52 mL, 3.7 mmol), DMAP (0.031 g, 0.26 mmol) and TsCl (0.50 g, 2.6 mmol). The reaction mixture was stirred at r.t. until a clear solution was obtained and TLC indicated the disappearance of starting material (24–48 h). The reaction mixture was diluted with CH_2Cl_2 (80 mL) and washed with 5% aq $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure to give a thick oil that was purified by flash chromatography on silica gel (CH_2Cl_2–EtOH, 100:2.5) to afford chromatographically and spectrally pure **10** (0.37 g, 74.0%).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.87–2.08 (m, 1 H, H-4′), 2.16 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 2.31–2.67 (m, 2 H, H-3′ and H-4′), 2.69–2.87 (m, 1 H, H-3′), 3.84–3.97 (m, 1 H, H-5′), 4.17–4.32 (m, 1 H, H-5′), 6.17–6.25 (m, 1 H, H-2′), 7.46–7.55 (m, 2 H, ArH), 8.10–8.18 (m, 2 H, ArH), 10.72 (s, 1 H, NH).

2-Acetylamino-8-bromo-6-[(4-methylphenyl)sulfonyloxy]-9(7)*H*-purine (15)

Compound **14** (0.15 g, 0.30 mmol) was dissolved in EtOH (10 mL) and heated at reflux temperature for 20 min. The solution was cooled in a refrigerator and the precipitated solid, consisting mostly of **1**, was removed by filtration. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel ($CHCl_3$–EtOH, 40:0.5) to afford **15**.

Table 1 Crystallographic Data of Compound **13**

Empirical formula	$C_{15}H_{17}BrN_6O_4$
Molecular weight	425.243
Crystal form	Prism
Crystal size (mm)	0.26 × 0.23 × 0.14
Crystal color	Colorless
Crystal system	Monoclinic
a (Å)	14.4399(7)
b (Å)	14.9685(7)
c (Å)	18.7679(13)
β (°)	104.063(2)
V (Å^3)	1869.08(5)
Space group	*P* $2_1/c$
Z	4
F(000)	1728
μ (mm^{-1})	2.121
Density (calc.) (g/cm^3)	1.436
$2\theta_{max}$ for data (°)	50.0
Diffractometer	Nonius Kappa CCD
Methods of collection	φ and ω scans
Index ranges	$-15 \le h \le 16$
	$-17 \le k \le 17$
	$-22 \le l \le 22$
Reflections collected	9386
Independent reflections	6482 (R_{int} = 0.055)
Reflections with $I > 2\sigma(I)$	3553
Method of solution	SIR97 [1]
Method of structure refinement	SHELXL97 [2]
Number of refined parameters	469
$(\Delta/\sigma)_{max}$	0.001
Final *R*-factor	0.0794
CCDC deposition number	297189

Yield: 0.029g (22.3%); mp 176–177 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.15 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3), 7.48 (d, *J* = 8.3 Hz, 2 H, ArH), 8.14 (d, *J* = 8.3 Hz, 2 H, ArH), 10.66 (s, 1 H, CH), 14.46 (br s, 1 H, NH).

Anal. Calcd for $C_{14}H_{12}BrN_5O_4S$: C, 39.45; H, 2.84; N, 16.43. Found: C, 39.81; H, 2.89; N, 16.08.

1-Benzyl-8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9-(tetrahydrofuran-2-yl)purine (16)

To a suspension of **10** (0.43 g, 1.2 mmol) in DMF (15 mL) were added K_2CO_3 (0.26 g, 1.9 mmol) and BnBr (0.22 g, 0.16 mL, 1.3 mmol). The reaction mixture was stirred for 24 h at r.t. then an additional amount of BnBr (0.05 g, 0.04 mL, 0.03 mmol) was added and the stirring was continued overnight. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give a residue that was purified by flash chromatography on silica gel ($CHCl_3$) and recrystallized from EtOH to afford **16**.

Yield: 0.36 g (67.9%); mp 168–170 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.95–2.11 (m, 1 H, CH-4′), 2.30–2.45 (m, 2 H, CH-4′ and CH-3′), 2.64–2.87 (m, 1 H, CH-3′), 3.02 (s, 3 H, CH_3), 3.15 (s, 3 H, CH_3), 3.85–3.95 (m, 1 H, CH-5′), 4.11–4.22 (m, 1 H, CH-5′), 5.37 (s, 2 H, NCH_2), 6.12–6.18 (m, 1 H, CH-2′), 7.18–7.31 (m, 5 H, ArH).

^{13}C NMR (400 MHz, DMSO-d_6): δ = 25.96 (C-4′), 29.86 (C-3′), 35.46 (N-CH_3), 41.17 (N-CH_3), 45.11 (C-10), 69.79 (C-5′), 86.78 (C-2′), 119.72 (C-5), 122.57 (C-8), 127.21 (C-14), 127.83 (C-12), 128.58 (C-13), 138.81 (C-11), 149.36 (C-4), 156.61 (C-6), 156.69 (C-2), 158.33 (C-17).

1-Benzyl-8-bromo-2-(*N*,*N*-dimethyliminoformamido)-6-oxo-9(7)*H*-purine (17)

Compound **16** (0.095 g, 0.2 mmol) was dissolved in EtOH (7 mL) and several crystals of PTSA were added. The reaction mixture was heated at reflux for 20 min then cooled in a refrigerator. The precipitated solid was collected by filtration and washed with EtOH to afford **17**.

Yield: 0.065 g (81.3%); mp 243–245 °C.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.99 (s, 3 H, CH_3), 3.14 (s, 3 H, CH_3), 5.38 (s, 2 H, NCH_2), 7.12–7.33 (m, 5 H, ArH), 8.50 (s, 1 H, CH), 13.32 (br s, 1 H, NH), 13.80 (br s, 1 H, NH).

Anal. Calcd for $C_{15}H_{15}BrN_6O$: C, 48.02; H, 4.03; N, 22.40. Found: C, 47.95; H, 3.85; N, 22.42.

References

(1) Legraverend, M.; Grierson, D. S. *Bioorg. Med. Chem.* **2006**, *14*, 3987.
(2) Robins, M. J.; Zou, R.; Guo, Z.; Wnuk, S. P. *J. Org. Chem.* **1996**, *61*, 9207.
(3) Madre, M.; Panchenko, N.; Zhuk, R.; Geenevasen, J. A.; van den Burg, A.; Koomen, G.-J. *Synthesis* **1999**, 775.
(4) (a) Zou, R.; Robins, M. J. *Can. J. Chem.* **1987**, *65*, 1436. (b) Robins, M. J.; Zou, R.; Guo, Z.; Wnuk, S. P. *J. Org. Chem.* **1996**, *61*, 9207. (c) Timar, Z.; Kovacs, L.; Kovacs, G.; Schmil, Z. *J. Chem. Soc., Perkin Trans. 1* **2000**, 19. (d) Dalpozzo, R.; de Nino, A.; Maiuolo, L.; Procopio, A.; de Munko, G.; Sindona, G. *Tetrahedron* **2001**, *57*, 4035. (e) Guillarme, S.; Legoupy, S.; Bourgougnon, N.; Aubertin, A.-M.; Huet, F. *Tetrahedron* **2003**, *59*, 9635.
(5) Huss, S.; Gosselin, G.; Imbach, J.-L. *J. Org. Chem.* **1988**, *53*, 499.
(6) Madre, M.; Zhuk, R.; Koomen, G.-J. *Nucleosides Nucleotides* **1997**, *16*, 1107.
(7) Reese, C. B.; Saffhill, R. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2937.
(8) Fan, Y.; Gaffney, B. L.; Jones, R. A. *Org. Lett.* **2004**, *6*, 2555.
(9) Ikaunieks, M.; Madre, M. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 755.
(10) Grotli, M.; Douglas, M.; Beijer, B.; Garcia, R. G.; Eritja, R.; Sproat, B. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2779.

(11) Vincent, S.; Mioskowski, C.; Lebeau, L. *J. Org. Chem.* **1999**, *64*, 991.
(12) Belyakov, S.; Ikaunieks, M.; Madre, M. *Acta Crystallogr., Sect. E* **2005**, *61*, 3452.
(13) (a) Bridson, P. K.; Markiewicz, W. T.; Reese, C. B. *J. Chem. Soc., Chem. Commun.* **1977**, 791. (b) Moon, B. J.; Huh, K. L. *Bull. Korean Chem. Soc.* **1991**, *12*, 196.
(14) Cruickshank, K. A.; Jiricny, J.; Reese, C. B. *Tetrahedron Lett.* **1984**, *25*, 681.
(15) Nonagawa, M.; Arai, T.; Endo, N.; Pack, S. P.; Kodaki, T.; Makino, K. *Org. Biomol. Chem.* **2006**, *4*, 1811.
(16) (a) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron* **1997**, *53*, 3035. (b) Lakshman, M. K.; Thomson, P. F.; Nuqui, M. A.; Hilmer, J. H.; Sevova, N.; Boggess, B. *Org. Lett.* **2002**, *4*, 1479.
(17) Štimac, A.; Muhič, D.; Kobe, J. *Nucleosides Nucleotides* **1994**, *13*, 625.
(18) Huang, Y.; Johnson, F. *Nucleosides, Nucleotides Nucleic Acids* **2002**, *21*, 435.
(19) Madre, M.; Panchenko, N.; Golbraikh, A.; Zhuk, R.; Pandit, U. K.; Geenevasen, J.; Koomen, G.-J. *Collect. Czech. Chem. Commun.* **1999**, *64*, 685.
(20) Quintanilla-Licea, R.; Colunga-Valladares, J. F.; Caballero-Quintero, A.; Rodriguez-Padilla, C.; Tamez-Guerra, R.; Gomez-Flores, R.; Waksman, N. *Molecules* **2002**, *7*, 662.