Synthetic Routes to \(N\)-9 Alkylated 8-Oxoguanines; Weak Inhibitors of the Human DNA Glycosylase OGG1

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**Abstract:** The human 8-oxoguanine DNA glycosylase OGG1 is involved in base excision repair (BER), one of several DNA repair mechanisms that may counteract the effects of chemo- and radiation therapy for the treatment of cancer. We envisage that potent inhibitors of OGG1 may be found among the 9-alkyl-8-oxoguanines. Thus we explored synthetic routes to 8-oxoguanines and examined these as OGG1 inhibitors. The best reaction sequence started from 6-chloroguanine and involved \(N\)-9 alkylation, C-8 bromination, and finally simultaneous hydrolysis of both halides. Bromination before \(N\)-alkylation should only be considered when the \(N\)-substituent is not compatible with bromination conditions. The 8-oxoguanines were found to be weak inhibitors of OGG1. 6-Chloro-8-oxopurines, byproducts in the hydrolysis of 2,6-halopurines, turned out to be slightly better inhibitors than the corresponding 8-oxoguanines.

**Keywords:** alkylation; cancer; DNA; enzyme inhibitors; guanine; halogenation
1. Introduction

Chemo- and radiotherapy are, in addition to surgery for removal of solid tumors, the two main treatment protocols currently available to improve the outcome of cancer patients in general, but treatment-related toxicity, the risk of secondary cancers, and the emergence of resistance limit their effectiveness [1]. Some chemotherapeutic drugs and radiotherapy work partly by imposing high concentrations of DNA damage on the genome of cancer cells, beyond the repair capacity of those cells. The drug-exposed cancer cells are heavily dependent on efficient DNA repair to survive. Consequently, inhibitors that reduce DNA repair activities should sensitize cancer cells to chemo- and/or radiotherapy [2–5].

Several DNA repair mechanisms counteract exogenous and endogenous processes that destabilize or directly damage genomes. The processes include, among others, base excision repair (BER), a mechanism that depends on enzymes that recognize small modifications in the native bases in DNA, resulting from alkylation, oxidation, deamination, or hydrolysis of the DNA bases. The pathway is initiated by a damage-specific DNA glycosylase that removes the altered base [6]. Some of these enzymes mainly remove oxidized bases, such as the human 8-oxoguanine DNA glycosylase (OGG1) that removes guanines that have been oxidized at the C8-position. The 8-oxoguanine base in the DNA is flipped into a lesion recognition pocket on the enzyme surface, exposing the Watson–Crick signature of guanine and the oxidized C8 position (Figure 1).

**Figure 1.** Structural details of 8oxoG base flipped into the lesion recognition pocket of OGG1 (Protein Data Bank deposition 1EBM [7]). The protein backbone is shown as a blue ribbon/helix. Selected amino acid side chains and the 8oxoG base are shown as ball-and-stick. Hydrogen bonds between the protein and 8oxoG are shown as dashed lines. Asp268 is the catalytic residue in OGG1. Symbols 5′ and 3′ indicate the position of the 5′ and 3′ phosphodiester links in the DNA.

We envisage that potent inhibitors of OGG1 may be found among the 9-alkyl-8-oxoguanines. The 8-oxo derivatives of guanosine or deoxyguanosine are probably not inhibitors of the glycosylases since they themselves may be substrates for the enzymes that cleave N,O-acetals in nucleic acids. As a continuance of our synthetic studies directed towards 9-substituted 8-oxoadenines [8,9], we herein present strategies for the synthesis of N-9 substituted 8-oxoguanines. Previous routes include rather tedious constructions of the guanine ring system [10–12], and hydrolysis of purine precursors; hydrolysis of
8-halopurines [13–16], or less conveniently hydrolysis of \( N-7 \) functionalizedpurines [11,17–19]. Results regarding inhibitory activity against the human DNA glycosylase OGG1 are also presented.

2. Results and Discussion

2.1. Chemistry

We found it most convenient to start the synthesis of 9-alkyl-8-oxopurines from commercially available purines, and in our opinion the best way to introduce the 8-oxo group would be by hydrolysis of an 8-halopurine. However, there still was the question of whether the halogen or the \( N-9 \) substituent should be introduced first and which protection/activation groups should be employed in the synthesis. Ideally, such groups should also be removed in the final hydrolysis step. Regioselectivity in \( N \)-alkylation of guanine derivatives was also an issue [20–27]. We chose to start from two guanine precursors, commercially available 2-amino-6-chloropurine (1a) and the \( O \)-carbamoylguanine 1b, easily available from guanine [28,29]. The synthetic routes explored are all summarized in Scheme 1.

**Reagents and conditions:**
(a) See Table 1; (b) See Table 2; (e) 1. Ac₂O, NaOAc, AcOH, 2. NaOH(aq), \( \Delta \); (d) 1. LDA, 2. (CCl₂Br)₂, THF, \(-78{\degree}C\); (e) Br₂, CHCl₃; (f) See [30]; (g) HCl(aq), EtOH.

**Scheme 1.** Synthetic routes to 8-oxoguanines 5.
Table 1. N-alkylation of guanine precursors 1a and 1b.

| Entry | R² | R⁶ | R          | Reagents and Conditions                              | Ratio 2:3:1 a | Yield (%) 2 b | Yield (%) 3 b |
|-------|----|----|------------|-----------------------------------------------------|---------------|---------------|---------------|
| 1     | Cl | NH₂| CH₂-c-hexyl| RBr, K₂CO₃, DMF, rt, 72 h                            | 80:20:0       | 67, 2a        | 10, 3a        |
| 2     | Cl | NH₂| CH₂-c-hexyl| ROH, DIAD, PPh₃, THF, 70 °C, 14 h                    | 93:7:0        | 76, 2a        | 5, 3a         |
| 3     | OCONPh₂ | NHAc | CH₂-c-hexyl | RBr, K₂CO₃, DMF, rt, 72 h                            | 81:19:0       | 45, 2e        | 7, 3e         |
| 4     | OCONPh₂ | NHAc | CH₂-c-hexyl | ROH, DIAD, PPh₃, THF, 70 °C, 14 h                    | 82:18:0       | 70, 2e        | 3, 3e         |
| 5     | Cl | NH₂| c-hexyl    | RI, K₂CO₃, DMF, rt, 72 h                             | 15:0:85       | –             | –             |
| 6     | Cl | NH₂| c-hexyl    | ROTs, K₂CO₃, DMF, rt, 72 h                           | – d           | 33, 2b        | –             |
| 7     | Cl | NH₂| c-hexyl    | ROH, DIAD, PPh₃, THF, 70 °C, 14 h                    | 8:4:88        | –             | –             |
| 8     | Cl | NH₂| c-hexyl    | ROH, DIAD, PPh₃, THF, ultrasound, 14 h               | 27:0:73       | 20, 2b        | –             |
| 9     | Cl | NH₂| c-hexyl    | ROH, DIAD, PPh₃, DMF, 150 °C, μW, 2 h                | 41:8:51       | –             | –             |
| 10    | OCONPh₂ | NHAc | c-hexyl    | ROTs, K₂CO₃, THF, rt, 72 h                           | – d           | 30, 2f        | –             |
| 11    | OCONPh₂ | NHAc | c-hexyl    | ROTs, K₂CO₃, DMF, 80 °C, 72 h                        | – de          | –             | –             |
| 12    | OCONPh₂ | NHAc | c-hexyl    | ROH, DIAD, PPh₃, THF, 70 °C, 14 h                    | – d           | 22, 2f        | –             |
| 13    | Cl | NH₂| c-pentyl   | RBr, K₂CO₃, DMF, rt, 72 h                            | 86:14:0       | 71, 2e        | 5, 3e         |
| 14    | Cl | NH₂| c-pentyl   | ROH, DIAD, PPh₃, THF, 70 °C, 14 h                    | 91:9:0        | 72, 2e        | 6, 3e         |
| 15    | OCONPh₂ | NHAc | c-pentyl   | RBr, K₂CO₃, DMF, rt, 72 h                            | 76:15:09      | 52, 2g        | –             |
| 16    | OCONPh₂ | NHAc | c-pentyl   | ROH, DIAD, PPh₃, THF, 70 °C, 14 h                    | 90:10:0       | 58, 2g        | –             |
| 17    | Cl | NH₂| c-pent-2-enyl | RBr, K₂CO₃, DMF, rt, 24 h                           | 23:16:61      | 18, 2d        | –             |
| 18    | Cl | NH₂| c-pent-2-enyl | ROH, DIAD, PPh₃, THF, 70 °C, 42 h                   | 55:18:27      | 40, 2d        | –             |
| 19    | Cl | NH₂| c-pent-2-enyl | RBr, K₂CO₃, DMF, rt, 24 h                           | 75:25:0       | 53, 2d        | 18, 3d        |

a From ¹H-NMR spectra of the crude products, the signals from H-8 in compounds 1, 2, and 3 were integrated; b Isolated yields; c Not isolated in pure form; d Difficult to determine due to overlapping signals in the ¹H-NMR spectra; e A complex mixture was formed; f Comparable results were obtained in DMF.
Table 2. Synthesis of 8-bromopurines 4.

| Entry | Starting Material * | Reagents and Conditions | Yield (%) 4 a,b |
|-------|---------------------|-------------------------|-----------------|
| 1     | 2a                  | Br₂, H₂O                | 79%, 4a         |
| 2     | 10                  | RBr, K₂CO₃, DMF         | 34%, 4a         |
| 3     | 10                  | ROH, DIAD, PPh₃, THF, 70 °C | 56%, 4a       |
| 4     | 2b                  | Br₂, H₂O                | 70%, 4b         |
| 5     | 2c                  | Br₂, H₂O                | 81%, 4c         |
| 6     | 2d                  | 1. LDA, 2. CCl₂BrCCl₂Br, THF, −78 °C | 32%, 4d |
| 7     | 10                  | ROH, DEAD, PPh₃, THF, 70 °C | 42%, 4d       |
| 8     | 10                  | ROAc, Pd(PPh₃)₄, NaH, DMF, 50 °C | 29%, 4d |

* The structures are shown in Scheme 1; b Isolated yields.

First we chose to N-alkylate the substrates 1 before C-8 halogenation and hydrolysis. Alkylations were conducted by various methodologies in order to find the conditions that gave the desired N-9 alkylated isomer 2 with high selectivity and in a good isolated yield (Scheme 1, Table 1). Relatively simple alkylating agents were chosen for the model reactions and we focused on alkylation with alkyl halides in the presence of base, Mitsunobu reactions, and Pd-catalyzed allylic alkylation.

The cyclohexylmethyl substituent could be introduced at N-9 either by reaction with alkyl bromide in the presence of a base [31,32] (Table 1, Entries 1 and 3) or with cyclohexylmethanol under Mitsunobu conditions (Table 1, Entries 2 and 4). The latter is often claimed to be more N-9 selective compared to classical alkylations of purines [33–35]. In all cases a mixture of the N-9 and N-7 alkylated isomers (2 and 3) was formed with good selectivity for the desired isomer 2. The isomers were identified from HMQC and HMBC-NMR, as described before [31].

The guanine precursor 1b, carrying a bulky substituent at C-6 that may sterically block N-7, is reported to react with high N-9 selectivity in other N-functionalization reactions [28,29,36–41]. Nevertheless, we found the regioselectivity in N-alkylation of purine 1b equal or slightly poorer compared to 6-chloroguanine 1a in all reactions performed in this study. In the alkylation of compound 1b, minor amounts of other relatively polar products were formed under both reaction conditions. These often made purification of the N-7 alkylated isomer 3 difficult. The identity of the byproducts could not be determined, but they may be formed as a result of cleavage of the O6-protecting group. Alkylation of N2, as observed by others [41], was not seen.

Introduction of the cyclohexyl group at N-9 turned out to be quite difficult (Table 1, Entries 5–12). Both starting materials (1a and 1b) did not react with cyclohexyl bromide (data not shown) and reacted slowly with cyclohexyl iodide or the corresponding tosylate, but compounds 2b and 2f could be isolated in modest yields (Table 1, Entries 5, 6, 10 and 11). It is, however, well known that cyclohexyl halides or pseudo halides may react sluggishly in substitution reactions [42]. The results were not significantly improved when the Mitsunobu reaction was employed (Table 1; Entries 7, 8, and 12), not even under ultrasound (Table 1, Entry 8) or microwave conditions (Table 1, Entry 9).

The cyclopentyl group could easily be installed at N-9 on both starting materials 1a and 1b by reaction with cyclopentyl bromide and base (Table 1, Entries 13 and 15) or by alkylation under Mitsunobu conditions (Table 1, Entries 14 and 16). The selectivity for N-9 was higher in the Mitsunobu reactions, but the isolated yields were comparable due to more tedious purification when Mitsunobu conditions, also producing phospine oxides and reduced azodicarboxylates, were employed.
Finally we introduced the cyclopent-2-enyl group at N-9 (Table 1, Entries 17–19). These reactions were only conducted at the guanine precursor 1a, since we so far had not observed any significant improvement in regioselectivity when compound 1b was employed and we had observed problems with compounds derived from purine 1b later in the planned synthetic sequence. In addition to alkylation with the halide and Mitsunobu reaction with the alcohol, we also attempted palladium catalyzed alkylation with the allylic acetate [43]. 3-Bromocyclopentene could only be generated as a 15% solution in CCl₄ and the reagent had a limited stability, probably partly due to traces of the radical initiator used in the synthesis left in the solution [44], which may explain the low yield of product 2d (Table 1, Entry 17). The Mitsunobu reaction between purine 1a and cyclopenten-2-ol was surprisingly slow, and full conversion was not achieved even after several days. Furthermore, the N-9/N-7 selectivity was only ca. 4:1 (Table 1, Entry 18). Pd-catalyzed allylic alkylation of purine 1a went to completion and gave the isomers 2d and 3d in a 4:1 ratio (Table 1, Entry 19).

The 6-chloropurines 2a, 2b, and 2c were readily brominated on C-8 simply by treatment of bromine in water (Scheme 1; Table 2; Entries 1, 4, and 5). For compound 2d, which has an alkene function, the bromide was introduced by C-8 lithiation followed by trapping with CCl₂BrCCl₂Br (Table 2, Entry 6) [9,32,45,46]. However, the yield was surprisingly low and also another route to bromide 4d was examined (see below). Finally hydrolysis of the dihalopurines 4, employing conditions used for hydrolysis of other 8-bromopurines [13–16,47], gave the 8-oxoguanines 5. Complete conversion was achieved in the hydrolysis compound 4a, whereas small amounts of the partly hydrolyzed chlorides 6 where present after hydrolysis of purines 4b–d even after prolonged reaction times.

Attempts to brominate the O-carbamoylguanine 2e failed (Scheme 1). Treatment with bromine or lithiation followed by trapping with CCl₂BrCCl₂Br only resulted in cleavage of the carbamoyl protecting group to give the guanine derivative 7. When compound 2e was treated with NBS, no reaction took place at all. Thus, no attempts were made to brominate the carbamoyl protected guanines 2f and 2g.

Since bromination of the cyclopentenylpurine 2d turned out to be a challenge, we also examined the possibility for introducing the 8-halo substituent before the N-9 alkyl group (Scheme 1). We chose to brominate the THP protected compound 8 [30] and removed the protection group under mild acidic condition, but direct bromination of purine 1a in a moderate yield has also been reported [48].

Alkylation of 8-bromo-6-chloropurin-2-amine (10) by bromomethylcyclohexane in the presence of K₂CO₃/DMF (Table 2, Entry 2) occurred slowly compared to alkylation of 2-amino-6-chloropurine (1a) under the same set of reaction conditions (for alkylation of compound 1a see Table 1). NMR analysis showed that approximately 50% of the starting material was intact even after 96 h reaction time and the desired product was isolated in a low yield. Also, ca. 4% of N-7 alkylated isomer was formed, as judged by NMR. When compound 10 was reacted under Mitsunobu (Table 2, Entry 3) conditions, high conversion (ca. 95%) and almost full selectivity towards the desired N-9 alkylated isomer 4a was achieved, as judged by ¹H-NMR. However, the product 4a was isolated only in 56% due to tedious separation from reduced DIAD. Since compound 10 reacted slower (conventional alkylation) or comparably (Mitsunobu alkylation) to compound 1a, it was concluded that there were no benefits associated with introducing the bromide before the N-alkyl group for the synthesis of 8-bromopurines 4a–c.

Also, synthesis of the 9-cyclopentenylpurine 4d by N-alkylation of compound 10 was examined (Table 2, Entries 7 and 8) since bromination of 2-amino-6-chloro-9-cyclopentenylpurine 2d turned out to give only a low yield of the desired product. Again, isolation of the desired product from alkylation
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under Mitsunobu conditions turned out to be troublesome. We tried this Mitsunobu alkylation using the water-soluble azodicarboxylate DMEAD (di-2-methoxyethyl azodicarboxylate) as well as DIAD [49]. Purification of the product was less complicated, but the conversion was low and ca. 40% of starting material 10 was recovered. Also, Pd-catalyzed alkylation turned out to be a very slow reaction and even after six days only 29% of the desired compound 4d could be isolated, together with 32% unconverted starting material 10.

2.2. Biology

As previously mentioned, our hypothesis was that N-alkyl-8-oxoguanines may inhibit the human 8-oxoguanine DNA glycosylase (OGG1). Other substituents in the purine 8-position are probably not tolerated, for instance 8-bromo- and 8-aminoguanines are reported to be enhancers for OGG1 activity [50]. Thus, the 8-oxoguanines 5 as well as the partly hydrolyzed 6-chloro-8-oxopurines 6 were tested against human DNA glycosylases OGG1 and NTH1. A general structure of the tested compounds is shown in Figure 2 and the results are presented in Tables 3 and 4, and Supplementary Figure S19.

![Image](https://example.com/image.png)

**Figure 2.** General structure of the compounds shown in Table 3.

| Compound | X    | R     | % Activity |
|----------|------|-------|------------|
| 5a       | OH   | CH₂-c-hexyl | 89 ± 5     |
| 5b       | OH   | c-hexyl    | 92 ± 2     |
| 6b       | Cl   | c-hexyl    | 70 ± 11    |
| 5c       | OH   | c-pentyl   | 101 ± 12   |
| 6c       | Cl   | c-pentyl   | 72 ± 9     |
| 5d       | OH   | c-pent-2-enyl | 92 ± 7    |
| 6d       | Cl   | c-pent-2-enyl | 84 ± 3    |

* The predominant 6-oxo tautomer of compounds 5 is shown in Scheme 1.

| Compound | X    | R     | % Activity |
|----------|------|-------|------------|
| 5a       | OH   | CH₂-c-hexyl | 96 ± 3     |
| 5b       | OH   | c-hexyl    | 123 ± 20   |
| 6b       | Cl   | c-hexyl    | 73 ± 37    |
| 5c       | OH   | c-pentyl   | 102 ± 16   |
| 6c       | Cl   | c-pentyl   | 108 ± 18   |
| 5d       | OH   | c-pent-2-enyl | 104 ± 21  |
| 6d       | Cl   | c-pent-2-enyl | 89 ± 13   |

* The predominant 6-oxo tautomer of compounds 5 is shown in Scheme 1.
Compounds 6b and 6c inhibit the OGG1 enzyme by ca. 30%, followed by compounds 5a, 5b, and 6d at ca. 10%–15%, all at 0.2 mM ligand concentration. Interestingly, the halogenated compounds seem in general to be better inhibitors than their 6-oxo derivatives. To check enzyme specificity, we tested the same seven compounds at the higher concentration of 0.5 mM against NTH1, a structural but not functional homolog of OGG1. Both enzymes have a deep pocket for binding of oxidized bases; in general, OGG1 repairs oxidized purines while NTH1 is involved in repair of oxidized pyrimidines. Compound 6b reduced the NTH1 activity by around 25% at 0.5 mM ligand concentration. An effect of varying the N-9 substituent is not so evident from the few compounds examined.

3. Experimental Section

3.1. General Information

$^1$H-NMR spectra were recorded at 300 MHz with a Bruker DPX 300, at 400 MHz with a Bruker DPX 400 or at 600 with a Bruker AVI 600 instrument (Bruker BioSpin AG, Fällanden, Switzerland). The $^{13}$C-NMR spectra were recorded at 75, 100, or 150 MHz with the Bruker instruments listed above. Assignments of $^1$H and $^{13}$C resonances are inferred from 1D $^1$H-NMR, 1D $^{13}$C-NMR, DEPT, or APT, and 2D NMR (HMQC, HMBC) spectroscopical data. $^1$H- and $^{13}$C-NMR spectra of all novel compounds can be found in the Supplementary Material (Figures S1–S18). HRMS (EI) was performed with a double-focusing magnetic sector VG Prospec Q instrument (Waters, Manchester, UK) and HRMS (ESI) with a TOF quadrupole Micromass QTOF 2 W instrument (Waters). Melting points were determined with a Büchi Melting point B-545 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. Dry DMF and THF were obtained from a solvent purification system, MB SPS-800 (MBraun, Garching, Germany). Acetic anhydride and diisopropylamine were distilled over CaH$_2$. DMSO was dried over activated 3 Å molecular sieves for four days. Potassium carbonate was oven dried at 150 °C under high vacuum for 12 h. A saturated aqueous solution of Br$_2$ was prepared by stirring water (20 mL) with Br$_2$ (0.200 mL) in a closed container for 15 min at ambient temperature. Sodium hydride (ca. 60% in mineral oil) was washed with dry pentane under inert atmosphere prior to use. All other reagents were commercially available and used as received. The following compounds were available by literature methods: Cyclohexyl tosylate [51], cyclopentenyl bromide [44], cyclopent-2-enol [52], cyclopentenyl acetate [53], 1b [29], 8 [30].

3.2. Synthesis

3.2.1. 2-Amino-6-chloro-9-(cyclohexylmethyl)-9H-purine (2a) and 2-Amino-6-chloro-7-(cyclohexylmethyl)-7H-purine (3a)

Method A: K$_2$CO$_3$ (1.63 g, 11.8 mmol) was added to a stirring solution of compound 1a (1.00 g, 5.90 mmol) in dry DMF (30 mL) at ambient temperature under N$_2$. After 20 min bromomethylcyclohexane (0.905 mL, 6.49 mmol) was added and the resulting mixture was stirred for 72 h, filtered, and evaporated. The isomers were separated by flash chromatography on silica gel, eluting with MeOH–CH$_2$Cl$_2$ (1:9) to yield 2a (1.05 g, 67%) and 3a (150 mg, 10%).
2a: colorless solid; mp 148–150 °C (lit. [54], 154–155 °C); 1H-NMR (DMSO-d$_6$, 400 MHz) $\delta$ 8.10 (s, 1H, H-8), 6.91 (s, 2H, NH$_2$), 3.88 (d, $J = 7.4$ Hz, 2H, NCH$_2$), 1.88–1.72 (m, 1H, H-1 in c-hex), 1.68–1.52 (m, 3H, c-hex), 1.51–1.42 (m, 2H, c-hex), 1.19–1.02 (m, 3H, c-hex) 1.00–0.85 (m, 2H, c-hex); 13C-NMR (DMSO-d$_6$, 100 MHz) $\delta$ 159.8 (C, C-2), 154.3 (C, C-4), 149.3 (C, C-6), 143.7 (CH, C-8), 123.3 (C, C-5), 48.8 (CH$_2$, NCH$_2$), 37.1 (CH, C-1 in c-hex), 29.9 (CH$_2$, C-3 and C-5 in c-hex), 25.8 (CH$_2$, C-4 in c-hex), 25.0 (CH$_2$, C-2 and C-6 in c-hex); HREIMS $m/z$ 265.1092 (calcd for C$_{12}$H$_{16}$ClN$_5$, 265.1094). Spectral data were in good agreement with those reported before [54].

3a: colorless solid mp 228–231 °C. 1H-NMR (DMSO-d$_6$, 400 MHz) $\delta$ 8.32 (s, 1H, H-8), 6.62 (s, 2H, NH$_2$), 4.10 (d, $J = 7.2$ Hz, 2H, NCH$_2$), 1.82–1.70 (m, 1H, H-1 in c-hex), 1.69–1.54 (m, 3H, c-hex), 1.50–1.41 (m, 2H, c-hex), 1.24–1.06 (m, 3H, c-hex), 1.03–0.89 (m, 2H, c-hex); 13C-NMR (DMSO-d$_6$, 100 MHz) $\delta$ 164.2 (C, C-4), 159.9 (C, C-2), 149.8 (CH, C-8), 142.3 (C, C-6), 114.9 (C, C-5), 51.8 (CH$_2$, NCH$_2$), 38.6 (CH, C-1 in c-hex), 29.6 (CH$_2$, C-3 and C-5 in c-hex), 25.8 (CH$_2$, C-4 in c-hex), 25.1 (CH$_2$, C-2 and C-3 in c-hex); HREIMS $m/z$ 265.1096 (calcd for C$_{12}$H$_{16}$ClN$_5$, 265.1094).

Method B: Compound 1a (200 mg, 1.18 mmol) was added to a solution of cyclohexylmethanol (141 mg, 1.24 mmol) and PPh$_3$ (325 mg, 1.24 mmol) in dry THF (10 mL) under N$_2$. The resulting suspension was treated with diisopropyl azodicarboxylate (DIAD) (0.244 mL, 1.24 mmol) and the reaction mixture was stirred at 70 °C for 7 h before cyclohexylmethanol (141 mg, 1.24 mmol), PPh$_3$ (325 mg, 1.24 mmol), and DIAD (0.244 mL, 1.24 mmol) were added. The mixture was stirred for another 7 h at 70 °C, cooled, treated with brine (10 mL), and extracted with CH$_2$Cl$_2$ (3 × 75 mL). The combined organic layers were washed with water (50 mL), dried (Na$_2$SO$_4$) and evaporated in vacuo. The isomers were separated by flash chromatography on silica gel eluting with EtOAc–Hexane (gradient; 70%–100% EtOAc) followed by MeOH–EtOAc (1:9) to yield 2a (240 mg, 76%) and 3a (16 mg, 5%).

3.2.2. 2-Amino-6-chloro-9-(cyclohexyl)-9H-purine (2b)

Method A: The title compound was prepared from compound 1a (200 mg, 1.18 mmol), K$_2$CO$_3$ (326 mg, 2.36 mmol) and cyclohexyl tosylate (450 mg, 1.77 mmol) in DMF (15 mL) as described for the synthesis of compounds 2a above. MeOH–EtOAc (1:9) was used for flash chromatography to yield 2b (98 mg, 33%). Colorless needles; mp 163–165 °C (lit. [55], 165 °C); 1H-NMR (DMSO-d$_6$, 400 MHz) $\delta$ 8.23 (s, 1H, H-8), 6.88 (s, 2H, NH$_2$), 4.28–4.12 (m, 1H, H-1 in c-hex), 2.01–1.75 (m, 7H, c-hex), 1.45–1.17 (m, 3H, c-hex); 13C-NMR (DMSO-d$_6$, 100 MHz) $\delta$ 159.5 (C, C-6), 153.5 (C, C-4), 149.3 (CH, C-8), 142.3 (C, C-6), 114.9 (C, C-5), 51.8 (CH$_2$, NCH$_2$), 38.6 (CH, C-1 in c-hex), 29.6 (CH$_2$, C-3 and C-5 in c-hex), 25.8 (CH$_2$, C-4 in c-hex), 25.1 (CH$_2$, C-2 and C-3 in c-hex); HREIMS $m/z$ 251.0934 (calcd for C$_{12}$H$_{14}$ClN$_5$, 251.0938). Spectral data were in good agreement with those reported before [55].

Method B: The title compound was prepared from compound 1a (1.00 g, 5.90 mmol), cyclohexanol [2 × (620 mg, 6.19 mmol)], PPh$_3$ [2 × (1.62 g, 6.19 mmol)] and DIAD [2 × (1.22 mL, 6.19 mmol) in THF (50 mL) as described for the synthesis of compounds 2a above. After each addition of cyclohexanol the mixture was subjected to sonication for 20 min using a sonicator probe. EtOAc–Hexane (gradient; 70%–100% EtOAc) was used for flash chromatography to yield 2b (295 mg, 20%).
3.2.3. 2-Amino-6-chloro-9-(cyclopentyl)-9H-purine (2c) and 2-Amino-6-chloro-7-(cyclopentyl)-7H-purine (3c)

**Method A:** The title compounds were prepared from compound 1a (1.00 g, 5.90 mmol), K₂CO₃ (1.63 g, 11.8 mmol) and bromocyclopentane (0.696 mL, 6.49 mmol) in DMF (50 mL) as described for the synthesis of compounds 2a and 3a above. MeOH–EtOAc (1:19) was used for flash chromatography to yield 2c (994 mg, 71%) and 3c (75 mg, 5%).

2c: colorless solid; mp 137–140 °C (lit. [55], 142 °C); ¹H-NMR (DMSO-d₆, 400 MHz) δ 8.20 (s, 1H, H-8), 6.86 (s, 2H, NH₂), 4.77–4.65 (m, 1H, H-1 in c-pent), 2.16–2.02 (m, 2H, c-pent), 2.00–1.75 (m, 4H, c-pent), 1.72–1.60 (m, 2H, c-pent); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 159.3 (C, C-2), 153.7 (C, C-4), 149.1 (C, C-6), 141.4 (CH, C-8), 123.5 (C, C-5), 55.1 (CH, C-1 in c-pent), 31.4 (CH₂, C-3 and C-4 in c-pent), 23.2 (CH₂, C-2 and C-5 in c-pent); HREIMS m/z 237.0777 (calcd for C₁₀H₁₂ClN₅, 237.0781).

Spectral data were in good agreement with those reported before [34,55,56].

3c: colorless solid; mp >230 °C (dec.); ¹H-NMR (DMSO-d₆, 400 MHz) δ 8.46 (s, 1H, H-8), 6.59 (s, 2H, NH₂), 5.11–5.01 (m, 1H, H-1 in c-pent), 2.24–2.10 (m, 2H, c-pent), 2.02–1.90 (m, 2H, c-pent), 1.86–1.62 (m, 4H, c-pent); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 164.3 (C, C-4), 159.7 (C, C-2), 146.6 (CH, C-8), 142.2 (C, C-6), 115.1 (C, C-5), 58.0 (CH, C-1 in c-pent), 32.6 (CH₂, C-3 and C-4 in c-pent), 23.1 (CH₂, C-2 and C-5 in c-pent); HREIMS m/z 237.0776 (calcd for C₁₀H₁₂ClN₅, 237.0781).

Spectral data were in good agreement with those reported before [34,55].

**Method B:** The title compounds were prepared from compound 1a (200 mg, 1.18 mmol), cyclopentanol [2 × (107 mg, 1.24 mmol)], PPh₃ [2 × (325 mg, 1.24 mmol)] and DIAD [2 × (244 µL, 1.24 mmol)] in THF (10 mL) as described for the synthesis of compounds 2a and 3a above. EtOAc–hexane (gradient; 70%–100% EtOAc) followed by MeOH–EtOAc (1:9) were used for flash chromatography to yield 2c (202 mg, 72%) and 3c (6 mg, 6%).

3.2.4. 2-Amino-6-chloro-9-(cyclopent-2-enyl)-9H-purine (2d) and 2-Amino-6-chloro-7-(cyclopent-2-enyl)-7H-purine (3d)

**Method A:** The title compound 2d was prepared from compound 1a (200 mg, 1.18 mmol), K₂CO₃ (490 mg, 3.54 mmol) and 3-bromocyclopentene (0.29 mL, ca. 80% pure, ca. 2.4 mmol) in DMF (20 mL) as described for the synthesis of compounds 2a and 3a above, except that the reaction time was 24 h. EtOAc–hexane (gradient; 50%–100% EtOAc) followed by MeOH–EtOAc (1:9) were used for flash chromatography to yield 2d (49 mg, 18%). Colorless solid; mp 154–154.5 °C (lit., [57] 166.0–166.7 °C); ¹H-NMR (DMSO-d₆, 400 MHz) δ 7.96 (s, 1H, H-8), 6.88 (s, 2H, NH₂), 6.26–6.18 (m, 1H, c-pent), 5.93–5.84 (m, 1H, c-pent), 5.51–5.41 (m, 1H, c-pent), 2.73–2.61 (m, 1H, c-pent), 2.47–2.36 (m, 2H, c-pent), 2.00–1.87 (m, 1H, c-pent); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 159.6 (C, C-6), 153.6 (C, C-4), 149.3 (C, C-2), 141.1 (CH, C-8), 137.3 (CH, C-2 in c-pent), 128.6 (CH, C-3 in c-pent), 123.6 (C, C-5), 59.4 (CH, C-1 in c-pent), 31.2 (CH₂, C-5 in c-pent), 30.4 (CH₂, C-4 in c-pent); HREIMS m/z 235.0624 (calcd for C₁₀H₁₀ClN₅, 235.0625).

Spectral data were in good agreement with those reported before [57].
Method B: The title compound 2d was prepared from compound 1a (340 mg, 2.01 mmol), cyclopent-2-enol [2 × (0.180 mL, 2.03 mmol)], PPh₃ [2 × (531 mg, 2.03 mmol)] and DIAD [2 × (0.442 mL, 2.03 mmol)] in THF (20 mL) as described for the synthesis of compounds 2a and 3a above. EtOAc–Hexane (gradient; 70%–100% EtOAc) followed by MeOH–EtOAC (1:9) were used for flash chromatography to yield 2d (187 mg, 40%).

Method C: A solution of compound 1a (100 mg, 0.590 mmol) and NaH (18 mg, 0.77 mmol) in dry DMSO (5 mL) was stirred at room temperature for 20 min under Ar atmosphere. The mixture was added to a solution of cyclopent-2-en-1-yl acetate (0.070 mL, 0.77 mmol) and Pd(PPh₃)₄ (103 mg, 0.0890 mmol) in dry DMSO (5 mL) and the resulting mixture was stirred at 50 °C for 48 h under Ar and evaporated in vacuo. The product was purified by flash chromatography as described in Method B to yield 2d (73 mg, 53%) and 3d (25 mg, 18%).

3d: colorless solid; mp 155–157 °C (dec.); ¹H-NMR (DMSO-d₆, 400 MHz) δ 8.15 (s, 1H, H-8), 6.61 (s, 2H, NH₂), 6.34–6.28 (m, 1H, c-pent), 6.03–5.96 (m, 1H, c-pent), 5.82–5.75 (m, 1H, c-pent) 2.61–2.34 (m, 3H, c-pent) 1.96–1.83 (m, 1H, c-pent); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 164.4 (C, C-4), 159.8 (C, C-2), 146.2 (CH, C-8), 142.3 (C, C-6), 138.3 (CH, C-2 in c-pent), 127.9 (CH, C-3 in c-pent), 114.8 (C, C-5), 62.5 (CH, C-1 in c-pent), 32.1 (CH₂, C-5 in c-pent), 31.0 (CH₂, C-4 in c-pent); HREIMS m/z 235.0631 (calcd for C₁₀H₁₀ClN₅, 235.0625). Spectral data were in good agreement with those reported before [57].

3.2.5. 2-Acetamido-9-(cyclohexylmethyl)-9H-purin-6-yl diphenylcarbamate (2e) and 2-Acetamido-7-(cyclohexylmethyl)-7H-purin-6-yl diphenylcarbamate (3e)

Method A: The title compounds were prepared from compound 1b (200 mg, 0.515 mmol), K₂CO₃ (142 mg, 1.03 mmol) and bromomethylcyclohexane (0.144 mL, 1.03 mmol) in DMF (7 mL) as described for the synthesis of compounds 2a and 3a above. MeOH–CH₂Cl₂ (1:9) followed by MeOH–CH₂Cl₂ (1:4) were used for flash chromatography to yield 2e (111 mg, 45%) and 3e (18 mg, 7%).

2e: colorless solid; mp 192–194 °C; ¹H-NMR (CDCl₃, 400 MHz) δ 7.97 (s, 1H, NH), 7.87 (s, 1H, H-8), 7.47–7.24 (m, 10H, Ph), 3.99 (d, J = 7.0 Hz, 2H, NCH₂), 2.58 (s, 3H, CH₃), 1.85 (m, 1H, H-1 in c-hex), 1.78–1.58 (m, 5H, c-hex), 1.30–1.07 (m, 3H, c-hex), 1.07–0.92 (m, 2H, c-hex); ¹³C-NMR (CDCl₃, 100 MHz) δ 171.1 (C, CONH), 156.2 (C, OCON), 155.4 (C, C-4), 152.2 (C, C-2), 150.6 (C, C-6), 144.4 (CH, C-8), 141.9 (C, Ph), 129.3 (CH, Ph), 127.2 (br, 2 × CH₂, Ph), 120.6 (C, C-5), 50.5 (CH₂, NCH₂), 38.4 (CH, C-1 in c-hex), 30.8 (CH₂, C-3 and C-5 in c-hex), 26.1 (CH₂, C-4 in c-hex), 25.6 (CH₂, C-2 and C-3 in c-hex), 25.3 (CH₃); HREIMS m/z 485.2311 (calcd for C₂₇H₂₉N₆O₃ + 1, 485.2301).

3e: colorless oil; ¹H-NMR (CDCl₃, 400 MHz) δ 8.10 (s, 1H, NH), 7.96 (s, 1H, H-8), 7.42–7.36 (m, 8H, Ph), 7.33–7.28 (m, 2H, Ph), 3.89 (d, J = 7.2 Hz, 2H, NCH₂), 2.63 (s, 3H, CH₃), 1.68–1.60 (m, 3H, c-hex), 1.45–1.35 (m, 2H, c-hex), 1.13–0.98 (m, 3H, c-hex), 0.92–0.72 (m, 3H, c-hex); ¹³C-NMR (CDCl₃, 100 MHz) δ 172.0 (C, CONH), 164.9 (C-4), 152.2 (C, C-2), 151.9 (C, OCON), 149.5 (C, C-6), 148.5 (CH, C-8), 141.5 (C, Ph), 129.6 (CH, Ph), 127.6 (br, 2 × CH, Ph), 111.9 (C, C-5), 53.8 (CH₂, NCH₂), 38.9 (CH, C-1 in c-hex), 30.3 (CH₂, C-3 and C-5 in c-hex), 26.0 (CH₂, C-4 in c-hex) 25.4 (CH₂, C-2 and C-6 in c-hex), 25.6 (CH₃); HREIMS m/z 485.2313 (calcd for C₂₇H₂₉N₆O₃ + 1, 485.2301).
Method B: The title compounds were prepared from compound 1b (200 mg, 0.515 mmol), cyclohexylmethanol [2 × (62 mg, 0.54 mmol)], PPh3 [2 × (142 mg, 0.540 mmol)] and DIAD [2 × (0.106 mL, 0.540 mmol)] in THF (10 mL) as described for the synthesis of compounds 2a and 3a above. EtOAc–Hexane (gradient; 70%–100% EtOAc) followed by MeOH–EtOAc (1:9) were used for flash chromatography to yield 2e (175 mg, 70%) and 3e (7 mg, 3%).

3.2.6. 2-Acetamido-9-(cyclohexyl)-9H-purin-6-yl diphenylcarbamate (2f)

Method A: The title compound was prepared from compound 1b (500 mg, 1.29 mmol), K2CO3 (329 mg, 2.38 mmol) and cyclohexyl tosylate (441 mg, 1.73 mmol) in THF (15 mL) as described for the synthesis of compounds 2a above. MeOH–EtOAc (1:19) was used for flash chromatography to yield 2f (180 mg, 30%). Off-white solid; mp 189–190 °C; 1H-NMR (DMSO-d6, 300 MHz) δ 10.67 (s, 1H, NH), 8.55 (s, 1H, H-8), 7.54–7.38 (m, 8H, Ph), 7.37–7.25 (m, 2H), 4.46–4.28 (m, 1H, H-1 in c-hex), 2.20 (s, 3H, CH3), 2.06–1.80 (m, 6H, CH in c-hex), 1.76–1.65 (m, 1H, c-hex), 1.51–1.14 (m, 3H, c-hex); 13C-NMR (DMSO-d6, 75 MHz) δ 168.8 (C, CONH), 155.0 (C, OCON), 154.3 (C, C-4), 151.7 (C, C-2), 150.3 (C, C-6), 144.1 (CH, C-8), 141.6 (C, Ph), 129.4 (CH, Ph), 127.1 (CH, Ph), 120.1 (C, C-5), 54.1 (CH, C-1 in c-hex), 31.9 (CH2, C-3 and C-5 in c-hex), 25.1 (CH2, C-2 and C-6 in c-hex), 24.7 (CH2, C-4 in c-hex), 24.6 (CH3); HREIMS m/z 470.2057 (calcd for C26H26N6O3, 470.2066).

Method B: The title compound was prepared from compound 1b (400 mg, 1.03 mmol), cyclohexanol [2 × (108 mg, 1.08 mmol)], PPh3 [2 × (284 mg, 1.08 mmol)] and DIAD [2 × (0.213 mL, 1.08 mmol)] in THF (10 mL) as described for the synthesis of compound 2a above. EtOAc–Hexane (gradient; 30%–100% EtOAc) was used for flash chromatography to yield 2f (107 mg, 22%) as an off-white solid.

3.2.7. 2-Acetamido-9-(cyclopentyl)-9H-purin-6-yl diphenylcarbamate (2g)

Method A: The title compound 2g was prepared from compound 1b (389 mg, 1.00 mmol), K2CO3 (277 mg, 2.00 mmol) and bromocyclopentane (0.120 mL, 1.10 mmol) in DMF (50 mL) as described for the synthesis of compounds 2a above. MeOH–EtOAc (1:19) was used for flash chromatography to yield 2g (238 mg, 52%). Colorless solid; mp 137–140 °C; 1H-NMR (DMSO-d6, 400 MHz) δ 10.62 (s, 1H, NH), 8.51 (s, 1H, H-8), 7.53–7.40 (m, 8H, Ph), 7.36–7.27 (m, 2H, Ph), 4.79–4.62 (m, 1H, H-1 in c-pent), 2.21 (s, 3H, CH3), 2.19–2.11 (m, 2H, c-pent), 2.10–1.83 (m, 4H, c-pent), 1.77–1.62 (m, 2H, c-pent); 13C-NMR (DMSO-d6, 100 MHz) δ 168.8 (C, CONH), 155.0 (C, OCON), 154.6 (C, C-4), 151.7 (C, C-2), 150.2 (C, C-6), 144.4 (CH, C-8), 141.6 (C, Ph), 129.4 (CH, Ph), 127.2 (CH, Ph), 120.3 (C, C-5), 56.1 (CH, C-1 in c-pent), 31.7 (CH2, C-3 and C-4 in c-pent), 24.5 (CH3), 23.5 (CH2, C-2 and C-5 in c-pent), one Ph signal was hidden; HREIMS m/z 456.1903 (calcd for C25H24N6O3, 456.1910).

Method B: The title compound 2g was prepared from compound 1b (389 mg, 1.00 mmol), cyclopentanol [2 × (91 mg, 1.1 mmol)], PPh3 [2 × (276 mg, 1.05 mmol)] and DIAD [2 × (0.207 mL, 1.05 mmol)] in THF (10 mL) as described for the synthesis of compounds 2a above. EtOAc–Hexane (gradient; 70%–100% EtOAc) followed by MeOH–EtOAc (1:9) were used for flash chromatography to yield 2g (264 mg, 58%).
3.2.8. 2-Amino-8-bromo-6-chloro-9-(cyclohexylmethyl)-9H-purine (4a)

Method A: Sat. aq. Br2 (50 mL) was added dropwise to a rapidly stirred suspension of 2a (1.50 g, 5.64 mmol) in water (20 mL) over 10 min at ambient temperature. The flask was closed and the reaction mixture was stirred for 74 h. The flask was left open in the hood until all Br2 was evaporated before the water was removed in vacuo. The product was purified by flash chromatography on silica gel, eluting with EtOAc–Hexane (1:1) to yield 4a (1.55 g, 79%). Yellow solid; mp 169–170 °C. 1H-NMR (DMSO-d6, 400 MHz) δ 7.07 (s, 2H, NH2), 3.86 (d, J = 8.0 Hz, 2H, NCH2), 1.96–1.81 (m, 1H, H-1 in c-hex), 1.72–1.44 (m, 5H, c-hex), 1.24–0.92 (m, 5H, c-hex); 13C-NMR (DMSO-d6, 100 MHz) δ 159.7 (C, C-2), 155.0 (C, C-4), 148.0 (C, C-6), 129.5 (C, C-8), 123.3 (C, C-5), 49.6 (CH2, NCH2), 36.9 (CH, c-hex), 30.0 (CH2, c-hex), 25.7 (CH2, c-hex), 25.1 (CH2, c-hex); HREIMS m/z 343.0198 (calcd for C12H15BrClN5, 343.0199).

Method B: K2CO3 (231 mg, 1.67 mmol) was added to a stirring solution of compound 10 (207 mg, 0.833 mmol) in dry DMF (15 mL) at ambient temperature under N2. After 20 min, bromomethylcyclohexane (0.175 mL, 1.25 mmol) was added and the resulting mixture was stirred for 80 h before K2CO3 (115 mg, 0.835 mmol) and bromomethylcyclohexane (0.175 mL, 1.25 mmol) was added and the mixture was stirred for additional 16 h and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with EtOAc–Hexane (2:3) to yield 4a (98 mg, 34%).

Method C: The title compound was prepared from compound 10 (175 mg, 0.704 mmol), cyclohexylmethanol [2 × (0.091 mL, 0.74 mmol)], PPh3 [2 × (276 mg, 0.740 mmol)] and DIAD [2 × (0.146 mL, 0.740 mmol)] in THF (10 mL) as described for the synthesis of compounds 2a above. EtOAc–Hexane (gradient; 20%–100% EtOAc) was used for flash chromatography to yield 4a (136 mg, 56%).

3.2.9. 2-Amino-8-bromo-6-chloro-9-(cyclohexyl)-9H-purine (4b)

The title compound was prepared from compound 2b (250 mg, 0.993 mmol) and saturated aqueous Br2 (12 mL) in water (5 mL) as described for the synthesis of compound 4a above. EtOAc–Hexane (1:1) was used for flash chromatography to yield 4b (229 mg, 70%). Yellow solid; mp 181–183 °C; 1H-NMR (DMSO-d6, 600 MHz) δ 6.98 (br s, 2H, NH2), 4.35–4.22 (m, 1H, H-1 in c-hex), 2.46–2.27 (m, 2H, c-hex), 1.92–1.75 (m, 4H, c-hex), 1.74–1.62 (m, 1H, c-hex), 1.45–1.29 (m, 2H, c-hex), 1.28–1.12 (m, 1H, c-hex); 13C-NMR (DMSO-d6, 150 MHz) δ 159.1 (C, C-2), 154.5 (C, C-4), 148.2 (C, C-6), 128.6 (C, C-8), 123.7 (C, C-5), 57.6 (CH, C-1 in c-hex), 29.7 (CH2, C-3 and C-5 in c-hex), 25.3 (CH2, C-2 and C-6 in c-hex), 24.6 (CH2, C-4 in c-hex); HRESIMS m/z 330.0131 (calcd for C11H14BrClN5 + 1, 330.0121).

3.2.10. 2-Amino-8-bromo-6-chloro-9-(cyclopentyl)-9H-purine (4c)

The title compound was prepared from compound 2c (880 mg, 3.70 mmol) and sat. aq. Br2 (35 mL) in water (10 mL) as described for the synthesis of compound 4a above. EtOAc–Hexane (1:1) was used for flash chromatography to yield 4c (950 mg, 81%). Yellow solid; mp 172–174 °C; 1H-NMR (DMSO-d6, 600 MHz) δ 6.97 (s, 2H, NH2), 4.85–4.77 (m, 1H, H-1 in c-pent), 2.33–2.17 (m, 2H, c-pent), 2.11–1.87 (m, 4H, c-pent), 1.71–1.59 (m, 2H, c-pent); 13C-NMR (DMSO-d6, 150 MHz) δ 159.1 (C, C-2), 154.3 (C, C-4), 148.2 (C, C-6), 129.3 (C, C-8), 123.9 (C, C-5), 57.7 (CH, C-1 in c-pent), 29.7 (CH2, C-3 and C-5 in c-pent), 29.7 (CH2, C-2 and C-6 in c-pent), 24.4 (CH2, C-4 in c-pent); HREIMS m/z 314.9880 (calcd for C10H11BrClN5 + 1, 330.0121).
3.2.11. 2-Amino-8-bromo-6-chloro-9-(cyclopent-2-enyl)-9H-purine (4d)

**Method A:** A solution of diisopropylamine (0.145 mL, 1.03 mmol) in dry THF (3 mL) was stirred at −78 °C under Ar. n-BuLi (0.536 mL, 1.00 mmol, 1.87 M in hexane) was added dropwise. After stirring for 30 min, a solution of compound 2d (118 mg, 0.500 mmol) in THF (1.5 mL) was added. After additional stirring for 1 h at −78 °C, a solution of CBrCl2CBrCl2 (326 mg, 1.00 mmol) in THF (1.5 mL) was added dropwise and the resulting mixture was stirred at −78 °C for 1 h, and then 10 min without cooling. Saturated aqueous NH4Cl (5 mL) was added and the resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried (MgSO4), and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with EtOAc–Hexane (1:1) to yield 4d (50 mg, 32%). Buff solid; mp 157–158 °C (dec.); 1H-NMR (DMSO-d6, 600 MHz) δ 6.95 (s, 2H, NH2), 6.15–6.13 (m, 1H, c-pent), 5.74–5.72 (m, 1H, c-pent), 5.69–5.60 (m, 1H, c-pent) 2.90–2.79 (m, 1H, c-pent), 2.48–2.36 (m, 2H, c-pent), 2.22–2.14 (m, 1H, c-pent); 13C-NMR (DMSO-d6, 150 MHz) δ 159.3 (C, C-2), 154.4 (C-4), 148.0 (C, C-6), 136.5 (CH, C-3 in c-pent), 128.0 (C, C-8), 127.7 (CH, C-2 in c-pent), 123.5 (C-5), 62.1 (CH, C-1 in c-pent), 32.0 (CH2, C-5 in c-pent), 27.9 (CH2, C-4 in c-pent); HREIMS m/z 312.9734 (calcd for C10H9BrClN5, 312.9730).

**Method B:** Compound 10 (64 mg, 0.26 mmol) was added to a cooled solution of cyclopent-2-en-1-ol (44 mg, 0.51 mmol) and PPh3 (135 mg, 0.515 mmol) in anhydrous THF (5 mL) under Ar. The resulting suspension was treated with diethyl azodicarboxylate (DEAD, 0.080 mL, 0.51 mmol) and the resulting mixture was stirred at ambient temperature for 1 h and at 70 °C for 15 h. The mixture was cooled, treated with brine (50 mL), and extracted with CH2Cl2 (3 × 50 mL). The combined organic layer was washed with water (10 mL), dried (Na2SO4), and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with EtOAc–Hexane (3:7) to yield 4d (34 mg, 42%).

**Method C:** A solution of compound 10 (110 mg, 0.423 mmol) and NaH (16 mg, 0.67 mmol) in dry DMF (10 mL) was stirred at ambient temperature for 20 min under Ar. Pd(PPh3)4 (77 mg, 0.067 mmol) and cyclopent-2-en-1-yl acetate (84 mg, 0.66 mmol) were added, and the resulting mixture was stirred at 55 °C. After three days Pd(PPh3)4 (77 mg, 0.067 mmol) and cyclopent-2-en-1-yl acetate (84 mg, 0.66 mmol) were added. The reaction mixture was stirred for three more days and evaporated under in vacuo. The product was purified by flash chromatography on silica gel eluting with EtOAc–Hexane (gradient 50%–100% EtOAc) followed by MeOH–EtOAc (1:9) to yield 4d (41 mg, 29%).

3.2.12. 9-(Cyclohexylmethyl)-8-oxoguanine (5a)

A mixture of compound 4a (263 mg, 0.763 mmol), NaOAc (319 mg, 3.89 mmol), glacial AcOH (9 mL), and Ac2O (1.5 mL, 17 mmol) was stirred at reflux under N2 for 16 h, before the mixture was cooled and evaporated in vacuo. The residue was suspended in water (3 mL) and stirred at ambient temperature while the pH was adjusted to 13 by dropwise addition of 10M NaOH (aq). The resulting solution was refluxed for 20 min, cooled to 0 °C, and stirred while the pH was brought down to 7 by dropwise addition of 6M HCl (aqueous). The precipitate was collected and dried in vacuo. The product was purified by flash chromatography on silica gel eluting with MeOH–CH2Cl2 (1:4) to yield 5a (160 mg, 80%). Pinkish solid; mp 297–300 °C; 1H-NMR (DMSO-d6, 400 MHz) δ 10.57 (s, 1H, NH), 10.48 (s, 1H, NH), 6.43 (s, 2H, NH2), 3.42 (d, J = 7.4 Hz, 2H, NCH2), 1.85–1.71 (m, 1H, H-1 in c-hex), 1.69–1.48 (m, 5H, c-hex),
1.19–1.06 (m, 3H, c-hex), 0.98–0.85 (m, 2H, c-hex); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) δ 153.4 (C, C-6), 152.6 (C, C-8), 150.9 (C, C-2), 148.2 (C, C-4), 98.0 (C, C-5), 44.9 (CH$_2$, NCH$_2$), 36.3 (CH, C-1 in c-hex), 30.1 (CH$_2$, C-3 and C-5 in c-hex), 25.9 (CH$_2$, C-4 in c-hex), 25.1 (CH$_2$, C-2 and C-6 in c-hex); HREIMS $m/z$ 263.1380 (calcd for C$_{12}$H$_{17}$N$_5$O$_2$, 263.1382).

3.2.13. 9-(Cyclohexyl)-8-oxoguanine (5b) and 2-Amino-6-chloro-9-cyclohexyl-7H-purin-8(9H)-one (6b)

The title compounds were prepared from compound 4b (186 mg, 0.563 mmol), NaOAc (231 mg, 2.81 mmol), glacial AcOH (7 mL), and Ac$_2$O (1.20 mL, 12.7 mmol) as described for the synthesis of compound 5a above, except that the reflux time with NaOH was 4 h. MeOH–EtOAc (1:9) was used for flash chromatography to yield 5b (106 mg, 76%) and 6b (7 mg, 11%).

5b: colorless solid; mp 367–368 °C; $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ 10.57 (s, 1H, NH), 10.45 (s, 1H, NH), 6.37 (s, 2H, NH$_2$), 4.04–3.91 (m, 1H, H-1 in c-hex), 2.28–2.11 (m, 2H, c-hex), 1.86–1.71 (m, 2H, c-hex), 1.69–1.52 (m, 3H, c-hex), 1.36–1.04 (m, 3H, c-hex); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) δ 152.9 (C, C-6), 151.8 (C, C-8), 150.9 (C, C-2), 147.7 (C, C-4), 98.1 (C, C-5), 51.2 (CH, C-1 in c-hex), 29.5 (CH$_2$, C-3 and C-5 in c-hex), 25.5 (CH$_2$, C-2 and C-6 in c-hex), 24.8 (CH$_2$, C-4 in c-hex); HREIMS $m/z$ 249.1222 (calcd for C$_{11}$H$_{15}$N$_5$O$_2$, 249.1226).

6b: yellow solid mp 320–321 °C; $^1$H-NMR (DMSO-$d_6$, 300 MHz) δ 11.20 (br s, 1H, NH), 6.54 (s, 2H, NH$_2$), 4.12–3.98 (m, 1H, H-1 in c-hex), 2.27–2.12 (m, 2H, c-hex), 1.88–1.75 (m, 2H, c-hex), 1.73–1.58 (m, 3H, c-hex), 1.39–1.09 (m, 3H, c-hex); $^{13}$C-NMR (DMSO-$d_6$, 75 MHz) δ 158.3 (C, C-8), 152.5 (C, C-2), 135.5 (C, C-6), 109.8 (C, C-5), 51.7 (CH, C-1 in c-hex), 29.1 (CH$_2$, C-3 and C-5 in c-hex), 25.4 (CH$_2$, C-2 and C-6 in c-hex), 24.8 (CH$_2$, C-4 in c-hex); HREIMS $m/z$ 267.0877 (calcd for C$_{11}$H$_{14}$ClN$_5$O, 267.0887).

3.2.14. 9-(Cyclopentyl)-8-oxoguanine (5c) and 2-Amino-6-chloro-9-cyclopentyl-7H-purin-8(9H)-one (6c)

The title compounds were prepared from compound 4c (250 mg, 0.790 mmol), NaOAc (325 mg, 3.96 mmol), glacial AcOH (10 mL), and Ac$_2$O (3.00 mL, 31.6 mmol) as described for the synthesis of compound 5a above, except that the refluxing time with NaOH was 6 h. MeOH–EtOAc (1:9) was used for flash chromatography to yield 5c (130 mg, 70%) and 6c (12 mg, 6%).

5c: colorless solid; mp 309–310 °C; $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ 10.58 (s, 1H, NH), 10.47 (s, 1H, NH), 6.35 (s, 2H, NH$_2$), 4.46–4.42 (m, 1H, H-1 in c-pent), 2.17–2.01 (m, 2H, c-pent), 1.94–1.73 (m, 4H, c-pent), 1.63–1.50 (m, 2H, c-pent); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) δ 152.9 (C, C-6), 151.9 (C, C-8), 150.9 (C, C-2), 147.8 (C, C-4), 98.2 (C, C-5), 51.8 (CH, C-1 in c-pent), 29.0 (CH$_2$, C-2 and C-5 in c-pent), 24.3 (CH$_2$, C-3 and C-4 in c-pent); HREIMS $m/z$ 235.1067 (calcd for C$_{10}$H$_{13}$N$_5$O$_2$, 235.1069).

6c: colorless solid; mp 321–322 °C (dec.); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ 11.20 (s, 1H, NH), 6.52 (s, 2H, NH$_2$), 4.70–4.46 (m, 1H, c-pent), 2.20–2.01 (m, 2H, c-pent), 1.98–1.74 (m, 4H, c-pent), 1.68–1.50 (m, 2H, c-pent); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) δ 158.3 (C, C-4), 152.6 (C, C-8), 152.2 (C, C-6), 135.5 (C, C-2), 109.9 (C, C-5), 52.1 (CH, C-1 in c-pent), 28.7 (CH$_2$, C-2 and C-5 in c-pent), 24.3 (CH$_2$, C-3 and C-4 in c-pent); HREIMS $m/z$ 253.0727 (calcd for C$_{10}$H$_{12}$ClN$_5$O, 253.0734).
3.2.15. 9-(Cyclopent-2-enyl)-8-oxoguanine (5d) and 2-Amino-6-chloro-9-(cyclopent-2-enyl)-7H-purin-8(9H)-one (6d)

The title compounds were prepared from compound 4d (210 mg, 0.668 mmol), NaOAc (274 mg, 3.34 mmol), glacial AcOH (8 mL), and Ac₂O (2.78 mL, 29.4 mmol) as described for the synthesis of compound 5a above, except that the refluxing time with NaOH was 30 h and the heating bath was kept at 160 °C in the first reaction step. Glacial AcOH was used for the final neutralization and EtOAc followed by MeOH–EtOAc (1:9) were used for flash chromatography to yield 5d (119 mg, 71%) and 6d (9 mg, 5%).

5d: colorless solid; mp 322–325 °C (dec.); ¹H-NMR (DMSO-d₆, 400 MHz) δ 10.60 (s, 1H, NH), 10.49 (s, 1H, NH), 6.34 (s, 2H, NH₂), 5.99–5.96 (m, 1H, H-3 in c-pent), 5.62–5.59 (m, 1H, H-2 in c-pent), 5.30–5.21 (m, 1H, H-1 in c-pent), 2.79–2.63 (m, 1H, H-5a in c-pent), 2.40–2.26 (m, 1H, H-5b in c-pent), 2.24–2.13 (m, 1H, H-4a in c-pent), 2.13–2.03 (m, 1H, H-4b in c-pent); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 153.0 (C, C-6), 151.8 (C, C-8), 151.0 (C, C-2), 147.7 (C, C-4), 134.5 (CH, C-3 in c-pent), 129.0 (CH, C-2 in c-pent), 98.3 (C, C-5), 56.8 (CH, C-1 in c-pent), 31.8 (CH₂, C-4 in c-pent), 27.4 (CH₂, C-5 in c-pent); HREIMS m/z 233.0914 (calcd for C₁₀H₁₁N₅O₂, 233.0913).

6d: yellow solid; mp 310–310.5 °C; ¹H-NMR (DMSO-d₆, 300 MHz) δ 11.18 (s, 1H, NH), 6.48 (s, 2H, NH₂), 6.06–6.02 (m, 1H, H-3 in c-pent), 5.65–5.61 (m, 1H, H-2 in c-pent), 5.37–5.27 (m, 1H, H-1 in c-pent), 2.84–2.69 (m, 1H, H-5a in c-pent), 2.43–2.05 (m, 3H, c-pent); ¹³C-NMR (DMSO-d₆, 75 MHz) δ 158.3 (C, C-8), 152.4 (C, C-4), 152.1 (C, C-2), 135.4 (C, C-6), 135.3 (CH, C-3 in c-pent), 128.1 (CH, C-2 in c-pent), 109.9 (C, C-5), 57.2 (CH, C-1 in c-pent), 31.8 (CH₂, C-4 in c-pent), 27.0 (CH₂, C-5 in c-pent); HREIMS m/z 251.0568 (calcd for C₁₀H₁₀ClN₅O, 251.0574).

3.2.16. N-[9-(Cyclohexylmethyl)-6-oxo-6,9-dihydro-1H-purin-2-yl]acetamide (7)

Method A: Br₂ (33 mg, 0.21 mmol) was added slowly to a stirred solution of compound 2e (20 mg, 0.41 mmol) in CHCl₃ (4 mL) and the resulting mixture was stirred for 6 h at ambient temperature. The reaction mixture was evaporated to dryness and the product was purified by flash chromatography on silica gel eluting with MeOH–EtOAc (1:19) to yield 7 (10 mg, 84%). Off-white solid; mp 271–273 °C (dec.); ¹H-NMR (DMSO-d₆, 400 MHz) δ 12.01 (s, 1H, N₂H) 11.63 (s, 1H, NH), 7.95 (s, 1H, H-8), 3.90 (d, J = 7.4 Hz, 2H, NCH₂), 2.17 (s, 3H, CH₃), 1.88–1.74 (m, 1H, c-hex), 1.70–1.54 (m, 3H, c-hex), 1.53–1.44 (m, 2H, c-hex), 1.21–1.07 (m, 3H, c-hex), 1.01–0.87 (m, 2H, c-hex); ¹³C-NMR (DMSO-d₆, 100 MHz) δ 173.5 (C, CON₂), 154.9 (C, C-6), 148.8 (C, C-4), 147.6 (C, C-2), 140.2 (C, C-8), 120.0 (C, C-5), 48.9 (CH₂, NCH₂), 37.4 (CH, C-1 in c-hex), 29.9 (CH₂, C-3 and C-5 in c-hex), 25.8 (CH₂, C-4 in c-hex), 25.0 (CH₂, C-2 and C-6 in c-hex), 23.8 (CH₃); HREIMS m/z 289.1534 (calcd for C₁₄H₁₅N₅O₂, 289.1539).

Method B: The title compound was prepared from compound 2e (20 mg, 0.41 mmol), diisopropylamine (0.012 mL, 0.83 mmol), n-BuLi (0.060 mL, 0.83 mmol, 1.4 M in hexane) and CBrCl₂CBrCl₂ (27 mg, 0.83 mmol) in THF (tot. vol. 3 mL) as described for the synthesis of compound 4d above, except that the reaction was stirred at −78 °C for 2 h after the addition of CBrCl₂CBrCl₂. The product was purified by flash chromatography as described above to yield 7 (7 mg, 59%).
3.2.17. 8-Bromo-6-chloro-N,N,9-bis(tetrahydro-2H-pyran-2-yl)-9H-purin-2-amine (9)

The title compound was prepared from compound 8 (1.00 g, 2.96 mmol), diisopropylamine (0.84 mL, 5.9 mmol), n-BuLi (4.23 mL, 5.20 mmol, 1.4 M in hexane), and CBrCl₂CBrCl₂ (1.93 g, 5.92 mmol) in THF (tot. vol. 30 mL) as described for the synthesis of compound 4d above, except that the reaction was stirred at −78 °C for 2 h after the addition of CBrCl₂CBrCl₂. EtOAc–Hexane (1:1) was used for flash chromatography to yield 9 (987 mg, 80%). Colorless solid; mp 145–147 °C (dec.); 1H-NMR (DMSO-d₆, 300 MHz) δ 8.23 (s, 1H, NH), 5.52 (dd, J = 11.0, 2.4 Hz, 1H, CH in THP), 5.13–5.02 (m, 1H, CH in THP), 4.10–3.77 (m, 1H, OCH₂ in THP), 3.69–3.56 (m, 1H, OCH₂ in THP), 3.49–3.39 (m, 1H, OCH₂ in THP), 3.14–2.90 (m, 1H, THP), 2.06–1.29 (m, 11H, CH₂ in THP); 13C-NMR (DMSO-d₆, 75 MHz) δ 157.2 (C, C-2), 154.2 (C, C-8), 148.3 (C, C-6), 129.5 (C, C-4), 124.3 (C, C-5), 84.4 (CH, N9-THP), 80.2 (CH, THP), 68.0 (CH₂, OCH₂ in THP), 65.7 (CH₂, OCH₂ in THP), 30.1, 27.6, 24.9, 24.5, 22.6 and 22.5 (all CH₂, THP); HREIMS m/z 415.0417 (calcd for C₁₅H₁₉BrClN₅O₂, 415.0411).

3.2.18. 2-Amino-8-bromo-6-chloro-1H-purine (10)

A mixture compound 9 (150 mg, 0.360 mmol), 96% EtOH (10 mL) and 9.6 M HCl (0.5 mL), was stirred at ambient temperature for 30 min and neutralized by the addition of solid KHCO₃. The resulting mixture was evaporated in vacuo and the product was isolated by flash chromatography on silica gel eluting with MeOH–CHCl₃ (1:50:) to yield 10 (80 mg, 90%) as a yellow solid; mp >300 °C (dec.). 1H-NMR (DMSO-d₆, 400 MHz) δ 13.65 (s, 1H, NH), 6.88 (s, 2H, NH₂); 13C-NMR (DMSO-d₆, 100 MHz) δ 159.7, 156.2, 147.1, 126.5, 124.0; HREIMS m/z 246.9257 (calcd for C₅H₃BrClN₅, 246.9260). Spectral data were in good agreement with those reported before [48].

3.3. DNA Glycosylase Activity Assay

The enzyme OGG1 (residues 12–327) was diluted to the desired concentration (60 pM) using a protein dilution buffer (15% glycerol, 1 mM EDTA, 25 mM HEPES pH 7.9, 1 mM DTT, 0.1 µg/µL BSA). Enzyme, compound 5 or 6 (0.2 mM), and 5'-32P end-labeled duplex DNA containing an 8-oxo-G/C base pair were mixed in a 10 µL reaction volume of 50 mM MOPS pH 7.5, 1 mM EDTA, 5% glycerol, and 1 mM DTT. The sequence of the damaged strand in the DNA substrate used is 5'-GCATGCCTGCA CGG-8oxoG-CATGGCCAGATCCCCGGGTACCGAG-3', which was annealed with a complementary strand containing a C opposite 8oxoG. The reactions were incubated for 10 min at 37 °C, followed by addition of 2.5 µL 0.5 M NaOH and incubation for 20 min at 70 °C, in order to stop the reaction and ensure complete strand cleavage. Then 0.5 M HCl/0.25 M MOPS pH 7.5 (2.5 µL) was added to each sample to neutralize the pH. Formamide DNA loading buffer (15 µL) was added to the reaction mixtures and the samples were incubated at 95 °C for 5 min to denature the DNA. The reaction products were analyzed on 20% denaturing urea gels. The gels were transferred to 3M paper and dried at 80 °C for 45 min. The dry gels were placed in a storage phosphor screen overnight, and subsequently scanned on a Typhoon 9410 Variable Mode Image. ImageQuant TL Version 2003.02 (Amersham Biosciences, Piscataway, NJ, USA) was used to analyze the results. For human NTH1, the same procedure was followed, except that the DNA substrate contained a 5-hydroxyuracil/G base pair instead of the 8oxoG/C pair in the OGG1
substrate. The concentration of NTH1 was 3 nM to make sure the activity in the assay was within the linear range. Compounds were screened at 0.5 mM concentration.

4. Conclusions

Synthetic routes to 8-oxoguanines have been examined. The best reaction sequence from chloroguanine 1a to the target compounds was found to be N-9 alkylation, C-8 bromination, and finally simultaneous hydrolysis of both halides. Bromination before N-alkylation should only be considered in cases where the N-substituent is not compatible with bromination conditions, since a bromide in the purine 8-position lowers the reactivity in N-alkylations. In most cases, alkylation with an alkyl halide in the presence of a base compared favorably to reactions under Mitsunobu conditions. 2-Amino-6-chloropurine (1a) turned out to be a superior guanine precursor compared to the O-carbamoylguanine 1b. The latter did not result in improved N-9 selectivity in the alkylation and was not compatible with standard reaction conditions for C-8 bromination.

Enzymatic assays show that partly hydrolyzed 6-chloro-8-oxopurines 6 are somewhat better OGG1 inhibitors than the 8-oxoguanines 5. However, an inhibitory effect was only observed when using at least 0.2 mM concentration of the compounds, suggesting that the R-group should be extended even further to make more interactions with the enzyme’s substrate recognition pocket. Further, testing of the same compounds at a 2.5-fold higher concentration against human NTH1, which is a structural homolog of OGG1, showed that the synthesized compounds do not inhibit NTH1 at 0.5 mM, except possibly for a weak effect for compound 6b. To develop these compounds into more potent inhibitors of OGG1, one possibility is to try compounds with more ribose-like R-groups. In the present study, the R-group contains a cyclic hydrocarbon only, and it would also be interesting to replace this with carbocyclic 2′-deoxyribose derivatives, as in antiviral drugs like abacavir and entecavir. In these nucleoside analog drugs, the R-group is not particularly larger than the R-group in our study, but it contains 5′ and/or 3′ hydroxyl groups. Since the structure of the OGG1 enzyme is known [7], molecular modeling will be included in the search for more potent OGG1 inhibitors in the future.

Supplementary Material

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/09/15944/s1.

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Author Contributions

LLG and BD designed the research. TRM performed the synthetic organic chemistry, and MEYA and PSA the biological experiments. All authors contributed to writing the paper and read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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