Synthesis of Lipophilic N^9-Benzylguanine Derivatives

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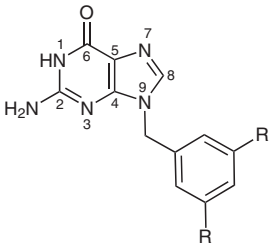
Abstract: Lipophilic N^9-benzylguanine derivatives were synthesized from the arylalkylation of 2-*N*-acetylguanine with substituted benzyl bromides.

Key words: 2-*N*-acetylguanine, benzylation, nucleobases, regioselectivity, HMBC

Benzylguanine derivatives have demonstrated outstanding potential in a wide range of biological systems. O^6-Benzylguanine is a well-studied anticancer drug that binds and inhibits the DNA repair enzyme O^6-alkylguanine DNA alkyltransferase and is used to enhance the effects of other chemotherapeutic agents.[1,2] *N*-Benzylguanine derivatives have also exhibited diverse biological activity; N^9-benzylguanine derivatives have demonstrated potent activity as purine nucleoside phosphorylase inhibitors, HIV integrase inhibitors, antitumor agents, and antiviral agents.[3–8] In addition to the diverse biological activities, lipophilic guanine derivatives have served as important model compounds to investigate guanine oxidation mechanisms and as precursors for model ion channel formation and G-quadruplex structures.[9–13] Our interests in N^9-benzylguanine derivatives center around studying the self-assembling properties of these compounds to form G-quartet structures and the mechanisms by which G-quartet and G-quadruplex secondary structures affect oxidation rates and oxidation products of guanine.[14–19]

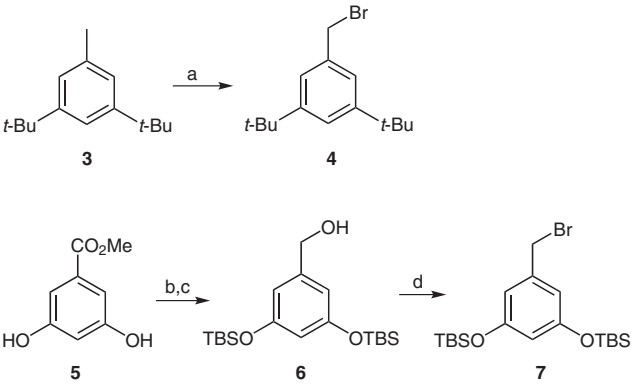
The potential of such interesting chemical and biological properties establishes the necessity for effective syntheses of novel guanine analogs. In this light, we are interested in synthesizing lipophilic N^9-benzylguanine derivatives. We envisioned N^9-benzylguanine derivatives **1** and **2**, resulting from the coupling of 2-*N*-acetylguanine and benzyl bromide derivatives (Figure 1).

Lipophilic benzyl bromide derivatives **4** and **7** were synthesized by standard protocols (Scheme 1). Treatment of 3,5-di-*tert*-butyl-toluene (**3**) with *N*-bromosuccinimide (NBS) at 80 °C afforded 3,5-di-*tert*-butylbenzyl bromide (**4**).[20] Methyl 3,5-dihydroxybenzoate (**5**) was protected using TBSCl, and reduction of the ester with LAH yielded 3,5-bis(*tert*-butyldimethylsilyloxy)benzyl alcohol (**6**). Subsequent treatment with trioctyl phosphine and carbon



1 R = *t*-Bu
2 R = OTBS

Figure 1 N^9-Benzylguanine derivatives



Scheme 1 *Reagents and conditions*: (a) NBS, AIBN, CCl_4, 80 °C, 24 h, 85%; (b) TBSCl, imid., DMF, 24 h, 97%; (c) LAH, Et_2O, r.t. to reflux, 2 h, 83%; (d) $P(C_8H_{17})_3$, CBr_4, Et_2O, 0 °C to r.t., 1 h, 65%.

tetrabromide gave 3,5-bis(*tert*-butyldimethylsilyloxy)benzyl bromide (**7**) in sufficient yield.[21]

With the benzyl bromide derivatives in hand, we were interested in studying the arylalkylation of 2-*N*-acetylguanine (**8**) under various reaction conditions (Scheme 2). When **4** was reacted with **8** in *N*,*N*-dimethylformamide (DMF) at 80 °C, a 1:1 mixture of 2-*N*-acetyl-N^9-(3,5-di-*tert*-butylbenzyl)guanine (**9**)[22] and 2-*N*-acetyl-N^7-(3,5-di-*tert*-butylbenzyl)guanine (**10**)[23] were isolated in 65% yield. The addition of base (potassium carbonate) at a variety of temperatures (r.t., 80, 100 °C) did not dramatically affect selectivity, but generally resulted in lower yields of **9** and **10**. N-Deacetylation of **9** with sodium hydroxide afforded N^9-(3,5-di-*tert*-butylbenzyl)guanine (**1**) in 71% yield.[24]

The reaction of 2-*N*-acetylguanine with **7** in DMF at 80 °C resulted in the formation of four products, namely, 2-*N*-acetyl-N^9-[3,5-bis(*tert*-butyldimethylsilyloxy)benzyl]-guanine (**11**),[25] 2-*N*-acetyl-N^7-[3,5-bis(*tert*-butyldimethyl-

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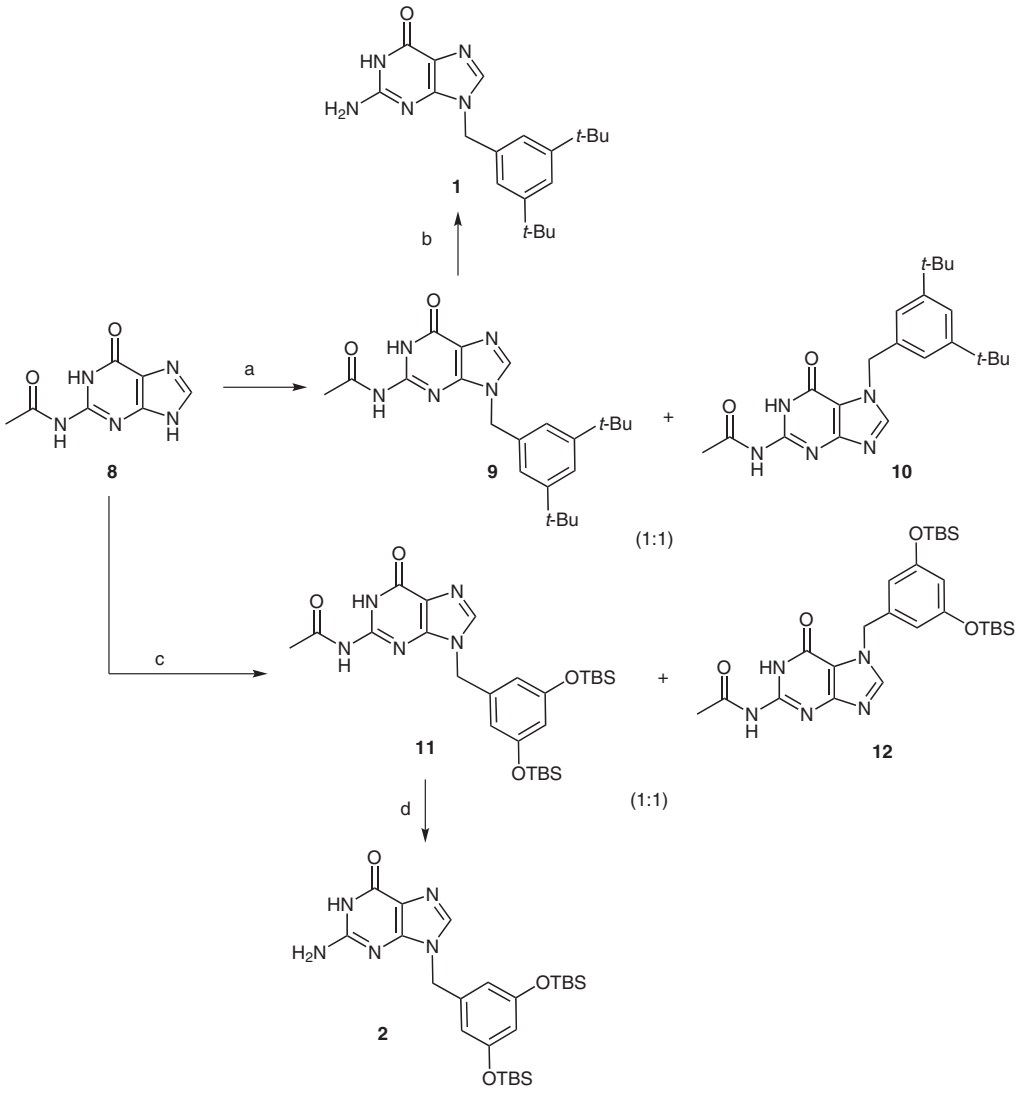

Scheme 2 *Reagents and conditions*: (a) **4**, DMF, 80 °C, 24 h, 65%; (b) NaOH, DMF, 1 h, 71%; (c) **7**, K_2CO_3, DMF, r.t., 24 h, 67%; (d) NH_2NH_2, MeOH, 1 h, 66%.

silyloxy)benzyl]guanine (**12**),[26] and the monosilylated derivatives 2-*N*-acetyl-N^9-[3-(*tert*-butyldimethylsilyloxy)-5-hydroxybenzyl]guanine and 2-*N*-acetyl-N^7-[3-(*tert*-butyldimethylsilyloxy)-5-hydroxybenzyl]guanine in 20, 20, 14, and 14% yields, respectively. Treatment of 2-*N*-acetylguanine with **7** in DMF in the presence of potassium carbonate at room temperature minimized formation of the monosilylated derivatives, and **11** and **12** were isolated in a 1:1 ratio in 67% yield. N-Deacetylation of **11** proved to be more difficult than expected because standard amide deprotection methods resulted in the formation of a mixture of monosilylated and desilylated products. However, reaction of **11** with hydrazine resulted in the successful formation of N^9-[3,5-bis(*tert*-butyldimethylsilyloxy)benzyl]guanine (**2**).[27] Through the use of equivalent molar amounts of N^2-acetylguanine and benzyl bromide, we detected no evidence of diarylakylated products.

Characterization of regioisomers **9**–**12** was accomplished by ^1H and ^{13}C NMR spectroscopic analysis, heteronuclear multiple bond correlation (HMBC) measurements, and by mass spectrometry. The N^9- and N^7-regiosiomers were identified on the basis of the chemical shifts of the methylene protons (NCH_2Ar) and C-5 peaks, and on the HMBC 2-D NMR spectra. The benzylic proton peaks were found at δ = 5.06–5.27 and 5.41–5.53 ppm for the N^9-and N^7-regioisomers, respectively, which is consistent with similar products.[28,29] HMBC NMR experiments confirmed our assignments for products **9**–**12** and **1** and **2**. A typical partial HMBC spectrum is shown in Figure 2; H-8 exhibits cross-peaks corresponding to C-5 and C-4 (and a residual one-bond coupling to C-8), while the benzylic hydrogens are found to couple to C-4 (in addition to C-8, Ar-H1′, and Ar-H2′) and not to C-5, which is indicative of the N^9-regioisomer for compound **2**.[9] Compound **1** exhibited a similar HMBC correlation for H-8 and NCH_2-Ar.

In conclusion, lipophilic N^9-benzylguanine derivatives **1** and **2** were synthesized for the first time. This synthetic pathway represents a practical and efficient approach to the synthesis of N^9-benzylguanine analogs, and optimizes

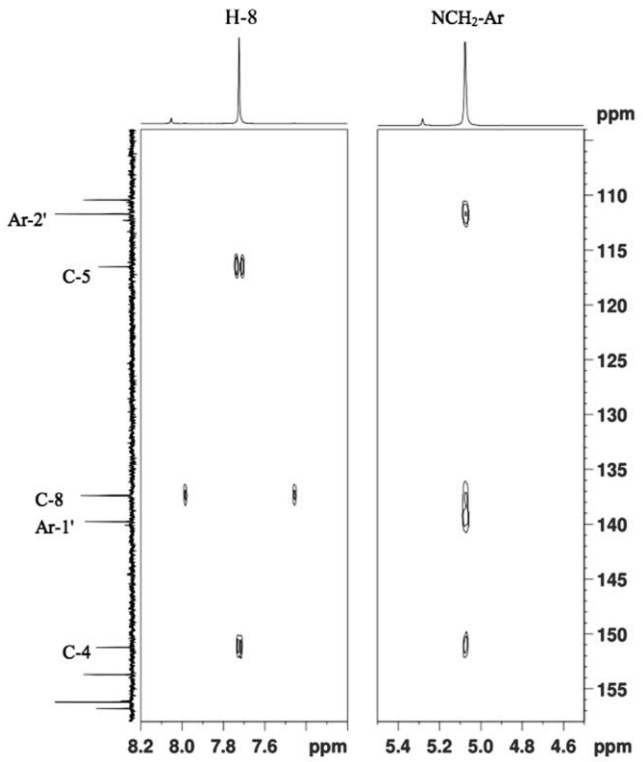

Figure 2 Partial HMBC spectrum of **2**. The cross-peak between NCH_2-Ar and C-4 indicates formation of the N^9-regioisomer. H-8 exhibits cross-peaks to C-4 and C-5, while the benzylic protons demonstrate cross-peaks to C-4 and to the aromatic carbons.

yields for the coupling reaction of 2-*N*-acetylguanine with substituted benzene derivatives and N-deacetylation. HMBC experiments provided conclusive evidence for the structure of each regioisomer. Our future plans involve using these derivatives to investigate the self-assembly of G-quartet structures and examine low-temperature guanine oxidation intermediates.

References and Notes

(1) Hegi, M. E.; Liu, L.; Herman, J. G.; Stupp, R.; Wick, W.; Weller, M.; Mehta, M. P.; Gilbert, M. R. *J. Clin. Oncol.* **2008**, *26*, 4189.

(2) Ljungman, M. *Chem. Rev.* **2009**, *109*, 2929.

(3) Castilho, M. S.; Postigo, M. P.; de Paula, C. B. V.; Montanari, C. A.; Oliva, G.; Andricopulo, A. D. *Bioorg. Med. Chem.* **2006**, *14*, 516.

(4) Murthy, D.; Brakta, M.; Ellis, L. O. *Med. Chem. Res.* **2003**, *12*, 13.

(5) Nair, V.; Uchil, V.; Neamati, N. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1920.

(6) Chern, J. W.; Wise, D. S.; Shewach, D. S.; Daddona, P. E.; Townsend, L. B. *Eur. J. Med. Chem.* **1994**, *29*, 3.

(7) Kasibhatla, S. R.; Hong, K.; Biamonte, M. A.; Busch, D. J.; Karjian, P. L.; Sensintaffar, J. L.; Kamal, A.; Lough, R. E.; Brekken, J.; Lundgren, K.; Grecko, R.; Timony, G. A.; Ran, Y.; Mansfield, R.; Fritz, L. C.; Ulm, E.; Burrows, F. J.; Boehm, M. F. *J. Med. Chem.* **2007**, *50*, 2767.

(8) Chen, L.; Kode, N.; Murthy, D.; Phadtare, S. *Med. Chem. Res.* **2005**, *14*, 445.

(9) McCallum, J. E. B.; Kuniyoshi, C. Y.; Foote, C. S. *J. Am. Chem. Soc.* **2004**, *126*, 16777.

(10) Sheu, C.; Kang, P.; Khan, S.; Foote, C. S. *J. Am. Chem. Soc.* **2002**, *124*, 3905.

(11) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 474.

(12) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 6439.

(13) Davis, J. T.; Spada, G. P. *Chem. Soc. Rev.* **2007**, *36*, 296.

(14) Marlow, A. L.; Mezzina, E.; Spada, G. P.; Masiero, S.; Davis, J. T.; Gottarelli, G. *J. Org. Chem.* **1999**, *64*, 5116.

(15) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060.

(16) Ma, L.; Iezzi, M.; Kaucher, M. S.; Lam, Y.-F.; Davis, J. T. *J. Am. Chem. Soc.* **2006**, *128*, 15269.

(17) Szalai, V. A.; Singer, M. J.; Thorp, H. H. *J. Am. Chem. Soc.* **2002**, *124*, 1625.

(18) Kawanishi, S.; Oikawa, S. *Ann. N. Y. Acad. Sci.* **2004**, *1019*, 278.

(19) Kawai, K.; Fujitsuka, M.; Majima, T. *Chem. Commun.* **2005**, *11*, 1476.

(20) Bollinger, J. M.; Comisarow, M. B.; Cupas, C. A.; Olah, G. A. *J. Am. Chem. Soc.* **1967**, *89*, 5687.

(21) Cardona, L.; Fernandez, I.; Garcia, B.; Pedro, J. R. *Tetrahedron* **1986**, *42*, 2725.

(22) ***N*²-Acetyl-*N*⁹-(3,5-di-*tert*-butylbenzyl)guanine (9)**: Purification was achieved through flash chromatography using a CombiFlash® system (regioisomer **9** being more polar than regioisomer **10**) with a gradient of CH_2Cl_2–MeOH (100:0→50:50) and subsequent recrystallization in EtOH–H_2O (32%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.07 (s, 1 H, H-8), 7.31 (s, 1 H, ArH), 7.08 (s, 2 H, ArH), 5.27 (s, 2 H, CH_2), 2.16 (s, 3 H, CH_3), 1.23 (s, 18 H, 2 × *t*-Bu). ^{13}C NMR (400 MHz, DMSO-d_6): δ = 173.5, 155.0, 150.8, 148.7, 147.9, 139.8, 135.9, 121.3, 121.1, 119.9, 46.7, 34.5, 31.1, 23.8. HRMS (ESI+): *m*/*z* [MH]$^+$ calcd: 396.2400; found: 396.2408.

(23) ***N*²-Acetyl-*N*⁷-(3,5-di-*tert*-butylbenzyl)guanine (10)**: Purification was achieved through flash chromatography using a CombiFlash® system (regioisomer **9** being more polar than regioisomer **10**) with a gradient of CH_2Cl_2–MeOH (100:0→50:50) and subsequent recrystallization in EtOH–H_2O (32%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (s, 1 H, H-8), 7.39 (s, 1 H, ArH), 7.23 (s, 2 H, ArH), 5.53 (s, 2 H, CH_2), 2.37 (s, 3 H, CH_3), 1.29 (s, 18 H, 2 × *t*-Bu). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.4, 156.8, 153.5, 151.8, 148.0, 142.8, 134.5, 122.7, 122.0, 112.2, 51.3, 34.9, 31.4, 24.1. HRMS (ESI+): *m*/*z* [MH]$^+$ calcd: 396.2400; found: 396.2408.

(24) ***N*⁹-(3,5-Di-*tert*-butylbenzyl)guanine (1)**: Purification was achieved through flash chromatography using a CombiFlash® system with a gradient of CH_2Cl_2–MeOH (100:0→50:50) and subsequent recrystallization in EtOH–H_2O (71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.67 (s, 1 H, NH), 7.76 (s, 1 H, H-8), 7.28 (s, 1 H, ArH), 7.11 (s, 2 H, ArH), 6.48 (s, 2 H, NH_2), 5.13 (s, 2 H, CH_2), 1.22 (s, 18 H, 2 × *t*-Bu). ^{13}C NMR (400 MHz, DMSO-d_6): δ = 157.1, 153.8, 151.4, 150.8, 137.7, 136.5, 121.5, 121.2, 116.5, 46.5, 34.6, 31.3. HRMS (ESI+): *m*/*z* [MH]$^+$ calcd: 354.2294; found: 354.2294.

(25) ***N*²-Acetyl-*N*⁹-[3,5-bis(*tert*-butyldimethylsilyloxy)-benzyl]guanine (11)**: Purification was achieved through flash chromatography using a CombiFlash® system (regioisomer **11** being more polar than regioisomer **12**) with a gradient of CH_2Cl_2–MeOH (100:0→50:50) and subsequent recrystallization in EtOH–H_2O (33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (s, 1 H, H-8), 6.27 (s, 3 H, ArH), 5.06 (s, 2 H, CH_2), 2.36 (s, 3 H, CH_3), 0.94 (s, 18 H, 2 × *t*-Bu), 0.16 (s, 12 H, 4 × CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 171.7, 157.2, 156.8, 149.3, 147.3, 139.3, 137.3, 120.5,

112.8, 112.0, 47.4, 25.7, 24.5, 18.3, –4.31. HRMS (ESI+): *m*/*z* [MH]$^+$ calcd: 544.2775; found: 544.2778.

(26) ***N*^{2}-Acetyl-*N*^{7}-[3,5-bis(*tert*-butyldimethylsilyloxy)-benzyl]-guanine (12)**: Purification was achieved through flash chromatography using a CombiFlash® system (regioisomer **11** being more polar than regioisomer **12**) with a gradient of CH_2Cl_2–MeOH (100:0→50:50) and subsequent recrystallization in EtOH–H_2O (33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (s, 1 H, H-8), 6.44 (s, 2 H, ArH), 6.30 (s, 1 H, ArH), 5.41 (s, 2 H, CH_2), 2.37 (s, 3 H, CH_3), 0.94 (s, 18 H, 2 × *t*-Bu), 0.16 (s, 12 H, 4 × CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.5, 157.2, 156.5, 153.4, 148.4, 142.8, 137.1, 113.3, 112.3, 112.1, 50.8, 25.7, 24.6, 18.3, –4.31. HRMS (ESI+): *m*/*z* [MH]$^+$ calcd: 544.2775; found: 544.2777

(27) ***N*^{9}-[3,5-Bis(*tert*-butyldimethylsilyloxy)benzyl]guanine (2)**: Purification was achieved through flash chromatography using a CombiFlash® system with a gradient of CH_2Cl_2–MeOH (100:0→50:50) and subsequent recrystallization in EtOH–H_2O (66%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.58 (s, 1 H, NH), 7.73 (s, 1 H, H-8), 6.43 (s, 2 H, NH_2), 6.29 (s, 2 H, ArH), 6.17 (s, 1 H, ArH), 5.08 (s, 2 H, CH_2), 0.89 (s, 18 H, 2 × *t*-Bu), 0.12 (s, 12 H, 4 × CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 156.8, 156.2, 153.7, 151.2, 139.8, 137.4, 116.5, 111.7, 110.4, 45.3, 25.5, 17.9, –4.6. HRMS (ESI+): *m*/*z* [MH]$^+$ calcd: 502.2670; found: 502.2667.

(28) Madre, M.; Panchenko, N.; Zhuk, R.; Geenevasen, J. A.; Van den Burg, A.; Koomen, G.-J. *Synthesis* **1999**, 775.

(29) Kjellberg, J.; Johansson, N. G. *Tetrahedron* **1986**, *42*, 6541.