Benzylation of adenine under basic conditions. Synthesis, NMR spectroscopy and X-ray crystallography, characterization of N9- and N3-benzyladenine

Dominique M. S. Buyens, Paidamwoyo Mangondo, Ignacy Cukrowski and Lynne A. Pilcher*

Department of Chemistry
Faculty of Natural and Agricultural Sciences, University of Pretoria,
Lynnwood Road, Hatfield, Pretoria 0002, South Africa
Fax: +27 12 420 4687
E-mail address: lynne.pilcher@up.ac.za

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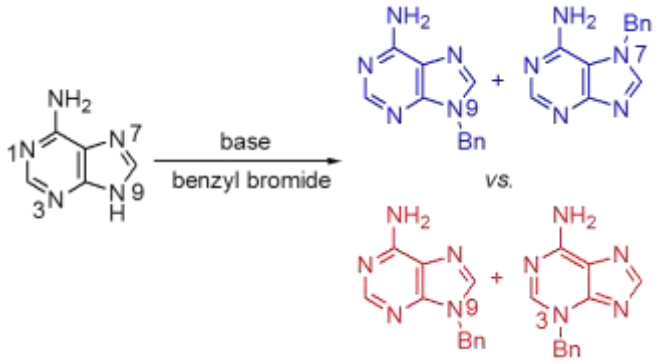

The benzylation of adenine yields the N9/N3 regio-isomers, not the often reported N9/N7 regio-isomers, in a ratio dependent on solvent.

Keywords: Adenine • benzylation reaction • NMR spectroscopy • nucleophilic substitution • regio-selectivity

ABSTRACT

The preferred sites for the benzylation of adenine under basic conditions were proven to be the N9 and N3 positions. Formation of the N9-benzyladenine product is favoured in polar aprotic solvents, such as DMSO, whereas the proportion of N3-benzyladenine formed increases as the proportion of polar protic solvents, such as water, increases. X-ray crystal structures were obtained for both N9- and N3-benzyladenine. ^{1}H-^{13}C HMBC NMR spectroscopy revealed diagnostic correlations used to assign the ^{1}H and ^{13}C NMR chemical shifts confirming that the solution structures in three different solvents were the same as the isolated crystals. ^{13}C NMR assignment for N9-, N3- and N7- benzyladenine was confirmed by computation using ADF.

Introduction

As an important building block in nature, adenine has been used as a scaffold to synthesize many medicinal compounds. N9-alkyladenine derivatives, specifically N9-benzyladenine derivatives exhibit potent cyclic nucleotide phosphodiesterase (PDE) inhibition, with high selectivity for PDE-4 [1,2]. Other N9-substituted adenine derivatives have antiviral activity for DNA viruses and retroviruses [3-5] and others are cytotoxic to tumour cell lines [4,6]. N9 substituted-8-oxoadenine derivatives have been shown to have interferon (IFN) inducing activity and have been synthesised from N9-substituted adenines, such as N9-benzyladenine [7,8].

The synthesis of N9-benzyladenine has been reported often over the last 30 years, commonly synthesised by the alkylation of adenine with benzyl bromide under basic conditions [5,8-12]. In addition to the N9-benzyladenine compound, many authors report the concurrent formation of a minor structural benzyladenine isomer as a by-product. The majority of papers have identified this minor isomer to be N7-benzyladenine [10,11] while two groups identified this isomer as N3-benzyladenine [8,9]. It is commonly reported that the reaction of adenine with an alkyl halide at elevated temperatures in the presence of base, yields the N9 and N7 regio-isomers, whereas in the absence of base yields N3-alkylated adenine [13-19] and it has been reasoned that the formation of the N9/N7 regio-isomers can be explained in terms of the dominance of the adenine tautomers, N9-H and N7-H [20]. Our aim was therefore to synthesize sufficient quantities of the minor benzyladenine regioisomer to allow for a full characterization by X-ray crystallography and two-dimensional NMR techniques.

Results and Discussion

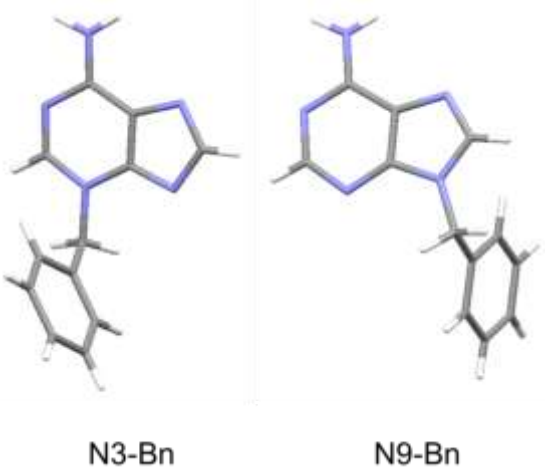

Figure 1. Capped stick representation of the crystal structures of N9- and N3-benzyladenine.

To prepare the benzyladenine products, adenine was treated with a base such as NaH or tBuOK in DMSO to generate the adenine anion which was reacted with benzyl bromide at elevated temperatures. The two regioisomers were formed in varying ratios from 95:5 to 70:30 for the major N9-benzyladenine to the minor isomer. Following chromatographic separation of the products and crystallization from CH_2Cl_2 and methanol (1:1) X-ray crystal structures were obtained for both compounds (crystallographic data in PART D in the ASI),

confirming the structure of the major product and allowing the minor product to be identified as N3-Bn. (Figure 1).

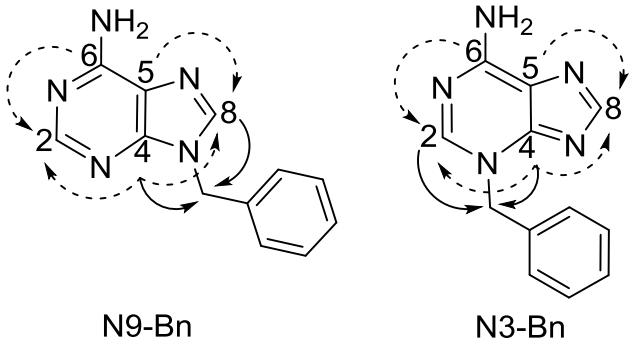

Figure 2. Diagnostic uniquely (solid arrows) and common (dotted arrows) HMBC ^{1}H-^{13}C correlations for N9- and N3-benzyladenine.

Two-dimensional ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC) spectroscopy and standard H,C-heteronuclear multibond coherence (HMBC) spectroscopy were used to characterize the solution structures of the two products. The ^{1}H and ^{13}C NMR chemical shifts of the two products were assigned in three different solvents (Tables 1-2). The ^{1}H-^{13}C HMBC correlations clearly indicated the placement of the benzyl group on the ring (Figure 2). Detailed explanations for the interpretation of the NMR data are given in Part A in the ASI.

Table 1 ^{1}H NMR chemical shifts (ppm) for N9-Bn and N3-Bn (400 MHz)

C2-H (1H, s)	C8-H (1H, s)	NH_2 (2H, s)	Ph (5H, m)	CH_2 (2H, s)	Solvent
N9-Bn					
8.16	8.26	7.23–7.33 (7H)		5.36	[D_6]DMSO
8.38	7.78	6.02	7.25–7.38	5.36	$CDCl_3$
8.22	8.15	-	7.28–7.37	5.43	[D_4]MeOD
N3-Bn					
8.57	7.78	7.94	7.27–7.47	5.51	[D_6]DMSO
8.26	8.18	10.34, 6.70	7.39	5.58	$CDCl_3$
8.60	8.21	-	7.46–7.33	5.63	[D_4]MeOD

The ^{1}H NMR chemical shifts for the purine protons of both the N9-Bn and N3-Bn varied significantly in the three different solvents. The ^{1}H NMR shifts for N3-Bn in $CDCl_3$ were very similar to those for N9-Bn in [D6]DMSO; whereas the N3-Bn shifts in [D6]DMSO were very similar to those of N9-Bn in $CDCl_3$, having the C2-H and C8-H shifts reversed in both cases. The relative position of the C8 signal in the ^{13}C NMR spectra for N3-Bn in different solvents varied considerably showing a chemical shift range of 7.8 ppm.

Table 2 ^{13}C NMR chemical shifts (ppm) for N9-Bn and N3-Bn (100 MHz)

C2	C4	C5	C6	C8	$\underline{C}H_2$	Ph	Solvent
N9-Bn							
152.8	149.6	118.8	156.1	141.0	46.3	127.6, 127.9,128.8, 137.2 (C_0)	[D_6]DMSO
152.2	150.1	119.3	154.8	141.0	47.6	128.0, 128.8, 129.3, 135.5 (C_0)	$CDCl_3$
154.1	150.8	119.9	157.4	142.8	48.2	128.9, 129.4, 130.1, 137.8 (C_0)	[D_4]MeOD
N3-Bn							
143.5	149.6	120.1	154.9	152.3	52.1	128.0, 128.1,128.6, 136.1 (C_0)	[D_6]DMSO
146.2	148.4	115.0	153.8	144.5	53.7	128.8, 129.8, 129.9, 132.9 (C_0)	$CDCl_3$
147.5	150.3	117.1	156.2	150.0	54.3	129.4, 130.0, 130.7, 136.2 (C_0)	[D_4]MeOD

Theoretical prediction of ^{13}C NMR spectra. Concurrent to obtaining sufficient quantities of the minor benzyladenine isomer for structural analysis, we predicted the ^{13}C NMR spectra for N9-Bn and the two reported structural isomers (Table 3). The ^{13}C NMR values are in good agreement for all three isomers N9-, N3- and N7-Bn (Table 3) with the exception of C6 in N3-Bn ($\Delta\delta$ -2.1 ppm). The weaker prediction for C6 in N3-Bn correlates to the solvent dependent variation of chemical shift for this carbon observed in the experimental ^{13}C NMR.

Table 3 Predicted and Experimental ^{13}C NMR chemical shifts (ppm) for benzyladenine isomers in DMSO-d_6.

		C2	C4	C5	C6	C8
N9-Bn	Predicted	152.1	150.1	119.8	154.8	142.3
	Experimental	151.3	149.3	118.6	154.9	141.4
	Difference	-0.8	-0.8	-1.2	0.1	0.0
N3-Bn	Predicted	143.2	150.6	121.6	152.8	153.4
	Experimental	143.5	149.6	120.1	154.9	152.2
	Difference	-0.3	-1.0	-1.5	2.1	-1.2
N7-Bn	Predicted	153.4	160.7	110.2	1521.5	147.9
	Literature [21]	152.6	159.3	110.8	151.8	147.5
	Difference	-0.8	-1.4	0.6	0.3	-0.4

Although the prediction for C4 in N7-Bn shows a slight difference ($\Delta\delta$ 1.4 ppm) when compared to reported experimental values, the trend of C4 being significantly more downfield than the other carbons for this isomer is recovered. The computed ^{13}C NMR shifts successfully predicted the upfield shift of C5 in N7-Bn relative to the other benzyladenine isomers. The data obtained confirms our assignment of ^{13}C NMR chemical shifts and shows that the data for the minor product fits the N3-structural isomer.

Solvent effect during synthesis.

In conducting the reaction to obtain sufficient quantities of the minor isomer for full NMR analysis, we observed that ratios of the products were variable and an increase in the N3 product corresponded to increased moisture in the reaction solvent. The effect of solvent was studied systematically (Table 4).

Table 4 Reaction conditions for the benzylation of adenine using benzyl bromide and KO*t*Bu /NaH

No.	Solvent	N9-Bn:N3-Bn
1	DMF (dried)[a]	100:0
2	DMSO (dried)[a]	100:0
3	DMSO (wet)	95:5–70:30[b]
4	Acetonitrile	86:14
5	Ethanol	50:50
6	*tert*-butanol	25:75
7	DMSO, water (33%)	50:50
8	DMSO, water (50%)	40:60

All reactions were stirred for 4 hours at refluxing temperature.
[a] NaH used as a base, [b] N9:N3 ratio varied depending on the dryness of the DMSO.

The N9-Bn:N3-Bn ratio in favour of N9-Bn is obtained when polar aprotic solvents, such as DMSO, DMF and acetonitrile, are used (Table 4 No. 1–4). When stringently dried polar aprotic solvents (DMSO or DMF) were used, N9-Bn was formed as the only product (Table 4 No. 1 and 2). Using polar protic solvents reverses the ratio to favour the formation of N3-Bn (Table 4, No. 5 and 6). All reactions shown in Table 4 were repeated several times, each producing consistent N9-Bn:N3-Bn ratios, except reactions repeated in No. 3, where the series of reactions were performed over a time period using the same batch of DMSO.
The relationship between polar aprotic solvents favouring N9-Bn and polar protic solvents favouring the N3-Bn could be due to competition between S_N2 *vs.* S_N1 mechanistic pathways respectively, having the formation of N3-Bn as a result of S_N1 substitution [22,23]. The use of bases which could form alcohols and water, such as KO*t*Bu and NaOH, respectively, could have provided an S_N1 microsolvation environment, which enabled the formation of N3-Bn. In addition, it has been suggested that the adenine anion could accept a proton from the polar protic solvent, yielding neutral adenine which undergoes N3-alkylation [23].

Conclusions

This research was conducted to add to the growing data to show that adenine reacts at the N9 and N3 positions [8,24]. NMR data has routinely been misinterpreted, but a thorough analysis in three solvents leads to unambiguous interpretation, and is well supported by computational predictions using ADF (RB3LYP/ATZP/COSMO. The ratio of N9-:N3-benzyladenine can be varied to favour either product through the use of solvent, with N9-benzyladenine favoured in polar aprotic solvents and N3-benzyladenine favoured in polar protic solvents. Our work agrees well with the recently (2015) published work by Gao *et al*. [25], who determined that the alkylation of adenine with R-propylenecarbonate in the presence of pulverized sodium hydroxide in addition to the N9 regioisomer yields the N3-subsitutent, (R)-3-(2′-hydroxyprop-1-yl) adenine, not the N7-regio-isomer as reported by Raić *et al*. [26] Thus, surely the preference for N3 is probably general for all alkylation reactions, not just the benzylation.

EXPERIMENTAL

Instrumentation

The ^{1}H NMR spectra were recorded at 400.13 MHz with a Bruker 400 AVANCE ultrashield +. The decoupled ^{13}C NMR spectra were recorded at 101 MHz using the Bruker 400 AVANCE ultrashield +. ^{1}H NMR spectra were calibrated using residual non-deuterated solvent signals ([D_6]DMSO at 2.50 ppm, [D_4]MeOD at 3.31 ppm and $CDCl_3$ at 7.24 ppm). The ^{13}C NMR spectra were calibrated using solvent signals ([D_6]DMSO at 39.5 ppm, [D_4]MeOD at 49.2 ppm and $CDCl_3$ at 77.2 ppm). All melting points were performed on single crystals using a Stuart melting point apparatus SMP10 and are uncorrected. All flash column chromatography was performed with silica gel 60 from Merck, Darmstadt, Germany (No. 64271). X-ray diffraction to obtain the crystal structures reported was performed on a Bruker D8 Venture (copper radiation). IR spectra were recorded in KBr using a Perkin Elmer Spectrum RXI FT-IR spectrometer. Mass spectra were recorded using a Waters® Synapt G2 high definition mass spectrometry(HDMS) system (Waters Inc., Milford, Massachusetts, USA) in ESI positive mode operating at a capillary voltage of 2.8 kV using FIA (5 μL).

Synthesis

N9-benzyladenine and N3-benzyladenine KO*t*Bu (0.436g, 3.89 mmol) and adenine (0.499g, 3.70 mmol) were added to DMF (30ml) and stirred for 30 minutes at room temperature, forming a white precipitate (adenine$^{-}K^{+}$ salt). Benzyl bromide (0.44ml, 5.8 mmol) was added and the solution was heated to 125 °C and stirred for 24 hours. The reaction mixture was filtered and the solvent was removed from the filtrate by a N_2(g) stream at 50 °C. The precipitate was rinsed with ethyl acetate and the products were isolated by gradient flash chromatography, ethyl acetate: CH_2Cl_2: hexane (1:1:0.5); ethyl acetate: CH_2Cl_2: hexane (1:1:0.5), methanol (10%); ethyl acetate: CH_2Cl_2: hexane (1:1:0.5), methanol (12.5%). The pure compounds were recrystallized from CH_2Cl_2 and methanol (1:1). **N9-benzyladenine**: White. Yield: 30% (0.251 mg). m.p.: 235–236 °C (lit. [8] 233–235 °C). IR (KBr, cm^{-1}): 3399, 3298, 3091, 1645, 1596, 1572, 1485, 1325, 1300, 1246, 731. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C) δ = 8.26 (s, 1H, C8-H), 8.16 (s, 1H, C2-H), 7.38–7.17 (m, 7H, NH_2, C_6H_5), 5.37 (s, 2H, CH_2). ^{13}C NMR (101 MHz, [D_6]DMSO, 25 °C) δ = 156.1 (1C, C6), 152.8 (1C, C2), 149.6 (1C, C4), 141.0 (1C, C8), 137.2 (1C, C_0, C_6H_5), 128.8 (2C, C_6H_5), 127.9 (1C, C_6H_5), 127.6 (2C, C_6H_5), 118.8 (1C, C5), 46.3 (1C, CH_2), MS (+ESI): *m/z* 226.11 $(M+H)^{+}$. **N3-benzyladenine**: White. Yield: 4% (0.030 mg). m.p.: 279–281 °C, (lit. [21] 280–281 °C). IR (KBr, cm^{-1}): 3319, 3099, 1658, 1617, 1567, 1455, 1405, 1226, 1176, 1014, 656. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C) δ = 8.57 (s, 1H, C2-H), 7.96 (s, 2H, NH_2), 7.78 (s, 1H, C8-H), 7.50–7.40 (m, 2H, C_6H_5), 7.39–7.23 (m, 3H, C_6H_5), 5.51 (s, 2H, CH_2). ^{13}C NMR (101 MHz, [D_6]DMSO, 25 °C) δ = 154.9 (1C, C6), 152.3 (1C, C8), 149.6 (1C, C4), 143.5 (1C, C2), 136.1 (1C, C_0, C_6H_5), 128.6 (2C, C_6H_5), 128.1 (2C, C_6H_5), 128.1 (1C, C_6H_5), 120.1 (C5), 52.1 (CH_2), MS (+ESI): *m/z* 226.11 $(M+H)^{+}$.

Crystal data for N9-Bn. CCDC ref no. 1451434. $C_{12}H_{11}N_5$, M_{rel} = 225.26, T = 150 K, monoclinic, space group P $2_1/c$, **a** 11.7874(4) Å **b** 12.4129(4) Å **c** 7.1279(2) Å, **α** 90 **β** 90.7760(11) **γ** 90, V = 1042.83 $Å^3$, R-factor (%) = 3.87.

Crystal data for N3-Bn. CCDC ref no. 1451435. $C_{12}H_{11}N_5$, M_{rel} = 225.26, T = 150 K, monoclinic, space group P $2_1/c$, **a** 7.2996(4) Å **b** 11.7426(6) Å **c** 12.4520(7) Å, **α** 90 **β** 100.998 **γ** 90, V = 1047.74 $Å^3$, R-factor (%) = 3.41.
The above synthetic procedure was repeated using the relevant bases and solvents reported in this article. The DMSO and DMF used for obtaining N9-benzyladenine as a single product were dried over activated molecular sieves, 4 Å.

Computational methods
The crystal structure of the N9-Bn and self-constructed structures of the N7- and N3-Bn were optimized in Amsterdam Density Functional (ADF) [27,28] 2010 software. The B3LYP level of theory was used in conjunction with an augmented triple-ζ basis set with valence shell polarization (ATZP) [28,29], in the COSMO [30,31] (conductor-like screening model) implicit solvation model using DMSO as a solvent. NMR [27,32-34] calculations were performed using ADF software to obtain the ^{13}C NMR chemical shifts. The calculated ^{13}C NMR spectra for adenine, N9-, N3- and N7-Bn were calibrated using the DMSO solvent signal (ppm) as a reference to give the absolute computed values.

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Additional supporting information (ASI)
Contains all ^1H, ^{13}C, HMBC and HSQC spectra for N9-Bn and N3-Bn and the computational prediction of the ^{13}C NMR spectra.
CCDC-1451434 and CCDC- 1451435 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailingdata_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

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