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Migrating ^{1}H NMR peaks in the benzylation of adenine reveal the disruptive Kornblum oxidation in DMSO

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Abstract

The alkylation of adenine using alkyl halides under basic conditions in dimethyl sulfoxide (DMSO), a common reaction to achieve N9-alkylated adenine derivatives, is often low yielding with unreacted adenine and complicated reaction mixtures. Herein, we report the reaction monitoring of the alkylation of adenine in DMSO in the presence of NaH using benzylic halides via real-time ^{1}H NMR spectroscopy. NMR analysis revealed that under these generally used reaction conditions, the adeninate anion starting material is protonated as the anionic nucleophile abstracts a labile proton from an alkoxy sulfonium ion intermediate formed via the Kornblum oxidation reaction. To prevent the protonation of the adeninate anion, the reaction was performed in the presence of a mop-up base DBU. Simultaneously increasing the concentration of the alkyl halide and the mop-up base in a 1:1 ratio resulted in a complete reaction; however, increasing the temperature of the reaction promoted depletion of the starting material by protonation and hence reduced conversion to products. This result implies that heating of such electrophiles in DMSO should be avoided. The addition of a mop-up base can help resolve the complication of protonation arising from the Kornblum oxidation reaction in alkylation reactions under similar conditions.

1 | INTRODUCTION

Adenine (AdeH) and its anion (Ade^{-}) are poorly soluble in most organic solvents [1]. Thus, solvents for the reactions of these compounds are limited to dimethyl sulfoxide (DMSO) and DMF. The direct alkylation of adenine using an alkyl halide in the presence of a base (such as NaH, NaOH, KOH, or K_2CO_3) is a common reaction carried out in these two solvents to achieve N9-alkylated adenine derivatives [2–6], which have an important biological activity such as potent cyclic nucleotide phosphodiesterase inhibition, antiinflammatory activity, and antiviral activity [7–11]; however, this reaction has long presented the problem of complex mixtures (N9-, N3-, and N7-alkylated adenine regioisomers) and low yields [2–4, 6, 12–16].

Nuclear magnetic resonance (NMR) spectroscopy is a powerful technique to monitor chemical reactions in situ. Real-time reaction monitoring via NMR has been used to gain chemical and mechanistic insights into reactions [17, 18], detect reactive intermediates and other chemical species in solution [19], determine the thermodynamics and kinetics of a reaction [20–22], and for the optimization reactions [23].

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We were engaged in real-time monitoring by ^{1}H NMR spectroscopy of the reaction between benzylic halides and adenine in DMSO under basic conditions to explore the origin of the preference for the N9 and N3 benzylated products and to gain insight into the chemical processes taking place in solution. We observed an unexpected peak migration of the Ade$^-$ starting material in the ^{1}H NMR spectra. This migration was attributed to the protonation of the Ade$^-$ and the products despite the basic medium. This protonation renders Ade$^-$ unreactive as neutral adenine is formed. We show that this protonation, which contributes to reduced yields, can be avoided by adding a mop-up base at ambient temperature.

2 | RESULTS AND DISCUSSION

The reactions of benzyl bromide (Figures 1, **1**), pentafluorobenzyl bromide (**2**) and (1-bromoethyl)benzene (**3**) with the pre-formed adeninate anion in DMSO were monitored by real-time ^{1}H NMR spectroscopy. The N9- (major) and N3- (minor) benzylated adenine derivatives

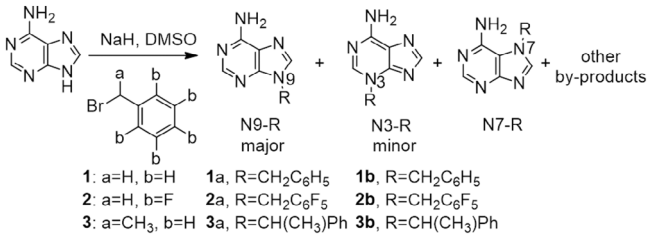

FIGURE 1 Alkylation of adenine in anhydrous dimethyl sulfoxide with NaH using benzyl bromide (**1**), pentafluorobenzyl bromide (**2**) or (1-bromoethyl)benzene (**3**).

were identified, Table 1. Trace amounts of N7 and other by-products were also observed in the spectra. This is in agreement with the general trend in the alkylation pattern of adenine [2, 6, 13, 24–26]. The real-time ^{1}H NMR spectra of the reactions revealed migrating peaks identified as corresponding to the protonation of the N3-benzylated adenine products of **1** and **2** (Figure 2, chemical shifts at the start of the reaction are indicated by the red asterisk). This is evident by the migration of the C2-H and C8-H resonances of N3-benzyl adenine (N3-Bn) in the ^{1}H NMR spectra of the reaction of Ade$^-$ with benzyl bromide (Figure 2A), where the proton peaks become deshielded, shifting from 8.57 to 8.63 and 7.76 to 7.86 ppm. The effect is more pronounced in the reaction of Ade$^-$ with pentafluorobenzyl bromide, having the C2-H and C8-H of the N3-pentafluorobenzyladenine (N3-PFB) migrate from 8.53 and 7.70 to 8.89 and 8.37, respectively (Figure 2B).

The absence of the Ade$^-$ peaks and the presence of the N-alkylated adenine product peaks in the very first ^{1}H NMR spectra obtained shows that the benzylation reaction between Ade$^-$ and benzylic halide **1** and **2** was already complete within the first few minutes. Yet, typically, the reaction duration is extended to 16 h or more in an attempt to improve conversion to the product.

The alkylation reaction using reagent **3** resulted in an overall slower reaction, which can be ascribed to the bulkier methyl group at the site of nucleophilic attack, increasing the activation barrier for the reaction. This slow reaction rate allowed two regions in the ^{1}H NMR spectra to be identified, labeled as regions 1 and 2 in Figure 3.

Region 1 indicates that the benzylation reaction is taking place alongside the protonation of Ade$^-$ (chemical

TABLE 1 ^{1}H NMR chemical shifts for adenine and the major and minor products for the alkylation of the adeninate anion using benzylic halides, and the ratios of N9- and N3-R adenine derivatives (N9:N3), in anhydrous dimethyl sulfoxide-d_6.

	N9:N3	C8-H	C2-H	C_6H_5	CH_2	CH	CH_3
Adenine		8.11	8.09				
Benzylic halide							
Benzyl bromide (**1**)							
N9-benzyladenine, N9-Bn (**1a**)	1:0.4	8.26	8.16	7.23–7.33	5.36	—	—
N3-benzyladenine, N3-Bn (**1b**)		7.76	8.57	7.27–7.47	5.51	—	—
Pentafluorobenzyl bromide (**2**)							
N9-pentafluorobenzyladenine, N9-PFB (**2a**)	1:0.3	8.20	8.11	—	5.51	—	—
N3-pentafluorobenzyladenine, N3-PFB (**2b**)		7.70	8.53	—	5.67	—	—
(1-bromoethyl)benzene (**3**)							
N9-(1-phenylethyl)-adenine, N9-PE (**3a**)	1:0.5	8.40	8.11	7.39–7.25	—	5.82	1.94
N3-(1-phenylethyl)-adenine, N3-PE (**3b**)		7.75	8.57	7.49–7.27	—	6.10	2.02

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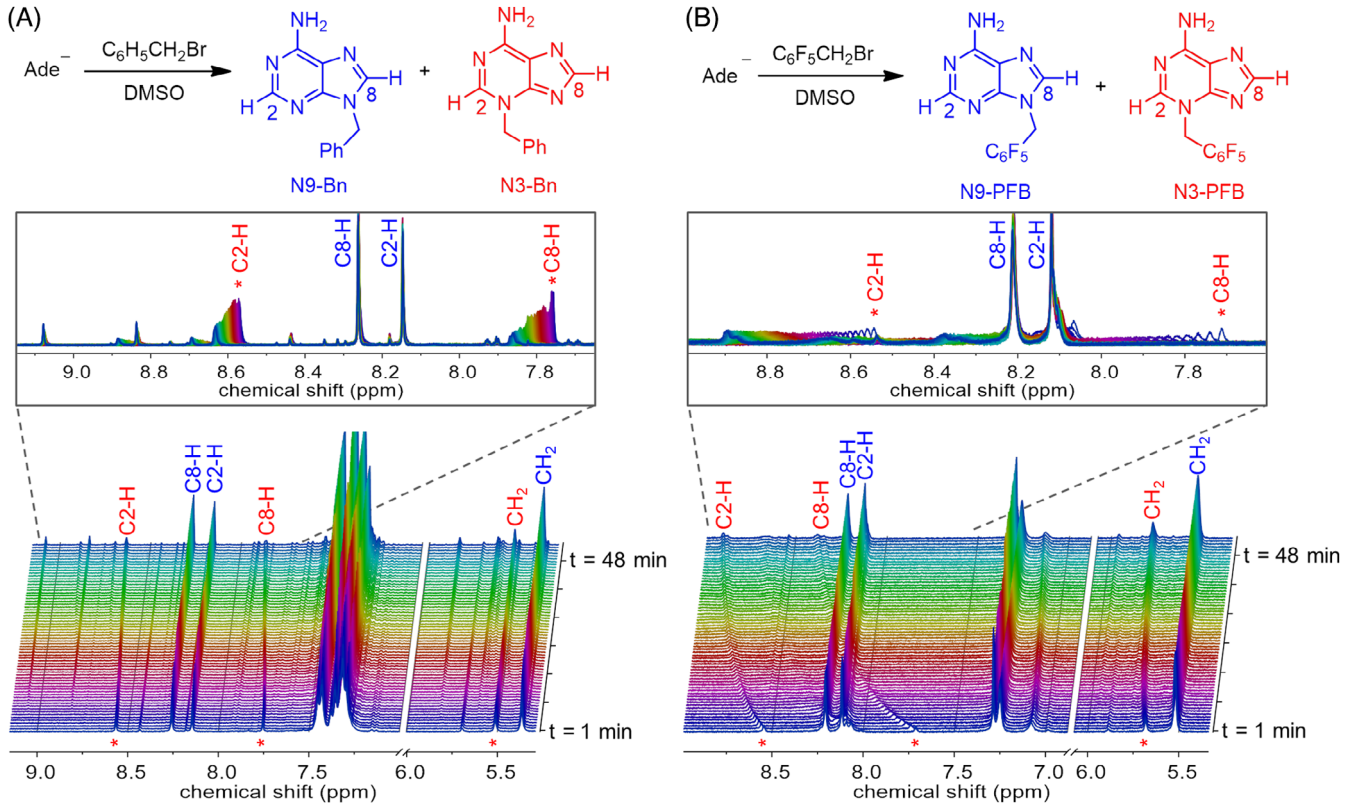

FIGURE 2 1D ^{1}H NMR stacked spectra for the time course array of the alkylation of the adeninate anion (44 mM) with (A) benzyl bromide (4.4 equivalents) and (B) pentafluorobenzyl bromide (4.7 equivalents) in anhydrous dimethyl sulfoxide-d_6 at 22°C for 52 min (data were collected every 15.5 s). Proton peaks of the N9- (blue) and N3-alkylated adenine (red) are shown. Chemical shifts of N3-Bn and N3-PFB at the start of the reaction are indicated by the red asterisk. Every fourth scan is shown.

shifts at the start of the reaction are indicated by the black asterisk in Figure 3) whereby the C2-H and C8-H purine protons shift from 7.80 and 7.50 ppm, respectively, to under the C2-H of N9-(1-phenylethyl)-adenine product at 8.11 ppm as neutral adenine is formed (AdeH). In region 2, a subsequent protonation of N3-(1-phenylethyl)-adenine occurs only after Ade$^-$ is depleted by protonation and alkylation. The C2-H and C8-H proton peaks become deshielded, shifting from 8.57 and 7.75 to 8.82 and 8.17, respectively, and smaller deshielding of the benzylic CH peak from 6.10 to 6.15 ppm. It is to be noted that the N3-isomer is protonated before N9-alkylated adenine in all three reactions. The protonation of Ade$^-$ most likely occurs in the reaction of Ade$^-$ with benzylic halides **1** and **2**; however, the reaction rates were too fast, and therefore, only the protonation of the N3-benzylated adenine was observed.

The change in the extent of protonation depending on the structure of the benzylic halide indicated that the source of the proton is from a side reaction involving the alkylating reagent. The side reaction is proposed to be the Kornblum oxidation reaction: the oxidation of primary and secondary alkyl halides by DMSO to yield aldehydes or ketones, respectively [27, 28]. Although known for a long time, the reaction is not usually considered in the case of substitution reactions of alkyl halides in DMSO. The benzylic halides **1**, **2**, and **3** undergo S_N2 nucleophilic substitution by a DMSO solvent molecule, Figure 4, displacing the halide and forming an alkoxy sulfonium ion intermediate (**4**). In the presence of a base (Ade$^-$ in this research), a proton at the site of the nucleophilic attack is abstracted, resulting in an aldehyde for reagents **1** and **2** and a ketone for reagent **3**, as well as neutral adenine and dimethyl sulfide.

The aldehydes resulting from the Kornblum oxidation reaction for alkylating reagents **1** and **2**, benzaldehyde and pentafluorobenzaldehyde, are evident in the ^{1}H NMR spectra by the presence of the aldehyde peak at 10.02 and 10.14 ppm, respectively, (Figure S4A,B). The formation of methylphenyl ketone from the alkylating reagent **3** is observed in the ^{1}H NMR spectra with the proton peaks present at 2.58 (CH_3) and phenyl protons at 7.97 and 7.95 (Figure 3), which correspond to those reported for methylphenyl ketone in DMSO-d_6 [29]. Furthermore, evidence for the formation of the alkoxy sulfonium ion intermediate was present in the mass spectrum

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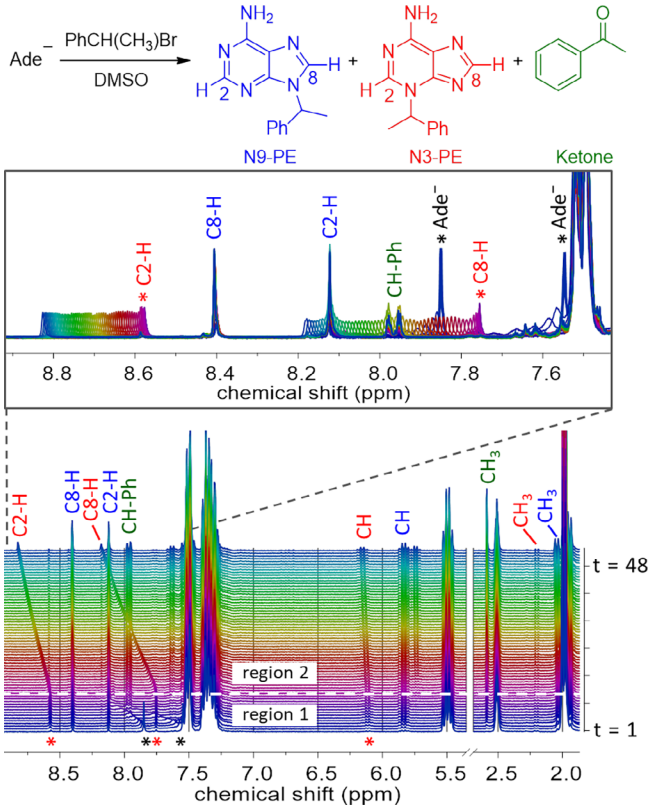

FIGURE 3 1D ^{1}H NMR stacked spectra for the time course array of the alkylation of the adeninate anion (44 mM) with (1-bromoethyl)benzene (4.6 equivalents) in anhydrous dimethyl sulfoxide-d_6 at 22°C for 52 min (data were collected every 15.5 s). Proton peaks of the N9-PE (blue), N3-PE (red), Ade$^-$ (black), and methylphenyl ketone (green) are shown. Chemical shifts of N3-PE and Ade$^-$ at the start of the reaction are indicated by the red and black asterisk, respectively. Every fourth scan is shown.

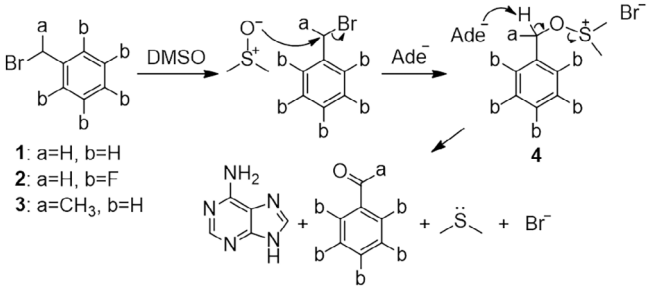

FIGURE 4 Kornblum oxidation reaction [27, 28] involving dimethyl sulfoxide and benzylic halides **1**, **2**, and **3** in the presence of the adeninate anion base.

obtained by flow injection analysis in real time for the reaction of **3** with Ade$^-$ in DMSO (Figure S5).

A comparison of the reaction outcome between the alkylation of adenine with the different benzylic halides (Figures 2 and 3) highlights the interplay between the electrophile's reactivity towards S_N2 reactions and

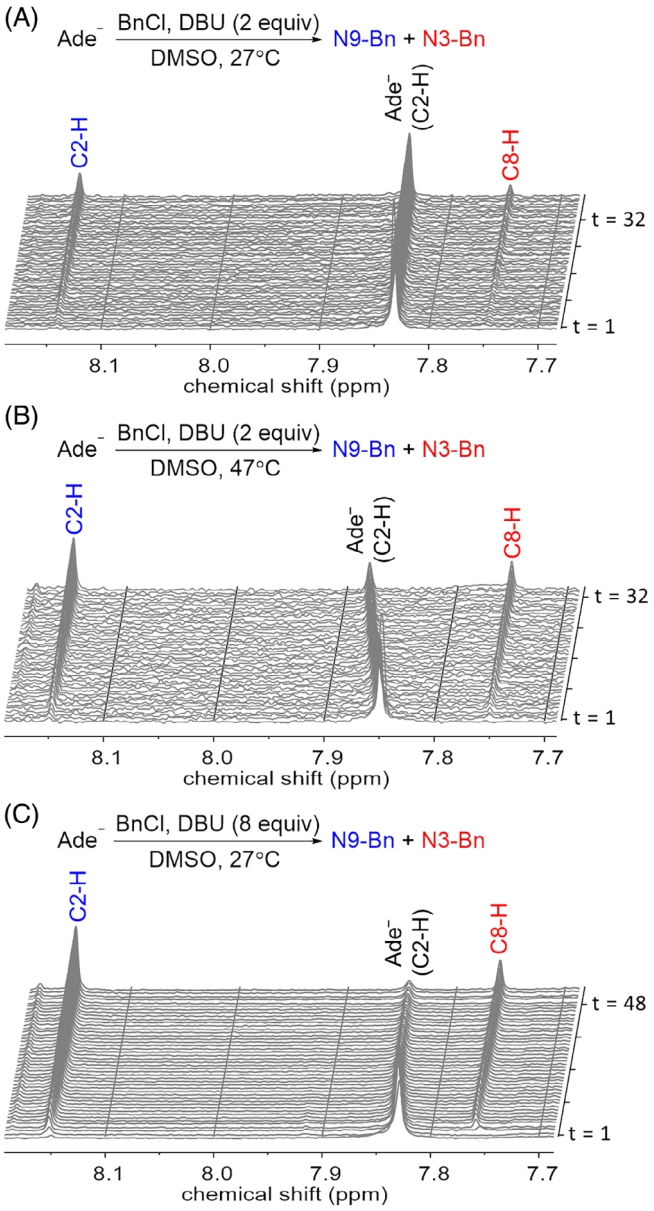

FIGURE 5 1D ^{1}H NMR stacked spectra for the time course array of the reaction of the adeninate anion (13.5 mM) with BnCl in dimethyl sulfoxide-d_6 solution in the presence of DBU at (A) 27°C with two equivalents. BnCl and DBU, (B) 47°C with two equivalents. BnCl and DBU and (C) 27°C with eight equivalents. BnCl and DBU, for 34 (A and B) and 52 min (C) (data were collected every 15.5 s). The proton peak of the C2-H of N9-Bn (blue), C8-H of N3-Bn (red), and C2-H of Ade$^-$ (black) are shown. Every fourth scan is shown.

the acidity of the alkoxy sulfonium ion intermediate it generates. The electronegativity of the fluorine atoms, with their larger inductive effect, renders the benzylic protons of the pentafluorobenzyl alkoxy sulfonium intermediate more acidic. This would result in increased protonation of the products. Since the chemical shifts in the ^{1}H NMR spectra indicate a rapid equilibrium between

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the protonated and unprotonated forms, the increased migration of peaks for this reaction represents increased protonation. These results fit the proposed mechanism. It is noticeable that the adeninate anion is protonated first, followed by the N3-alkylated product, corresponding to decreasing basicity for these compounds. The N9-alkylated product was not protonated during the timeframe of the experiments.

We were curious to know if adding a base could prevent the protonation of the Ade^- nucleophile and the reaction products. Two bases were investigated, namely 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (Figure 5) and triethylamine (TEA, Figure S7).

The addition of excess NaH to compete for the labile proton on the alkoxy sulfonium ion was not considered because DMSO is deprotonated by NaH and can subsequently react with the alkyl halide [30]. Furthermore mixtures of DMSO and NaH are hazardous [31–33].

We changed from benzylic bromide reagents to using the less reactive benzyl chloride to give us the opportunity to observe the slower reaction. Changing to benzyl chloride did not affect the ratio of the N9-, N3-, and N7-benzyl products formed.

We studied the effect of temperature and benzyl chloride concentration on the degree of protonation of Ade^- in the presence of the added base. For example, when the reaction was performed at 27°C using two equivalents of benzyl chloride and DBU, the ^{1}H NMR spectra showed no protonation of Ade^-. This indicated the efficiency of DBU as a mop-up base (Figure 5A). Similar results were obtained for TEA. When the temperature of the reaction was increased from 27 to 47°C we observed slight protonation of Ade^- (Figure 5B). Thus, at higher temperatures, the rate of the Kornblum oxidation was increased, and Ade^- was once again protonated, but to a much lower extent than in the absence of DBU. This is interesting as the reaction is usually performed at elevated temperatures with long reaction times. The increase in protonation with the increase in temperature was much larger for the TEA base, whereby a far more significant amount of protonation of Ade^- occurred, Figure S7B. By contrast, increasing the equivalents of benzyl chloride and DBU (in a 1:1 ratio) from two equivalents to an extreme eight equivalents at 27°C showed a negligible amount of protonation of Ade^- (Figure 5C) demonstrating that DBU can be used to quench the excess protons made available by the excess reagent. Under these conditions, almost full conversion of the starting material was achieved within the timeframe of the ^{1}H NMR run of 52 min compared to the standard reported reaction times of more than 16 h. The product yields were 62 (N9), 24 (N3), and 5% (N7) at 91% conversion of starting material, as determined by NMR.

We do not suggest that eight equivalents of alkyl halide and DBU need to be added to obtain the complete conversion of the starting material, as this is wasteful, and other complications can arise. It should be noted that high concentrations of DBU should be avoided as it can act as a competitive nucleophile in the presence of halogenated compounds (Figure S8). Our results here give insight into how increasing the equivalents of alkyl halide and DBU (in a 1:1 ratio) affects the extent of protonation of Ade^-. Using lower equivalents of the alkyl halide (i.e., two equivalents) with longer reaction times in the presence of equal equivalents of DBU at room temperature will prevent protonation of Ade^- and its alkylated products from giving a more complete and cleaner reaction.

3 | CONCLUSION

The alkylation of adenine using an alkyl halide in the presence of a base in DMSO or DMF solution is a common reaction to achieve N9-alkylated adenine derivatives. We have shown that the Ade^- starting material is depleted by protonation due to the Kornblum oxidation reaction, which occurs as a side reaction in DMSO. Similar complications arise in DMF when using benzylic halides, such as benzyl bromide and NaH [31, 34–36]. The depletion of the adeninate anion by protonation is only partly responsible for the low yields of the alkylation of adenine using primary and secondary alkyl halides, which undergo the Kornblum oxidation. The formation of the minor regioisomers further reduces the percent yield of the generally desired N9-alkylated adenine. To overcome the regioselectivity issue, researchers have investigated alternative synthetic routes [37–39].

To enhance the way we approach the alkylation of the adeninate anion using alkyl halides in DMSO solution and to ensure full conversion of the starting material, a mop-up base, such as DBU, should be used to compete for the labile proton of the alkoxy sulfonium ion intermediate, which results from the unavoidable Kornblum oxidation reaction. This prevents depletion of the adeninate anion starting material by protonation. Increasing the alkyl halide concentration in a 1:1 ratio with the mop-up base results in the completion of the reaction without enhancing the protonation of the adeninate anion. On the other hand, increasing the temperature of the reaction, as is often done when attempting to drive a reaction to completion, only results in further depletion of the starting material by protonation.

The depletion of the starting material under these conditions is most likely not limited to Ade^- but probably occurs with other anion nucleophiles during

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alkylation reactions. Other reactions involving the direct alkylation of anionic nucleophiles with primary and secondary alkyl halides in DMSO should be studied to investigate whether there is protonation interference from the Kornblum oxidation reaction, leading to reduced yields.

4 | EXPERIMENTAL SECTION

4.1 | General

The ^{1}H NMR spectra were recorded at 300 MHz with a Bruker 300 AVANCE Ultrashield Plus. The ^{1}H NMR spectra were calibrated using the DMSO-d_6 solvent peak at 2.50. The ^{13}C NMR spectra were recorded at 101 MHz using the Bruker 400 AVANCE Ultrashield Plus. The ^{13}C NMR spectra were calibrated using the DMSO-d_6 solvent peak at 39.5 ppm. All reagents were purchased from Sigma-Aldrich. All samples were prepared and sealed within the glove box using the anhydrous DMSO-d_6. Sodium hydride was used as a base to avoid the introduction of water into the reactions. Reagents were weighed using the Mettler Toledo XP6 Excellence Plus XP Micro Balance. For the ^{1}H NMR temperature experiments at 27, 32, and 47°C, the NMR tubes were placed inside the NMR machine and allowed to heat up to the desired temperature of either 27, 32, or 47 K for 10 min. The room temperature was 22°C for ambient runs. The ratio of N9-:N3-:N7- of benzylic adenine derivatives was obtained by the ratio of the integration of the CH_2 peak of each isomer.

4.2 | Mass spectrometry

Analysis was performed using flow injection analysis (FIA); the flow rate was set to 0.4 mL/min, and the injection volume was 5 μL. Ultra purity methanol (Romil-UpS™, Microsep, South Africa) spiked with 0.1% formic acid (Fluka® Analytical, Sigma–Aldrich, South Africa), B2, and water with 0.1% formic acid, A1, were used as elution solvents. An isotopic flow (A1:B2; 50:50 [%v/v]) was used through the entire run of 1 min. Compound detection was performed using a Waters® Synapt G2 high-definition mass spectrometry (HDMS) system (Waters Inc., Milford, MA). Samples were analyzed using FIA. The system is comprised of a Waters Acquity Ultra Performance Liquid Chromatography (UPLC®) system hyphenated to a quadrupole-time-of-flight (QTOF) instrument. The system was operated with MassLynx™ (version 4.1) software (Waters Inc.) for data acquisition and processing. An internal lock mass control standard, 2 ng/μL solution leucine enkephalin (m/z 555.2693), was directly infused into the source through a secondary orthogonal electrospray ionization (ESI) probe allowing intermittent sampling. The internal control was used to compensate for instrumental drift, ensuring good mass accuracy. The source conditions were as follows: the capillary voltage for ESI was 2.6 kV for positive ionization. The source temperature was set at 120°C, the sampling cone voltage at 30 V, the extraction cone voltage at 4.0 V, and the cone gas (nitrogen) flow at 30.0 L/h. The desolvation temperature was set at 350°C with a gas (nitrogen) flow of 600.0 L/h. Mass spectral scans were collected every 0.3 s. The raw data were collected in the form of a centroid profile. Mass-to-charge ratios (m/z) between 50 and 1200 Da were recorded.

4.3 | Real-time ^{1}H NMR spectroscopy runs

4.3.1 | Preparation for benzylic bromide reactions

The anhydrous DMSO-d_6 was prepared as follows: Powdered 4 Å molecular sieves were heated at 200°C under vacuum for 4 days and placed inside a glove box. The sieves were added to DMSO-d_6 and left for 24 h. The solvent was analyzed via ^{1}H NMR to confirm the absence of water. The molecular sieves were removed by passing the solvent through a 25 mm hydrophilic polyamide syringe filter.

Reaction with benzyl bromide

NaH (0.64 mg, 0.027 mmol) was added to an NMR tube containing a heterogeneous mixture of 500 μL DMSO-d_6 and adenine (3.01 mg, 0.0222 mmol). The generated H_2 gas was allowed to escape. To the homogeneous solution, 1,4-Di-tert-butylbenzene (0.64 mg, 0.0034 mmol) was added as an internal standard. The NMR tube was sealed and removed from the glove box. The reaction was initiated by the addition of benzyl bromide (12 μL, 0.100 mmol). The tube was shaken to mix the reagents. The ^{1}H NMR spectra were recorded immediately at 22°C, taking about a minute to obtain the first spectra. Scans were collected every 15.5 s for 52 min. ^{1}H NMR chemical shifts for N9- and N3-benzyladenine match those reported in reference [13]. Trace amounts of N7-Bn are evident by proton chemical shifts at: 8.44 (CH), 8.18 (CH), and 5.70 (CH_2).

N9-benzyladenine (**1a**): ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ: 8.26 (s, 1H, C8-H), 8.16 (s, 1H, C2-H), 7.33–7.23 (m, 7H, NH_2, C_6H_5), 5.36 (s, 2H, CH_2). N3-benzyladenine (**1b**): ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ: 8.57 (s, 1H, C2-H), 7.76 (s, 1H, C8-H), 7.47–7.27 (m, 7H, NH_2, C_6H_5), 5.51 (s, 2H, CH_2).

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Reaction with 2,3,4,5,6-pentafluorobenzyl bromide

NaH (0.65 mg, 0.027 mmol) was added to an NMR tube containing a heterogeneous mixture of 500 μL DMSO-d_6 and adenine (3.03 mg, 0.0224 mmol). The generated H_2 gas was allowed to escape. To the homogeneous solution, 1,4-Di-tert-butylbenzene (0.71 mg, 0.0037 mmol) was added as an internal standard. The NMR tube was sealed and removed from the glove box. The reaction was initiated by the addition of 2,3,4,5,6-pentafluorobenzyl bromide (16 μL, 0.106 mmol). The tube was shaken to mix the reagents. The ^{1}H NMR spectra were recorded immediately at 22°C, taking about a minute to obtain the first spectra. Scans were collected every 15.5 s for 52 min.

N9-pentafluorobenzyladenine (**2a**): ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ: 8.20 (s, 1H, C8-H), 8.11 (s, 1H, C2-H), 5.51 (s, 2H, CH_2). N3-pentafluorobenzyladenine (**2b**): ^{1}H NMR (300 MHz, DMSO-d_6, 25°C) δ: 8.53 (s, 1H, C2-H), 7.70 (s, 1H, C8-H), 5.67 (s, 2H, CH_2).

Reaction with (1-bromoethyl)benzene

NaH (0.64 mg, 0.027 mmol) was added to an NMR tube containing a heterogeneous mixture of 500 μL DMSO-d_6 and adenine (2.99 mg, 0.0221 mmol). The generated H_2 gas was allowed to escape. To the homogeneous solution, 1,4-Di-tert-butylbenzene (0.64 mg, 0.0034 mmol) was added as an internal standard. The NMR tube was sealed and removed from the glove box. The reaction was initiated by the addition of (1-bromoethyl)benzene (14 μL, 0.103 mmol). The tube was shaken to mix the reagents. The ^{1}H NMR spectra were recorded immediately at 22°C, taking about a minute to obtain the first spectra. Scans were collected every 15.5 s for 52 min.

N9-(1-phenylethyl)-adenine (**3a**): ^{1}H NMR (400 MHz, DMSO-d_6, 25°C) δ: 8.40 (s, 1H, C8-H), 8.11 (s, 1H, C2-H), 7.39–7.25 (m, 7H, NH_2, C_6H_5), 5.82 (q, 1H, CH), 1.94 (d, 3H, CH_3). N3-(1-phenylethyl)-adenine (**3b**): ^{1}H NMR (400 MHz, DMSO-d_6, 25°C) δ: 8.57 (s, 1H, C2-H), 7.93 (s, 2H, NH_2), 7.75 (s, 1H, C8-H), 7.49–7.47 (m, 2H, C_6H_5), 7.36–7.27 (m, 3H, C_6H_5), 6.10 (q, 1H, CH), 2.02 (d, 3H, CH_3).

4.3.2 | Preparation for benzyl chloride reactions

A stock solution was prepared within the glove box: NaH (9.19 mg, 0.383 mmol) was added to a heterogeneous mixture of adenine (43.1 mg, 0.319 mmol) and anhydrous DMSO-d_6 (5000 μL). The solution was stirred until bubbles of H_2 gas ceased. Anisole (34 μL, 0.322 mmol) was added as an internal standard. Solutions with a concentration of 13.5 mM of adenine were prepared from the stock solution in NMR tubes with a final volume of 400 μL using DMSO-d_6. Each reaction contained 13.6 mM of anisole as an internal standard. To each tube, either 1,8-Diazabicyclo(5.4.0)undec-7-ene at two equivalents. (1.61 μL, 0.0108 mmol) or at eight equivalents (6.44 μL, 0.0432 mmol), or triethylamine at two equivalents (1.50 μL, 0.0108 mmol) or at eight equivalents (6.02 μL, 0.0432 mmol), was added. The NMR tubes were sealed and removed from the glove box. Before running the experiments, the NMR tubes were placed inside the NMR machine and allowed to heat up to the desired temperature of 22 (TEA), 27, or 47°C. The reactions with two equivalents of DBU or TEA were initiated by the addition of benzyl chloride (1.24 μL, 0.0108 mmol). The ^{1}H NMR spectra were recorded immediately, either at 22 (TEA), 27, or 47°C, taking about a minute to obtain the first spectra. Scans were collected every 15.5 s for 34 or 52 min. The reactions with eight equivalents of DBU or TEA were initiated by the addition of benzyl chloride (4.98 μL, 0.0432 mmol). The ^{1}H NMR spectra were recorded immediately, either at 22 (TEA), 27, or 47°C, taking about a minute to obtain the first spectra. Scans were collected every 15.5 s for 52 min.

The NMR percent yield of N9-, N7-, and N3-Bn for the reaction with eight equivalents of DBU was calculated as follows:

$$\text{Yield} = \frac{\text{mol product}}{\text{mol starting material}} \times 100. \quad (1)$$

The concentration of N9-, N7-, and N3-Bn was calculated using the integration of the methyl peak of anisole and the CH_2 peak of N9-, N7- and N3-Bn, Equation (2),

$$C = CCF \times \frac{AI}{NN}, \quad (2)$$

where C is the concentration, CCF is the concentration conversion factor, AI is the absolute integral, and NN is the number of protons. CCF is calculated when C is the known concentration of anisole (13.6 mM), AI is the methyl group integral, and NN is 3.

4.4 | Mass spectrometry run

4.4.1 | Reaction with (1-bromoethyl)benzene

A stock solution was prepared with adenine (8.53 mg, 0.0631 mmol) and NaH (1.82, 0.0757 mmol) in DMSO. From the stock solution, a solution with a final concentration of 0.631 mM (0.631 μmol) was prepared in an LC/MS vial, using DMSO as the diluting solvent to a final volume of 1 mL. The reaction was initiated by the addition of (1-bromoethyl)benzene (0.86 μL, 6.31 μmol). The

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mass spectra were recorded immediately at ambient temperature (22°C).

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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