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

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Supporting Information

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^{1}H NMR spectra for the benzylation of the adeninate anion using benzyl bromide, 2,3,4,5,6-pentafluorobenzyl bromide and (1-bromoethyl)benzene

Presented below are the full ^{1}H NMR spectra from 0 ppm taken at the start of the reaction (t=1 minute) and at the end of the reaction (t=52 minutes) for the alkylation of the adeninate anion in DMSO-d_6 with 1,4-Di-tert-butylbenzene as an internal standard, using benzyl bromide (Figure S1), 2,3,4,5,6-pentafluorobenzyl bromide (Figure S2) and (1-bromoethyl)benzene (Figure S3) as the alkylating reagent.

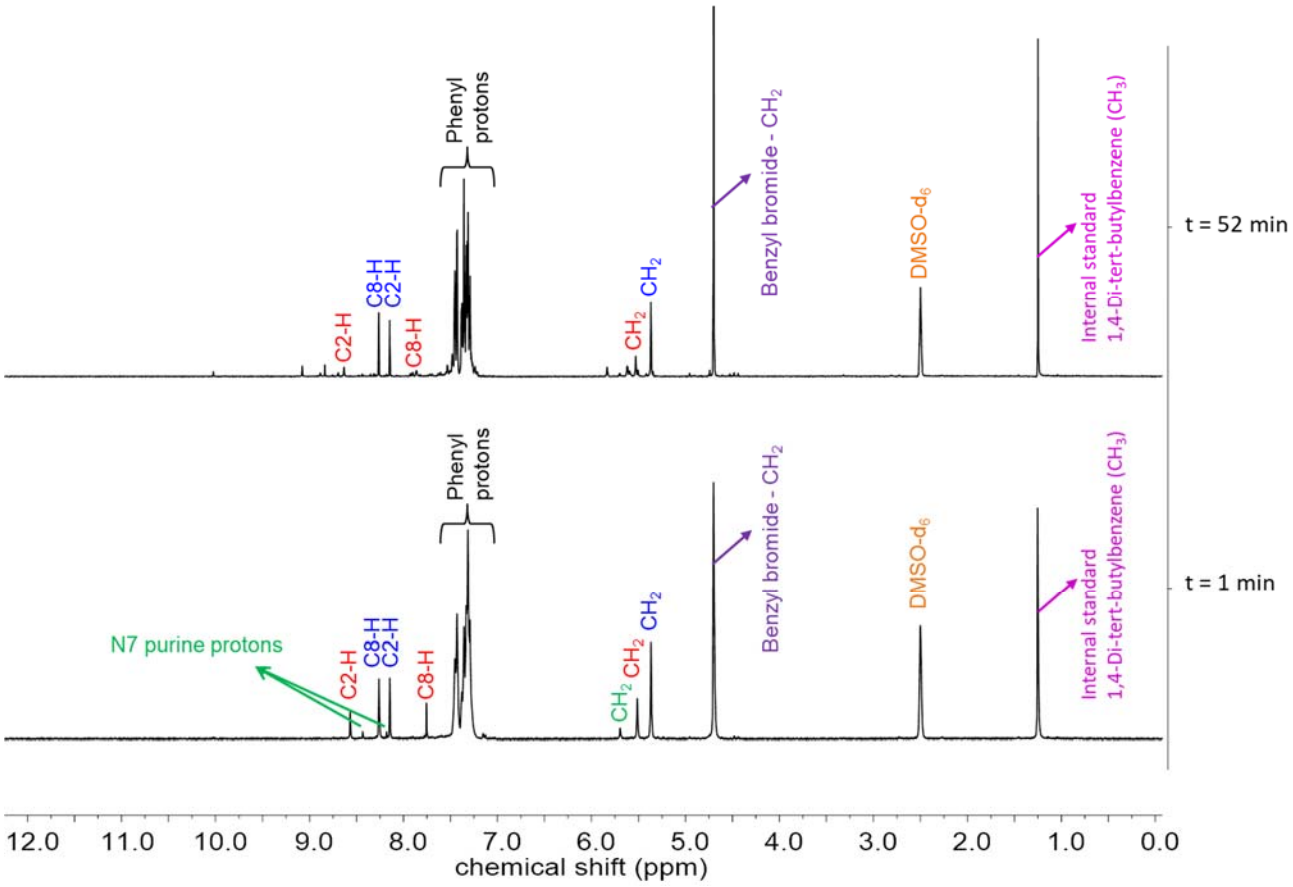

Figure S1: 1D ^{1}H NMR spectra at t=1 and t= 52 minutes for the time course array of the alkylation of the adeninate anion (44 mM) with benzyl bromide (4.4 equiv.) in anhydrous DMSO-d_6 at 22 °C (Figure 2A main text). Proton peaks of the N9-Bn (blue), N3-Bn (red) and N7-Bn (green) are shown, along with DMSO-d_6 (orange), benzyl bromide (purple) and the internal standard 1,4-Di-tert-butylbenzene (pink).

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).

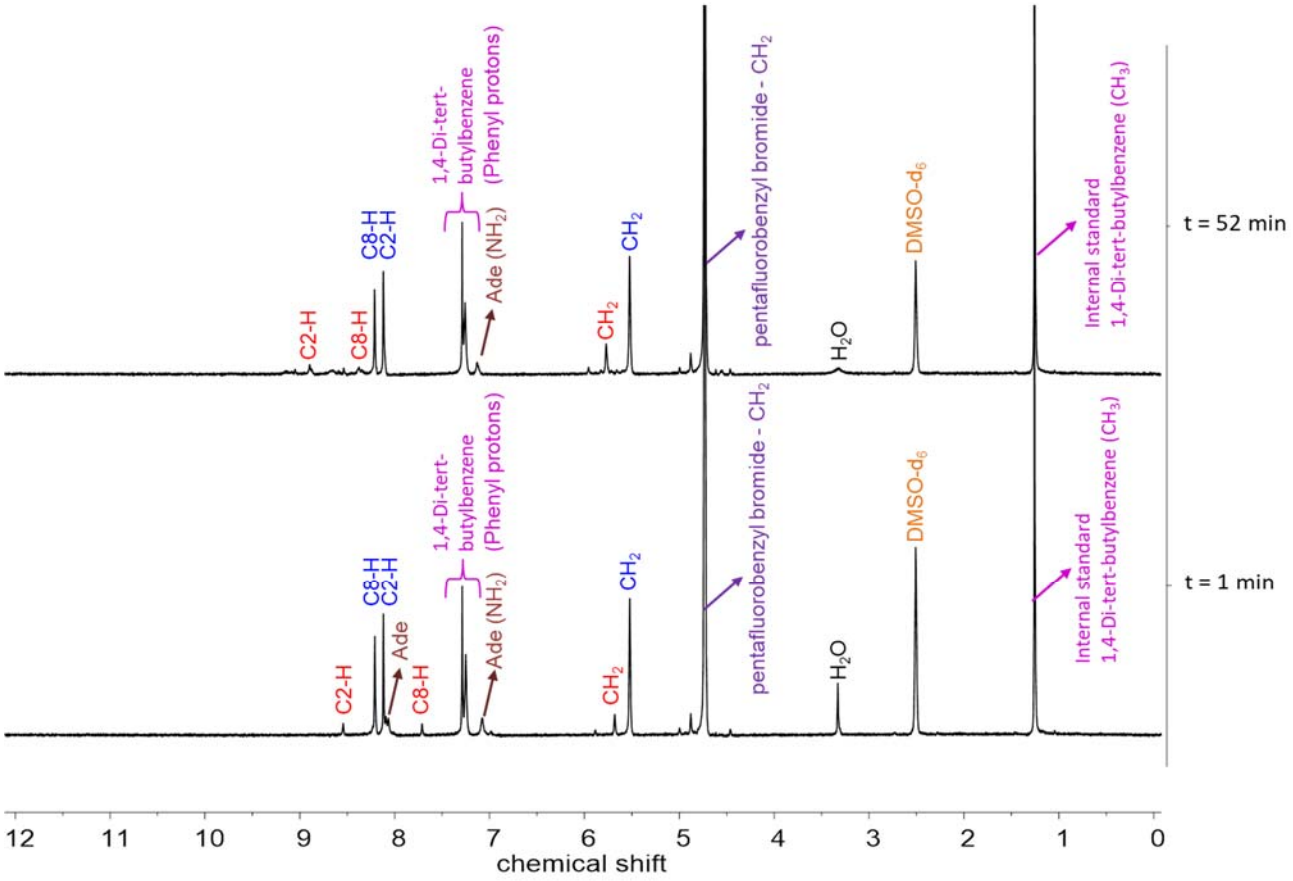

Figure S2: 1D ^{1}H NMR spectra at t=1 and t= 52 minutes for the time course array of the alkylation of the adeninate anion (44 mM) with pentafluorobenzyl bromide (4.7 equiv.) in anhydrous DMSO-d_6 at 22 °C (Figure 2B main text). Proton peaks of the N9- PFB (blue), N3-PFB (red), and adenine (brown) are shown, along with DMSO-d_6 (orange), pentafluorobenzyl bromide (purple) and the internal standard 1,4-Di-tert-butylbenzene (pink).

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)

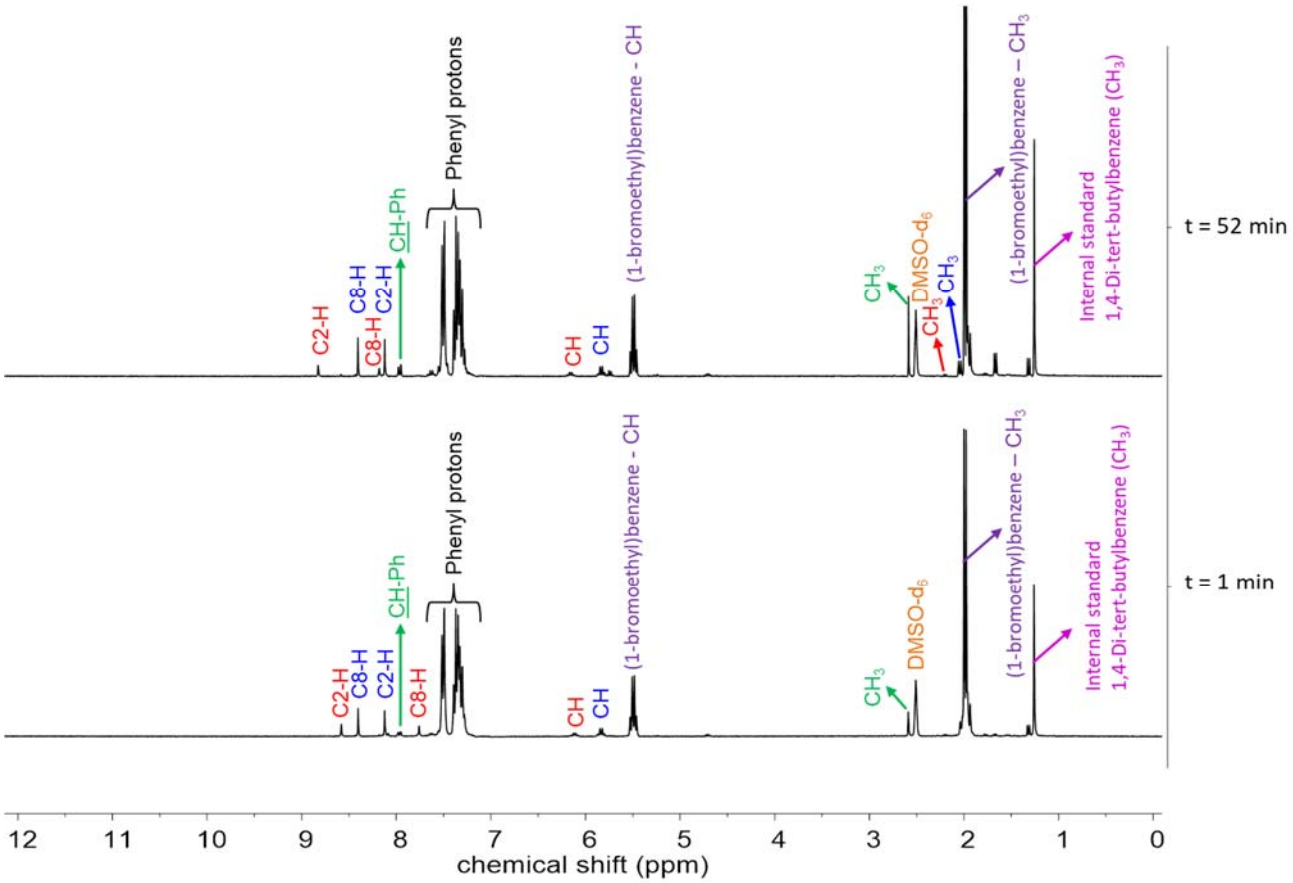

Figure S3: 1D 1H NMR spectra at t=1 and t= 52 minutes for the time course array of the alkylation of the adeninate anion (44 mM) with (1-bromoethyl)benzene (4.6 equiv.) in anhydrous DMSO-d6 at 22 °C (Figure 3 main text). Proton peaks of the N9-PE (blue), N3-PE (red), and methylphenyl ketone (green) are shown, along with DMSO-d_6 (orange), (1-bromoethyl)benzene (purple) and the internal standard 1,4-Di-tert-butylbenzene (pink).

For confirmation of the proton peaks for N9-PE and N3-PE, the products were purified and analysed using HMBC and HSQC: The DMSO–d_6 solvent from the reaction mixture was removed from the filtrate by an N_2(g) stream at 30 °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 ^{1}H NMR spectra were recorded at 400 MHz with a Bruker 400 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.

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). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C) δ = 156.02 (1C, C6), 152.42 (1C, C2), 149.25 (1C, C4), 141.73 (1C, C_0, C_6H_5), 139.15 (1C, C8), 128.65 (2C, C_6H_5), 127.65 (1C, C_6H_5), 126.25 (2C, C_6H_5), 118.93 (1C, C5), 53.17 (1C, CH), 20.59 (1C, 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). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C) δ = 154.74 (1C, C6), 152.39 (1C, C8), 149.43 (1C, C4), 141.74 (1C, C2), 140.12 (1C, C_0, C_6H_5), 128.61 (2C, C_6H_5), 128.04 (1C, C_6H_5), 126.93 (2C, C_6H_5), 120.42 (1C, C5), 58.51 (1C, CH), 19.04 (1C, CH_3).

^{1}H NMR spectra for benzaldehyde and 2,3,4,5,6-pentafluorobenzaldehyde

The ^{1}H NMR spectrum for the reaction between adenine, NaH and either benzyl bromide or pentafluorobenzyl bromide in DMSO-d_6 is shown in Figure S4 A and B, respectively. The formation of the benzaldehyde and 2,3,4,5,6-pentafluorobenzaldehyde from the Kornblum oxidation reaction, Figure 4 in the main text, is evident by the appearance of the peaks at 10.02 (Figure S4 A) and 10.14 ppm (Figure S4 B), respectively.

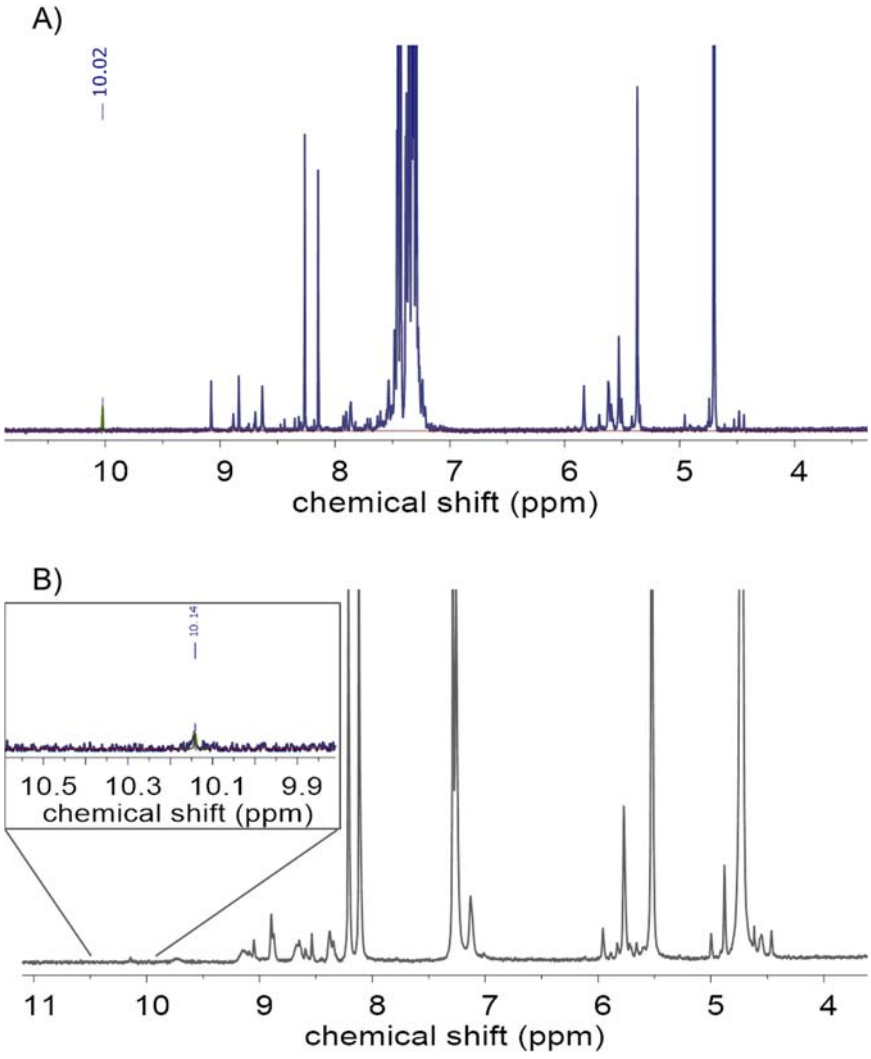

Figure S4: The ^{1}H NMR chemical shifts for the aldehyde compound resulting from A) benzyl bromide and B) pentafluorobenzyl bromide in anhydrous DMSO-d_6

Mass spectrum for alkoxy sulfonium ion intermediate

The mass spectrum of the reaction between the adenine, NaH and (1-bromoethyl)benzene in DMSO solution is shown in Figure S5.

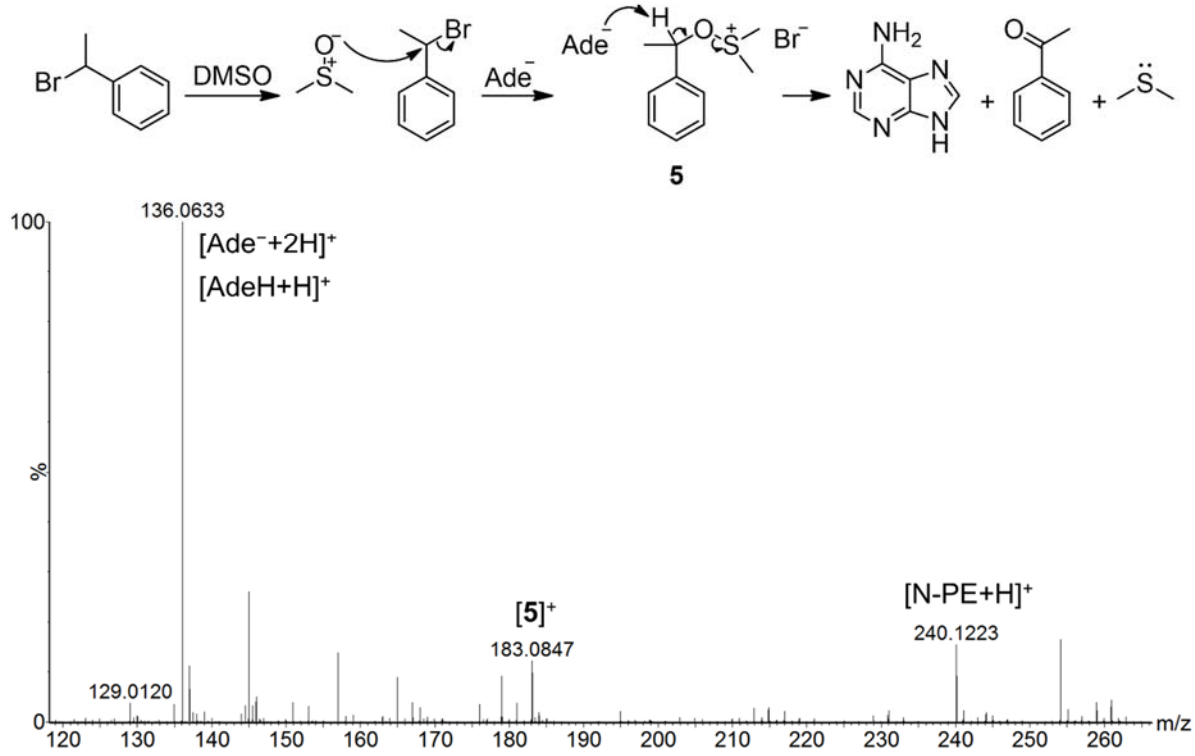

Figure S5: High resolution mass spectrum (positive ion mode) of the reaction between adenine, NaH and (1-bromoethyl)benzene in DMSO solution. The monoisotopic mass found for the adeninate anion 136.0633 $[Ade^{-}+2H]^{+}$, adenine 136.0633 $[Ade+H]^{+}$, alkoxy sulfonium ion intermediate (**5**) 183.0847 $[\mathbf{5}]^{+}$, and the N-(1-phenylethyl)-adenine 240.1223 $[N\text{-}PE+H]^{+}$ are shown.

The reaction yields the N9- and N3-(1-phenylethyl)-adenine (N-PE), which are indistinguishable from each other in the mass spectrum (HRMS (ESI/Q-TOF) m/z: $[M + H]^{+}$ Calcd for $C_{13}H_{14}N_5$ 240.1249; Found 240.1223, shown as $[N\text{-}PE + H]^{+}$ in Figure S5). The reaction occurs alongside

the Kornblum oxidation reaction (reaction scheme in Figure S5) in which the alkoxy sulfonium ion intermediate (**5**) is observed in the mass spectrum (HRMS (ESI/Q-TOF) m/z: $[M]^+$ Calcd for $C_{10}H_{15}OS$ 183.0844; Found 183.0847, shown as $[5]^+$). The adeninate anion (Ade^-) abstracts a proton from **5**, resulting in neutral adenine, AdeH. Ade^- and AdeH are indistinguishable from each other in the mass spectrum (HRMS (ESI/Q-TOF) m/z: $[M + 2H]^+$ and $[M + H]^+$ Calcd for $C_5H_7N_5$ 136.0623; Found 136.0633, shown as $[Ade^-+2H]^+$ and $[Ade+H]^+$.

^{1}H NMR spectra for the benzylation of the adeninate anion using benzyl chloride with DBU as a mop-up base.

Presented below, Figure S6, is the full ^{1}H NMR spectrum from 0 ppm taken at the end of the reaction (t=52 minutes) for the alkylation of the adeninate anion in DMSO-d_6 with anisole as an internal standard, using 8 equiv. BnCl and DBU at 27 °C (Figure 5C in the main text).

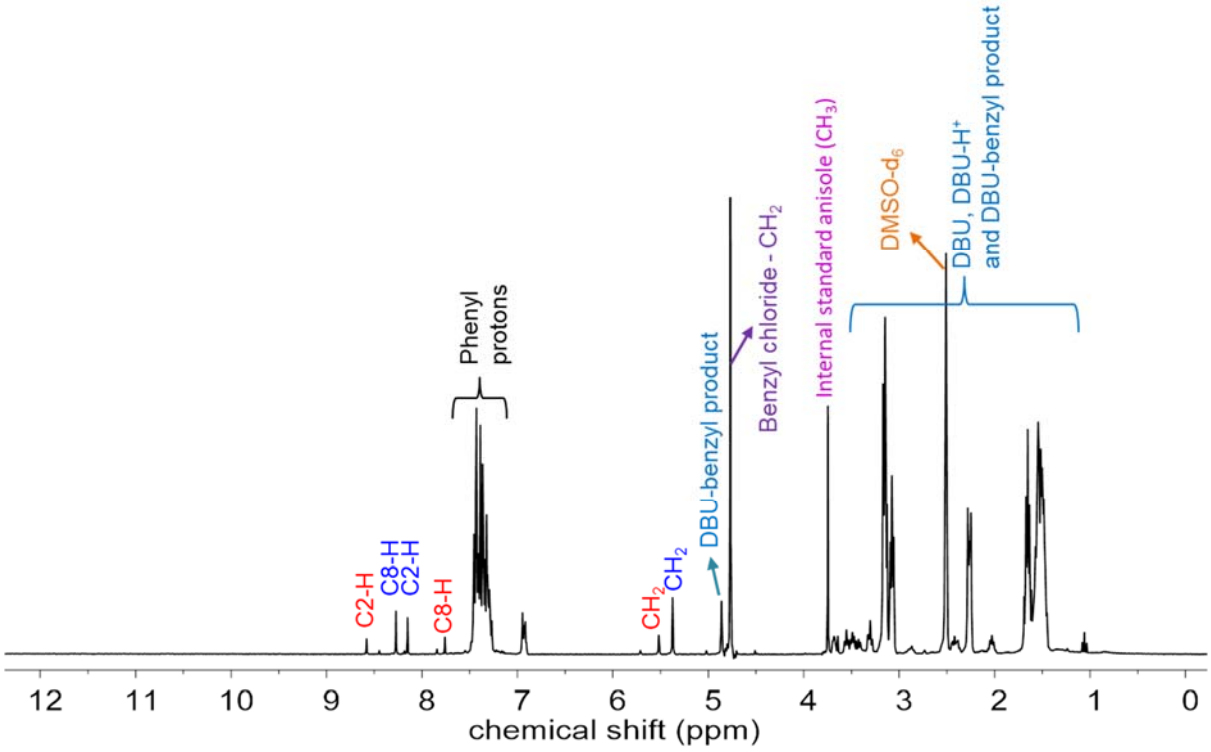

Figure S6: 1D ^{1}H NMR spectrum at t= 52 minutes for the time course array of the alkylation of the adeninate anion (13.5 mM) with benzyl chloride (8 equiv.) and DBU (8 equiv.) in anhydrous DMSO-d_6 at 27 °C. Proton peaks of the N9-Bn (blue) and N3-Bn (red) are shown, along with DMSO-d_6 (orange), benzyl chloride (purple), the internal standard anisole (pink), and DBU, DBU-H$^+$ and the DBU-benzyl product (blue), refer to Figure S8 for the DBU-benzyl product ^{1}H NMR.

Dynamic ^{1}H NMR spectra for TEA reactions

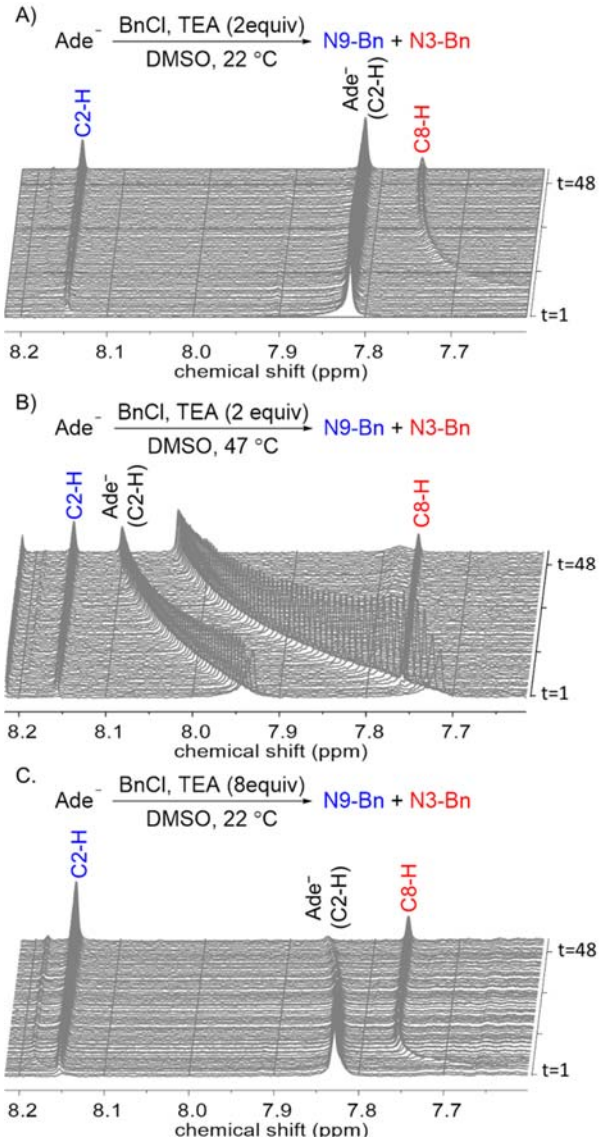

Figure S7: Dynamic ^{1}H NMR spectra of the reaction of AdeH, NaH and BnCl in DMSO-d_6 solution in the presence of TEA at A. 22 °C with 2 equiv. BnCl and TEA, B. 47 °C with 2 equiv. BnCl and TEA, and C. 22 °C with 8 equiv. BnCl and TEA, for 52 minutes (data was collected every 15.5 seconds). Every fourth scan is shown.

DBU as a nucleophilic base

At high concentrations of DBU, it is reasonable to suspect that DBU itself can act as a nucleophilic base. The chemical features of DBU, being sterically hindered, render it a good non-nucleophilic base, however, its nucleophilic nature has been revealed in the presence of halogenated compounds.[1,2] Briefly shown here DBU does indeed act as a nucleophile in the presence of BnCl in anhydrous DMSO-d_6, Figure S8. The resulting product of nucleophilic attack from DBU with BnCl has the CH_2 peak occur at 4.86 ppm. This peak is absent when TEA is used as a base. However, the reaction between DBU and BnCl is much slower in comparison to that of the benzylation of Na-Ade. There is only a 5% decrease in BnCl within 30 minutes for a 1:1 eq. of BnCl:DBU in DMSO.

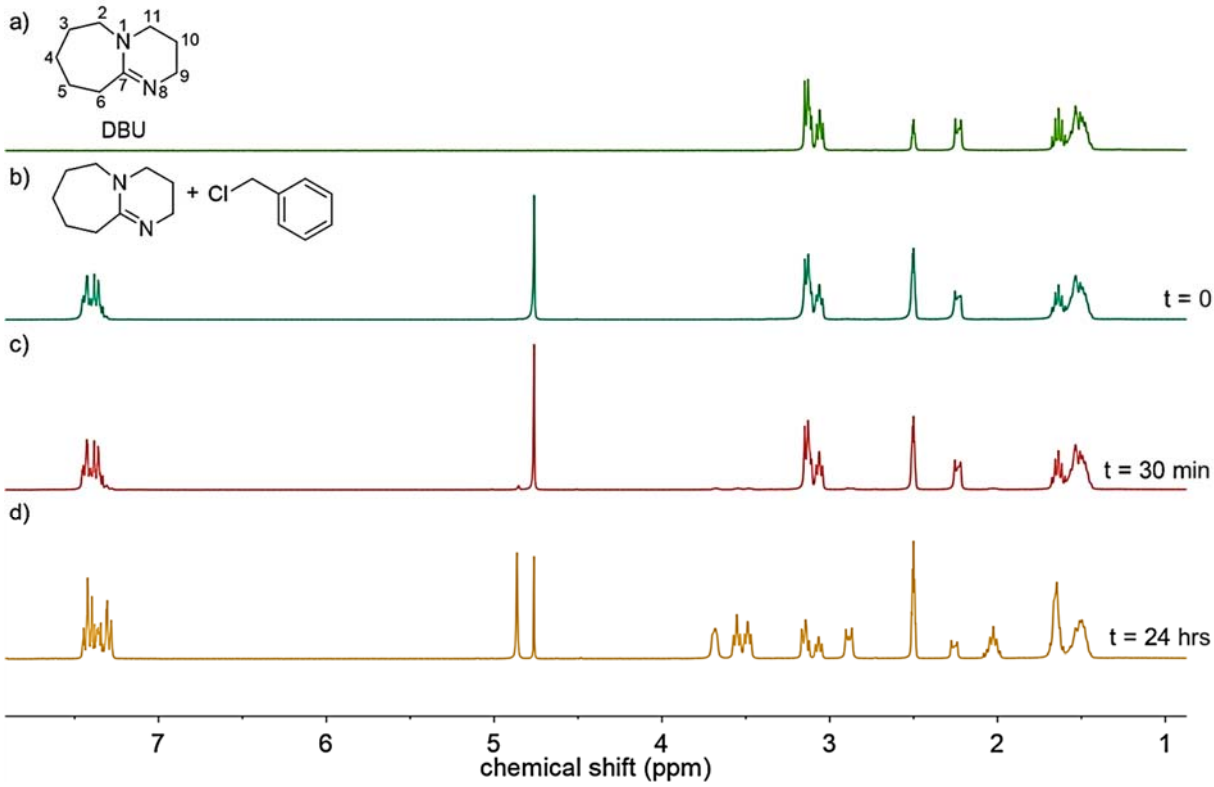

Figure S8: ^{1}H NMR of a solution of a) DBU (63.9 mM), and DBU and BnCl (65.17 mM) at b) t=0, c) t=30 minutes and d) t=24 hours, at 22 °C.

References:

(1) Baidya, M.; Mayr, H. Nucleophilicities and Carbon Basicities of DBU and DBN. *Chem. Commun.* **2008**, No. 15, 1792. https://doi.org/10.1039/b801811a.

(2) Ghosh, N. DBU (1,8-Diazabicyclo[5.4.0]Undec-7-Ene) - A Nucleophilic Base. *Synlett* **2004**, *2* (3), 574–575. https://doi.org/10.1055/s-2004-815436.