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A facile one-pot synthesis of 8-oxo-7,8-dihydro-(2′-deoxy)adenosine in water

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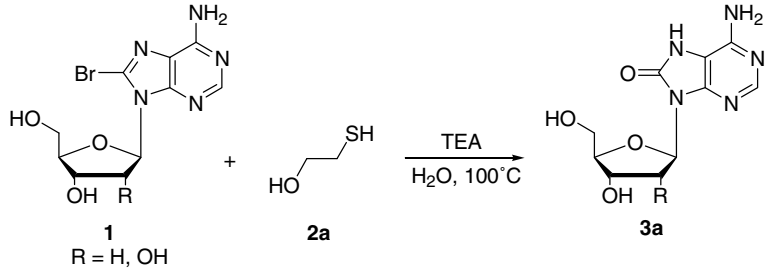
Abstract—Reaction of 2-mercaptoethanol with 8-bromo-2′-deoxyadenosine and 8-bromo-adenosine in aqueous solution and in the presence of triethylamine gave the 8-oxo-adenine derivatives in very good yields. Some mechanistic details are reported.

8-Oxo-7,8-dihydro-2′-deoxyadenosine (8-oxo-dAdo) is one of the products of oxidative or radiation-induced DNA damage.[1] Economical and efficient synthesis of a specific DNA lesion and its incorporation into a defined sequence of oligonucleotides has been an outstanding approach to investigate the biological consequences. 8-Oxo-dAdo is commercially available as the free nucleoside although it is rather expensive. Synthetically, this lesion is available from 8-bromo-2′-deoxyadenosine (8-Br-dAdo) through substitution of the C8 bromide by a benzyloxy group in DMSO and hydrogenolysis of the benzyl group (10% Pd/C) in an overall 70% yield.[2]

The analogous ribonucleoside (8-oxo-Ado), obtained in 84% yield by a two-step reaction from 8-Br-Ado using NaOAc in AcOH–Ac_2O followed by NaOH in ethanol, was recently employed as a key intermediate in the synthesis of phosmidosine and phosmidosine analogs, important nucleotide antibiotics as well as potent anticancer drugs.[3] Exploring radical-based reactivity in water in our laboratory,[4] we discovered by serendipity a facile entry to 8-oxo-dAdo and 8-oxo-Ado by a common approach that can be also extended to the thio-analogous. Herein, we present our synthetic method and the related mechanistic investigation.

To a 1.0 mM suspension of 8-Br-dAdo (**1**, R = H) in water 3 M equiv of 2-mercaptoethanol (**2a**) and 10 M equiv of triethylamine (TEA) were added, then the resulting clear solution was heated at 100 °C for 2 h. HPLC-MS analysis[5] of the reaction mixture revealed the formation of 8-oxo-dAdo (**3a**, R = H) as the only reaction product (Scheme 1). Subsequent water elimination gave the crude product **3a** in 95% yield. Further



Scheme 1. Formation of 8-oxo-dAdo (and 8-oxo-Ado) from reaction of 8-Br-dAdo (and 8-Br-Ado) with 2-mercaptoethanol.

Keywords: DNA damage; Nucleosides; 8-Oxo-7,8-dihydro-2′-deoxyadenosine; Mercaptoethanol; 8-Bromo-2′-deoxyadenosine.
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purification was achieved by reverse-phase chromatography on C18 silica gel column by elution with water-acetonitrile. Compound **3a** was identified by comparison of ^{1}H NMR and HPLC-MS spectra with those of an authentic commercial sample. Similar results were obtained from 8-Br-Ado (**1**, R = OH) under identical conditions. HPLC-MS analysis of the reaction mixture showed the formation of 8-oxo-Ado (**3a**, R = OH) in nearly quantitative yield (Scheme 1). The crude compound was separated in 93% yield and purified as described above for 8-oxo-dAdo.

Mechanistic details for the formation of the 8-oxo-adenine derivatives were obtained by independent experiments carried out with 8-Br-dAdo (**1**, R = H). In order to obtain evidence of a possible reaction intermediate, we monitored the reaction with thiol **2a** by HPLC-MS analysis at different time intervals. Actually, we found that the disappearance of starting 8-Br-dAdo is accompanied by the initial formation of sulfide **4a**[6] (Scheme 2). As shown in Figure 1, the yield of this product reached the maximum after 30 min (60%), then progressively decreased with the concomitant formation of the 8-oxo-dAdo **3a**. In a repeated experiment, 8-Br-dAdo was reacted with thiol **2a** for 30 min, then the reaction mixture was purified by column chromatography on silica gel to obtain an almost pure sample of the above sulfide **4a** characterized by MS, ^{1}H and ^{13}C NMR analysis.

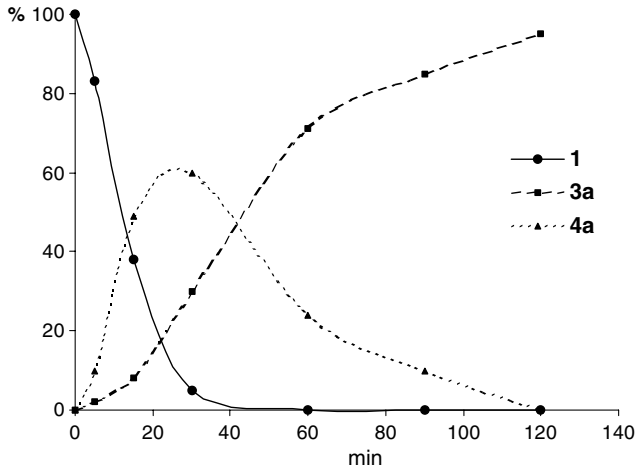
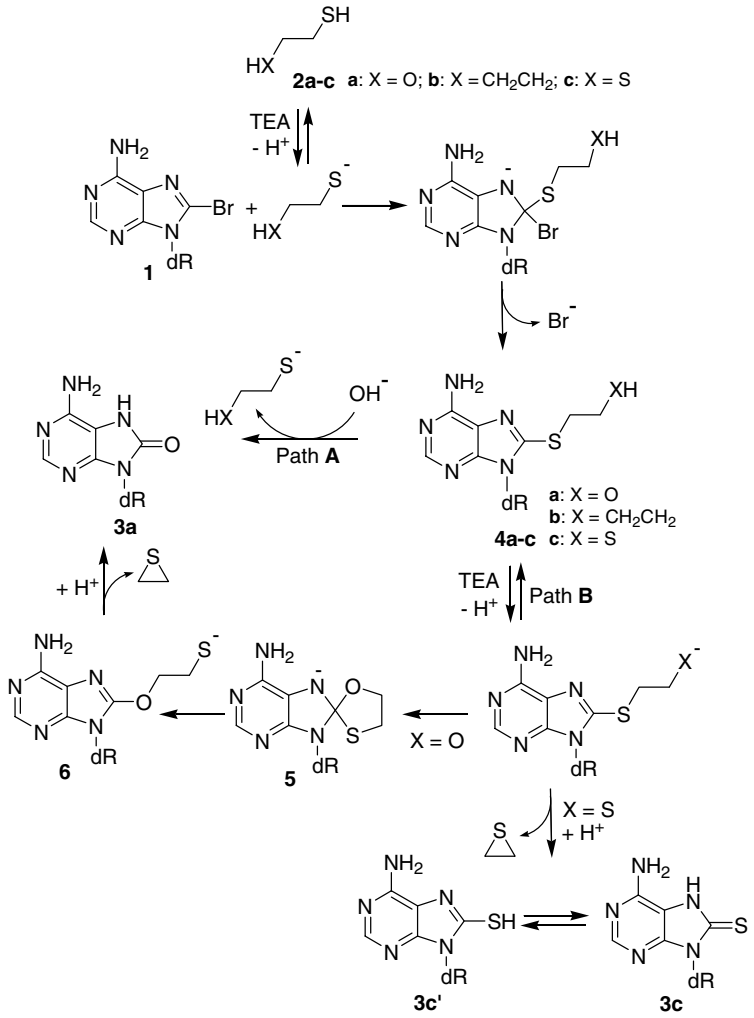


Figure 1. Product yields (%) versus time (min) of the reaction of **1**, R = H with **2a**.



Scheme 2. Mechanistic details for the reaction of 8-oxo-Ado with thiols **2a**–**c**.

Formation of product **3a** from **1** and **2a** requires TEA; the reaction in the absence of base led to total decomposition of 8-Br-dAdo within 2 h. The principal product obtained was 8-bromoadenine together with sulfide **4a** being formed in only 15% yield; compound **3a** was completely absent. Importantly, 8-bromoadenine was found to result from simple ribose hydrolysis as determined by a blank reaction lacking both thiol and TEA.

These findings indicated that 8-oxo-dAdo **3a** was formed through the intermediacy of sulfide **4a** under basic conditions. In turn, sulfide **4a** can be easily accounted for through a nucleophilic aromatic substitution of the bromide ion by the thiolate ion (Scheme 2). In principle, the formation of **3a** from **4a** could be explained through two different reaction pathways: (path **A**) intermolecular aromatic substitution by the water solvent (or hydroxy ion) with displacement of the 2-hydroxythiolate ion, or (path **B**) 1,4-sulfur-to-oxygen migration of the C8 carbon atom, possibly occurring in a two-step process through an initial nucleophilic addition of the β-oxygen to the C8–N double bond with the intermediacy of the *spiro*-compound **5**. The resulting thiolate ion **6** could afford the oxo-derivative **3a** through intramolecular nucleophilic aromatic substitution by the β-sulfur atom at the α-carbon atom, with displacement of a thiirane molecule (Scheme 2).

Significant support of the proposed pathway **B** has been obtained. First, formation of product **3a** from **1** and **2a** in ^{18}O water did not lead to isotopic incorporation. This excludes the possibility that water is the source of the C8 moiety.[7] This observation was supported by the reaction of **1** with butane thiol **2b**, which led to quantitative formation of sulfide **4b**.[8]

Further evidence supporting the mechanism **B** came from the reaction with 1,2-ethanedithiol (**2c**). After 2 h reaction time the mixture was treated with 10% HCl_{aq} and extracted with ethyl acetate. Solvent evaporation gave a tautomeric **3c/3c′** mixture[9,10] in a 20:80 ratio, as indicated by HPLC-MS and ^1H NMR analysis, in 75% overall yield (Scheme 2). The **3c/3c′** ratio changed from 20:80 to 80:20 by subsequent silica gel column chromatography by elution with ethyl acetate/methanol. HPLC-MS analysis of the reaction mixture at different reaction times gave no evidence of a possible intermediate **4c**, thus indicating the fact that the intramolecular nucleophilic aromatic substitution leading to **3c/3c′** is a fast process. This result was not surprising, since the sulfur anion is expected to be a better leaving group that the oxygen anion. The formation of **3c/3c′** in fairly good yields appears to be of interest from a synthetic point of view. In fact, the one-pot reaction herein reported can represent a convenient synthetic method alternative to the two-step synthesis from 8-Br-dAdo recently reported in the literature.[11]

In conclusion, we have reported a new facile synthetic methodology for the preparation of 8-oxo-adenine derivatives **3a**, R = H or OH, in very high yield (93–95%) through a one-pot reaction in water solution of 8-bromo-(2′-deoxy)adenosine (**1**, R = H or OH) with 2-mercaptoethanol (**2a**) at 100 °C in the presence of TEA. This procedure has been successfully extended to the synthesis of 8-thio-2′-deoxyadenosine derivative (**3c/3c′**).

Acknowledgements

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5. HPLC analyses were performed in all cases on a Zorbax MS C18 column (4.6 × 150 mm, 5 μm) with a linear gradient H_2O/acetonitrile from 100:0 to 50:50 at a flow rate 0.4 ml/min, detection at $\lambda = 260$ nm.
6. Compound **4a** was obtained as an almost pure sample by silica gel column chromatography by elution with ethyl acetate/methanol. ^1H NMR (400 MHz, DMSO-d_6), δ 8.02 (1H, s; H2), 7.20 (2H, br s), 6.20 (1H, dd, $J_1 = 8.4$, $J_2 = 6.0$ Hz; H1′), 5.40 (1H, br s; OH), 5.30 (1H, br s; OH), 4.40 (1H, m; H3′), 3.86 (1H, dt, $J_d = 2.4$, $J_t = 4.4$ Hz; H4′), 3.67 (2H, t, $J = 6.4$ Hz; O–CH_2) 3.63 (1H, dd, $J_1 = 12.0$, $J_2 = 4.4$ Hz; H5′), 3.47 (1H, dd, $J_1 = 12.0$, $J_2 = 4.4$ Hz; H5″), 3.37 (2H, ABX_2 system, $J_{AB} = 14.0$, $J_{AX} = J_{BX} = 6.4$ Hz; inner line separation 2.0 Hz; S-CH_2), 3.04 (1H, ddd, $J_1 = 13.5$, $J_2 = 8.4$ Hz; $J_3 = 6.0$ Hz; H2′), 2.10 (1H, ddd, $J_1 = 13.5$, $J_2 = 6.0$ Hz; $J_3 = 2.0$ Hz; H2, ″). ^{13}C NMR (75 MHz, DMSO-d_6), δ 35.52 (CH_2), 38.05 (CH_2), 60.17 (CH_2), 62.77 (CH_2), 71.87 (CH), 85.49 (CH), 88.73 (CH), 120.00 (q), 149.27 (q), 151.16(q), 151.89 (CH), 154.80 (q). MS ES(+) 328 (M+1), MS^2 212].
7. In order to investigate the possible role of the solvent in the formation of **3a** the reaction of **1** with **2a** and TEA was also carried out in methanol as a solvent. However, the reaction in methanol was found much slower. After 7 h heating at 100 °C HPLC-MS analysis showed the presence of starting material **1** in 20% yield, together with sulfide **4a** as the major product (50% yield), small amounts of 8-oxo derivative **3a** (3%) and significant amounts (27%) of 8-bromoadenine.
8. Compound **4b** was obtained as an almost pure sample by silica gel column chromatography by elution with ethyl acetate/methanol. ^1H NMR (400 MHz, DMSO-d_6), δ 8.00 (1H, s; H2), 7.00 (2H, br s), 6.20 (1H, dd, $J_1 = 8.4$, $J_2 = 6.0$ Hz; H1′), 5.4 (1H, br s; OH), 5.3 (1H, br s; OH), 4.40 (1H, m; H3′), 3.87 (1H, dt, $J_d = 2.0$, $J_t = 4.5$ Hz; H4′), 3.62 (1H, dd, $J_1 = 12.5$, $J_2 = 4.5$ Hz; H5′), 3.48 (1H, dd, $J_1 = 12.5$, $J_2 = 4.5$ Hz; H5″), 3.28 (2H, ABX_2 system, $J_{AB} = 11.2$, $J_{AX} = J_{BX} = 7.2$ Hz; inner line separation 3.2 Hz; S-CH_2), 3.00 (1H, ddd, $J_1 = 13.5$, $J_2 = 8.4$ Hz; $J_3 = 5.5$ Hz; H2′), 2.10 (1H, ddd, $J_1 = 13.5$, $J_2 = 6.0$ Hz; $J_3 = 2.0$ Hz; H2″), 1.66 (2H, quintuplet, $J = 7.2$ Hz;

CH_2), 1.40 (2H, sextuplet, $J = 7.2$ Hz; CH_2), 0.88 (3H, t, $J = 7.2$ Hz; CH_3); ^{13}C NMR (75 MHz, DMSO-d_6), δ 14.08 (CH_3), 21.83 (CH_2), 31.47 (CH_2), 32.46 (CH_2), 38.05 (CH_2), 62.67 (CH_2), 71.90 (CH), 85.49 (CH), 88.73 (CH), 120.06 (q), 149.22 (q), 151.08 (q), 151.90 (CH), 154.82 (q). MS ES(+) 340 (M+1), MS^2 224].

9. Thione **3c** was identified by comparison of the ^1H NMR spectrum with that reported in the literature. See Ref. 11.
10. Compound **3c**′ was characterized in mixture with **3c** by ^1H NMR and HPLC-MS analysis. ^1H NMR (400 MHz, DMSO), (D_2O shake) δ 8.0 (1H, s, H2), 6.75 (1H, dd, $J_1 = 9.0$, $J_2 = 6.4$ Hz; H1′), 4.40 (1H, m; H3′), 3.63 (1H, A part of an ABX system, $J_{AB} = 12.5$, $J_{AX} = 3.6$ Hz; H5′), 3.50 (B part of an ABX system, $J_{AB} = 12.5$, $J_{BX} = 3.6$ Hz; H5″), 2.80 (1H, m; superimposed to 1H, ddd, $J_1 = 13.2$, $J_2 = 9.0$, $J_3 = 5.6$ Hz; H2′), 2.0 (1H, dd, $J_1 = 13.2$, $J_2 = 9.0$, $J_3 = 6.4$ Hz; H2″); MS (ES+) 284 (M+1); MS^2 250; MS^3 168.
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