Sulfanyl radical promoted C4′–C5′ bond scission of 5′-oxo-3′,4′-didehydro-2′,3′-dideoxynucleosides†

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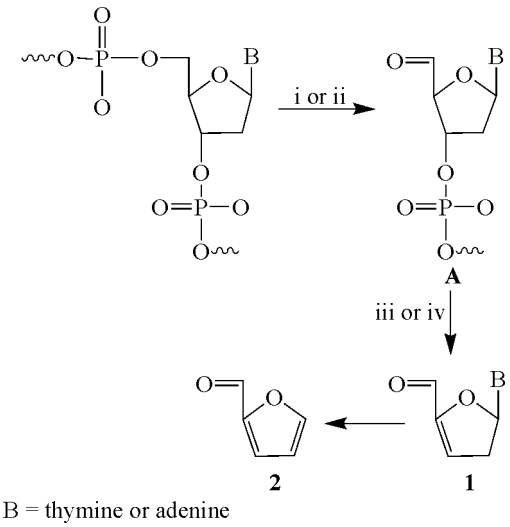
Treatment of C5′-aldehydes 4a,b under mildly basic conditions leads to the formation of 3′,4′-didehydroaldehydes 1a,b and furfural 2. Sulfanyl radical addition to 1a,b eventually gives rise to the lactones 11a,b through C4′–C5′ bond scission of the 1,2-dioxetane intermediates 10a,b.

5′-Oxo-derivatives represent an interesting class of nucleoside analogs from both a synthetic[1–3] and biological[4,5] point of view. The biological relevance of the 5′-oxonucleosides came to light when C5′-aldehyde termini **A** were found as oxidatively damaged products of DNA, deriving from strand breakage mediated by natural or chemical agents, such as enediynes[4] and metal porphyrins.[5]

Pratviel *et al.*[5a] characterized the 3′,4′-unsaturated-5′-aldehydes of 2′-deoxyadenosine and thymidine **1** among products derived from metalloporphyrin-mediated DNA cleavage after thermal treatment. These aldehydes have been shown to be the precursors of furfural **2**, which was identified as the eventual sugar degradation product[5b] (Scheme 1). Earlier, the 3′,4′-unsaturated-5′-aldehyde of thymidine had been suggested by Goldberg *et al.*[4a] as a plausible intermediate for the formation of furfural **2** by nuclease/basic treatment of products derived from neocarzinostatin-mediated DNA strand breakage (Scheme 1).

However, apart from the above-cited reports, the 3′,4′-didehydro-2′,3′-dideoxy-5′-aldehydes **1** remain virtually uninvestigated. Therefore, we considered that understanding their chemical behaviour could be of interest to chemists and biochemists, in order to elucidate the course of potential DNA damage. For this purpose, we planned a synthetic route to the C5′-aldehydes **1** and started to study their chemical reactivity. In this paper we report the synthesis of C5′-aldehydes **1a**,**b** and preliminary results concerning their reactivity under radical conditions. We found that sulfanyl radical addition to the C3′ atom under oxygenated conditions eventually led to products deriving from an unprecedented, and unexpected, C4′–C5′ bond scission.

The 3′,4′-didehydro-2′,3′-dideoxy-5′-aldehydes **1a**,**b** were synthesized as follows. The *O*3′-benzoyl derivatives **3a**[6] and **3b**[2b] were prepared according to known procedures. The C5′-aldehydes **4a**,**b** were obtained by oxidation of **3a**,**b** through a DMSO-based method using a modified Moffatt procedure.[7] The previously



Scheme 1 *Reagents and conditions*: i: NCS, 2-mercaptoethanol; ii: Mn-TMPyP/$KHSO_5$; iii: enzyme digestion followed by basic treatment at pH 12; iv: 90 °C.

unreported aldehyde **4a** was obtained in 80% yield as a 10 : 90 mixture of **4a** and its hydrated form **4a**′ by silica gel column chromatography of the reaction mixture. Similarly, the previously reported,[2b] but neither isolated nor characterized, C5′-aldehyde **4b** was obtained in 90% yield as an 80 : 20 mixture of **4b** and its hydrated form **4b**′.

The oxidation of **3b** was also performed using a modified Swern method, as reported by Matsuda.[2b] Work-up and flash column chromatography led to a 70 : 30 mixture of **4b** and **4b**′ mixture in 75% yield.

The subsequent hydro-benzoyloxy elimination leading to **1a**,**b** was performed under mildly basic conditions by treatment of a dichloromethane solution of the mixture of the appropriate aldehyde **4a**,**b** and its hydrated form **4a**′,**b**′ with 4 molar equivalents of TEA at room temperature. In both cases the crude mixture was chromatographed on a silica gel column to give the 3′,4′-didehydro-5′-aldehydes **1a**,**b** in excellent yield (85%).

The C5′-aldehyde **1b** could also be obtained in a one-pot process in good yield (65% isolated yield) by oxidation of the adenosine derivative **3b** under Swern conditions,[2b] followed by final treatment of the reaction mixture with 6 molar equivalents of TEA.

The hydro-benzoyloxy elimination reaction was found to be the crucial step. In fact, the yield of the aldehydes **1a**,**b** was strongly dependent on the reaction time of the C5′-aldehydes **4a**,**b** with TEA. Prolonged reaction time caused a progressive decrease of the yield of **1a**,**b** with concomitant formation of furfural **2**. The formation of **1a**,**b** and **2** *vs.* time was monitored by ^{1}H NMR. As

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† Electronic supplementary information (ESI) available: NMR and MS spectral data of compounds **1a**,**b**, **4a**,**b**, and **11a**,**b**. See DOI: 10.1039/b609995e

shown in Fig. 1, the disappearance of starting **4a**,**b** was complete within 10 min, leading to **1a**,**b**, and thereafter furfural **2** appeared at the expense of the aldehyde **1a**,**b** at a rate independent of the nature of the base unit.

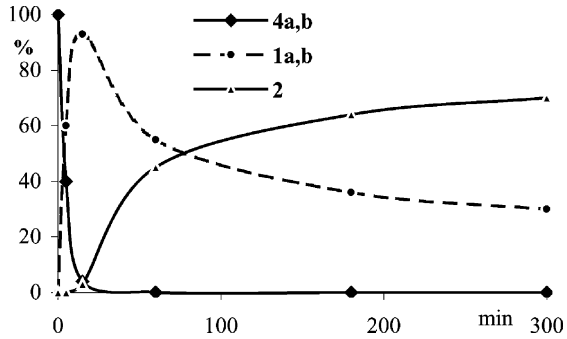
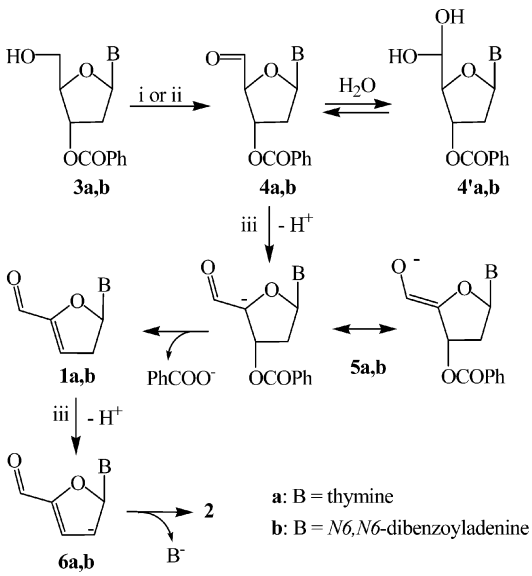


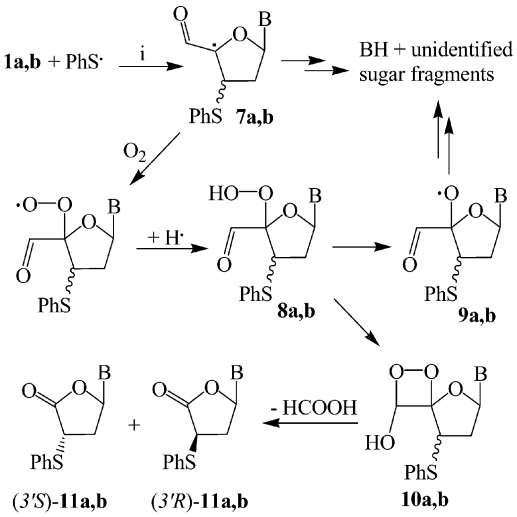
Fig. 1 Formation of products **1a**,**b** and **2** *vs.* time from reaction of C5′-aldehydes **4a**,**b** with TEA.

From a mechanstic standpoint, the formation of furfural **2** from aldehydes **4a**,**b** is easily explained through two consecutive base-catalysed elimination (E2cb) reactions. The formation of the carbanion/enolate **5a**,**b** followed by elimination of the benzoate ion leads to the aldehydes **1a**,**b**. Further elimination of the H2′ proton gives the resonance-stabilised ion **6a**,**b**, from which furfural **2** arises by loss of the base ion (Scheme 2). Interestingly, β-hydroacyloxy eliminations are well known under pyrolytic conditions, whereas they are unprecedented under basic conditions.



Scheme 2 *Reagents and conditions*: i: DCC, $Cl_2CHCOOH$, DMSO, ii: $(COCl)_2$, DMSO, TEA, CH_2Cl_2; iii: TEA, CH_2Cl_2.

In order to explore the behaviour of the 3′,4′-unsaturated C5′-aldehydes under radical stress we first reacted the aldehydes **1a**,**b** with benzenethiol under radical conditions. The reactions were carried out[8] with 1.2 molar equivalents of benzenethiol in a sealed tube at 80 °C in the presence of dioxygen and in the presence or absence of 0.2 molar equivalents of AIBN as initiator. Complete disappearance of **1a**,**b** occurred within 2 h. Silica gel column chromatography of the reaction mixture from **1a** furnished thymine as the major product, and a 50 : 50 3′*S*/3′*R* diastereomeric mixture of the lactone **11a** in 35% yield. Analogously, column chromatography of the reaction mixture from **1b** furnished adenine as major product, as well as a 70 : 30 3′*S*/3′*R* diastereomeric mixture of the lactone **11b** in 30% yield (Scheme 3). The absolute configuration of the diastereomeric lactones **11a** and **11b** was established on the basis of NOE experiments.



a: B = thymine; **b**: B = *N6,N6*-dibenzoyladenine

Scheme 3 *Reagents and conditions*: i: PhSH, AIBN or O_2, PhF, 82 °C.

The role played by dioxygen in the formation of **11a**,**b** was proved by reacting **1b** with benzenethiol in refluxing fluorobenzene in the presence of equimolar amounts of AIBN. Under these hypoxic conditions the lactone **11b** was formed in poor yields (<5%), with adenine as the main reaction product.

On the basis of these experimental results, we can propose the following mechanism for the formation of **11a**,**b**. Benzenesulfanyl radicals, produced by hydrogen atom abstraction either by cyanoisopropyl radicals or dioxygen, add to the C3′ carbon atom in a regioselective manner. In principle, the resulting radical **7a,b** could react in two different ways: i) hydrogen atom abstraction from benzenethiol; however, no hydro-sulfenylation adduct was detected, not even when the reaction of **1b** was repeated in the presence of a large excess (5 molar equivalents) of benzenehiol;[9] ii) trapping by dioxygen to give peroxyl radicals, from which the hydroperoxide **8a**,**b** can be formed by hydrogen atom abstraction from benzenethiol. Actually, this reaction was expected, since peroxyl radicals, and hydroperoxides, have been claimed as intermediates in DNA strand breakage promoted by formation of C4′-radicals under aerobic conditions and in the presence of thiol. It has been reported that subsequent O–O bond scission leads to oxy radicals, responsible for the formation of the observed decomposition products.[10]

We inferred that hydroperoxide **8a**,**b**, in addition to the O–O bond scission leading to the oxy radical **9a**,**b**, and then to the free base and unidentified sugar fragments,[11] can give the 3-hydroxy-1,2-dioxetane **10a**,**b** through nucleophilic addition to the α-carbonyl carbon atom.

1,2-Dioxetanes, an interesting class of chemiluminescent products, are known to undergo C–C and O–O bond scission leading

to two carbonyl compounds through a [2 + 2] *retro*-Diels–Alder reaction.[12] In this way, the lactones **11a**,**b** can be easily formed from **10a**,**b** with concomitant elimination of formic acid. To our knowledge this is the only example of a C4′–C5′ bond scission in nucleoside chemistry, even though intramolecular addition of a hydroperoxide to the carbonyl carbon atom of an α-formyl group, followed by fragmentation of the resulting 3-hydroxy-1,2-dioxetane, was reported some time ago.[13]

It is worth noting that the lactone **11b**, in contrast to **11a**, was formed with 70 : 30 stereoselectivity. This finding suggests that benzenesulfanyl radicals approach the C3′–C4′ double bond of **1a** nonstereoselectively, while they approach **1b** preferentially from the side opposite to the adenine base. This different behaviour might be due to the greater steric hindrance of the adenine unit with respect to the thymine.

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8 *Method A*: A 10.0 mM solution of the appropriate C5′-aldehyde **1a** or **1b** (0.30 mmol), benzenethiol (0.60 mmol, 0.06 mL) and AIBN (50 mg, 0.30 mmol) in fluorobenzene (30 mL) was kept in a sealed tube in a thermostated bath at 82 °C for 2 h. *Method B*: A 10.0 mM solution of the appropriate C5′-aldehyde **1a** or **1b** (0.30 mmol) and AIBN (50 mg, 0.30 mmol) in fluorobenzene (30 mL) was refluxed under argon for 10 min, and then benzenethiol (0.60 mmol, 0.06 mL) was added. The resulting solution was refluxed for 2 h. In both cases the solvent was removed under reduced pressure, the residue was analysed by HPLC-MS and ^{1}H NMR and then chromatographed on a silica gel column by gradual elution with hexane–ethyl acetate.

9 We might explain this finding by assuming that polar factors could play a determining role, since both sulfanyl radicals and radicals **7a**,**b** are expected to be electrophilic in character. In fact, it is generally assumed that the hydrogen atom transfer reaction is disfavored when the attacking radical and the displaced radical show the same philicity. See: B. P. Roberts and A. J. Steel, *J. Chem. Soc., Perkin Trans. 2*, 1994, 2155; B. P. Roberts, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2719.

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11 As far as the formation of the free base is concerned, we cannot exclude the possibility that the free base and the unidentified sugar fragments arise from hydroperoxides **8** through initial O–O bond scission in competition with the intramolecular nucleophilic addition to the α-carbonyl atom. However, the finding that adenine is the almost exclusive product when **1b** was reacted under hypoxic conditions suggested that another route is available for its formation. Since both aldehydes **1a** and **1b** were found to be thermally stable, we could infer that the free base might arise from radical **7a**,**b** through some decomposition reaction in competition with the trapping by dioxygen.

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