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Selective Oxidation of Uracil and Adenine Derivatives by the Catalytic System $MeReO_3/H_2O_2$ and $MeReO_3$/Urea Hydrogen Peroxide

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Abstract—Methyltrioxorhenium (MTO) is a useful and selective catalyst for the oxidation of uracil and purine derivatives using environmentally friendly hydrogen peroxide (H_2O_2, 30% water solution) or hydrogen peroxide/urea adduct (UHP) as oxygen atom donors. In particular, the MTO/UHP system constitutes a convenient combination to convert uracil derivatives into the biologically relevant 5,6-oxiranyl-5,6-dihydrouracils in good yields. Purine derivatives are selectively oxidized to the corresponding 1-oxides, the best yield being obtained in the presence of pyrazine-2-carboxylic acid (PCA). The oxidation of the plasmid pBG1 is also reported as the first example of double-strand DNA cleavage mediated by the catalytic system MTO/H_2O_2.

Because of possible biological implications, the oxidative transformations of nucleic acids and their components have been extensively studied and provide exciting and complex challenges for chemists and biochemists.[1] The focus of these studies is on chemical events originating both on the sugar moiety and on the purine and pyrimidine nucleobases.[2] These transformations may render the polynucleotide subject to cleavage[3] or may originate unnatural covalent cross-links with proteins.[4] To date, much attention has focused on the insertion of oxygen into the C–H σ bond of the deoxyribose moiety because this event can lead to direct strand scission.[5] On the other hand, purine and pyrimidine heterocycles are reactive targets for electrophilic oxidizing agents. These oxidants rarely lead to direct strand scission but perform regio-specific and site-specific (that is sequence-specific) modifications that allow the polymer cleavage or the formation of covalent bonds with proteins only in a second chemical or biochemical step.

In the oxidation of pyrimidine nucleobases, 5,6-oxiranyl-5,6-dihydro-uracil derivatives are of particular significance because they are supposed to be responsible for the formation of protein–nucleic acids cross-links.[4] All the synthetic attempts to prepare these derivatives failed producing undesired side-products, namely 1,2-diols, rearranged products, or overoxidation products.

As a part of a project aimed at obtaining new and selective modifications of nucleic acid components,[6] we have reported the first synthesis of 5,6-uracil and thymine epoxides by the use of dimethyldioxirane (DMDO) as a stoichiometric oxidant.[7] These epoxides were found to be highly reactive with nucleophiles and they were used as synthetic intermediates for the preparation of C-6 substituted-5,6-dihydrouracil derivatives, inhibitors of the Sendai virus.[8] This procedure is a new synthetic entry to C-6 substituted uracils that are not usually prepared in good yields using the methods reported for uracil derivatives.[9] Moreover, we have reported an improvement of the synthesis of 1,3-dimethyluracil epoxides,[10] and the first described stereoselective synthesis of 5,6-epoxides of thymidine nucleosides, by the use of manganese tetraphenylporphyrins as catalysts and DMDO as an oxidant.[11]

With the aim of finding a new and efficient procedure for the synthesis of uracil and thymine epoxides with the less expensive and environmentally friendly hydrogen peroxide (H_2O_2), our attention was next turned to the use of methyltrioxorhenium ($MeReO_3$, MTO) as a catalyst.[12] MTO forms monoperoxo $[MeRe(O)_2(O_2)]$ and bis(peroxo) $[MeRe(O)(O_2)_2]$ complexes with H_2O_2 as active catalytic forms. A detailed structure of $[MeRe(O)(O_2)_2]$ diglyme (diethyleneglycol dimethyl ether) adduct, in which two η^2-coordinated peroxo ligands are nearly coplanar with the Re–C bond, has been rigorously established.[13] Theoretical studies have been addressed to elucidate the reactivities and the geometrical features of the transition state of

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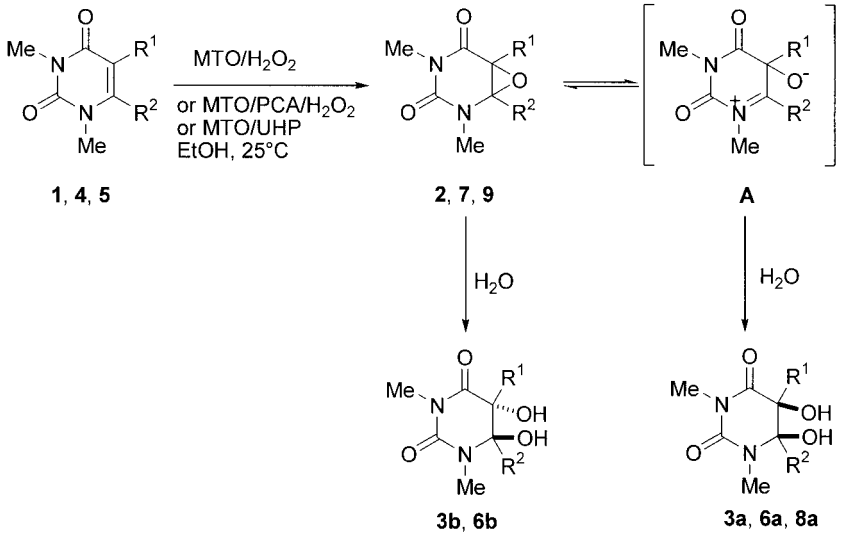
epoxidation of alkenes by these complexes.[14] The use of MTO as catalyst for the epoxidation of simple alkenes has been reported; the formation of *trans*-1,2-diols being a typical side reaction.[15] Additives such as amines, for example 4,4′-dimethyl-2,2′-bipyridine, quinine, cinchonine, pyridine or substituted pyridine derivatives suppress the formation of 1,2-diols,[16] but, in some cases, the catalyst turnover is also decreased significantly.[17] Moreover, the catalytic potential of the combination of MTO and urea/hydrogen peroxide adduct (UHP) in non-aqueous media has been reported,[18] as well as an interesting host–guest chemistry observed in the urea matrix in the oxidation of triorganosilanes.[19]

Herein, we present the MTO catalyzed oxidation of uracil derivatives to the corresponding 5,6-epoxides with H_2O_2 and UHP as an environmentally friendly oxygen source. Our results demonstrate that the MTO/UHP is to date the best oxidizing system to obtain uracil epoxides in high yields and selectivities. The transformation of adenine derivatives and adenine nucleosides into the corresponding 1-oxides as well as the oxidative cleavage of the plasmid pBG1 were also reported as representative models of MTO mediated purine and DNA transformations, respectively.

Results

The oxidations of uracil derivatives were performed on 1 mmol of the appropriate substrate with H_2O_2 (3.0 equiv.; 30% water solution) at 25°C in the presence of catalytic amount of MTO (0.05 equiv.) in different solvents (5 mL) depending on the specific reaction conditions. Treatment of 1,3-dimethyluracil **1** in EtOH afforded a mixture of two easily chromatographically separable products, 1,3-dimethyl-5,6-oxiranyl-5,6-dihydrouracil **2** as the main product, and *cis*-1,3-dimethyl-5,6-dihydroxy-5,6-dihydrouracil **3a** as by-product, in 45 and 22% yields, respectively (Scheme 1, Table 1, Entry 1). In the absence of MTO no reaction took place under otherwise identical reaction conditions. The corresponding *trans*-diol **3b** was not observed in the reaction mixture. In accord with data previously reported,[20] it is reasonable to suggest that the formation of the *cis*-diol **3a**, inconsistent with the S_N2 mechanism, may proceed through an α-stabilized nitrogen cationic intermediate (compound **A**, Scheme 1) in which a positive charge is localized on the N(1)-position of the uracil ring. Bond formation of C-6 with moisture would now be expected to proceed with energetically favorable cisoid (gauche) stereochemistry[21] to yield **3a**.[22] As shown earlier for vanadium[23] and MTO-catalyzed oxidations of



1, **2**, **3a-b**: R^1=R^2=H. **4**, **6a-b**, **7**: R^1=Me, R^2=H. **5**, **8a**, **9**: R^1=H, R^2=Me.

Scheme 1.

Table 1. Oxidation of uracil derivatives

Entry	Substrate	Product(s)	Oxidant	Conversion (%)[a]	Yield(s) (%)[a,b]
1	**1**	**2** (**3a**)	MTO/H_2O_2	75	45 (22)
2	**1**	**2** (**3a**)	MTO/UHP	89	82 (7)
3	**1**	**3a** (**3b**)	MTO/H_2O_2/PCA	94	75 (10)
4	**4**	**6a** (**6b**)	MTO/H_2O_2	75	57 (18)
5	**4**	**6a** (**6b**)	MTO/H_2O_2/PCA	95	83 (9)
6	**4**	**7** (**6a**)	MTO/UHP	87	89 (4)
7	**5**	**8a**	MTO/H_2O_2	78	63
8	**5**	**8a**	MTO/H_2O_2/PCA	93	89
9	**5**	**9** (**8a**)	MTO/UHP	87	87 (7)

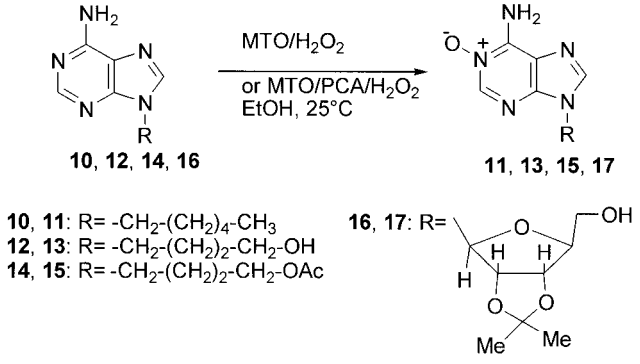
[a] Conversions and yields calculated after purification of the reaction mixture.
[b] Yield(s) based on converted substrate.

saturated and aromatic hydrocarbons,[24] the addition of pyrazine-2-carboxylic acid (PCA) to the reaction mixture increase the conversion of the reaction (the optimal ratio MTO:PCA being 1:4), but only diols **3a** and **3b** were obtained (Scheme 1, Table 1, Entry 3). Probably, the presence of PCA, that stabilizes the rhenium peroxides increasing their reactivity,[25] also catalyzes the ring opening of the initially formed epoxide. On the other hand, MTO oxidation of **1** using the UHP adduct as primary oxidant (3.0 equiv./mol) in a nonaqueous medium (CH_2Cl_2) afforded the epoxide **2** in good yield beside a low amount of **3a** (Scheme 1, Table 1, Entry 2).

Attention was next turned to study the oxidation of 1,3,5-trimethyl uracil (1, 3-dimethyl thymine) **4** and 1,3,6-trimethyl uracil **5** under previously reported experimental conditions. The oxidation of **4** with MTO/H_2O_2 in EtOH afforded a mixture of *cis*- and *trans*-1,3,5-trimethyl-5,6-dihydro-5, 6-dihydroxy uracils **6a** and **6b** in 57 and 18% yields, respectively (Scheme 1, Table 1, Entry 4). The yield of diol **6a** increased significantly when the oxidation of **4** was performed in the presence of a slight excess of PCA (Scheme 1, Table 1, Entry 5). In the latter two cases no traces of the 5,6-epoxide were found in the reaction mixture. The oxidation of **4** performed with MTO/UHP in CH_2Cl_2 gave the desired 1,3,5-trimethyl-5,6-dihydro-5, 6-oxiranyl uracil **7** in high yield (Scheme 1, Table 1, Entry 6). Treatment of **5** with MTO/H_2O_2 in EtOH both in presence or absence of PCA afforded the *cis*-1,3,6-trimethyl-5,6-dihydro-5, 6-dihydroxy uracil **8a** as the only recovered product in acceptable yields (Scheme 1, Table 1, Entries 7–8), as well as unreacted substrate. Finally, MTO oxidation of **5** performed with MTO/UHP in CH_2Cl_2 gave 1,3,6-trimethyl-5,6-dihydro-5,6-oxiranyl uracil **9** and *cis*-diol **8a** in 87 and 7% yields, respectively, (Scheme 1, Table 1, Entry 9). *cis*-Diols **3a**, **6a**, and **8a** were found positive to meta-periodate/benzidine analysis,[26] and their *cis*-stereochemistry was further confirmed by comparison with authentic samples prepared by reaction of compounds **1**, **4**, and **5**, with $KMnO_4$ or OsO_4 according to methods described in the literature.[27]

It is interesting to note that *cis*-diols **1**, **4**, and **5** were recovered as the main nucleophile ring opening products even if *trans*-1,2-diols are usually observed as side-products in MTO epoxidations of alkenes. Low amounts of the *trans*-diols were obtained only in the oxidation of compounds **1** and **4** in the presence of PCA. On the basis of these data it is possible that the reaction pathway involving the α-stabilized nitrogen cationic intermediate might be the main operative side-process for the MTO oxidation of uracil derivatives. The highest conversions of uracil derivatives were obtained in the presence of PCA (Table 1) but under these experimental conditions 5,6-epoxides were not isolated. The results above demonstrate unequivocally that the MTO/UHP combination constitutes a convenient and efficient oxidation system based on H_2O_2 to catalytically convert uracil derivatives into the corresponding 5,6-epoxides in acceptable to good yields.

The oxidation of 9-(*n*-hexan-1′-yl)adenine **10**, 9-(4′-hydroxybutan-1′-yl)adenine **12**, 9-(4′-acetoxybutan-1′-yl)adenine **14**, and 2′,3′-di-*O*-isopropylideneadenosine **16**, was than performed with the purpose to evaluate the selectivity and efficacy of MTO as catalyst in the H_2O_2 oxidation of purine derivatives. The reactions were performed on 1 mmol of the appropriate substrate with an excess of H_2O_2 (3.0 equiv.; 30% water solution) in EtOH or CH_2Cl_2 (5 mL) at 25°C in the presence of catalytic amount of MTO (0.05 equiv.). Treatment of compound **10** in EtOH afforded the 1-oxide derivative **11** in 83% yield, as the only recovered product (Scheme 2, Table 2, Entry 1). No traces of products of 8-hydroxylation were found in the reaction mixture, showing a different selectivity of the MTO/H_2O_2 system with respect to DMDO.[28] In a similar way, the oxidations of compounds **12**, **14**, and **16** gave the corresponding 1-oxide derivatives **13**, **15**, and **17**, in good yields (Scheme 2, Table 2, Entries 2–4). In the oxidation of the purine nucleoside **16** no C(5′)-OH oxidation or C(1′)-H oxygen atom insertion on the ribosyl moiety were observed in spite of the reported reactivity of alcohols and alkanes with MTO.[27] Noteworthy, caffeine (not shown), which is not oxidable at the 1-position, was not reactive under our experimental conditions even in the presence of a large excess of H_2O_2 and for a longer reaction time. The oxidation of compound **10** was also performed in EtOH in the



Scheme 2.

Table 2. Oxidation of adenine derivatives

Entry	Substrate	Product(s)	Oxidant	Conversion (%)[a]	Yield (%)[a,b]
1	**10**	**11**	MTO/H_2O_2	87	83
2	**12**	**13**	MTO/H_2O_2	81	85
3	**14**	**15**	MTO/H_2O_2	82	89
4	**16**	**17**	MTO/H_2O_2	78	93
5	**10**	**11**	MTO/H_2O_2/PCA	>98	>98
6	**10**	**11**	MTO/UHP	>98	>98

[a] Conversions and yields calculated after purification of the reaction mixture.
[b] Yield based on converted substrate.

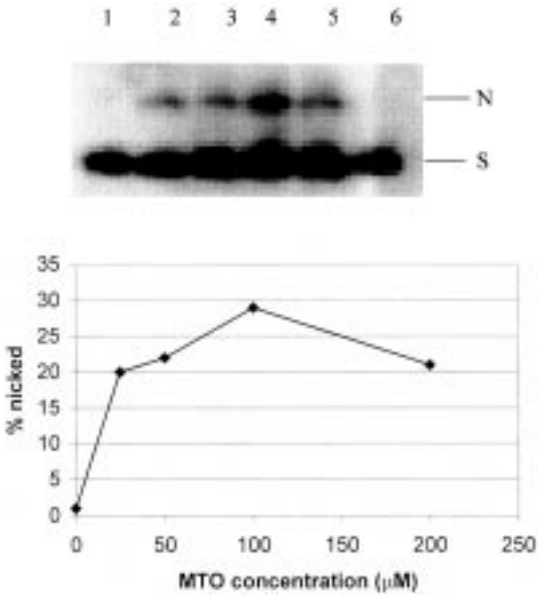


Figure 1. Oxidation assay on plasmid pBG1 (1.2 μg/μL), using methyltrioxorhenium (MTO) as catalyst and H_2O_2 as oxygen atom donor. Panel (A): DNA cleavage was detected by observing changes in the relative amounts of the two forms of plasmid pBG1, the nicked form (N) and the supercoiled form (S). Panel (B): Data were obtained from densitometric scans of photographs of 1.4% agarose gels. Five different concentrations of MTO were used: (1) 25 μM, (2) 50 μM, (3) 100 μM, (4) 200 μM, (5) 250 μM.

presence of PCA (the optimal ratio MTO:PCA being 1:4) to give the 1-oxide derivative **11** in quantitative yield (Scheme 2, Table 2, Entry 5). A similar result was obtained with the MTO/UHP combination in CH_2Cl_2 (Scheme 2, Table 2, Entry 6). The structures of 1-oxides **11**, **13**, **15**, and **17** were confirmed by comparison with authentic samples prepared by reaction of the corresponding substrates with a mixture of H_2O_2 and acetic acid according to methods described in literature.[29]

Adenine 1-oxides are important intermediates in the synthesis of 2-deuterioadenines, substituted or unsubstituted at the C-9 position, using the 'fission and reclosure' technology[30] and are easily transformed into the corresponding 4-aminoimidazole-5-carboxamidoximes by simple treatment with alkali.[31] Furthermore, the antiviral activity of adenine 1-oxide derivatives has been re-investigated and significant activity against vaccinia virus has been reported.[32] Oxidation of adenine derivatives to the corresponding 1-oxides can be achieved with different oxidants, however, methods depending on *meta*-chloroperbenzoic acid (*m*-CPBA) seem to be the most common. Such peracid-based procedures require more than a stoichiometric amount of oxidant and are less efficient for electron-deficient substrates. Furthermore, *m*-CPBA cannot be stored in pure form because of the explosion hazards. With MTO the synthesis of adenine 1-oxides is performed efficiently using H_2O_2 as environmental friendly oxidizing agent, and does not generate by-products other than water, so that workup is simple.

The oxidation of the plasmid pBG1 was then studied as an example of MTO mediated DNA trasformation. DNA cleavage was detected by observing changes in the relative amounts of the two forms of plasmid pBG1, the nicked form (N) and the supercoiled form (S), measured from densitometric scans of photographs of 1.4% agarose gels. The oxidations were carried out on 2 μl of plasmid (1.2 μg/μl) at 25°C with a small excess of H_2O_2 (30% water solution) using different concentrations of MTO. Data presented are for triplicate runs. Shown in Fig. 1 (Panels A and B), the amount of nicked DNA formed by the action of the MTO increases up to a certain concentration (about 100 μM) of the catalyst. Noteworthy, a further increase of the MTO concentration seems to determine a decrease of the nicked DNA. Negligible cleavage occurred under these conditions in the absence of MTO. Recently, Wang and coworkers reported a dentrimental effect of a high nitrogen/MTO mole ratio on the catalytic oxidation of alkenes by silica supported MTO complex of γ-(2,2′-dipyridyl)-aminopropylsiloxane. This effect was attributed to the further coordination of MTO to nucleophilic atoms adjacent to the reactive center.[33] Even if the silica-supported MTO system is rather different from the more simple homogeneous MTO catalyst, it is reasonable to hypothesize that the less efficient DNA cleavage observed at high MTO concentrations (>100 μM) might be due to a saturation process of the active sites on the polynucleotide. To the best of our knowledge, this is the first reported observation of double-strand DNA cleavage mediated by the MTO/H_2O_2 catalytic system.

In conclusion, MTO is a useful catalyst for the oxidation of purine and pyrimidine derivatives using H_2O_2 or UHP as primary oxidants. Different products were obtained in the oxidation of uracil derivatives depending on the reaction conditions. In particular, biologically relevant 5,6-epoxides were obtained in good yields when UHP was used as oxygen atom donor. *cis*-Diols were obtained as by-products in significant amounts in the absence of UHP. Low amounts of the *trans*-diols were obtained only in the oxidation of compounds **1** and **4** in the presence of PCA. Purine derivatives are selectively oxidized by MTO to the corresponding 1-oxides, the best yield being obtained in the presence of PCA. The reaction did not proceed in the absence of an oxidable nitrogen atom in the 1-position on the purine ring even in the presence of a large excess of H_2O_2 and for a longer reaction time. All reactions were performed efficiently using an environmentally friendly oxidizing agent, that does not generate by-products other than water. At concentration <100 μg MTO/H_2O_2 nicked effectively the plasmid pBG1 even if a further increase of the MTO concentration seems to determine a decrease of the efficiency of the cleavage. Further studies are in course in our laboratory to elucidate the reaction pathway and the sequence-selectivity of oxidation of double-stranded DNA with the MTO/H_2O_2 system.

Experimental

NMR spectra were recorded on a Bruker (200 MHz) spectrometer and are reported in δ values. Mass spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV. Elemental analyses were performed by a Carlo Erba 1106 analyzer. Infrared spectra were recorded on a Perkin Elmer 298 spectrophotometer using NaCl

plates. All solvents are ACS reagent grade and were redistilled and dried according to standard procedures. Chromatographic purifications were performed on columns packed with Merck silica gel 60, 230–400 mesh for flash technique. Thin layer chromatography was carried out using Merck platten Kieselgel 60 F254.

Starting compounds: 1,3-Dimethyluracil **1**, 1,3,5-trimethyluracil **4** and 1,3,6-trimethyluracil **5** were synthesized according to the procedure reported by Allen.[34] Adenine derivatives **10**, **12**, **14**, and **16** were synthesized according to the procedure reported by Holy.[35]

9-(*n*-Hexan-1′-yl)adenine (10). Colourless oil; [Found: C, 60.41; H, 7.76; N, 31.89. $C_{11}H_{17}N_5$ requires C, 60.25; H, 7.81; N, 31.94%]; ν_{max} (Nujol) 3300, 3150, 2890, 1680, 1600, 1490, 1400 cm^{-1}; δ_H (200 MHz, $CDCl_3$/CD_3OD):0.68 (3H, m, CH_3), 1.13 (8H, m, CH_2), 1.66 (2H, m, NCH_2), 7.70 (1H, s, CH), 8.09 (1H, s, CH); δ_c (200 MHz, $CDCl_3$/CD_3OD):13.48, 22.12, 25.97, 29.70, 30.89, 43.84, 118.76, 140.32, 149.27, 152.27, 153.37; *m/z* (EI) 219 (M^+).

9-(4′-Hydroxybutan-1′-yl)adenine (12). Colourless oil; [Found: C, 52.21; H, 6.32; N, 33.81. $C_9H_{13}N_5O$ requires C, 52.16; H, 6.32; N, 33.79%]; ν_{max} (Nujol) 3450, 3280, 3180, 2890, 1670, 1610, 1480 cm^{-1}; δ_H (200 MHz, $CDCl_3$/CD_3OD) 1.38 (2H, m, CH_2), 1.76 (2H, m, CH_2), 3.14 (2H, t, *J*=6.9 Hz, NCH_2), 4.05 (2H, t, *J*=6.9 Hz, OCH_2,), 7.77 (1H, s, CH), 8.04 (1H, s, CH); δ_c (200 MHz, $CDCl_3$/CD_3OD) 26.76, 29.07, 43.77, 61.22, 119.22, 140.46, 149.82, 152.49, 155.48; *m/z* (EI) 207 (M^+).

9-(4′-Acetoxybutan-1′-yl)adenine (14). Colourless oil; [Found: C, 53.07; H, 6.06; N, 28.13. $C_{11}H_{15}N_5O_2$ requires C, 53.0; H, 6.06; N, 28.09%]; ν_{max} (Nujol) 3300, 3150, 2890, 1770, 1680, 1600, 1480 cm^{-1}; δ_H (200 MHz, $CDCl_3$/CD_3OD) 1.60 (2H, m, CH_2), 1.84 (2H, m, CH_2), 1.87 (3H, s, CH_3), 4.03 (2H, t, *J*=6.0 Hz, NCH_2), 4.18 (2H, t, *J*=8.0 Hz, OCH_2), 6.38 (2H, br. s.,NH_2), 7.76 (1H, s, CH), 8.25 (1H, s, CH); δ_c (200 MHz, $CDCl_3$/CD_3OD) 20.77, 25.67, 26.65, 43.44, 63.38, 119.16, 140.17, 149.72 (C), 152.56, 155.37, 171.10; *m/z* (EI) 249 (M^+).

2′,3′-Isopropylidene adenosine (16). White solid, mp 221–222°C; [Found: C, 50.80; H, 5.57; N, 22.89. $C_{13}H_{17}N_5O_4$ requires C, 50.81; H, 5.57; N, 22.79%]; ν_{max} (Nujol) 3460, 3300, 3150, 2890, 1680, 1600, 1475 cm^{-1}; δ_H (200 MHz, $CDCl_3$/CD_3OD) 1.43 (3H, s, CH_3), 1.66 (3H, s, CH_3), 3.79 (2H, m, $H_{5'}$,$H_{5''}$), 4.40 (1H, m, $H_{4'}$), 5.10 (1H, m, $H_{3'}$), 5.32 (1H, m, $H_{2'}$), 6.20 (1H, d, *J*=4.0 Hz, $H_{1'}$), 8.25 (1H, s, CH), 8.38 (1H, s, CH); δ_c (200 MHz, $CDCl_3$/CD_3OD) 25.53, 27.60, 63.56, 82.96, 85.31, 88.10, 92.10, 92.99, 115.26, 141.74, 149.15, 153.63, 164.0; *m/z* (EI) 307 (M^+).

Oxidation of uracil and adenine derivatives. General procedure

Protocol (A): A solution of MTO (0.05 equiv.) in EtOH (5 mL) was mixed with H_2O_2 (30% water solution; 3.0 equiv.) and stirred under an argon atmosphere for 10 min at 25°C. The substrate (1 mmol) was added and the solution stirred for 18 h at 25°C, after which 5 mL of water was added. The mixture was extracted with CH_2Cl_2 (3×15 mL). The combined organic phases were washed with water (2×5 mL) and brine (5 mL), dried over Na_2SO_4 and then concentrated. The crude was purified by flash-chromatography (CH_2Cl_2–MeOH=from 9.9:0.1 to 9.0:1.0) to give the product in acceptable yields.

Protocol (B): A solution of MTO (0.05 equiv.) and pyrazine-2-carboxylic acid (PCA; 0.2 equiv.) in EtOH (5 mL) was mixed with H_2O_2 (30% water solution; 3.0 equiv.) and stirred under an argon atmosphere for 10 min at 25°C. The substrate (1 mmol) was added and the solution stirred for 18h at 25°C, after which 5 ml of water was added. The work-up and purification procedure are previously reported (see protocol A).

Protocol (C): A solution of MTO (0.05 equiv.) in CH_2Cl_2 (5 mL) was mixed with the urea/hydrogen peroxide adduct (282 mg, 3.0 mmol) and stirred under an argon atmosphere at 25°C for 10 min. The substrate (1.0 mmol) was added and the solution stirred for 18 h at 25°C. The work-up and purification procedure are previously reported (see protocol A).

Descriptions of ^1H NMR, ^{13}C NMR, IR, elemental analyses, and mass spectra of uracil derivatives **2**, **3a**–**b**, **6a**–**b**, **7**, **8a**, and **9** have been previously published by us and are fully reported in Refs. 8 and 10. Some relevant data for epoxides **2**, **7**, and **9**, are reported here only for the reader utility.

1,3-Dimethyl-5,6-oxiranyl-5,6-dihydro uracil (2). Colourless oil; δ_H (200 MHz, $CDCl_3$) 3.12 (3H, s, CH_3), 3.16 (3H, s, CH_3), 4.30 (H-5, d, *J*=3.0 Hz), 4.92 (H-6, d, *J*=3.0 Hz); δ_c (200 MHz, $CDCl_3$): 171.1, 153.2, 80.2, 68.7, 34.6, 27.5; *m/z* (EI) 156 (M^+).

1,3,5-Trimethyl-5,6-oxiranyl-5,6-dihydro uracil (7). Colourless oil; δ_H (200 MHz, $CDCl_3$) 1.37 (3H, s, CH_3), 3.0 (3H, s, CH_3), 3.08 (3H, s, CH_3), 4.44 (H-6, s, CH); δ_c (200 MHz, $CDCl_3$) 173.92, 152.34, 84.92, 71.84, 35.33, 28.07, 23.22; *m/z* (EI) 170 (M^+).

1,3,6-Trimethyl-5,6-oxiranyl-5,6-dihydro uracil (9). Colourless oil; δ_H (200 MHz, $CDCl_3$) 2.20 (3H, s, CH_3), 3.30 (3H, s, CH_3), 3.36 (3H, s, CH_3), 5.59 (H-6, s, CH); δ_c (200 MHz, $CDCl_3$): 152.62, 151.41, 101.22, 84.04, 33.02, 28.43, 17.59; *m/z* (EI) 170 (M^+).

9-(*n*-Hexan-1′-yl)adenine-1-oxide (11). Colourless oil; (161 mg, 87%); [Found: C, 56.20; H, 7.28; N, 29.66. $C_{11}H_{17}N_5O$ requires C, 56.15; H, 7.28; N, 29.76%]; ν_{max} (Nujol) 3290, 3140, 2890, 1680, 1600, 1490, 1400, 1280 cm^{-1}; δ_H (200 MHz, $CDCl_3$ /CD_3OD) 0.85 (3H, m, CH_3), 1.47 (8H, m, CH_2), 3.08 (2H, d, *J*=8.0 Hz, NCH_2,), 8.09 (1H, s, CH), 8.12 (1H, s, CH); δ_c (200 MHz, $CDCl_3$/CD_3OD) 14.20, 23.05, 27.66, 28.65, 32.25, 43.23, 117.83, 146.64, 152.68, 156.68, 159.90; *m/z* (EI) 235 (M^+).

9-(4′-Hydroxybutan-1′-yl)adenine-1-oxide (13). Colourless oil; [Found: C, 48.43; H, 5.86; N, 33.45. $C_9H_{13}N_5O_2$ requires C, 48.42; H, 5.86; N, 31.37%];%]; ν_{max} (Nujol) 3445, 3270, 3185, 2890, 1670, 1610, 1480, 1300 cm^{-1}; δ_H (200 MHz, $CDCl_3$/CD_3OD) 1.38 (2H, m, CH_2), 1.73 (2H, m, CH_2), 3.18 (2H, t, *J*=6.90 Hz, NCH_2,), 4.09 (2H, t,

J=6.90 Hz, OCH_2,), 7.79 (1H, s, CH), 8.21 (1H, s, CH); δ_c (200 MHz, $CDCl_3/CD_3OD$) 26.30, 29.27, 43.64, 61.31, 119.29, 140.58, 150.10, 153.22, 155.48; *m/z* (EI) 223 (M^+).

9-(4′-Acetoxybutan-1′-yl)adenine-1-oxide (15). Colourless oil; [Found: C, 49.85; H, 5.69; N, 26.43. $C_{11}H_{15}N_5O_3$ requires C, 49.80; H, 5.69; N, 26.40%]; ν_{max} (Nujol) 3300, 3160, 2885, 1770, 1680, 1600, 1480, 1270 cm^{-1}; δ_H (200 MHz, $CDCl_3/CD_3OD$) 1.32 (2H, m, CH_2), 1.35 (2H, m, CH_2), 1.38 (3H, s, CH_3), 3.45 (2H, t, J=6.0 Hz, NCH_2), 3.67 (2H, t, J=6.0 Hz, OCH_2), 7.63 (1H, s, CH), 7.92 (1H, s, CH); δ_c (200 MHz, $CDCl_3/CD_3OD$)18.92, 24.42, 25.28, 42.35, 62.39, 118.1, 142.57, 142.60, 143.13, 148.10, 149.15, 170.20; *m/z* (EI) 265 (M^+).

2′,3′-Isopropylidene adenosine-1-oxide (17). Colourless oil; [Found: C, 48.31; H, 5.29; N, 21.69. $C_{13}H_{17}N_5O_5$ requires C, 48.29; H, 5.29; N, 21.66%];%]; ν_{max} (Nujol) 3458, 3280, 3150, 2890, 1680, 1600, 1475, 1290 cm^{-1}; δ_H (200 MHz, $CDCl_3/CD_3OD$) 1.69 (3H, s, CH_3), 3.53 (2H, s, $H_{5'}$, $H_{5''}$), 4.17 (1H, s, $H_{4'}$), 4.81 (1H, s, $H_{3'}$), 5.06 (1H, s, $H_{2'}$), 5.98 (1H, s, $H_{1'}$), 8.25 (1H, s, CH), 8.36 (1H, s, CH); δ_c (200 MHz, $CDCl_3/CD_3OD$) 25.43, 27.41, 62.75, 82.10, 85.10, 87.70, 92.05, 92.20, 114.9, 143.5, 144.4, 150.1; *m/z* (EI) 323 (M^+).

Oxidation of the plasmid pBG1

The oxidation of the plasmid pBG1 was carried out on 2 μl of plasmid (1.2 μg/μL) with an excess of H_2O_2 in the presence of different amounts of MTO for 3 min at 25°C. The reaction was stopped with 20 μl of STOP mixture (5 mM EDTA and 0.2% SDS); 4 μL of 3 M Na–acetate pH=8. 360 μL of 90% ethanol were added, the samples were kept at −20°C for 30 min and then centrifuged for 30 min. The supernatant was removed, 100 μL of 70% ethanol were added and centrifuged again for 10 min. The supernatant was again removed and the samples dried in a speed vacuum centrifuge. The pellets were then resuspended in 20 μL of water, 5 μL of which were used for analysis on electrophoresis 1.4% agarose gel, after adding 3 μL of loading buffer (0.25% blue bromophenol, 0.25% xilencianol and 30% glycerol). DNA cleavage was detected by observing changes in the relative amounts of the two forms of plasmid pBG1, the nicked form (N) and the supercoiled form (S), measured from densitometric scans of photographs of 1.4% agarose gels. Data presented are for triplicate runs.

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