Pergamon

Tetrahedron Vol. 51, No. 12, pp. 3607-3616, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00076-3

Ozonation of Thionucleosides. A New Chemical Transformation of 4-Thiouracil and 6-Thioguanine Nucleosides to Cytosine and Adenosine Counterparts.

Raffaele Saladino,*[a] Claudia Crestini,[a] Francesca Occhionero,[b] and Rosario Nicoletti*[b]

[a]Dipartimento Agrochimico Agrobiologico Università degli studi diViterbo "La Tuscia", via San Camillo de Lellis, 01100 Viterbo, Italy.

[b]Dipartimento di Chimica Organica Università degli studi di Roma "La Sapienza", p.le Aldo Moro 5, 00185 Roma, Italy.

Abstract: The ozonation of 4-thiopyrimidine and 6-thiopurine nucleosides in presence of amines afforded selectively and under mild experimental conditions several cytidine and adenosine nucleosides. The same reaction carried out in presence of alcohols afforded O^4- or O^6-alkylated derivatives of the nucleosides.

Several studies have been made on the reactions of nucleic acids with ozone[1] in connection with damage to biological systems including chromosome aberrations in human cells[2] and mutational changes of *Escherichia coli*.[3] Although thiopyrimidine and thiopurine nucleosides occur as components of transfer ribonucleic acids (t-RNA),[4] little is known about the function of these thioderivatives. Favre[5] showed that the oxidative formation of a covalent cross-link between 4-thiouridine-8 and cytidine-13 in many t-RNAs from *Escherichia coli* play an important role in controlling the t-RNA conformation and in the bacterial growth inhibition.[6] On the other hand, one of the general method for the synthesis of cytidine nucleosides consists in the oxidative transformation of 4-thiouracil nucleosides[7]in the presence of ammonia derivatives. However these procedures require severe experimental conditions, and in some cases the ribose moiety and possibly other groups of the molecule, are altered under these conditions.[8] Thus, there is considerable interest in the study of the structural properties[9] and oxidative modifications of thionucleosides, especially with regard to the selective oxidation of the thioamide group.[10]

Recently, in the course of our studies about the chemistry of nucleic acids components,[11] we have employed the oxidation of substituted thiouracils[12] and pyrimidine-2-thione[13] with ozone in alcohols for site-specific introduction of alkoxyl groups at C-2 uracil and pyrimidine residues, respectively. By these first results we have observed that the behaviour of the thioamide moiety towards ozone is multifarious, depending on the nature of the substrate as well as on the actual reaction conditions. As an extension of these studies, we clarifie here the chemistry of thiopyrimidine and thiopurine nucleosides with ozone and we describe a new, selective and

efficient method for the synthesis of several pyrimidine and purine nucleosides such as cytidine and adenosine derivatives.

2', 3', 5'-Tri-O-acetyl-4-thiouridine[14] **1** was allowed to react in CH_2Cl_2 at 0°C with an ozone-oxygen stream introduced at a flow of 660 ml/min (27 mg of O_3/min). Under these conditions a rapid decolorization of the mixture was observed and the reaction was completed in only one minute to give 2', 3', 5'-tri-O-acetyl-uridine **2** in good yield (Scheme 1, Table, Entry 1).[15]

Entry	Substrate	Method[a]	Nucleophile	Product(s)	Reaction Time (min)	Yield(%)
1	1	A		2	1	69
2	1	B		2 (3)	5	53 (36)
3	1	B		3	10	66
4	1	C	MeOH	4a (2)	1	56 (28)
	1	C	EtOH	4b (2)	1	65 (18)
5	5	A		6	1	83
6	5	B		6 (7)	5	44 (35)
7	5	C	MeOH	8a (6)	2	47 (29)[b]
	5	C	EtOH	8b (6)	2	39 (35)[c]
8	1	D	NH_3[d]	9a	2	63
	1	D	$MeNH_2$	9b	2	58
	1	D	$(Et)_2NH$	9c	2	69
	1	D	Pyrrolidine	9d	2	65
9	10	A		11	1	83
10	10	C	MeOH	12a	1	66
	10	C	EtOH	12b	1	72
11	10	D	NH_3	13	5	92
12	14	D	NH_3	15	5	95

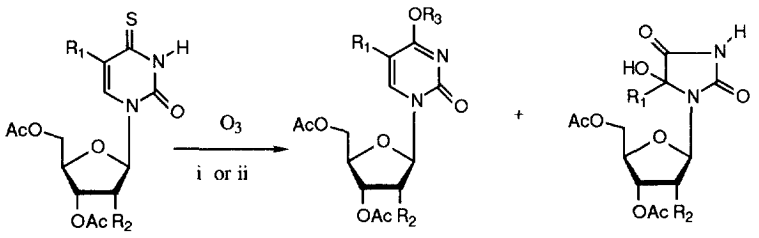
Table: Ozonation of thionucleosides. All ozonations were carried out using 1 mmol of substrate and an ozone-oxygen stream introduced at a flow of 660 mL/min (27 mg of O_3/min).[a] Method A: ozone, CH_2Cl_2, 0°C. Method B: ozone, CH_2Cl_2, 25°C. Method C: ozone,CH_2Cl_2-alcohol (1:1 v/v), 0°C. Method D: ozone, CH_2Cl_2, amine (1.8 equiv/mol), 0°C. [b] Compound **7** was recovered in 13% yield. [c] Compound **7** was recovered in 18% yield. [d] 2N methanol solution.

The ribose moiety of **1** may be degraded by ozone through a 1,3-dipolar insertion mechanism on the acetal carbon-hydrogen bond,[16] even if more slowly than the base portion,[1b] but we did not detect any trace of this degradation in our reaction mixture. Moreover, results of the ozonation of **1** appear to be dependent on experimental parameters such as reaction time and temperature; other parameters such as the concentration of the substrate appear to have little effect.

Thus, the ozonation of **1** performed in CH_2Cl_2 at 25°C with variable times gave a separable mixture of **2** and 2', 3', 5'-tri-O-acetyl-5-hydroxy-1-(β-D-ribosyl)hydantoin **3**. The ratio of the products was dependent on the reaction time, the latter 5-hydroxyhydantoin derivative **3**, probably formed by the ozonolysis of the $C_{5,6}$-

double bond of **2**,[17] being the main product after 10 minutes (Scheme 1, Table, Entries 2 and 3).[18] The same reaction carried out in CH_2Cl_2 at 0°C in presence of alcohols (methanol and ethanol) as nucleophiles[12] gave the corresponding O^4-alkyluridine derivatives **4a-b** as main products and **2** as by-product (Scheme 1, Table, Entry 4).[19]

A similar sequence of reactions was carried out with 3', 5'-di-O-acetyl-4-thiothymidine **5**. Compound **5** prepared starting from thymidine as described by Fox[14] reacted under the previously described conditions with ozone in CH_2Cl_2 at 0°C to give 2', 3'-di-O-acetyl-thymidine **6** as only recovered product for short reaction times (one minute), and a mixture of **6** and 3', 5'-di-O-acetyl-5-hydroxy-5-methyl-1(β-D-ribosyl)hydantoin **7** in CH_2Cl_2 at 25 °C for extended reaction times (Scheme 1, Table, Entries 5 and 6). The ozonation of **5** performed in CH_2Cl_2 at 0 °C in presence of alcohols gave the expected O^4-alkythymidine derivatives **8a-b**, but in lower yields than in the case of the previously described O^4-alkyluridine derivatives; furthermore, we observed the presence of compounds **6** and **7** as by-products even for short reaction times (Scheme 1, Table, Entry 7). These data suggest that the electrophilic ozone attack on the $C_{5,6}$-double bond of the uracil ring of **6** may be assisted, in the latter case, by the electron-releasing C-5 methyl group, in accord with the data reported by Matsui[17] on the effect of C-5 substituents on the ozonolysis of substituted uracils.



1, 5 **2, 6, 4a-b, 8a-b** **3, 7**

a: R_3=Me; b: R_3=Et

1, 3, 4a-b R_1=H, R_2=OAc. **2** R_1=R_3=H, R_2=OAc
5, 7, 8a-b R_1=Me, R_2=H. **6** R_1=Me, R_2=R_3=H

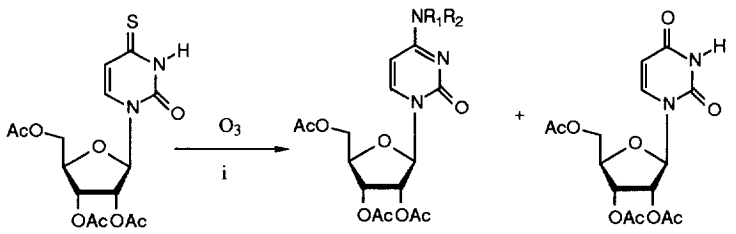
i: Ozone-oxygen flow of 660 ml/min (27 mg of O_3/min), CH_2Cl_2, O°C or 25°C. ii: Ozone-oxygen flow of 660 ml/min, CH_2Cl_2-alcohol (1:1 v/v), O°C.

Scheme 1

Attention was next turned to the use of 2', 3', 5'-tri-O-acetyl-4-thiouridine **1** as starting material for the synthesis of cytidine nucleosides. Amines are very reactive toward ozone,[20] and in some cases they appear to be more reactive[21] than olefins to give amine oxides as major products,[22] but to the best of our knowledge there are no reports in literature dealing with the behaviour of thioamide containing compounds toward ozone in presence of easily oxidable amines.

Compound **1** was then allowed to react with ozone in CH_2Cl_2 at 0°C in presence of a small excess (1.8 equiv./mol) of amine (ammonia, methylamine, diethylamine and pyrrolidine) to give the corresponding cytidine derivatives **9a-d** as main products in satisfactory yields (Scheme 2, Table, Entry 8). It is interesting to note that the transformation of the thioamide group present in **1** was very selective. In fact, no hydantoin derivative **3** and

only small amount of **2** were detected, showing that the possible desulfurization and/or the $C_{5,6}$-double bond ozonolysis were not competitive reactions under these experimental conditions.



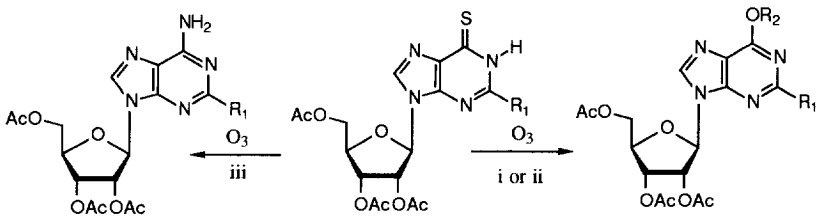
1 **9a-d** **2**

9a $R_1=R_2=H$. **9b** $R_1=H$, $R_2=Me$. **9c** $R_1=R_2=Et$.
9d $R_1=R_2= -CH_2-(CH_2)_2-CH_2-$

i: Ozone-oxygen flow of 660 ml/min (27 mg of O_3/min), CH_2Cl_2, amine (1.8 equiv./mol) O °C.

Scheme 2

By the use of the same general approach, 2-acetamido-6-thio-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)purine **10** was allowed to react with ozone to give N^2-acetyl-2', 3', 5'-tri-O-acetyl-guanosine **11** in CH_2Cl_2 at 0°C, O^6-alkyl-2-acetamido-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)purine derivatives **12a-b** in CH_2Cl_2 at 0°C in presence of alcohols (methanol and ethanol), and 2-acetamido-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)adenine **13** in CH_2Cl_2 at 0°C in presence of ammonia (2N solution in methanol, 1.8 equiv./mol) [Scheme 3, Table, Entries 9-11].



13, 15 **10, 14** **11, 12a-b**

a: R_2=Me, b: R_2=Et

10, 11, 12a-b, 13 R_1=NHAc. **14, 15** R_1=H

i: Ozone-oxygen flow of 660 ml/min (27 mg of O_3/min), CH_2Cl_2, O°C. ii: Ozone-oxygen flow of 660 ml/min, CH_2Cl_2-alcohol (1:1 v/v), O°C. iii: Ozone-oxygen flow of 660 ml/min, ammonia (1.8 equiv./mol; 2N MeOH solution), CH_2Cl_2, O°C.

Scheme 3

In a similar way, 6-thio-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)purine **14** reacted with ozone and ammonia (2N solution in methanol, 1.8 equiv./mol) in CH_2Cl_2 at 0°C to give 2', 3', 5'-tri-O-acetyl-adenosine **15** in satisfactory yield (Scheme 3, Table, Entry 12). The oxidative functionalization of the thioamide moiety was very selective also in the ozonation of thiopurine nucleosides and in all experiments we did not detect any trace of possible rearranged products, showed by Shapiro[23] in the ozonation of caffeine, formed through initial $C_{5,6}$-double bond ozonolysis. These results demonstrate that the ozonation of thiopyrimidine and thiopurine derivatives is a very efficient and selective procedure for the synthesis of several cytidine and adenosine derivatives.

Work is in progress in our laboratories to study the use of this procedure for the site-specific modifications of oligonucleotides containing thionucleosides.

Experimental

NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer and are reported in δ values. Infrared spectra were recorded on a Perkin Elmer 298 spectrophotometer using NaCl plates. Microanalyses were performed by C. Erba 1106 analyzer. Mass spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV. Melting points were obtained on a Reichert Kofler apparatus and are uncorrected. All solvents were 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

Commercially available 2-acetamido-6-thio-9-(2',3',5,-tri-O-acetyl-β-D-ribosyl)purine **10** (Aldrich, Co.) was used without further purification. 2',3',5'-tri-O-acetyl-4-thiouridine **1** and 3',5'-di-O-acetyl-4-thiothimidine **5** were synthesized according to the procedure reported by Fox;[14] 6-thio-9-(2',3',5'-tri-O-acetyl-β-D-ribosyl)purine **14** was synthesized according to the procedure reported by Lewis[24].

Ozonation of thiopyrimidine and thiopurine nucleosides **1**, **5**, **10** and **14**. General procedure.

2 mmol of substrate were dissolved in 15 ml of the appropriate solvent and placed in a 100 ml, three-necked flask equipped with a magnetic stirrer, a gas inlet tube, and a bubble flow meter. The substrate, dissolved in 5 ml of the appropriate solvent [CH_2Cl_2 or CH_2Cl_2-alcohol (1:1 v/v)], and when necessary in the presence of amines (1.8 equiv./mol), was allowed to react with an ozone-oxygen stream introduced at a flow of 660 ml/min (27 mg of O_3/min) until its disappearance in the solution (TLC, $CHCl_3/CH_3OH$=9.0:1.0 as eluent). The resulting mixture was purged with nitrogen for 30 min, transferred to a round bottomed flask and concentrated in vacuo. When necessary, the residue was purified by flash-chromatography using chloroform/methanol as eluent.

2', 3', 5'-Tri-O-acetyl-uridine **2**- (510 mg, 69%), m. p. 127-129 °C [lit.[25], 128-129 °C]. I.R. ($CHCl_3$) νmax: 3385 (NH), 1750 (C=O), 1680 (C=O) and 1636 cm^{-1} (C=C). δ_H [$CDCl_3$, 200 MHz] 2.10 (9H, s, CH_3), 4.35 (3H, m, H-4', 5', 5''), 5.30 (2H, m, H-2', 3'), 5.75 (1H, d, J 5.0 Hz, H-5), 6.05 (1H, m, H-1'), 7.35 (1H, d, J 5.0 Hz, H-6); m/z 370 (M^+, 12%).

2', 3', 5'-tri-O-Acetyl-5-hydroxy-1-(β-D-ribosyl)hydantoin **3**- (494 mg, 66%), oil. I.R. ($CHCl_3$) νmax: 1760 (C=O) and 1680 cm^{-1} (C=O). δ_H [$CDCl_3$, 200 MHz] 2.19 (9H, s, CH_3), 4.10 (3H, m, H-4', 5', 5''), 5.25 (2H, m, H- 2', 3'), 5.60 (2H, m, H-1', 5); δ_C [$CDCl_3$, 200 MHz] 22.57 (CH_3), 22.82 (CH_3), 22.94 (CH_3), 66.25 (CH_2), 73.69 (CH), 76.93 (CH), 81.74 (CH), 85.85 (CH), 156.90 (CH), 166.51 (C), 167.64 (C), 175.74 (C), 175.86 (C), 176.92 (C); m/z 374 (M^+, 12%).

4-Methoxy-1-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)uracil **4a**- (430 mg, 56%), oil. I.R. ($CHCl_3$) νmax: 1760 (C=O), 1680 (C=0), and 1630 cm^{-1} (C=C). δ_H [$CDCl_3$, 200 MHz] 2.09 (9H, s, CH_3), 3.93 (3H, s, CH_3), 4.34 (3H, m, H-4', 5', 5''), 5.31 (2H, m, H-2', 3'), 5.90 (1H, d, J 7.5 Hz, H-5), 6.14 (1H, d, J 4.2 Hz, H-1'), 7.64 (1H, d, J 7.5 Hz, H-6); m/z 384 (M^+, 12%).

4-Ethoxy-1-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)uracil **4b**- (517 mg, 65%), oil. I.R. ($CHCl_3$) ν_{max}: 1760 (C=O), 1680 (C=O), and 1630 cm^{-1} (C=C). δ_H [$CDCl_3$, 200 MHz] 1.32 (3H, m, CH_3), 2.09 (9H, s, CH_3), 4.37 (5H, m, CH_2, H-4', 5', 5''), 5.32 (2H, m, H-2', 3'), 5.90 (1H, d, J 7.2 Hz, H-5), 6.18 (1H, d, J 3.2 Hz, H-1'), 7.65 (1H, d, J 7.2 Hz, H-6) ; m/z 398 (M^+, 18%).

2', 3'-Di-O-acetyl-thymidine **6**- (541 mg, 83%), m. p. 126-128 °C [lit.[26], m. p. 126-128 °C]; I.R. ($CHCl_3$) νmax: 3380 (NH), 1730 (C=O), 1680 (C=0) and 1630 cm^{-1} (C=C). δ_H [$CDCl_3$, 200 MHz] 1.92 (3H, s, CH_3), 2.09 (6H, s, CH_3), 2.44 (2H, m, H-2', 2''), 4.33 (3H, m, H-4', 5', 5''), 5.19 (1H, m, H-3'), 6.25 (1H, m, H-1'), 7.24 (1H, s, H-6); m/z 326 (M^+, 12%).

3', 5'-Di-O-acetyl-5-hydroxy-5-methyl-1(β-D-ribosyl)hydantoin **7**- (231 mg, 35%), oil; I.R. ($CHCl_3$) ν_{max}: 1760 (C=O) and 1680 cm^{-1} (C=O). δ_H [$CDCl_3$, 200 MHz] 1.60 (3H, d, J 2 Hz, CH_3), 2.09 (6H, s, CH_3), 3.10 (2H, m, H-2', 2''), 4.20 (3H, m, H-4', 5', 5''), 5.20 (1H, m, H-3'), 5.55 (1H, q, $J_{1',2'}$ 6 Hz, $J_{1',2''}$ 8.5 Hz, H-1'); δ_C [$CDCl_3$, 200 MHz] 13.90 (CH_3), 20.69 (CH_3), 20.77 (CH_3), 22.59 (CH_2), 62.97 (CH_2), 63.74 (CH), 74.56 (CH), 81.74 (CH), 86.08 (CH), 128.97 (C), 131.03 (C), 160.03 (C), 170.78 (C), 171.81 (C); m/z 330 (M^+, 18%).

4-Methoxy-1-(2'-deoxy-3', 5'-di-O-acetyl-β-D-ribosyl)thymine **8a**- (320 mg, 47%), oil; I.R. ($CHCl_3$) νmax: 1760 (C=O), 1680 (C=0), and 1630 cm^{-1} (C=C). δ_H [$CDCl_3$, 200 MHz] 1.92 (3H, s, CH_3), 2.08 (6H, s,

CH_3), 2.20 (2H, m, H-2', 2"), 4.33 (3H, m, H-4', 5', 5"), 5.17 (1H, m, H-3'), 6.31 (1H, q, $J_{1',2'}$ 8.13 Hz, $J_{1',2"}$ 5.64 Hz, H-1'), 7.49 (1H, s, H-6); m/z 340 (M^+, 55%).

4-Ethoxy-1-(2'-deoxy-3', 5'-di-O-acetyl-β-D-ribosyl)thymine **8b**- (276 mg, 39%), oil; I.R. ($CHCl_3$) νmax: 1760 (C=O), 1680 (C=0), and 1630 cm^{-1} (C=C). δ_H [$CDCl_3$, 200 MHz] 1.39 (3H, t, J 7 Hz, CH_3) 1.93 (3H, s, CH_3), 2.11 (6H, s, CH_3), 2.68 (2H, m, H-2', 2"), 4.30 (3H, m, H-4', 5', 5"), 4.45 (2H, m, CH_2), 5.20 (1H, m, H-3'), 6.45 (1H, q, $J_{1',2'}$ 8.09 Hz, $J_{1',2"}$ 5.58 Hz, H-1'), 7.51 (1H, s, H-6); m/z 354 (M^+, 21%).

2', 3', 5'-Tri-O-acetyl-cytidine **9a**- (465 mg, 63%), m.p. 165-167 °C (from CH_3OH); I.R. ($CHCl_3$) ν_{max} 3400 (NH), 1740 (C=O) and 1650 cm^{-1} (C=C); δ_H [$CDCl_3$, 200 MHz] 2.05 (6H, s, CH_3), 2.07 (3H, s, CH_3), 4.27 (3H, m, H-4', 5', 5"), 5.39 (2H, m, H-2', 3'), 5.84 (1H, d, J 3.7 Hz, H-1'), 5.93 (1H, d, J 7.6 Hz, H-5), 7.35 (1H, d, J 7.6 Hz, H-6); δ_C [$CDCl_3$, 200 MHz] 20.26 (CH_3), 2048 (CH_3), 20.53 (CH_3), 62.97 (CH_2), 69.95 (CH), 73.40 (CH), 79.10 (CH), 89.97 (CH), 96.12 (CH), 141.15 (CH), 155.88 (C), 166.39 (C), 169.80 (C), 169.92 (C), 170.64 (C); m/z 369 (M^+, 27%).

2', 3', 5'-Tri-O-acetyl-N^4-methyl-cytidine **9b**- (444 mg, 58%), oil; I.R. ($CHCl_3$) ν_{max} 3400 (NH), 1740 (C=O) and 1645 cm^{-1} (C=C); δ_H [$CDCl_3$, 200 MHz] 2.13 (6H, s, CH_3), 2.15 (3H, s, CH_3), 2.64 (3H, s, NCH_3), 4.36 (3H, m, H-4', 5', 5"), 5.34 (2H, m, H-2', 3'), 5.80 (1H, d, J 8.10 Hz, H-5), 6.04 (1H, d, J 2.4 Hz, H-1'), 7.40 (1H, d, J 8.10 Hz, H-6); δ_C [$CDCl_3$, 200 MHz] 20.05 (CH_3), 20.08 (CH_3), 20.12 (CH_3), 26.0 (NCH_3), 62.87 (CH_2), 69.80 (CH), 73.7 (CH), 79.90 (CH), 88.80 (CH), 99.90 (CH), 141.60 (CH), 155.88 (C), 169.70 (C), 169.80 (C), 169.92 (C), 170.10 (C); m/z 383 (M^+, 13%).

2', 3', 5'-Tri-O-acetyl-N^4,N^4-di-ethyl-cytidine **9c**- (587 mg, 69%), oil; I.R. ($CHCl_3$) ν_{max} 3400 (NH), 1750 (C=O) and 1650 cm^{-1} (C=C); δ_H [$CDCl_3$, 200 MHz] 1.35 (6H, m, CH_3), 2.07 (6H, s, CH_3), 2.11 (3H, s, CH_3), 3.0 (4H, m, CH_2), 4.34 (3H, m, H-4', 5', 5"), 5.32 (2H, m, H-2', 3'), 5.91 (1H, d, J 7.3 Hz, H-5), 6.14 (1H, d, J 4.6 Hz, H-1'), 7.65 (1H, d, J 7.3 Hz, H-6); δ_C [$CDCl_3$, 200 MHz] 10.49 (CH_3), 20.0 (CH_3), 20.15 (CH_3), 20.31 (CH_3), 41.81 (CH_2), 54.38 (CH_2), 62.71 (CH), 69.73 (CH), 73.45 (CH), 79.58 (CH), 88.95 (CH), 96.75 (CH), 115.38 (C), 142.25 (C), 170.01 (C), 169.86 (C), 170.15 (C), 170.26 (C); m/z 425 (M^+, 67%).

2', 3', 5'-Tri-O-acetyl-4-(1-Pyrrolidinyl)cytidine **9d**- (550 mg, 65%), oil; I.R. ($CHCl_3$) ν_{max} 1750 (C=O) and 1630 cm^{-1} (C=C); δ_H [$CDCl_3$, 200 MHz] 1.80 (4H, m, CH_2), 2.04 (9H, s, CH_3), 3.45 (4H, m, CH_2), 4.0 (3H, m, H-4', 5', 5"), 4.35 (2H, m, H-2', 3'), 5.65 (1H, d, J 6.8 Hz, H-5), 5.80 (1H, d, J 3.5 Hz, H-1'), 7.65 (1H, d, J 6.8 Hz, H-6); δ_C [$CDCl_3$, 200 MHz] 18.80 (CH_2), 20.80 (CH_2), 24.48 (CH_3), 25.27 (CH_3),

28.52 (CH_3), 46.88 (CH_2), 46.95 (CH_2), 61.83 (CH_2), 63.66 (CH), 70.83 (CH), 82.41 (CH), 92.10 (CH), 92.95 (CH), 112.33 (CH), 136.12 (C), 139.58 (C), 156.72 (C), 156.89 (C), 157.0 (C); m/z 423 (M^+, 57%).

N^2-Acetyl- 2', 3', 5'-tri-O-acetyl-guanosine **11**- (749 mg, 83%), oil. I.R. ($CHCl_3$) ν_{max}: 1750 (C=O), 1680 (C=O), and 1630 cm^{-1}(C=C). δ_H [$CDCl_3$, 200 MHz] 2.05 (3H, s, CH_3), 2.15 (6H, s, CH_3), 2.50 (3H, s, CH_3), 4.45 (3H, m, H-4', 5', 5"), 5.70 (1H, m, H-3'), 5.95 (2H, m, H-1', 2'), 7.70 (1H, s, H-8), 9.35 (1H, broad singlet, NH); m/z 451 (M^+, 45%).

2-Acetamido-6-methoxy-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)purine **12a**- (614 mg, 66%), oil. I.R. ($CHCl_3$) ν_{max}: 1760 (C=O), 1680 (C=O), and 1630 cm^{-1}(C=C). δ_H [$CDCl_3$, 200 MHz] 2.06 (9H, s, CH_3), 2.52 (3H, s, CH_3), 4.11 (3H, s, CH_3), 4.42 (3H, m, H-4', 5', 5"), 5.68 (1H, m, H-3'), 5.90 (1H, m, H-2'), 6.05 (1H, d, J 4.5 Hz, H-1'), 7.90 (1H, s, H-8), 8.03 (1H, broad singlet, NH); m/z 465 (M^+, 33%).

2-Acetamido-6-ethoxy-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)purine **12b**- (690 mg, 72%), oil. I.R. ($CHCl_3$) ν_{max}: 1760 (C=O), 1680 (C=O), and 1630 cm^{-1}(C=C). δ_H [$CDCl_3$, 200 MHz] 1.47 (3H, t, J 7 Hz, CH_3), 2.07 (6H, s, CH_3), 2.15 (3H, s, CH_3), 2.51 (3H, s, CH_3), 4.20 (3H, m, H-4', 5', 5"), 4.57 (2H, q, J 7 Hz, CH_3), 5.65 (1H, m, H-3'), 5.89 (1H, m, H-2'), 6.04 (1H, d, J 4.53 Hz, H-1'), 7.95 (1H, s, H-8), 8.03 (1H, broad singlet, NH); m/z 479 (M^+, 57%).

2-Acetamido-9-(2', 3', 5'-tri-O-acetyl-β-D-ribosyl)adenine **13**- (828 mg, 92%), 148-150 °C (from EtOH); I.R. ($CHCl_3$) ν_{max} 3200 (NH) and 1750 cm^{-1} (C=O); δ_H [$CDCl_3$, 200 MHz] 1.95 (3H, s, CH_3), 2.10 (3H, s, CH_3), 2.15 (3H, s, CH_3), 2.35 (3H, s, CH_3), 4.50 (3H, m, H-4', 5', 5"), 5.95 (3H, m, H-1', 2', 3'), 6.65 (2H, b.s., NH_2), 7.75 (1H, s, H-8), 9.70 (1H, b.s., NH); m/z 450 (M^+, 22%).

2', 3', 5'-Tri-O-acetyl-adenosine **15**- (747 mg, 95%), m.p. 173-174 °C (from EtOH) [lit.[27], m.p. 174 °C]. I.R. ($CHCl_3$) ν_{max} 3200 (NH) and 1730 cm^{-1} (C=O); δ_H [$CDCl_3$, 200 MHz] 2.01 (6H, s, CH_3), 2.10 (3H, s, CH_3), 4.45 (3H, m, H-4', 5', 5"), 5.70 (1H, m, H-2'), 5.95 (1H, m, H-3'), 6.31 (1H, d, J 4.5 Hz, H-1'), 8.80 (1H, s, $H\text{-}2$), 8.98 ; m/z 393 (M^+, 22%).

Acknowledgements

We are grateful to Professor M. Botta (Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Italy) for useful discussions; financial support from Italian MURST is gratefully acknowledged.

References and Notes

1. (a) Ishizaki, K.; Shinriki, N.; Ueda, T. *Chem. Pharm. Bull.* **1984**, 34, 3601-3606. (b) Borek, C.; Ong, A.; Cleaver, J. E. *Toxicol. Ind. Health* **1988**, 4, 547-553. (c) Nompex, P.; Dore, M.; De Laat, J.; Bawa, M.; Legube, B. *Ozone: Sci. Eng.* **1991**, 13, 265-286.
2. (a) Gooch, P. C.; Creasia, D. A.; Brewen, J. G. *Environ. Res.* **1976**, 12, 188. (b) Guerrero, R. R.; Rounds, D. E.; Olson, R. S.; Hackney, J. D. *Environ. Res.* **1979**, 18, 336 and references cited therein.
3. (a) Hajnsdorf, E.; Dubreuil, Y. L.; Bezerra, R. Favre, A.; Expert-Bezancon, A. *Photochem. Photobiol.* **1987**, 45, 445-451. (b) Nagy-Gyorgy, P. S.; Chung, Y. S. *Mutagenesis* **1988**, 3, 257-261.
4. Keith, G.; Yusupov, M.; Brion, C.; Moras, D.; Kern, D. *Nucleic Acids Research* **1993**, 21, 4399.
5. Favre, A.; Yaniv, M.; Michelson, A. M. *Biochem. Biophys. Res. Comm.* **1969**, 37, 266-271.
6. Milder, S. J.; Kliger, D. S. *J. Am. Chem. Soc.* **1985**, 107, 7365-7373.
7. (a) Verheyden, J. P. H.; Wagner, D.; Moffat, J. G. *J. Org. Chem* **1971**, 2, 250-254 (c) Mitsuru, K.; Kiyoharu, M.; Masaaki, T. *Eur. Pat. Appl. EP* **1986**, 204, 264 (Chem. Abstr., 106, 214307m). (d) Saladino, R.; Crestini, C.; Bernini, R.; Frachey, G.; Mincione, E. *J. Chem. Soc., Perkin Trans. I* **1994**, 3053-3054.
8. Pleiss, M. G.; Cerutti, P. A. *Biochemistry* **1971**, 10, 3093-3099.
9. (a) Igarashi-Yamamoto, N.; Tajiri, A.; Hatano, S.; Shibuya, T.; Ueda, T. *Biochim. Biophys. Acta* **1981**, 656, 1. (b) Kung, P. P.; Jones, R. A. *Tetrahedron Lett.* **1991**, 32, 3919-3922.
10. (a) Hall, R. H. "The Modified Nucleosides in Nucleic Acids" Columbian University Press, Ney York and London **1971**, p. 347. (b) Koechetkov, N. K. and Budovskii, E. I. "Organic Chemistry of Nucleic Acids" E. I. Eds., Plenum Press, London and Ney York **1971**, part. B, p. 374. (c) Cantoni, G. L. and Davies, D. R. "Procedures in Nucleic Acids Research" Harper and Row Publishers, Ney York and London **1971**, Vol. 2, p. 643. (d) Field, L. in "Organic Chemistry of Sulfur", ed. Oae S., Plenum Press, New York, **1977**, chap. IV.
11. (a) Botta, M.; Saladino, R.; Lamba, D.; Nicoletti, R. *Tetrahedron* **1993**, 49, 6053-6070. (b) Saladino, R.; Lupattelli, P.; Mincione, E. *Tetrahedron Lett.* **1993**, 34, 6313-6316. (c) Botta, M.; Saladino, R.; Gentile, G.; Summa, V.; Nicoletti, R.; Verri, A.; Focher, F.; Spadari, S. *Tetrahedron* **1994**, 50, 3603-3618. (d) Botta, M.; Saladino, R.; Anzini, M.; Corelli, F. *Nucleosides & Nucleotides* **1994**, 13, 1769-1777.
12. Saladino, R.; Crestini, C.; Nicoletti, R. *Tetrahedron Lett.* **1993**, 34, 1631-1634.
13. Saladino R.; Crestini, C.; Mincione, E.; Nicoletti, R. *Tetrahedron* **1994**, 50, 3259-3272.
14. Fox, J. J.; Van Praag, D.; Wempen, I.; Doerr, L. I.; Cheong, L.; Knoll, J. E.; Eidinoff, M. L.; Bendich, A.; Brown, G. B. *J. Am. Chem. Soc.* **1959**, 81, 178.
15. An oxygen stream without ozone had no detectable effect on compound **1**.
16. Deslongchamps, P.; Atlani, D.; Fréhel, A.; Malaval, A.; Moreau, C. *Can. J. Chem.* **1974**, 52, 3651.
17. Matsui, M.; Kamiya, K.; Shibata, K.; Maramatsu, H.; Nakazumi, H. *J. Org. Chem.* **1990**, 55, 1396-1399.
18. To the best of our knownledge there is only one report on the ozonolysis of deprotected nucleosides in water (Masaki, M.; Inoue, T.; Shibata, K.; Maramatsu, H. *Bull. Chem. Soc. Jpn.* **1990**, 63, 296-297) but the authors obtained 5-hydroxyhydantoin derivatives in very low yields (9% and 16% yields for uridine and thymidine, respectively) probably due to undesidered side reactions on the unprotected ribose moiety.
19. In this case we did not detected any trace of the hydantoin derivative **3**.
20. (a) Razumowskii, S. D.; Zaikov, G. E. *Zh. Org. Chim.* **1972**, 8, 468. (b) Razumowskii, S. D.; Zaikov, G. E. *J. Org. Chem. USSR (Engl. Transl.)* **1972**, 8, 473. (c) Razumovskii, S. D.; Kefeli, A. A.; Zaikov, G. E. *Eur. Polym. J.* **1971**, 7, 275.

21. Kotani, T.; Tanaka, S.; Haneda, N.; Yamashita, S.; Furukawa, J. Nippon Gomu Kyokaishi 1963, 36, 795 (Chem. Abstr. 1964, 61, 5880).
22. Bailey, P. S.; Carter, T. P.; Southwick, L. M. *J. Org. Chem.* **1972**, 37, 2997.
23. Kolonko, K. J.; Shapiro, R. H.; Barkley, R. M.; Sievers, R. E. *J. Org. Chem.* **1979**, 44, 3769.
24. Lewis, L. R.; Robins, R. K.; Cheng, C. C. *J. Med. Chemistry* **1964**, 7, 200.
25. Brokes, J.; Beranek, J. *Coll. Czech. Chem. Comm.* **1974**, 39, 3100.
26. *Beil.* 24(3), 1299.
27. Bredereck, H. *Chem. Ber.* **1947**, 80, 401.

(*Received in UK* 22 *November* 1994; *revised* 24 *January* 1995; *accepted* 27 *January* 1995)