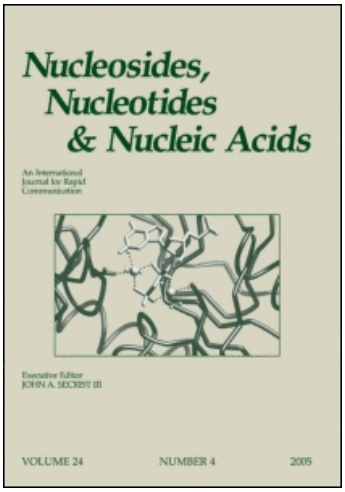

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CONVENIENT METHOD FOR THE PREPARATION OF 2′-DEOXYRIBOSYLUREA BY THYMIDINE OXIDATION AND NMR STUDY OF BOTH ANOMERS

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ABSTRACT

The synthesis of 2′-deoxyribosylurea by thymidine oxidation with potassium permanganate followed by alkaline hydrolysis of intermediates is described. The anomeric configuration of the resulting products was studied by NMR spectroscopy.

In the course of our studies on guanine modification by the oxidative nuclease, manganese(III)-bis-aqua-*meso*-tetrakis(4-*N*-methylpyridiniumyl)-porphyrin (Mn-TMPyP) associated to $KHSO_5$, we observed oxidized oligonucleotides with a mass corresponding to the transformation of one guanine into one urea residue after a 90 °C heating step of the damaged DNA[1]. We prepared 2′-deoxyribosylurea, as a model compound, to study the possibility of the detection and characterization of urea residues within oxidatively damaged DNA.

The acid-cata1yzed condensation of 2-deoxy-D-ribose with urea produced a mixture of α- and β-anomers of urea 2′-deoxyribofuranosides and pyranosides[2]. The degradation of 2′-deoxycytidine with hot formamide

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resulted in the formation of several products, including 2′-deoxyribosylurea, as recently described (yield not reported)[3]. According to a third published method[4], 5′-*O*-monomethoxytrityl thymidine (5′-MMTr-thymidine) was oxidized first with $KMnO_4$, and the resulting intermediate was oxidized with $Pb(OAc)_4$. Subsequent ammonolysis provided 5′-*O*-MMTr-2′-deoxyribosylurea (reported yield 33%). When we used lead tetraacetate for the oxidative cleavage of the products resulting of permanganate oxidation of 5′-MMTr-thymidine, a complex reaction mixture was obtained consisting 3 main components. The urea derivative content, after ammonia treatment, was $\sim 30\%$, whereas the main product ($\sim 60\%$) was supposed to be *N*-(2-deoxyribosyl)formamide[4–6]. The different products of the reaction mixture had close R_f values, making the product isolation difficult, and the yield of the desired urea derivative was low. In fact, this method would be more convenient for the preparation of the *N*-(2-deoxyribosyl)formamide, as already described[5].

It is known that the main product of permanganate oxidation of thymidine is thymidine glycol (5,6-dihydroxy-5,6-dihydrothymidine)[7–9] that can be further oxidized to fragmentation products, including urea derivatives[8]. In a similar way, thymidine glycol is also produced by radiolysis[11,12], by oxidation with osmium tetroxide[13] or Br_2[14]. It is relatively unstable and gives rise to ureas upon alkaline treatment[7,10,13,14]. The oxidative cleavage of thymidine glycol with $Pb(OAc)_4$ and the further ammonolysis of intermediates proceeds with formation of several products, thus lowering the yield of urea derivative[4,6]. We report here an efficient procedure for the preparative synthesis of 2′-deoxyribosylurea derivatives. Our approach was based on the fact that thymidine glycol hydrolysis at pH 12 produces 2′-deoxyribosylurea via alkali-catalyzed opening of oxidized thymine ring[13,14]. The proposed method consists of two steps: oxidation of 5′-protected thymidine with $KMnO_4$ followed by alkaline hydrolysis of intermediates (Fig. 1).

We carried out the permanganate oxidation of 5′-MMTr-thymidine **1** in acetonepyridine mixture, according to published procedure[4,5]. The loss of the aromatic character of the thymine base, due to the saturation of the C5-C6 bond, resulted in a sharp decrease of UV absorbance of products. The trityl group was therefore a convenient marker for TLC analyses. One main reaction product was observed by TLC, with some minor side products. Alkaline hydrolysis of the permanganate oxidation reaction intermediates was then studied under various reaction conditions. No hydrolysis was observed in pyridine-triethylamine-water (5/2/2; v/v/v) overnight at room temperature. We have found that in 0.1 M NaOH in aqueous ethanol the cleavage reaction was fast (less than 1 h to completion) and cleanly resulted in the corresponding 5′-MMTr-2′-deoxyribosylurea **2**. The alkaline hydrolysis of thymidine oxidation products afforded a simple and easily separable reaction mixture with the desired urea derivative as main component. The 5′-*O*-MMTr-2′-deoxyribosylurea **2** was isolated by silica gel chromatography

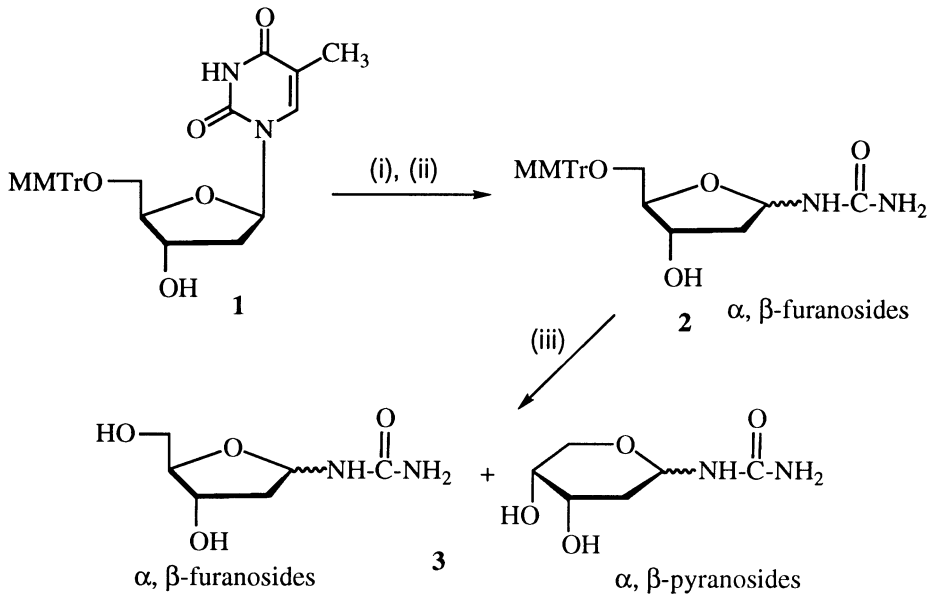

Figure 1. Reaction scheme for the preparation of 2′-deoxyribosylurea derivatives. (i) $KMnO_4$ (2 eq) in acetone-Py 3/1, 1.5 h, r.t.; (ii) 0.1 M NaOH in EtOH-water 3/1, 45 min, r.t., 57%; (iii) 2.5% $CHCl_2COOH$ in CH_2Cl_2, 5 min, 60%.

(yield 57%). Thymidine glycol was also efficiently hydrolyzed by anion-exchange resin Dowex 1-X8 (strongly basic, OH^--form), with the same rate as by NaOH solution. However, a significant amount of a side product was formed, with a higher R_f than that of **2**.

The ^1H NMR spectrum of 5′-*O*-MMTr-2′-deoxyribosylurea derivative **2** showed two families of resonances for the sugar protons, 3′-OH, NH and NH_2 in a ratio of 75/25, indicating the presence of two isomers. In accordance with published data[4,6], these two isomers were postulated to be the α- and *β*-anomers of the 5′-MMTr-2′-deoxyribosylurea derivative. The previously published preparation of 5′-MMTr-2′deoxyribosylurea by oxidation of 5′-MMTr-thymidine was reported to provide a mixture of the α- and *β*-anomers (rather than a *cis/trans* amide mixture[15]) in a 70/30 ratio, similar to that obtained in the present work[6]. Furthermore, the α-anomer had been identified as the major compound of this mixture of isomers, even when prepared from the *β*-anomer of 5′-MMTr-thymidine[6].

In the present study, the ^1H NMR spectrum and 1D ROESY of the 75/25 isomer mixture in DMSO-d_6 allowed us to characterize both anomers of the urea derivative **2**. Heating the sample at 55 °C in a mixture of D_2O/DMSO-d_6 (1/2, v/v) increased the percentage of the minor isomer in the mixture and facilitated its complete description (coupling constants). The ^1H NMR spectra of both anomers of 5′-protected 2′-deoxyribosylurea **2** are

described in details in the Experimental Section. Configurations were attributed from ROESY experiments. Since the H2′ and H2″ resonances of both isomers were observed at well-separated chemical shifts, it was possible to identify which isomer was in the α- or β-configuration by selective excitation experiments. For the major isomer of **2**, selective excitation of H2′ proton (2.34 ppm) gave a ROE with H1′ (5.70 ppm) and H3′ (4.15), and selective excitation of H2″ (1.72) showed a correlation with the anomeric NH (6.65) and 3′-OH (5.40) protons, indicating an α-configuration. For the minor isomer, by selective excitation of H2′ (1.85) ROES were detected with H3′ (4.15) and anomeric NH (6.67), whereas H2″ (1.95) was correlated to the H1′ (5.70) and 3′-OH (5.19) which is consistent with a β-configuration. These ROESY experiments confirmed therefore that the major compound was the α-anomer (Fig. 2).

^{13}C NMR spectrum of the mixture of isomers of urea derivative **2** was in agreement with this result. Assignment of resonances has been made using ^{1}H-^{13}C HMQC (Heteronuclear Multiple Quantum Coherence) sequence. Resonances of aromatic and urea carbons were found in the range 159–158 ppm (*C*=O and MeO-*C*), at 114.08 ppm (MeO-C-*C*) and 136–127 ppm (all other phenyl carbons). More clearly, all the signals of the sugar residue appeared as two sets of resonances, a minor one and a major one for each carbon. For example, C1′ signals were observed at 81.71 and 81.63 ppm for the α- and β-anomer, respectively.

After 5′-MMTr-2′-deoxyribosylurea **2** (α/β mixture, 75/25) was kept in solution either in frozen DMSO-d_6 at 4 °C, or at ambient temperature in D_2O/DMSO-d_6, for two weeks, the proton NMR spectrum showed that the ratio of α-/β-anomers changed to about 50/50. Pure anomers of 5′-MMTr-2′-deoxyribosylurea **2** cannot be obtained. Compound **2** is a 75/25 mixture of the two anomers immediately after the isolation, and its solution slowly equilibrates to a 50/50 anomeric mixture. It must be underlined however that further isomerization of **2** did not occur in solid state upon storage at −20 °C for 6 months.

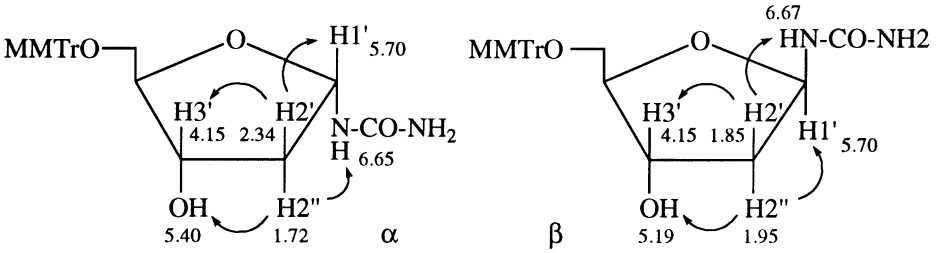

Figure 2. Overhauser effects (ROEs) observed for major (α) and minor (β) isomers of 2′-deoxyribosylurea derivative **2.**

The monomethoxytrityl group was removed from the 5′-protected deoxyribosylurea derivative **2** by acidic treatment leading to the formation of 2′-deoxyribosylurea **3**. The structure of this product was confirmed by proton NMR and mass spectroscopy. However, the ^{1}H NMR spectrum was rather complex, since 2′-deoxyribosylurea with free 5′-OH group undergoes isomerisation to pyranoside structure, consequently, α- and β-anomers of 2′-deoxy-D-ribofuranosyl- and -pyranosylurea were present in the equilibrium mixture[2,13]. Well-defmed signals of the urea residue NH_2 and NH groups were observed in the region 5.7–.5.9 (NH_2) and 6.5–6.8 ppm (NH). The NH_2 signals appeared as four singlets at 5.86, 5.78, 5.72 and 5.70 ppm in the ratio 14/48/10/28, respectively. The NH resonances were three doublets at 6.78, 6.63 and 6.58 ppm due to the coupling with H1′, in the ratio 48/28/24. This result is in agreement with the presence of four isomers, the two minor of them having NH resonances at the same chemical shift (6.58 ppm).

In conclusion, 2′-deoxyribosylurea derivatives, **2** and **3**, were obtained by a convenient method based on the alkaline hydrolysis of potassium permanganate oxidation intermediates of 5′-MMTr-thymidine, followed by acidic 5′-deprotection. However, 2′-deoxyribosylurea derivatives (**2** and **3**) always exist as a mixture of isomers[4,6]. The 5′-MMTr-2′-deoxyribosylurea **2** is a mixture of α- and β-anomers where α-anomer is the major component (75%) immediately after the preparation. In solution, these anomers tend to a 50/50 equilibrium. Interestingly, two preparation methods based on thymidine glycol intermediates give the similar unexpected α-anomer of urea derivative as major compound (this work and Ref. 4 and 6). This fact suggests that thymidine glycol may exist mainly as α-anomer under the used experimental conditions. Very few data on anomerization of thymidine glycol is available from the literature. Anomerization of 5,6-saturated pyrimidine nucleosides, including thymidine glycol, was reported under acidic conditions[16,17]. The presence of a hydroxyl group on the C6 of thymine probably promotes this process[16,18].

EXPERIMENTAL SECTION

All reagents and solvents were from commercial sources and used without further purification. Proton NMR spectra were recorded on Bruker AM-250 and AMX-400 spectrometers, and ^{13}C NMR spectrum on Bruker AMX-400, in DMSO-d_6 with tetramethylsilane as external standard. The 1D selective G ROESY experiments with a phase-alternated spin-lock were performed with a mixing time of 300 msec (RF field strength of 2.9 KHz). FAB mass spectra were obtained with Perkin-Elmer SCIEX API 100 spectrometer (DMF, *meta*-nitrobenzyl alcohol matrix, positive mode). TLC was performed on Kieselgel $60F_{254}$ plates (Merck) in the following systems:

chloroformmethanol 9/1 (A), chloroform-methanol 4/1 (B), acetonitrile-water 5/1 (C). Preparative column chromatography was carried out on Kieselgel 60 silica (Merck).

Nucleosides and other sugar-containing compounds were detected on TLC plates by spraying with a mixture anisaldehyde-HOAc-conc.H_2SO_4-EtOH 5/1/5/90 (v/v) with subsequent heating at 100 °C. Sugar derivatives are detected as blue spots[19].

Urea-containing species were detected with *p*-dimethylaminobenzaldehyde (DMABA, Ehrlich's reagent)[20]. TLC plates were sprayed with 1% ethanolic solution of this reagent, dried, kept in conc. HCl vapors for 30–40 sec and heated at 100 °C. Urea derivatives are revealed as yellow spots (this reagent also produces yellow-orange spots with MMTr group-containing compounds, by detritylation). To detect urea residues in trityl-containing products, the following procedure was used: after TLC run, the plate was kept in conc. HCl vapors for about 30–40 sec to cleave MMTr groups, then elution was performed with solvent A in which non-tritylated urea derivatives do not migrate. Then DMABA treatment revealed urea residues as usual.

The preparation of 5′-*O*-monomethoxytritylthymidine **1** was according to standard procedure[21] (yield 83%). R_f 0.63 (system A).

N-(5-*O*-monomethoxytrityl-2-deoxy-D-ribofuranosyl)-urea (2)

5′-*O*-monomethoxytritylthymidine **1** (514 mg, 1 mmol) was dissolved in 10 mL of acetone-pyridine mixture (3/1). Potassium permanganate (324 mg, 2.05 mmol) was added by portions during 20 min with stirring. The mixture was stirred for 1 hour until all starting nucleoside was consumed (TLC: main product R_f 0.50) (system A). Excess $KMnO_4$ was inactivated with 10% Na_2SO_3. The precipitate was filtered off and washed with acetone, the filtrate was then concentrated. Chloroform was added (50 mL), residual water was separated, organic phase was dried over Na_2SO_4 and evaporated under vacuum to give a light yellow oil. The oil was dissolved in 40 mL of ethanol-water (3/1) and a solution of NaOH (200 mg, 5 mmol) in the same solvent (10 mL) was added (final NaOH concentration 0.1 M). After 45 min, 10 mL of pyridine was added, and alkali solution was carefully neutralized with Amberlite IR-118 (Janssen) in pyridinium form (resin excess should be avoided). Resin was filtered, washed with ethanol, and filtrate was evaporated. Excess of pyridine was removed by co-evaporation with toluene. The urea derivative **2** was isolated by silica gel column chromatography. The column was washed with 3% methanol/chloroform and then with a gradient of 3–7% methanol in chloroform. Part of the recovered material had to be re-chromatographed in a gradient 3–6% of MeOH in chloroform. Fractions containing **2** were evaporated, the product was precipitated with dichloromethane/hexane, filtered, washed with hexane and dried under vacuum. Finally, 255 mg of white powder (yield 57%)

was obtained. R_f 0.42 (system A). The product was a mixture of α- and β-anomers of urea derivative **2** (α/β ratio 75/25). FAB MS: *m/z* 471 [$(M+Na)^+$], 460, 448 [M^+], 273 [$MMTr^+$], 154, 136.

The NMR study of **2** was performed on a α+β mixture. For clarity, proton spectra of both anomers are described separately.

^{1}H NMR for the α-anomer (250 MHz, DMSO-d_6): δ 7.33–7.53 (m, 12H, Ar), 7.03 (d, $J = 9.1$, 2H, Ar), 6.65 (d, ^3J(NH,H1′) = 10, 1H, NH), 5.90 (s, 2H, NH_2), 5.70 (ddd, ^3J(H1′,NH) = 10, ^3J(H1′,H2′) = 6.5, ^3J(H1′,H2″) = 4, 1H, H1′), 5.40 (d, ^3J(H3′,OH) = 4, 1H, OH), 4.14 (ddd, ^3J(H3′,H2′) = 6, ^3J(H3′,H2″) = ^3J(H3′,H4′) = 4, 1H, H3′), 4.02 (dd, ^3J(H4′,H5′ or H5″) = 8, ^3J(H4′,H3′) = 4, 1H, H4′), 3.86 (s, 3H, OCH_3), 3.03 (m, 2H, H5′ and H5″), 2.33 (ddd, ^2J(H2′,H2″) = 13, ^3J(H2′,H1′) = 6.5, ^3J(H2′,H3′) = 6, 1H, H2′), 1.72 (ddd, ^2J(H2″,H2′) = 13, ^3J(H2″,H1′) = ^3J(H2″,H3′) = 4, 1H, H2″).

^{1}H NMR for the β-anomer (250 MHz, DMSO-d_6): δ 7.33–7.53 (m, 12H, Ar), 7.03 (d, $J = 9.1$, 2H, Ar), 6.67 (d, ^3J(NH,H1′) = 10, 1H, NH), 5.74 (s, 2H, NH_2), 5.70 (ddd, ^3J(H1′,NH) = 10, ^3J(H1′,H2′) = 7, ^3J(H1′,H2″) = 6, 1H, H1′), 5.20 (d, ^3J(H3′,OH) = 4, 1H, OH), 4.14 (m, ^3J(H3′,H2′) = 6, ^3J(H3′,H2″) = 3.5, 1H, H3′), 3.86 (s, 3H, OCH_3), 3.80 (m, 1H, H4′), 3.03 (m, 2H, H5′ and H5″), 1.95 (ddd, ^2J(H2″,H2′) = 13, ^3J(H2″,H1′) = 6, ^3J(H2″,H3′) = 3.5, 1H, H2″), 1.83 (ddd, ^2J(H2′,H2″) = 13, ^3J(H2′,H1′) = 7, ^3J(H2′,H3′) = 6, 1H, H2′).

In DMSO-d_6 at 250 MHz, the H1′ resonances for α- and β-anomers of **2** were not well separated. In DMSO-d_6+D_2O, the signals of OH protons readily disappeared. The disappearance of the NH and NH_2 was slower, however, it was complete after 15 min at 55 °C making the H1′ patterns more clear. Furthermore, in DMSO-d_6/D_2O at 55 °C significant difference in the chemical shift values of H1′ protons was observed between the two anomers (5.62 and 5.58 ppm for the α- and β-anomer, respectively), whereas only minor changes were noted for other protons. Thus, H1′ signals allowed an accurate measurement of the coupling constants for both isomers. The same result was obtained in pure DMSO-d_6 at 25 °C at 400 MHz.

^{13}C NMR (100.62 MHz): δ 159.00, 158.45, 158.36, 145.27, 145.21, 135.95, 135.93, 130.91, 130.88, 128.85, 128.74, 127.68 and 114.08 (C-Phenyl and C=O), 86.44 (Ar_3C-, major isomer), 86.43 (Ar_3C-, minor) 84.59 (C4′, minor), 84.46 (C4′, major), 81.71 (C1′, major), 81.63 (C1′, minor), 72.34 (C3′, major), 72.24 (C3′, minor), 65.91 (C5′, minor), 65.25 (C5′, major), 55.89 (OCH_3), 40.50 (C2′, major), 40.45 (C2′, minor).

N-(2-deoxy-*D*-ribosyl)-urea (3)

The 5′-O-protected urea derivative **2** (25 mg, 0.055 mmol) was treated with 0.8 mL of 2.5% dichloroacetic acid in dichloromethane for 5 min. Diethyl ether was added (5 mL) and the precipitate was centrifuged and

washed three times with 1 mL of diethyl ether. The residue was dissolved in methanol (0.2 mL) (some heating was necessary), insoluble material was removed by centrifugation, and the product was precipitated with 2 mL of ether. Precipitate was centrifuged and washed with ether (3×1 mL). Then precipitation was repeated from 0.1 mL of methanol into 2 mL of ether. Product was collected by centrifugation, washed three times with 0.5 mL of ether and dried under vacuum. TLC showed the absence of any trityl-containing compounds, as well as any free urea. 6 mg of 2′-deoxyribosylurea **3** were obtained (yield 60%). R_f 0 (system A); 0.16 (B); 0.32, 0.35 (C, isomers). FAR MS: m/z 199 [$(M+Na)^+$], 177 [$(M+H)^+$], 154, 136, 117 [$(M-NHCONH_2)^+$]. ^{1}H NMR: δ 6.78 (d, $J = 9.5$, 0.48H, NH), 6.63 (d, $J = 9.6$, 0.28H, NH), 6.58 (d, $J = 10.0$, 0.24H, NH), 5.86 (s, 2×0.14H, NH_2), 5.78 (s, 2×0.48H, NH_2), 5.72 (s, 2×0.1H, NH_2), 5.70 (s, 2×0.28H, NH_2), 5.64–4.56 (m, 3H, H1′+OH; well-resolved OH doublets at 4.85 ($J = 5.4$), 4.70 ($J = 3.0$) and 4.56 ($J = 4.1$) ppm are observed), 3.4–4.2 (m, 4H, H3′, H4′, H5′, H5″), 1.6–2.0 (m, 2H, H2′, H2″).

ABBREVIATIONS

MMTr, monomethoxytrityl; DMABA, *p*-dimethylaminobenz-aldehyde; ROESY, rotating frame Overhauser enhancement spectroscopy.

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REFERENCES

1. Vialas, C.; Claparols, C.; Pratviel G.; B. Meunier J. Am. Chem. Soc. **2000**, *122*, 2157–2167.
2. Jensen, W.E.; Jones, A.S.; Ross, G.W.J. Chem. Soc. **1965**, 2463–2465.
3. Saladino, R.; Crestini, C.; Mincione, E.; Costanzo, G.; Di Mauro, E.; Negri, R. Bioorg. Med. Chem. **1997**, *5*, 2041–2048.
4. Guy, A.; Ahmad, S.; Téoule, R. Tetrahedron Lett. **1990**, *31*, 5745–5748.
5. Guy, A.; Duplaa, A.-M.; Ulrich, J.; Téoule, R. Nucleic Acids Res. **1991**, *19*, 5815–5820.
6. Baillet, S.; Behr, J.-P. Tetrahedron Lett. **1995**, *36*, 8981–8984.
7. Iida, S.; Hayatsu, H. Biochim. Biophys. Acta **1970**, *228*, 1–8.
8. Breimer, L.; Lindahl, T. Nucleic Acids Res. **1980**, *8*, 6199–6211.
9. Frenkel, K.; Goldstein, M.S.; Duker, N.J.; Teebor, G.W. Biochemistry **1981**, *20*, 750–754.

10. Iida, S.; Hayatsu, H. Biochim. Biophys. Acta **1971**, *240*, 370–375.
11. Cadet, J.; Teoule, R. Bull. Soc. Chim. **1975**, *3*–4, 885–890.
12. Téoule, R.; Bert, C.; Bonicel, A. Radiat. Res. **1977**, *72*, 190–200.
13. Ide, H.; Kow, Y.W.; Wallace, S.S. Nucleic Acids Res. **1985**, *13*, 8036–8052.
14. Ide, H.; Melamede, R.J.; Wallace, S.S. Biochemistry **1987**, *26*, 964–969.
15. Gervais, V.; Guy, A.; Téoule, R.; Fazakerley, G.V. Nucleic Acids Res. **1992**, *20*, 6455–6460.
16. Cadet, J.; Teoule, R. Tetrahedron Lett. **1972**, *31*, 3229–3232.
17. Cadet, J.; Teoule, R. Carbohydrate Res. **1973**, *29*, 345–361.
18. Vialas, C.; Pratviel, G.; Meyer, A.; Rayner, B.; Meunier, B.J. Chem. Soc., Perkin Trans. **1999**, *1*, 1201–1205.
19. Koster, H.; Schramm, G. Chem. Ber. **1969**, *102*, 3868–3876.
20. Hubener, H.J.; Bode, F.; Mollat, H.J.; Wehner, M.Z. Physiol. Chem. **1952**, *290*, 136–138.
21. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. J. Am. Chem. Soc. **1963**, *85*, 3821–3827.

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