Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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2-Deoxyribonolactone Lesions in X-irradiated DNA: Quantitative Determination by Catalytic Release of 5-Methylene-2-Furanone

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HPLC conditions. The following types of HPLC conditions were used in the study:

A. C18 μ-Bondapack 8 mm × 100 mm Radial Pak cartridge (Waters) washed with 40 mM ammonium acetate as a running phase and MeOH as an eluent (1-16.5% MeOH over 30 min, linear gradient, 2 mL/min, detection at 254 nm).

B. Gemini C18 10 mm × 250 mm semi-preparative column (Phenomenex) washed with 40 mM ammonium acetate and acetonitrile as an eluent (1-6% acetonitrile over 20 min, linear gradient, 4 mL/min, detection at 254 nm).

C. Luna C18 4.6 mm × 250 mm analytical column (Phenomenex) washed with 40 mM ammonium acetate and acetonitrile as an eluent (4-9.6% acetonitrile over 20 min, linear gradient, 1 mL/min, detection at 254 nm).

D. Gemini C18 4.6 mm × 250 mm analytical column (Phenomenex) washed with 40 mM ammonium acetate as a running phase, with acetonitrile as an eluent (1-8% acetonitrile over 20 min, non-linear gradient type 5 in the original Water's definition, 1 mL/min, detection at 254 nm).

E. Gemini C18 4.6 mm × 250 mm analytical column (Phenomenex) washed with 40 mM ammonium acetate as a running phase, with acetonitrile as an eluent (4-9.6% acetonitrile over 20 min, gradient type 5, 1 mL/min, detection at 254 nm).

F. Luna C18 4.6 mm × 250 mm analytical column (Phenomenex) washed with 40 mM ammonium acetate and acetonitrile as an eluent (1-9.6% acetonitrile over 20 min, linear gradient, 1 mL/min, detection at 254 nm).

G. Ion exchange chromatography on a Dionex DNAPac PA-100 column washed with 40 mM ammonium acetate/10% acetonitrile running phase and NaCl as an eluent (5-250 mM NaCl over 20 min, linear gradient, 1 mL/min, detection at 254 nm). These HPLC conditions were used for Figure 2D.

Purification of oligonucleotides. Commercially purchased DNA oligonucleotides (from Midland) were additionally purified by reversed-phase (RP) HPLC using HPLC conditions A or B.

Isolation of 4-7 from X-irradiated d(CGCG) films. Two fractions containing non-strand break d(CGCG) lesions (fraction 1 = 16-21 min, fraction 2 = 21-23.5 min; see Figure 1b) were collected by semi-preparative RP HPLC using HPLC conditions A from the aqueous solutions of the d(CGCG) films X-irradiated at 326 kGy and concentrated by spin-vacuuming to ~ 500 μL. At the second stage, the fractions 1 and 2 were run using analytical HPLC (see HPLC conditions C) and sub-fractions corresponding to individual peaks were collected (6 sub-fractions from fraction 1 and 8 sub-fractions from fraction 2 were collected, see Supporting Figure S1, A and B) and concentrated by spin-vacuuming to ~ 500 μL. Then each of the sub-fraction was tested for thermal decomposition by heating at 90^{o}C for 20 min in 50 mM sodium acetate buffer (pH 5.2) in the presence of 10 mM spermine tetrahydrochloride. The reaction mixtures were analyzed by RP HPLC (conditions D). The sub-fractions 1d, 2h, 1e, and 1b containing **4-7**, respectively, were selected based on their decomposition patterns which were in accordance with Scheme 2. Sub-fractions 1d and 1b containing **4** and **7**, respectively, were of sufficient purity as shown by their HPLC chromatograms (see Figure 3A, c and 3B, b) and did not require further purification, while sub-fractions 2h and 1e containing **5** and **6**, respectively, required additional purification by ion exchange chromatography using HPLC conditions G (see Supporting Figure S1, C and D). All isolated compounds

were characterized by thermal decomposition using HPLC conditions D and by molecular masses using MALDI-TOF. **4**: calculated -1079.19, found – 1080.56; **5** – calculated -1039.19, found -1040.54; **6** – calculated -1079.19, found – 1080.53; **7** - calculated – 1039.19, found – 1040.50.

Isolation of 8 from X-irradiated d(pCGCG) films. Three fractions containing non-strand break d(pCGCG) lesions (fraction 1=12-15.8 min, fraction 2 =15.8-16.7 min, fraction 3 = 16.7-17.8 min, see Supporting Figure S2, A) were collected by semi-preparative RP HPLC (conditions B) of an aqueous solution containing the X-irradiated (~300 kGy) d(pCGCG) film and concentrated by spin-vacuuming to ~ 500 μL. Then the fractions 1 – 3 were run through analytical HPLC (conditions F), and sub-fractions corresponding to individual peaks were collected (see Supporting Figure S2, B) and concentrated by spin-vacuuming to ~ 500 μL. Each of the sub-fractions were tested for thermal catalytic decomposition as described in the previous section for isolation of **4**-**7** from d(CCGG). The candidate fraction, 2a, was additionally purified by ion exchange chromatography using HPLC conditions G**,** and the isolated product (see Supporting Figure S2, C) was characterized as **8** by thermal decomposition using HPLC conditions D and by its molecular mass by using MALDI-TOF (calculated – 1159.16, found – 1160.28).

Identity of **3** and the corresponding phosphate fragments released upon decomposition of dL-containing tetramers was confirmed by coinjection with the reference compounds. Corresponding HPLC peaks for d(CGCp), d(pGCG), d(CGp), and d(pCG) were collected and additionally characterized by MALDI-TOF: d(pGCG), calculated – 965.16, found – 966.04; d(CGCp), calculated – 925.16, found – 926.33; d(pCG), calculated – 636.11, found – 637.09; d(CGp), calculated – 636.11, found – 637.29. The monomers d(Cp) and d(pG) were not characterized by MALDI-TOF since

their molecular masses are too low for the MALDI-TOF analysis. The peak for **3** isolated upon decomposition of **5**, **6**, or **8** was collected by using analytical HPLC, concentrated by spin-vacuuming and then extracted with diethyl ether for GC-MS analysis (HP 5980/5970 GC-MS with an J&W DB-5 (15m x 0.2mm x 0.2mm) column); calculated molecular mass - 96.02, found - 96.0.

Supporting Figures

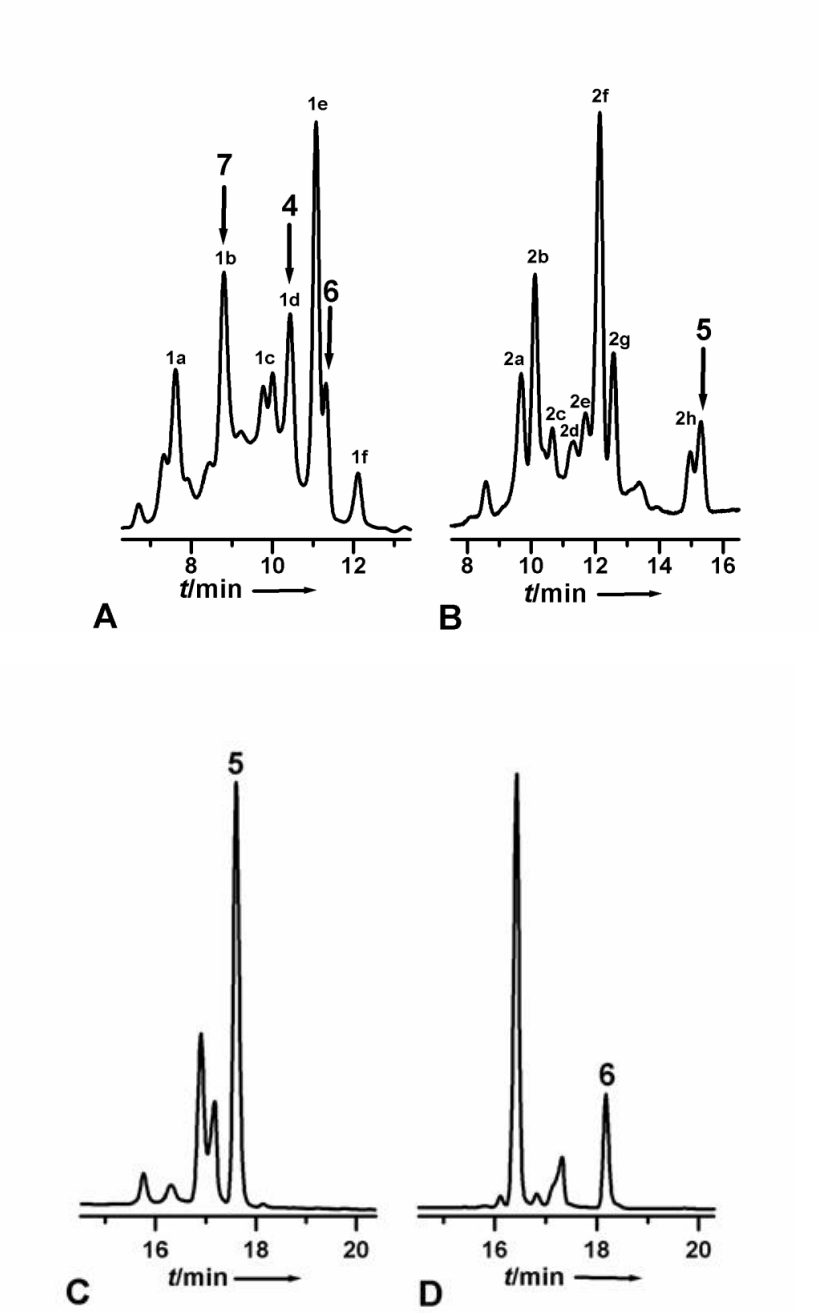

Fig. S1. Isolation of **4**-**7** from the X-irradiated d(CGCG) films. Panels **A** and **B:** fractions 1 in **A** and 2 in **B** are the second stage of isolation of **4**-**7** by analytical HPLC, using HPLC conditions C. Each sub-fraction 1a-1f and 2a-2h is labeled. Panels **C** and **D** show the third stage of isolation of **5** (in **C**) and **6** (in **D**) by ion exchange chromatography using HPLC conditions G. Positions of the peaks corresponding to **4**-**7** are shown in each chromatogram.

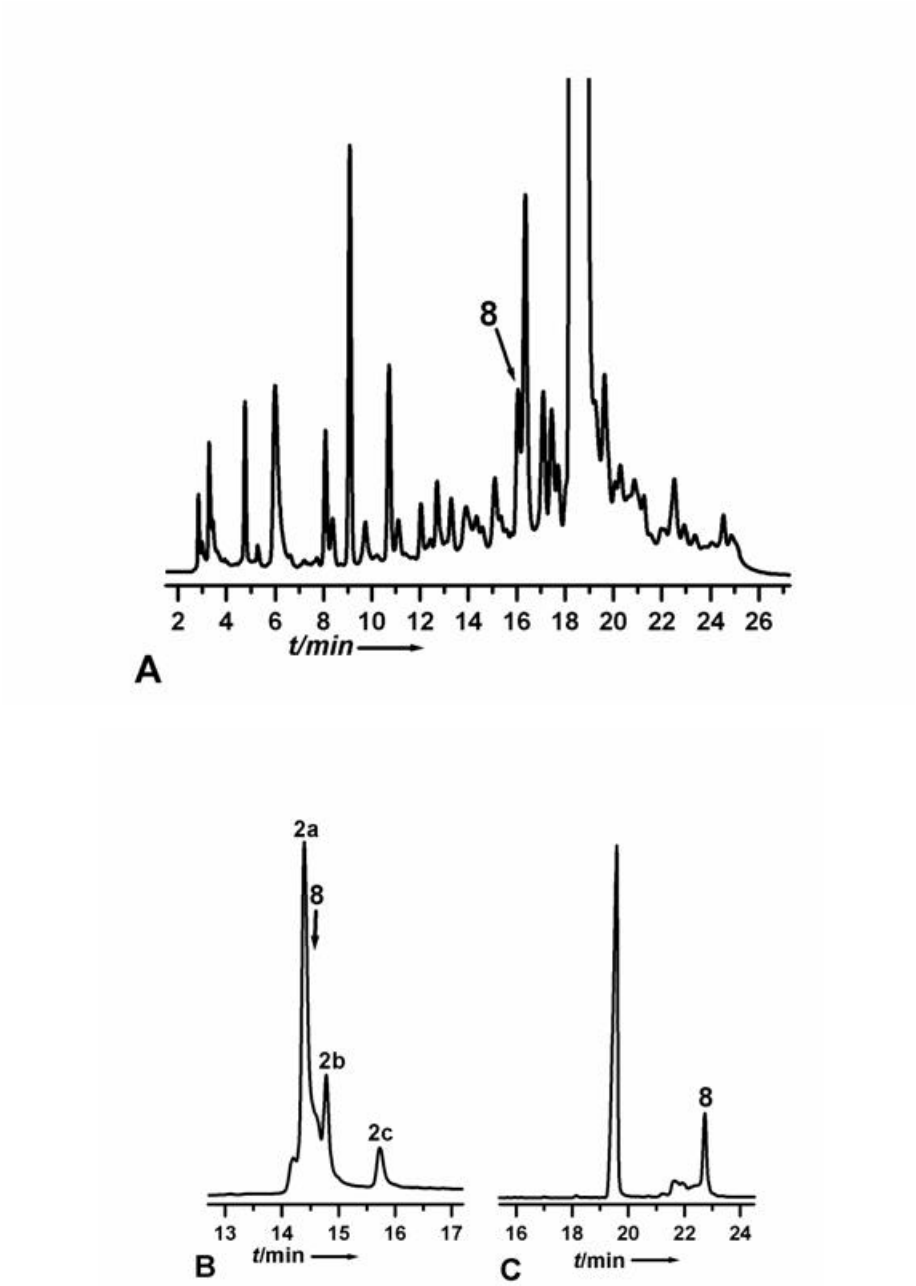

Fig. S2. Isolation of **8** from the X-irradiated d(pCGCG) films. **A**) the first stage of isolation by semi-preparative HPLC, using HPLC conditions B. **B**) the second stage of isolation by analytical HPLC, using HPLC conditions C from fraction 2. Each sub-fraction 2a-2c is labeled. **C**) the third stage of isolation by ion exchange chromatography, using HPLC conditions G. Positions of the peaks corresponding to **8** are shown in each chromatogram.