Supplementary Information

Alkylation of Complementary Ribonucleotides in Nanoreactors

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Dynamic Light Scattering measurements.

Determination of the particle size and polydispersity index was performed through Dynamic Light Scattering (DLS) technique using a Malvern Zetasizer Nano-ZS, consisting of an Avalanche photodiode (APD) detector and a 4mW He-Ne laser (λ = 633 nm). The scattering cell was thermostated at 298.0 ± 0.1 K with a Peltier element. All the experiments were performed at least in triplicate, at the scattering angle of 90°; other settings were: solvent (water) viscosity 0.887 mPa·s and solvent refractive index 1.33. For each experiment, fresh 100 µl aliquots were withdrawn from each sample; diluted 1:20 (v/v) with deionized water to avoid artifacts due to multiple scattering effects and left to equilibrate for 1-2 h at rest, at T=298 K. Then, before running DLS measurements, the sample solutions were filtered directly into dust-free light-scattering disposable cells through Millipore sterile membranes (cut-off 400 nm). The apparent hydrodynamic diameter D_{app} is obtained via the Stokes-Einstein equation:[1]

$$D_{app} = \frac{k_B T}{3\pi\eta D} \qquad 1)$$

where k_B is the Boltzmann constant, η is the solvent viscosity at temperature T and D is an average apparent diffusion coefficient of the particle in solution, assuming a spherical shape. Z-average diffusion coefficient $\overline{D}$ is defined according to $\overline{D} = \sum G_i D_i$ where G_i is the fraction of light scattered by the ith species assuming a spherical shape with a diffusion coefficient D_i.[2] Z-average hydrodynamic particle diameters (<Z>-diameters) and intensity-weighted size distributions were calculated by the cumulant[3] and CONTIN methods,[4] respectively, using the program provided by the manufacturer (DTS Version 6.01). The normalized autocorrelation function $G(\tau)$, was obtained from the average of 30 or more acquisitions. The intensity time-correlation function was obtained by averaging over a period of acquisition time, usually ~10 s, as a function of τ. The diffusion coefficient in equation 1 was evaluated by fitting the equation [**Error! Bookmark not defined.**]:

$$G(\tau) = 1 + \mathrm{A}\, exp\left(-2\mathrm{q}^2 \overline{D}\tau\right) \qquad 2)$$

to the measured correlation functions, treating $\overline{D}$ and the pre-exponential factor A as adjustable parameters. τ is the delay time and scattering vector q at angle θ is $\mathrm{q} = \frac{4\pi \mathrm{n}}{\lambda} sin\left(\frac{\theta}{2}\right)$, where n is the refractive index of pure water and λ the laser wavelength in vacuum. A polynomial fit to the logarithm of equation 2 leads to the size (the first cumulant) and its deviation (the second cumulant), where the polydispersity index PDI corresponds to the square of the normalized standard deviation of an underlying Gaussian size distribution. For samples with bimodal size distributions, the correlation data were force fitted to double-exponential relaxations with acceptable statistical accuracy.

Electrophoretic mobility measurements.

ζ potential measurements were performed with a Malvern Zetasizer Nano-ZS using the technique of Laser Doppler Velocimetry (LDV). In this technique, a voltage gradient is applied across a pair of electrodes at either end of the cell containing the particle dispersion. Charged particles are attracted to the oppositely charged electrode and their velocity can be measured and expressed in unit field strength as an electrophoretic mobility U_E. Before each experiment, fresh 100 μl aliquots were withdrawn from each sample, diluted 1:20 (v/v) with deionized water and left to equilibrate for 1-2 h at rest, at room temperature. The diluted samples were filled into disposable cell and the ζ -

potential was determined in triplicate for each type of particle systems. In order to check the Malvern device, a carboxy-modified polystyrene latex standard sample, with $\zeta = -68(\pm 6.8)$ mV, was used before each set of determinations as a control. The Malvern computer program was used to calculate the ζ potential automatically from the measured U_E by using the Henry equation:[5]

$$U_E = \frac{2\varepsilon\zeta}{3\eta} f(\kappa R) \qquad 3)$$

where ε is the dielectric constant, η is the viscosity of media, R is the hydrodynamic radius of particle and κR the ratio of particle size to Debye length. For the conversion of mobility into ζ potential the Smoluchowski factor $f(\kappa R) = 1.5$ was used, which in turn is valid under the limit $\kappa R \gg 1$. The instrument automatically optimizes the signal intensity within the range of 200–300 kcps, to keep the ratio of sample-to-reference count rates to be a constant, approximately. Effective voltage gradient was in the range 40-140 mV/mm. The pH measured in the aqueous dispersions did not vary appreciably within the experimental reaction time, falling in the neutral-alkaline range (pH ≈ 7-9). Accuracy of measured data was within ±5%.

HPLC-ESI-MS instrumentation and operating conditions.

The HPLC system adopted in the present work consisted of a Dionex (Sunnyvale, CA) P680 pumping system connected to a Supelcosil LC-18-DB column (250 × 2.1 mm id, particle diameter 5 µm, Supelco, Bellefonte, PA). A LCQ Classic ion trap mass spectrometer (ThermoElectron, San Jose, CA), equipped with a ESI interface, was connected to the outlet of the chromatographic column, thus enabling a simultaneous UV and MS detection. The divert/inject valve embedded in the LCQ mass spectrometer was used as loop injector (injection volume: 20 µL).

Chromatographic runs were performed at a flow rate of 0.2 mL/min by gradient elution with A) water and B) methanol, both containing 2.5 mM ammonium acetate. The following gradient elution program was used (all percentages are expressed as v/v values): linear from 10% to 90% solvent B for 30 min; isocratic at 90% B for 5 min; return to initial mobile phase composition in 5 minutes and column reconditioning for 20 minutes.

The LCQ spectrometer, controlled by the Xcalibur (ThermoElectron, San Jose, CA) software, was usually operated in the *negative ion* mode. The main electrospray interface and ion optics parameters were the following: spray voltage: -4.5 kV; sheath gas (nitrogen) flow rate: 0.9 L min^{-1}; capillary voltage: -45 V; heated capillary temperature: 190°C; tube lens offset voltage: -5 V; octapole 1 offset: 2.0 V; octapole 2 offset: 7.0 V; lens voltage: 35 V; octapole RF amplitude: 400.0 V; trap DC offset: 10.0 V.

Different MS events were set for each chromatographic run, namely a MS survey scan in the 50-1500 m/z range and a series of MS/MS full scan acquisitions. Due to the potential interference of UMP and its alkylated derivatives (*vide infra*), the MS/MS acquisitions were performed by isolating, in a 1 m/z unit-wide window, either the M or the M+1 isotopologues for each precursor ion of interest (the $[M-H]^-$ ions corresponding to residual CMP or its alkylation products). In all cases a collisional energy of 35% was adopted; the upper limit for the MS/MS spectra was always 10 m/z units higher than the precursor ion m/z value, whereas the lower limit was fixed automatically. All data elaborations (spectral averaging and ion current extractions from MS and MS/MS TIC traces) were performed with the Xcalibur software. Interpretation of MS/MS fragmentations was performed also with the aid of the Mass Frontier (HighChem Ltd., Bratislava, Slovakia) software.

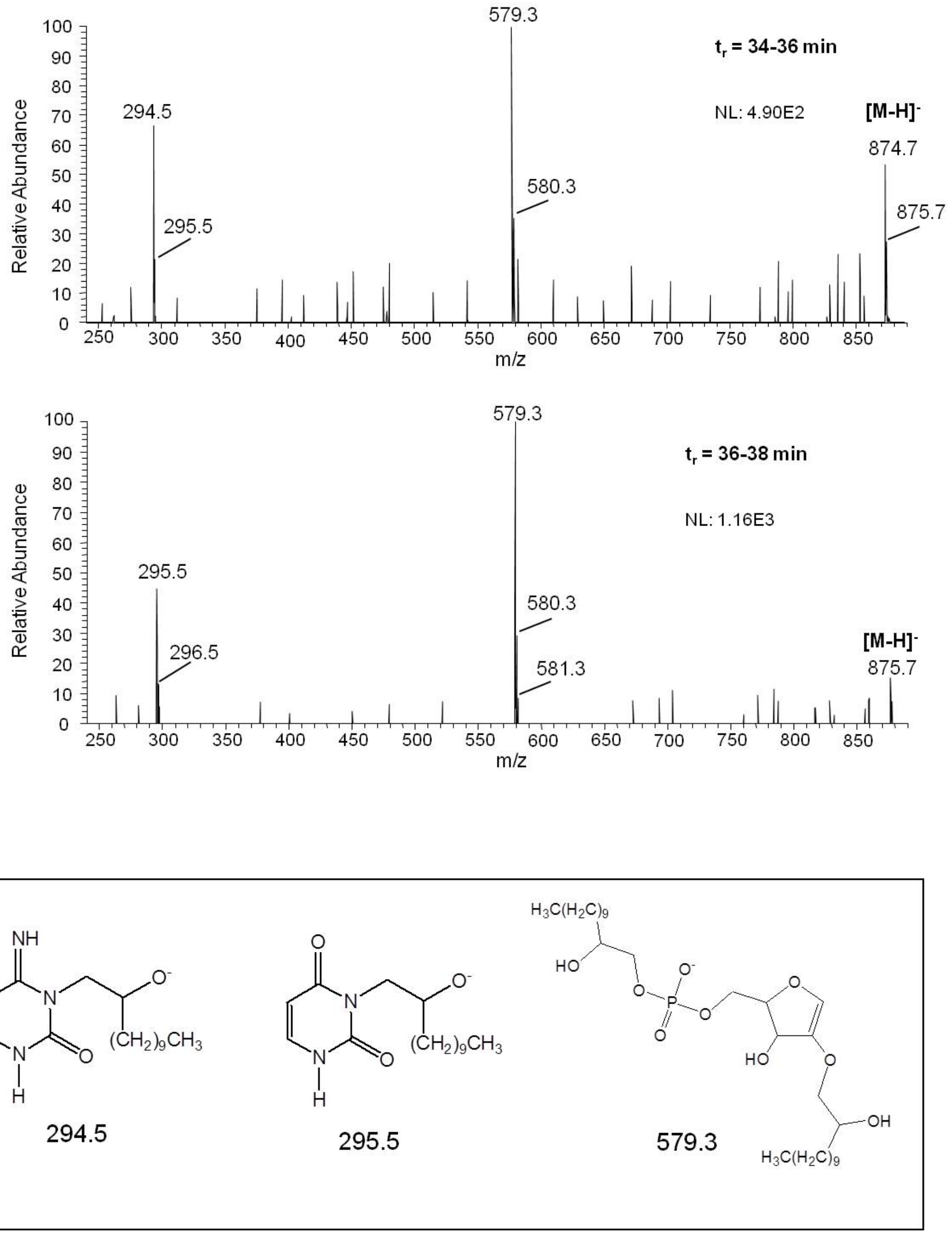

Fig. S1. MS/MS spectra obtained for the [M-H]$^-$ ions of the tri-alkylated derivatives of CMP (top) and UMP (bottom) ribonucleotides detected after 80 days of reaction with dodecyl-epoxide. The structures hypothesized for the main product ions observed are also reported. It is worth noting that: 1) the alkylation of the ribose ring may occur also on the OH group linked to carbon 3; 2) isomeric structures, alkylated either on C=O group(s) tautomerized to enols or on the NH_2 group (only for CMP), could be also considered for product ions at m/z 294.5 and 295.5; 3) only the nucleophilic attack to the less hindered carbon of the epoxide ring has been shown in the structures, yet the other carbon atom of the epoxide ring may be involved as an alternative site.

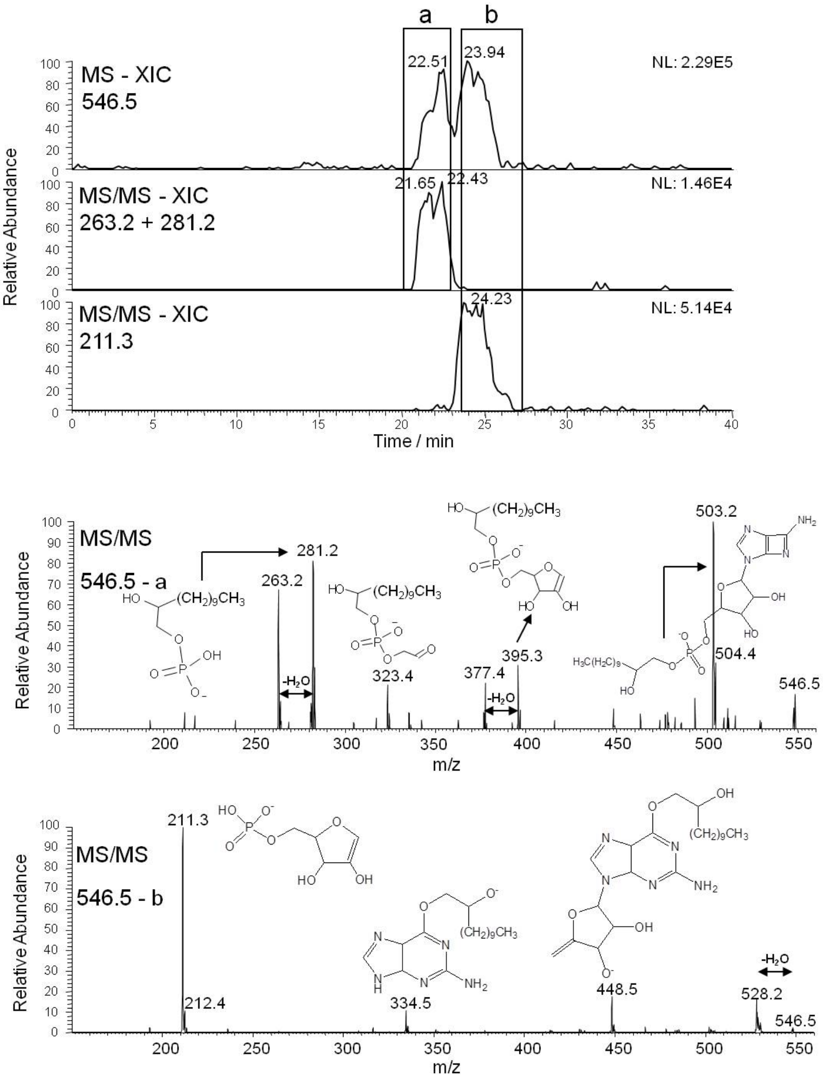

Figure S2. Top: comparison between eXtracted Ion Chromatograms (XIC) obtained from the MS- and the MS/MS - TIC traces relevant to an aliquot of the GMP-DE mixture after 20 days of reaction. The MS-XIC trace is referred to the m/z ratio of mono-alkylated GMP. The MS/MS-XIC traces are referred to the m/z ratios of, respectively, two product ions peculiar of GMP mono-alkylated on a phosphate OH group and a product ion typical of GMP mono-alkylated on guanine. Bottom: comparison between the MS/MS spectra averaged under the peaks located in the time intervals a and b, corresponding, respectively, to GMP mono-alkylated on a phosphate OH group or on a guanine site. The structures hypothesized for all the major product ions are reported to emphasize these assignments. In the case of guanine-alkylated GMP only alkylation on the tautomerized C=O group has been indicated, for the sake of brevity.

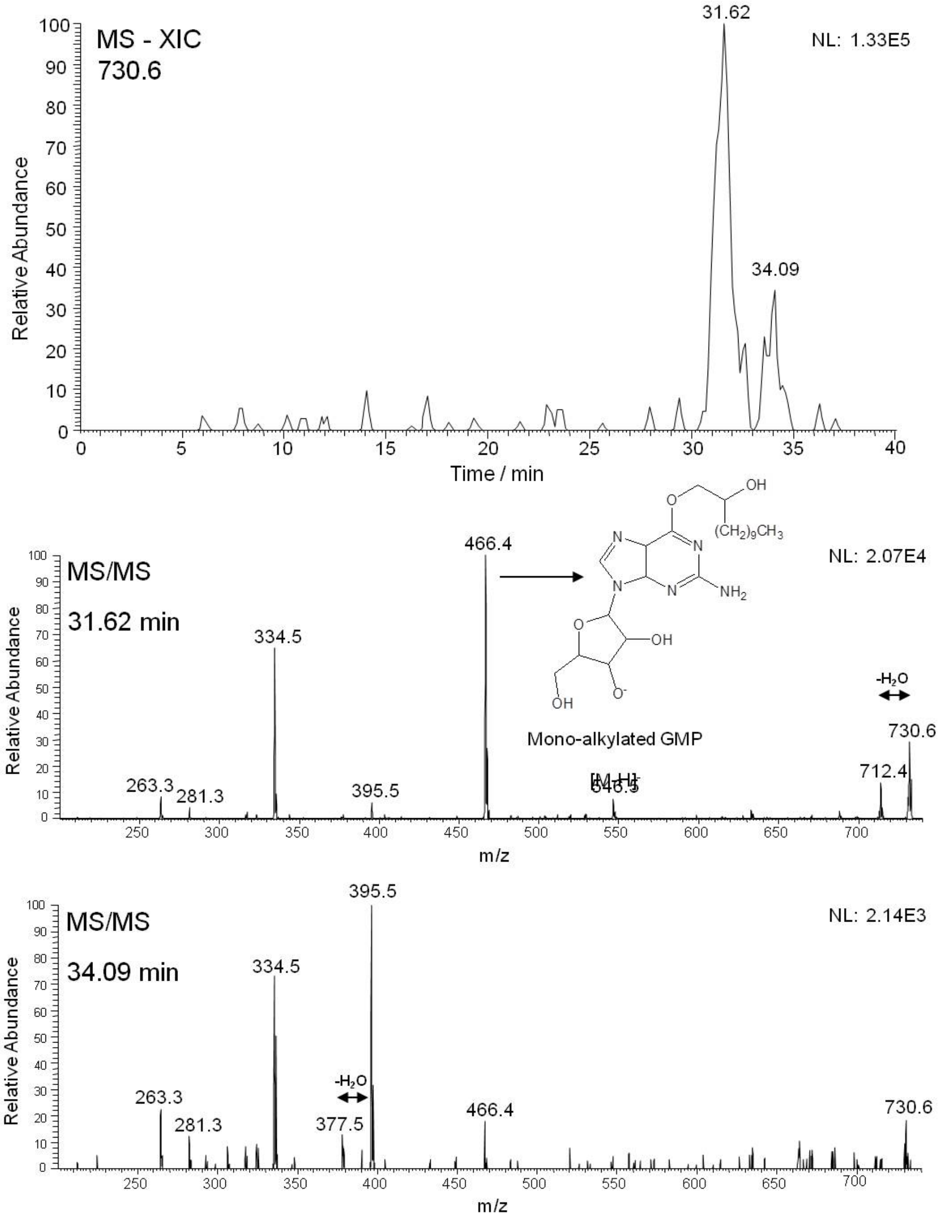

Figure S3. Top: eXtracted Ion Chromatogram (XIC) obtained from the MS- TIC trace relevant to an aliquot of the GMP-DE mixture after 20 days of reaction. The MS-XIC trace is referred to the m/z ratio of bi-alkylated GMP. Bottom: comparison between the MS/MS spectra averaged under the two peaks found in the MS-XIC trace for the m/z ratio 730.6. For the sake of clarity only one of the possible structures for the m/z 466.4 product ion is reported. The structures relevant to the other product ions are the same as those shown in Fig.S2.

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