Supporting Information

Determining the Molecular Basis for the pH-dependent Interaction among 2´-deoxynucleotides and 9H-pyrido[3,4-b]indole in its ground and electronic excited states

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Contents:

1. UV-vis spectrophotometric titration at pH 2.8, 5.5 and 10.5

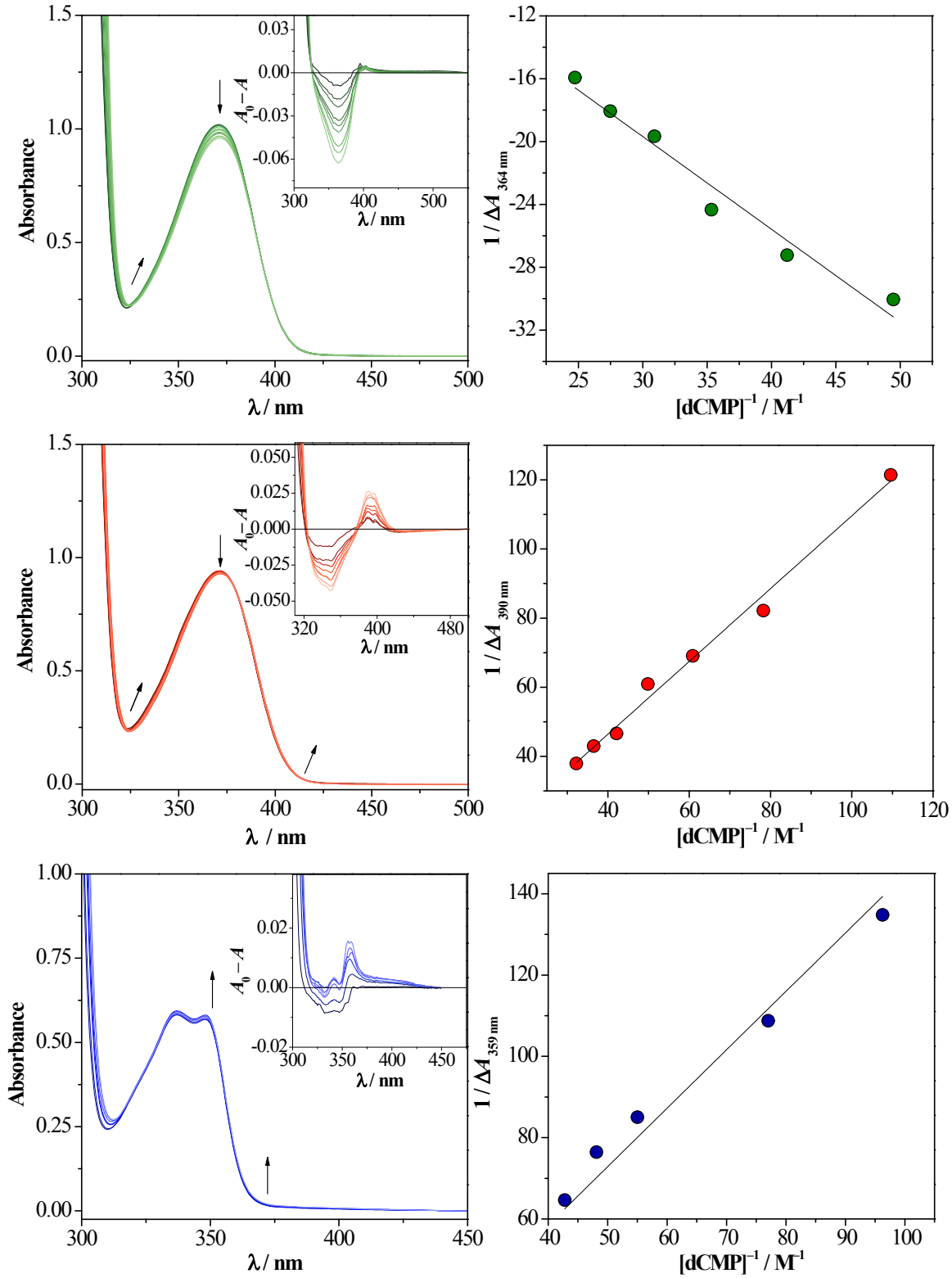

Figure SI.1. UV-vis absorption spectra of norharmane in the presence increasing amount of dCMP (see arrows). *Inset:* Experimental Difference spectra (ED). The right side of each figure shows a representative Benesi-Hildebrand plot for each case. **(a)** pH 2.8, $[nHoH^+]_0$ = 249 μM, **(b)** pH 5.5, $[nHoH^+]_0$ = 228 μM and (c) pH 10.5, $[nHoN]_0$ = 154 μM.

2. Results obtained by curve resolution techniques from UV-vis spectra of the nHo-dAMP complexes

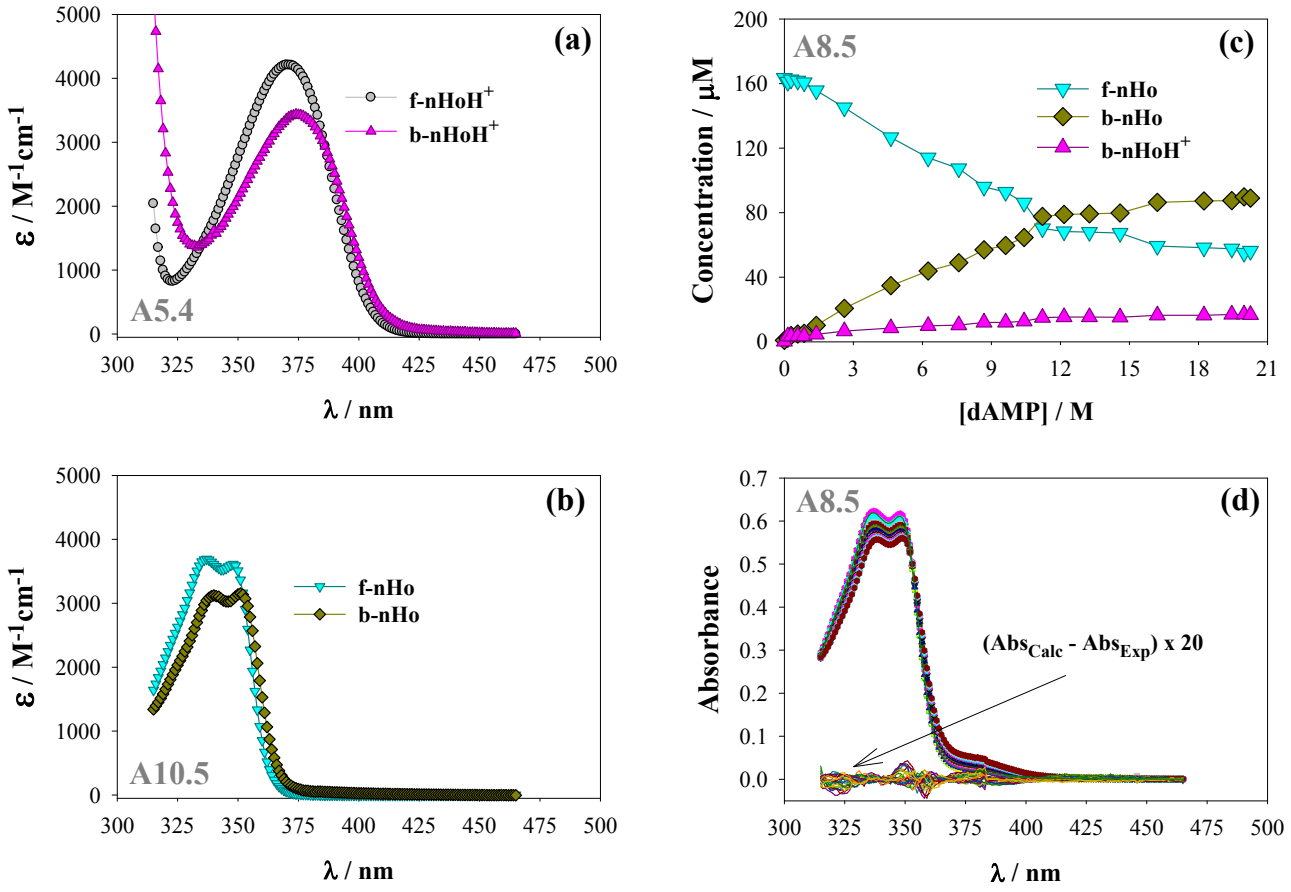

Figure SI.2. (a) Absorption spectra of free and bound forms of $nHoH^+$ at pH 5.0. Inset: Free and bound fractions of $nHoH^+$ against [dAMP]. **(b)** Absorption spectra of free and bound forms of nHo at pH 10.5. *Inset:* free and bound fractions of nHo against [dAMP]. **(c)** Concentration profiles of free nHo, bound nHo and bound $nHoH^+$ against [dAMP] resolved from the absorbance matrix recorded at pH 8.5. **(d)** Comparison between the experimental absorbance matrix A and the error matrix E, calculated as A - CS^T, at pH 8.5. Free norharmane forms: f-nHo and f-$nHoH^+$; Bound norharmane forms: b-nHo and b-$nHoH^+$.

3. Table SI.1. Data obtained from chemometric analysis.

System	ε / $M^{-1}cm^{-1}$	λ / nm	K_G^{calc}
G5.0	3230 (max)	375	84 ± 4
G10.5	3815 (max) 3690 (min) 3760 (max)	338 345 349	31 ± 2
A5.4	3438 (max)	374	75 ± 11
A10.5	3120 (max) 3025 (min) 3150 (max)	340 346 351	35 ± 2

where ε and λ are the molar absorption coefficient and the wavelength of the maximum of absorption of the complexes, respectively, obtained by the application of the ALS algorithm. K_G^{calc} represents the calculated binding constants values calculated for each nucleotide-norharmane complex.

4. Normalized UV-visible spectra of the complexes obtained by curve resolution techniques

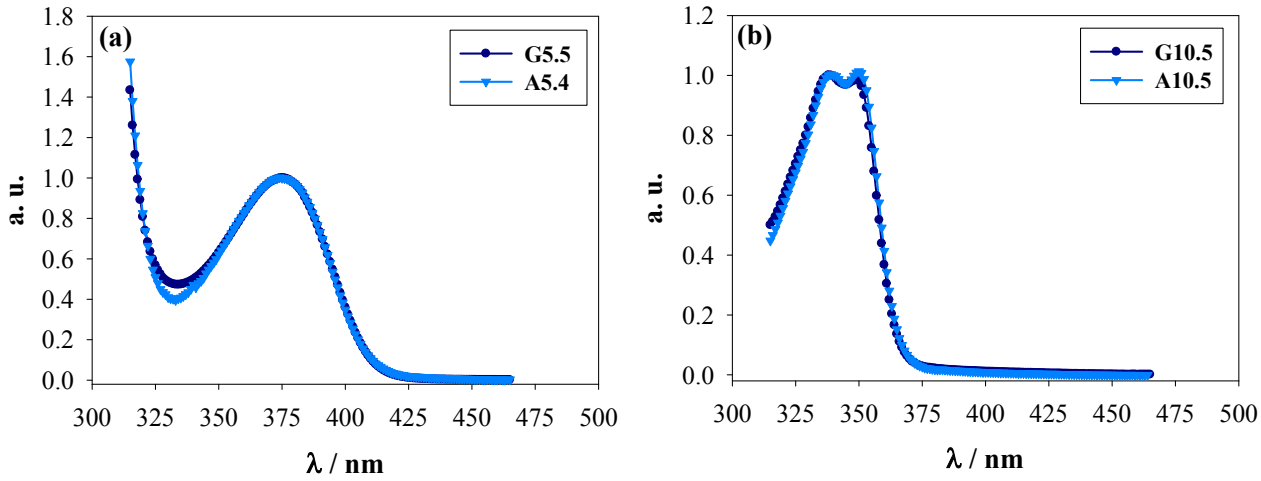

Figure SI.3. Comparison between the normalized UV-visible spectra of the complexes formed between purine nucleotides (dGMP and dAMP) and norharmane obtained under acidic **(a)** and alkaline **(b)** conditions.

5. Quenching of norharmane fluorescence by dCMP: steady state analysis

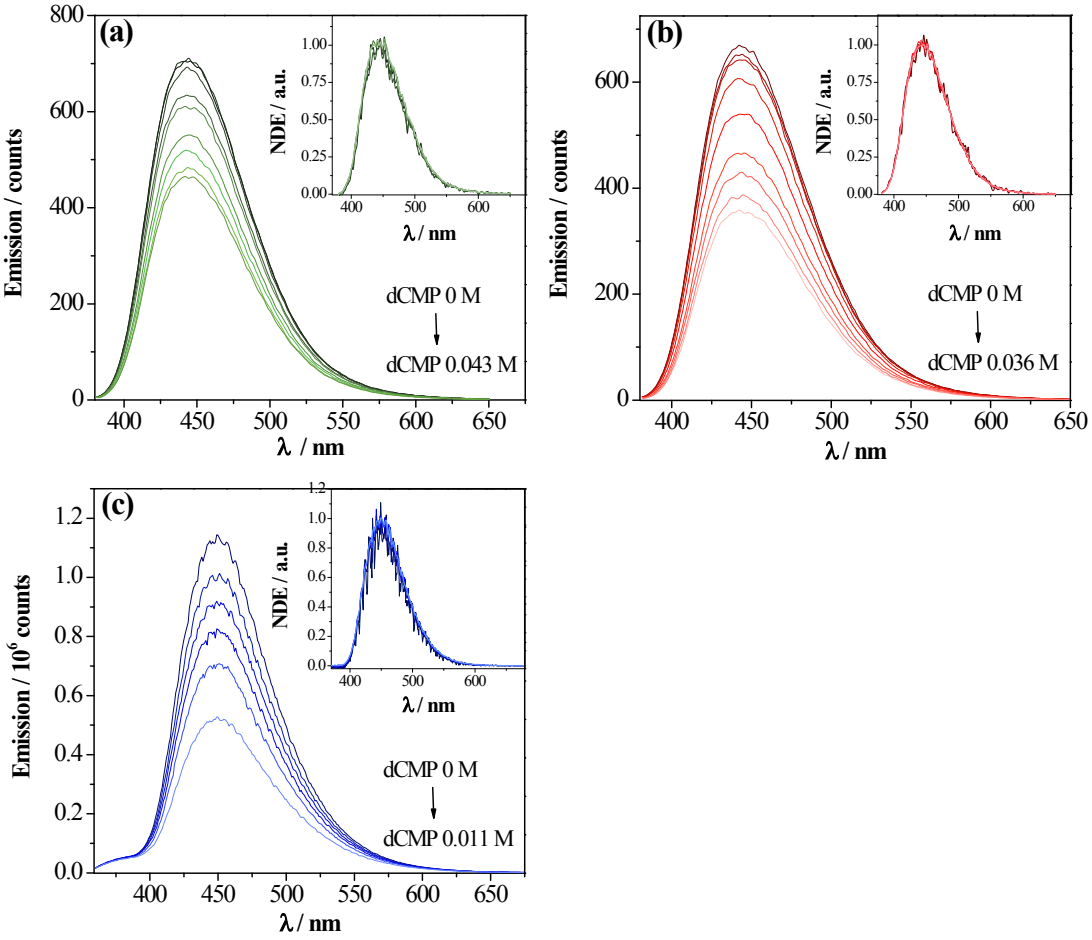

Figure SI.4. Corrected fluorescence spectra of norharmane aqueous solution (2.0×10^{-5} M) as a function of the dCMP concentrations: **(a)** pH 2.8, λ_{exc} = 370 nm, λ_{em} = 373-680 nm, **(b)** pH 5.5, λ_{exc} = 370 nm, λ_{em} = 373-680 nm, (c) pH 10.5, λ_{exc} = 349 nm, λ_{em} = 360-680 nm. *Insets*: Normalized Differential Emission Spectra.

6. Quenching of norharmane fluorescence by dCMP: time-resolved analysis

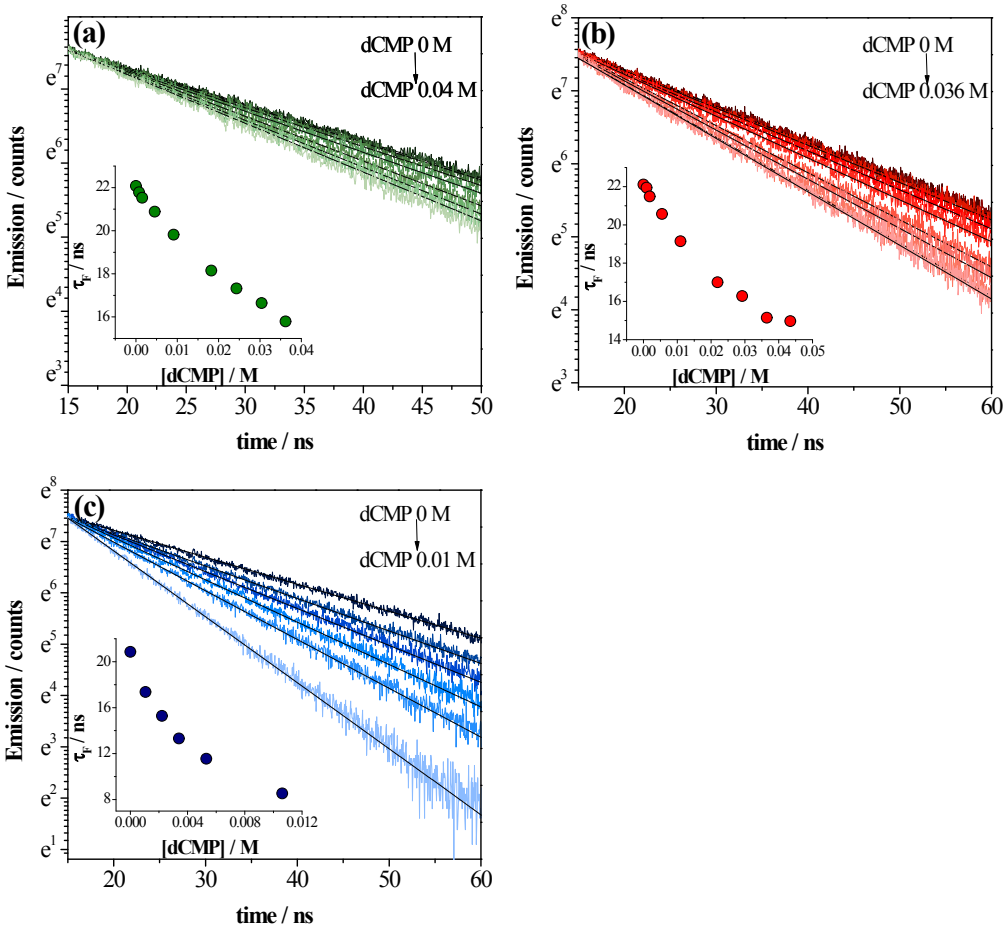

Figure SI.5. Fluorescence decays of norharmane aqueous solution (2.0×10^{-5} M) as a function of the dCMP concentrations at pH **(a)** 2.8, **(b)** 5.5 and **(c)** 10.5. λ_{exc} = 341 nm, λ_{em} = 450 nm. *Inset*: fluorescence lifetime as a function of [dCMP].

7. Stern-Volmer plots for quenching of norharmane fluorescence by dCMP

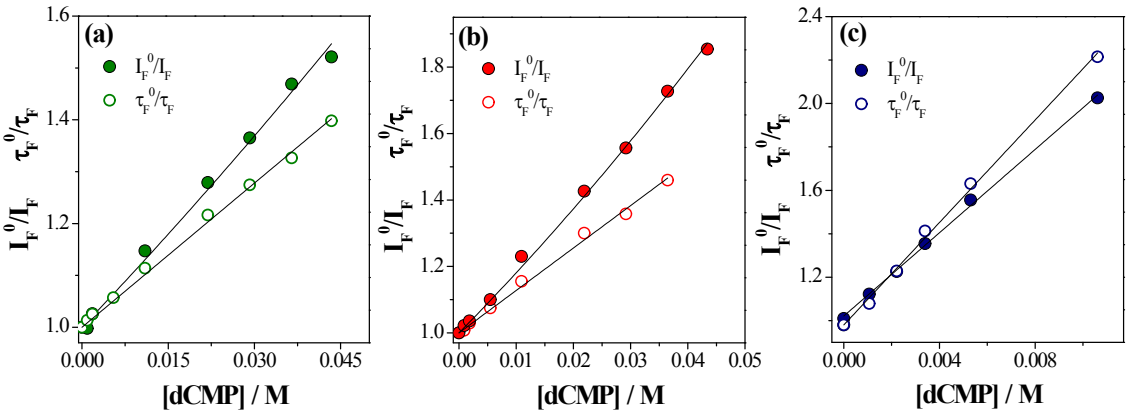

Figure SI.6. Quenching of norharmane fluorescence by dCMP at pH: **(a)** 2.5 **(b)** 5.5 and **(c)** 10.5. Stern–Volmer plots of the fluorescence intensities (I_F) and the fluorescence lifetimes (τ_F); λ_{exc} = 341 nm, λ_{em} = 450 nm.

8. Quenching of norharmane fluorescence by dAMP: steady state analysis.

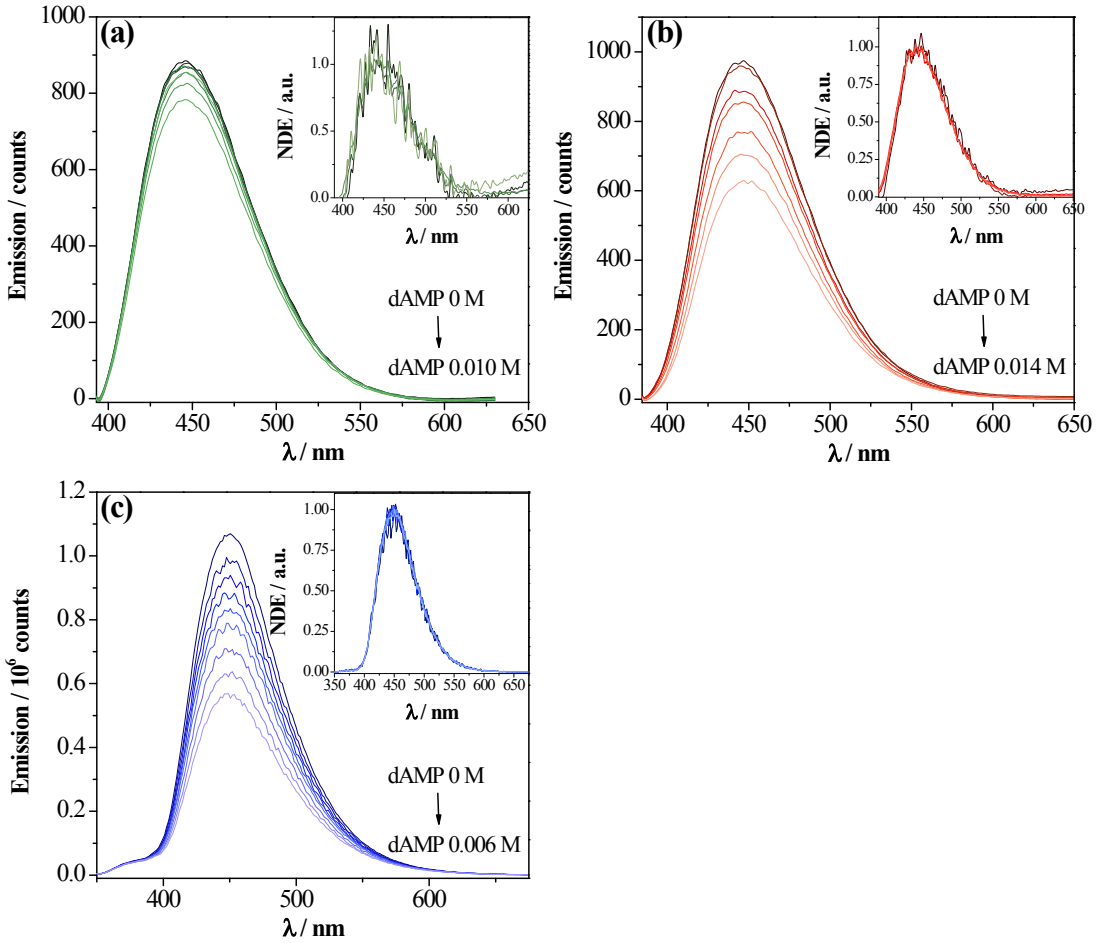

Figure SI.7. Corrected fluorescence spectra of norharmane aqueous solution (2.0×10^{-5} M) as a function of the dAMP concentrations: **(a)** pH 2.8, λ_{exc} = 370 nm, λ_{em} = 373-680 nm, **(b)** pH 5.5, λ_{exc} = 370 nm, λ_{em} = 373-680 nm, (c) pH 10.5, λ_{exc} = 349 nm, λ_{em} = 360-680 nm. *Insets*: Normalized Differential Emission Spectra.

9. Kinetic analysis of the dNMP bleaching photosensitized by norharmane in aqueous solution.

As a proof of principle, we have investigated the comparative damage on deoxynucleotides (dGMP, dAMP and dCMP) photosensitized by norharmane, under acidic and basic conditions (pH 5.0 and 10.5, respectively). Figure below shows that dCMP is photostable whereas purine deoxynucleotides are photosensitized, being dGMP the most photosensitive target. In addition, the damage is more pronounced in acidic than in alkaline media. These data agree with the nucleotides oxidation potential trend.

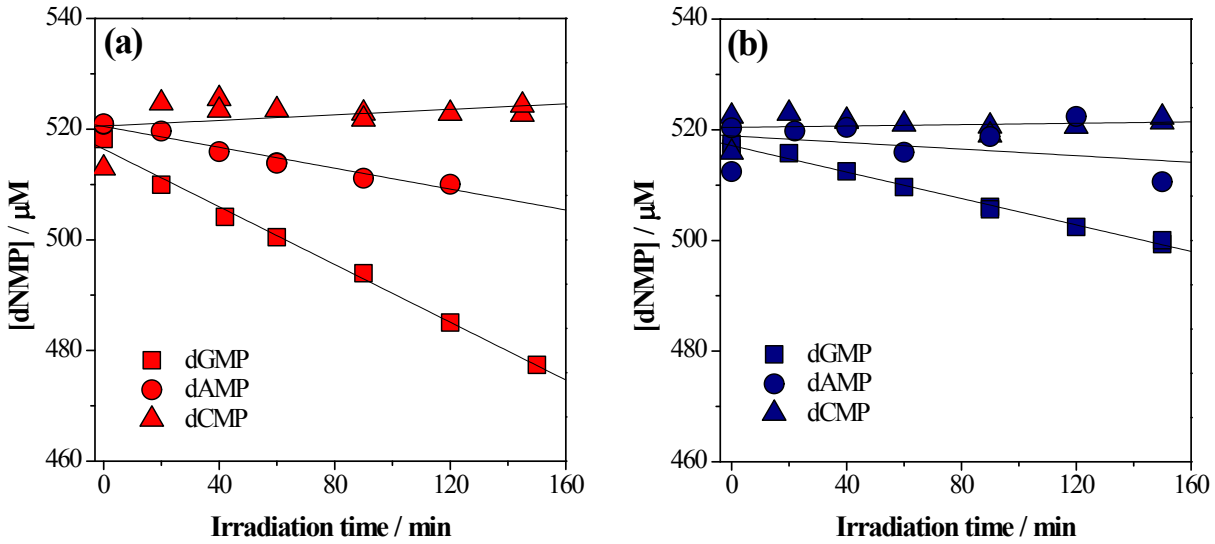

Figure SI.8. Evolution of the 2'-deoxynucleotide-5'-monophospate (dNMP) concentrations in air-equilibrated aqueous solutions at **(a)** pH 5.0 and **(b)** 10.5 as a function of elapsed UV irradiation time (concentrations were determined by HPLC analysis): λ_{exc} = 350 nm, photosensitizer used: norharmane.

10. Geometric features of the hydrogen bonds found for the stable conformers obtained for the norharmane–dGMP complex.

Table SI.2. The three G5.0, G8.5 and G10.5 systems are considered. Distances are in angstroms and angles in degrees. Only those conformers lying up to 2 kcal mol^{-1} above the most stable conformer of each system are shown. Kinetic analysis of the dNMP bleaching photosensitized by norharmane in aqueous solution.

Conformer	Bond type	d(X─H)	d(H···Y)	a(X─H···Y)
G5.0-1	(HPO4) O─H····O (OH)	1.025	1.756	166
	(NH2)N─H····O (HPO4)	1.045	1.845	169.8
G5.0-2	(HPO4) O─H····O (OH)	1.025	1.755	166
	(NH2)N─H····O (HPO4)	1.045	1.847	169.8
G5.0-3	(HPO4) O─H····O (OH)	1.02	1.721	174.3
	(NH2)N─H····O (HPO4)	1.042	1.88	163.2
G5.0-4	(NH2)N─H····O (HPO4)	1.05	1.795	155.6
	N9─H····O (HPO4)	1.041	1.716	173.3
	(HPO4) O─H····O (deoxyribose)	1.024	1.722	151.1
G5.0-5	N9─H····O (HPO4)	1.054	1.681	175.6
G5.0-6	N2─H····O (HPO4)	1.094	1.608	164.5
G8.5-1	(OH) O─H····O (PO4)	1.083	1.495	172
	(NH2)N─H····O (PO4)	1.047	1.823	171.4
	N9─H····O (deoxyribose)	1.034	1.959	169.6
G8.5-2	(OH) O─H····O (PO4)	1.091	1.479	171.2
	N9─H····O (deoxyribose)	1.031	1.881	173.4
G8.5-3	(OH) O─H····O (PO4)	1.091	1.48	170.9
	N9─H····O (deoxyribose)	1.031	1.881	173.2
G8.5-4	(OH) O─H····O (PO4)	1.09	1.481	170.8
	N9─H····O (deoxyribose)	1.031	1.88	172.8
G8.5-5	(OH) O─H····O (PO4)	1.09	1.483	169.9
	N9─H····O (deoxyribose)	1.031	1.89	172.8

G8.5-6	(OH) O─H···O (PO4)	1.09	1.483	170
	N9─H···O (deoxyribose)	1.031	1.887	173
G8.5-7	(OH) O─H···O (PO4)	1.091	1.48	171
	N9─H···O (deoxyribose)	1.031	1.879	173.1
G8.5-8	(OH) O─H···O (PO4)	1.092	1.478	171.3
	N9─H···O (deoxyribose)	1.031	1.882	173.5
G8.5-9	(OH) O─H···O (PO4)	1.092	1.479	171.1
	N9─H···O (deoxyribose)	1.031	1.878	173.1
G8.5-10	(OH) O─H···O (PO4)	1.092	1.479	171
	N9─H···O (deoxyribose)	1.031	1.88	173.2
G8.5-11	(OH) O─H···O (PO4)	1.091	1.482	170.1
	N9─H···O (deoxyribose)	1.031	1.889	174
G8.5-12	(OH) O─H···O (PO4)	1.091	1.479	171
	N9─H···O (deoxyribose)	1.031	1.881	173.3
G8.5-13	(OH) O─H···O (PO4)	1.091	1.479	170.9
	N9─H···O (deoxyribose)	1.031	1.881	173.2
G8.5-14	(OH) O─H···O (PO4)	1.089	1.49	168.6
	N9─H···O (deoxyribose)	1.03	1.87	176.3
G8.5-15	(OH) O─H···O (PO4)	1.09	1.489	168.2
	N9─H···O (deoxyribose)	1.03	1.87	176.2
G8.5-16	(OH) O─H···O (PO4)	1.083	1.512	161.4
	N9─H···O (deoxyribose)	1.031	1.873	177
G8.5-17	(OH) O─H···O (PO4)	1.078	1.515	169.4
	N9─H···O (PO4)	1.079	1.623	164.7
	(NH2)N─H···O (PO4)	1.044	1.973	159.8
G8.5-18	(OH) O─H···O (PO4)	1.077	1.518	168.8
	N9─H···O (PO4)	1.079	1.62	164.9

	(NH2)N─H····O (PO4)	1.045	1.958	159.5
G10.5-1	(OH) O─H····O (PO4)	1.089	1.486	170.1
	N9─H····O (deoxyribose)	1.033	1.87	173.3
G10.5-2	(OH) O─H····O (PO4)	1.089	1.486	170
	N9─H····O (deoxyribose)	1.033	1.87	172.7

11. Geometric features of the hydrogen bonds found for the stable conformers obtained for the norharmane–dCMP complex.

Table SI.3. The three C2.8, C5.5 and C10.5 systems are considered. Distances are in angstroms and angles in degrees. Only those conformers lying up to 2 kcal mol^{-1} above the most stable conformer of each system are shown.

Conformer	Bond type	d(X─H)	d(H···Y)	α(X─H···Y)
C2.8-1	N9─H···O (HPO4)	1.041	1.903	137.3
	N9─H···O (deoxyribose)	1.041	1.984	137.9
C5.5-1	N9─H···O (HPO4)	1.056	1.681	166.9
	(HPO4) O─H···O (deoxyribose)	1.016	1.895	137.6
C5.5-2	N9─H···O (HPO4)	1.036	1.742	170.8
	(HPO4) O─H···O (deoxyribose)	1.024	1.68	1.57.8
C5.5-3	N9─H···O (HPO4)	1.054	1.69	166.9
C5.5-4	(HPO4) O─H···O (OH deoxyribose)	1.027	1.729	165.8
C5.5-5	N9─H···O (HPO4)	1.036	1.741	170.8
	(HPO4) O─H···O (deoxyribose)	1.024	1.681	157.6
C5.5-6	2N─H···O (HPO4)	1.509	1.939	112.8
	(HPO4) O─H···O (OH deoxyribose)	1.026	1.769	163.7
C5.5-7	(HPO4) O─H···O (OH deoxyribose)	1.024	1.752	164.5
C5.5-8	(HPO4) O─H···O (OH deoxyribose)	1.023	1.758	165
C5.5-9	(HPO4) O─H···O (OH deoxyribose)	1.023	1.757	165.1
C5.5-10	N2─H···O (HPO4)	1.095	1.596	167.1
C5.5-11	N9─H···O (HPO4)	1.037	1.74	170.7
	(HPO4) O─H···O (deoxyribose)	1.022	1.736	161.2
C5.5-12	N9─H···O (HPO4)	1.05	1.721	159
C5.5-13	(HPO4) O─H···O (OH deoxyribose)	1.023	1.758	164.9
C5.5-14	(HPO4) O─H···O (OH deoxyribose)	1.036	1.667	161.7
C5.5-15	(HPO4) O─H···O (OH deoxyribose)	1.023	1.759	164.9
C5.5-16	N2─H···O (HPO4)	1.1	1.573	168.2
	(HPO4) O─H···O (OH deoxyribose)	1.025	1.766	139.1

C5.5-17	(HPO4) O─H···O (OH deoxyribose)	1.028	1.732	165.4
C5.5-18	N9─H···O (HPO4)	1.05	1.767	153
	(HPO4) O─H···O (OH deoxyribose)	1.033	1.677	162.7
C5.5-19	(HPO4) O─H···O (OH deoxyribose)	1.027	1.734	165.3
C5.5-20	(HPO4) O─H···O (OH deoxyribose)	1.037	1.646	163.5
C5.5-21	(HPO4) O─H···O (OH deoxyribose)	1.036	1.666	161.8
C5.5-22	N2─H···O (HPO4)	1.094	1.603	167
	(HPO4) O─H···O (OH deoxyribose)	1.025	1.731	162.9
C5.5-23	N2─H···O (HPO4)	1.06	1.89	127.5
	(HPO4) O─H···O (OH deoxyribose)	1.026	1.775	163.3
C5.5-24	(HPO4) O─H···O (OH deoxyribose)	1.027	1.727	157.1
C5.5-25	(HPO4) O─H···O (OH deoxyribose)	1.022	1.761	165
C5.5-26	N9─H···O (HPO4)	1.057	1.671	177.7
C5.5-27	(HPO4) O─H···O (OH deoxyribose)	1.027	1.707	168.5
C5.5-28	N9─H···O (HPO4)	1.058	1.678	170.6
C5.5-29	(HPO4) O─H···O (OH deoxyribose)	1.027	1.707	168.4
C5.5-30	(HPO4) O─H···O (OH deoxyribose)	1.027	1.732	165.5
C5.5-31	N2─H···O (HPO4)	1.096	1.588	159.4
	(HPO4) O─H···O (OH deoxyribose)	1.036	1.685	166.6
C5.5-32	N2─H···O (HPO4)	1.075	1.75	143.8
	(OH deoxyribose) O─H···O (HPO4)	1.031	1.717	161
C5.5-33	N2─H···O (HPO4)	1.096	1.587	159.7
	(OH deoxyribose) O─H···O (HPO4)	1.036	1.685	166.7
C5.5-34	(HPO4) O─H···O (OH deoxyribose)	1.026	1.729	157.6
C5.5-35	(HPO4) O─H···O (OH deoxyribose)	1.028	1.734	165.3

C5.5-36	N2─H···O (HPO4)	1.062	1.852	130
	(HPO4) O─H···O (OH deoxyribose)	1.028	1.757	164.7
C5.5-37	(OH deoxyribose) O─H···O (HPO4)	1.012	1.823	157.2
C5.5-38	(OH deoxyribose) O─H···O (HPO4)	1.012	1.824	157.2
C5.5-39	N9─H···O (HPO4)	1.058	1.659	174.8
C5.5-40	(OH deoxyribose) O─H···O (HPO4)	1.035	1.675	161.5
C5.5-41	N2─H···O (HPO4)	1.096	1.587	169.6
C10.5-1	(deoxyribose) O-H···O (PO4)	1.088	1.483	171.3
	N9-H···O (deoxyribose)	1.031	1.935	163.5
C10.5-2	(deoxyribose) O-H···O (PO4)	1.088	1.488	169.9
	N9-H···O (deoxyribose)	1.032	1.94	166.9
C10.5-3	(deoxyribose) O-H···O (PO4)	1.088	1.502	167.8
	N9-H···O (deoxyribose)	1.074	1.6	174.8
C10.5-4	(deoxyribose) O-H···O (PO4)	1.09	1.491	168.7
	N9-H···O (PO4)	1.072	1.613	172.6
C10.5-5	(deoxyribose) O-H···O (PO4)	1.089	1.496	168.1
	N9-H···O (PO4)	1.073	1.601	173.8
C10.5-6	(deoxyribose) O-H···O (PO4)	1.084	1.512	168.3
	N9-H···O (PO4)	1.073	1.601	169.9
C10.5-7	(deoxyribose) O-H···O (PO4)	1.09	1.484	170.6
C10.5-8	(deoxyribose) O-H···O (PO4)	1.083	1.496	171.1
	N9-H···O (deoxyribose)	1.035	1.872	162.4
C10.5-9	(deoxyribose) O-H···O (PO4)	1.089	1.485	170.3
C10.5-10	(deoxyribose) O-H···O (PO4)	1.096	1.463	170.1
	N9-H···O (PO4)	1.04	1.834	142
C10.5-11				
	(deoxyribose) O-H···O (PO4)	1.089	1.477	172.9
C10.5-12	(deoxyribose) O-H···O (PO4)	1.091	1.483	170
C10.5-13	(deoxyribose) O-H···O (PO4)	1.09	1.483	170.5
C10.5-14	(deoxyribose) O-H···O (PO4)	1.089	1.476	172.9
C10.5-15	(deoxyribose) O-H···O (PO4)	1.09	1.485	170.3
C10.5-16	(deoxyribose) O-H···O (PO4)	1.083	1.496	171.1
	N9-H···O (PO4)	1.035	1.873	162.4
C10.5-17	(deoxyribose) O-H···O (PO4)	1.089	1.476	173.1
C10.5-18	(deoxyribose) O-H···O (PO4)	1.091	1.48	170.5

C10.5-19	(deoxyribose) O-H···O (PO4)	1.091	1.483	170.4
C10.5-20	(deoxyribose) O-H···O (PO4)	1.089	1.476	173.1
C10.5-21	(deoxyribose) O-H···O (PO4)	1.089	1.479	172.4
C10.5-22	(deoxyribose) O-H···O (PO4)	1.083	1.497	170.6
	N9-H···O (deoxyribose)	1.035	1.869	163.1
C10.5-23	(deoxyribose) O-H···O (PO4)	1.055	1.604	148.5
	N9-H···O (PO4)	1.072	1.616	172.2

12. ^{1}H-NMR spectra of G5.0 and C2.8 systems.

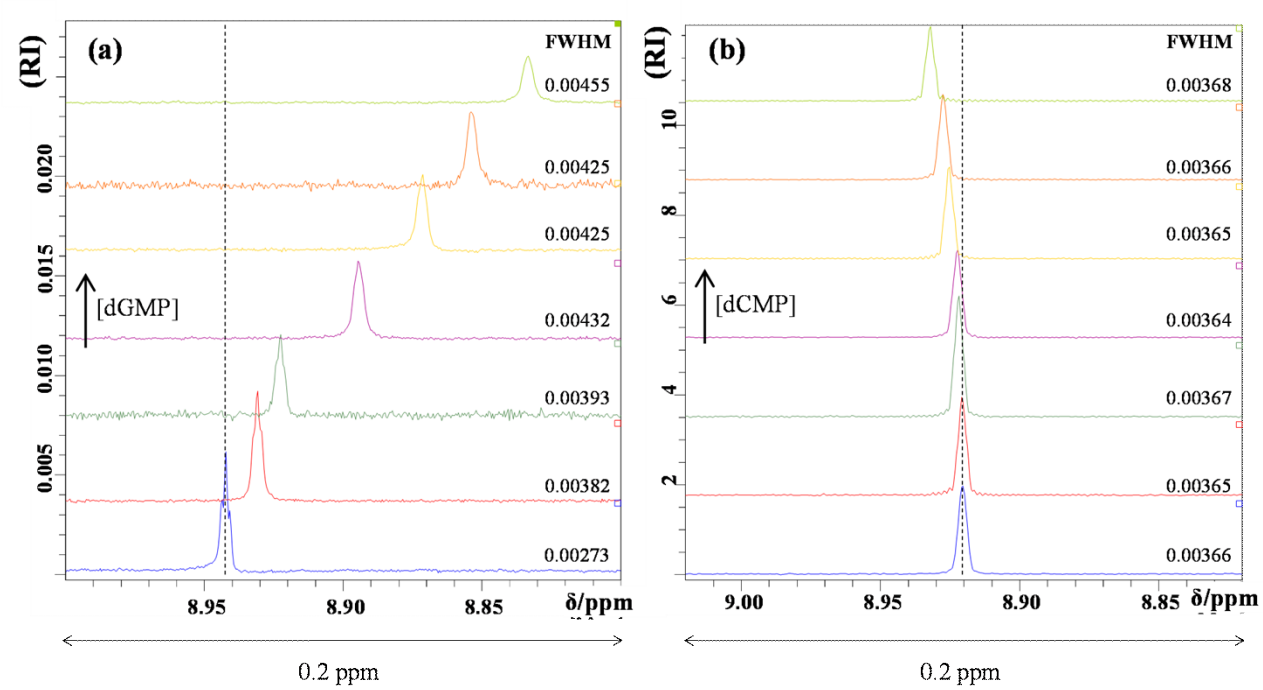

Figure SI.9. Representative example of the chemical shift of the norharmane C1-H signal as a function of dNMPs concentration in D_2O solution: **(a)** system G5.0 and (b) system C2.8. To show the changes, several ^{1}H-NMR spectra are displayed in the same figure. In each case, the dashed line was added as a reference; δ in ppm; FWHM: full with at half maximum in ppm.