Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information (ESI)

for

Acid-base properties of the nucleic-acid model 2'-deoxyguanylyl(5'→3')-2'-deoxy-5'-guanylate, d(pGpG)$^{3–}$, and of related guanine derivatives

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The following section numbers refer to those of the main paper; in this way it is made clear to which section the Supporting Information belongs. The reference numbers are those of the main paper; further references are numbered as 1S and 2S.

2S Results

The release of the first proton of the 5'-P(O)(OH)$_2$ group of $H_4[d(pGpG)]^+$ [eqn. (2)] could not be measured, but there are three ways to obtain an estimate based on the results listed in Table 1: (i) The acidity difference for the release of the final H^+ from the phosphate group in $H[d(pGpG)]^{2-}$ [eqn. (5)] and $H(dGMP)^-$ amounts to $\Delta\, pK_a = 0.27 \pm 0.03$ [= (6.56 ± 0.03) – (6.29 ± 0.01); Table 1, entries 5 and 9]; because experience shows[1S,33a] that the same difference also holds for the release of the primary phosphate proton, a value for $H_4[d(pGpG)]^+$ can be estimated based on the data for $H_3(dGMP)^+$, that is, $pK^{H}_{H_4[d(pGpG)]} = pK^{H}_{H_3(dGMP)} + \Delta\, pK_a = (0.35 \pm 0.2) + (0.27 \pm 0.03) = 0.62 \pm 0.20$. (ii) If one adds the difference (0.3 ± 0.4) between the pK_a values of $H_2(pUpU)^-$ (1.0 ± 0.3; Table 1, entry 6) and $H_2(UMP)$ (0.7 ± 0.3; Table 1, entry 7) to the value of $H_3(dGMP)^+$ (0.35 ± 0.2; Table 1, entry 9), one obtains for $pK^{H}_{H_4[d(pGpG)]} = (0.35 \pm 0.2) + (0.3 \pm 0.4) = 0.65 \pm 0.45$. (iii) By subtracting the difference between the pK_a values of $H_2(UMP)$ (0.7 ± 0.3; Table 1, entry 7) and $H_3(dGMP)^+$ (0.35 ± 0.2; Table 1, entry 9), which gives $\Delta\, pK_a' = 0.35 \pm 0.36$, from the value for $H_2(pUpU)^-$ (Table 1, entry 6), one obtains $pK^{H}_{H_4[d(pGpG)]} = pK^{H}_{H_2(pUpU)} - \Delta\, pK_a' = (1.0 \pm 0.3) - (0.35 \pm 0.36) = 0.65 \pm 0.47$. From these values we conclude that $pK^{H}_{H_4[d(pGpG)]} = 0.65 \pm 0.3$ and this estimate is listed in entry 5 of Table 1 (column 3).

That this estimated acidity constant is of a reasonable order, is confirmed by a different reasoning based on different data: It has recently been shown[2S] for several adenine-nucleotide analogues that $pK^{H}_{P(O)(OH)_2} = pK^{H}_{P(O)_2(OH)} - (5.68 \pm 0.05)$ [the corresponding difference for $H_3(AMP)^+$ also fits into this picture].[2S] Application of this result to guanine derivatives is possible if the distance effect between $(N1)H^+$ and $(N7)H^+$ is taken into account; evidently in the *anti* conformation the charge at $(N7)H^+$ of $H_3(GMP)^+$ is somewhat closer to the P(O)(OH)$_2$ group than that at $(N1)H^+$ in $H_3(AMP)^+$; this distance effect amounts to 0.14 ± 0.07 log units.[23] Hence, for the acidity constant of $H_4[d(pGpG)]^+$ one obtains $pK^{H}_{H_4[d(pGpG)]} = pK^{H}_{H[d(pGpG)]} - (5.68 \pm 0.05) - (0.14 \pm 0.07) = (6.56 \pm 0.03) - (5.68 \pm 0.05) - (0.14 \pm 0.07) = 0.74 \pm 0.09$. This value is within the error limits identical with the one estimated above and given in Table 1.

3S Discussion

Regarding the validity of the estimated value of 2.4 ± 0.2 for $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_3[d(pGpG)]}}$ (Table 1; entry 5; column 4) it may be noted that the *average* $\mathrm{p}K_{\mathrm{a}}$ value [2.69 = 0.5 × (2.4 + 2.98)] excellently agrees with the expected charge effect of singly negatively charged phosphate groups, as it amounts to 0.39 (= 2.69 – $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(dGuo)}}$ = 2.69 – 2.30) p*K* units. The *expected* charge effect follows from the comparison of $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_2(GMP)}} - \mathrm{p}K^{\mathrm{H}}_{\mathrm{H(Guo)}}$ = (2.48 ± 0.04) – (2.11 ± 0.04) = 0.37 ± 0.06 (Table 1; entries 1,8; column 4) and $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_2(dGMP)}} - \mathrm{p}K^{\mathrm{H}}_{\mathrm{H(dGuo)}}$ = (2.69 ± 0.03) – (2.30 ± 0.04) = 0.39 ± 0.05. The average of these expected effects, i.e., 0.38 ± 0.06 p*K* units, is evidently in perfect agreement with the above value, supporting thus the estimation made in the text in Section 3.

The acidity constants for $\mathrm{H_4(pGpG)^+}$ given in entry 12 of Table 1 have been estimated as follows: (i) The $\mathrm{p}K_{\mathrm{a}}$ value for $\mathrm{H_4[d(pGpG)]^+}$ was reduced for the effect of the 2'-OH group (see text in Section 3) to give $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_4\,(pGpG)}}$ = (0.65 ± 0.3) – (0.05 ± 0.02) = 0.6 ± 0.3. (ii) The effect of the 2'-OH group on the acidity of the $\mathrm{(N7)H^+}$ site amounts to $\Delta\,\mathrm{p}K_{\mathrm{a}}$ = 0.20 ± 0.06 (average of entries 1,2 and 8,9 in column 4 of Table 1); hence, one obtains $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_3(pGpG)}} = \mathrm{p}K^{\mathrm{H}}_{\mathrm{H_3[d(pGpG)]}} - \Delta\,\mathrm{p}K_{\mathrm{a}}$ = (2.4 ± 0.2) – (0.20 ± 0.06) = 2.2 ± 0.2 and for $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H_2(pGpG)}}$, due to the second $\mathrm{(N7)H^+}$ site, follows [2.98 (or 2.4 + 0.6 = 3.0) ± 0.13] – (0.20 ± 0.06) = 2.80 ± (0.14 + 0.02) = 2.80 ± 0.16 by also considering the statistical effect of 0.6 (and adding to the error limit 0.02 = 0.60 – 0.58). (iii) In the third paragraph of Section 3 we have seen that the effect of the 2'-OH group on the $\mathrm{P(O)_2(OH)^-}$ residue amounts to 0.04 ± 0.02 p*K* units, hence, $\mathrm{p}K^{\mathrm{H}}_{\mathrm{H(pGpG)}} = \mathrm{p}K^{\mathrm{H}}_{\mathrm{H[d(pGpG)]}}$ – (0.04 ± 0.02) = (6.56 ± 0.03) – (0.04 ± 0.02) = 6.52 ± 0.04. (iv) Similarly, the effect of the 2'-OH group on the deprotonation of the (N1)H sites amounts to 0.04 ± 0.05 (3σ) p*K* units which gives $\mathrm{p}K^{\mathrm{H}}_{\mathrm{pGpG}}$ = (9.54 ± 0.08) – (0.04 ± 0.05) = 9.50 ± 0.09 and $\mathrm{p}K^{\mathrm{H}}_{\mathrm{(pGpG-H)}}$ = [10.11 (or 9.54 + 0.6 = 10.14) ± 0.14] – (0.04 ± 0.05) = 10.10 ± (0.15 + 0.03) = 10.10 ± 0.18 (see (ii) for the explanation).

1S A. Fernández-Botello, R. Griesser, A. Holý, V. Moreno and H. Sigel, *Polyhedron*, 2003, **22**, 1067-1076.

2S A. Fernández-Botello, R. Griesser, A. Holý, V. Moreno and H. Sigel, *Inorg. Chem.*, 2005, **44**, 5104-5117.