Supplementary material for manuscript:

Experimental and theoretical study of thymine and cytosine derivatives: the crucial role of weak noncovalent interactions.

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**Materials and methods**

Elemental microanalyses were carried out using a Carlo Erba model 1108 or a Thermo Finnigan model Flash EA 1112 microanalyzers. IR spectra in the solid state (KBr or CsI pellets) were measured on a Bruker IFS 66 spectrometer. NMR spectra were recorded on a Bruker AMX300 spectrophotometer at room temperature. $^1$H chemical shifts in deuterated dimethyl sulfoxide (DMSO-$d_6$) were referenced to DMSO-$d_6$ $[\text{H-NMR, } \delta(\text{DMSO} = 2.47 \text{ ppm})]$. High Resolution Mass Spectroscopy with Electro Spray Ionization (ESI-HRMS) was focused on an AUTOSPEC 3000 with PEG-600 as standards for exact mass determination (2 mg/10 ml EtOH). Reagents were used as received from Sigma-Aldrich.

**Synthesis of N$^3$-hexylthymine (1)**

100 mmol of thymine in 950 mmol hexamethyldisilazane (HMDS) and some mg of ammonium sulfate anhydrous are heated under reflux, in nitrogen atmosphere, during 4 hours. Subsequent distillation of non-reacted HMDS and volatile by-products yields the corresponding O,O’-bistrimethylsilyloxythymine with NMR purity, which is used without any further purification (it precipitates on cooling).

A suspension of O,O’-bistrimethylsilyloxythymine (15 mmol) and 1-bromohexane (15 mmol) in 30 ml dry acetonitrile, under nitrogen, are heated at 130 °C in PARR® bomb during 36 hours. The resulting mixture is boiled with 50 ml methanol for 30 minutes and stirred overnight at room temperature. The corresponding N$^3$-hexylthymine appears (70% yield) from the methanol solution after a first precipitation of non-reacted thymine and white crystals, suitable for X-ray diffraction studies, were obtained after further recrystallisation in methanol. Anal. Found: C, 61.21; H, 8.69; N, 13.30 %. Calc. for C$_{11}$H$_{13}$N$_2$O$_2$·0.25H$_2$O: C, 61.51; H, 8.68; N, 13.04 %. IR (cm$^{-1}$): 549w, 684m, 765m, 894(br)m, 946m, 1007w, 1051w, 1102m, 1144m, 1219m, 1238m, 1257m, 1309w, 1361s, 1419w, 1463w, 1532s, 1574m, 1675vs(br), 1690vs, 2828m, 2857m, 2926s, 2954s, 3030s(br).

**Synthesis of N$^1$-hexylcytosine (2)**

100 mmol of cytosine in 950 mmol hexamethyldisilazane (HMDS) and some mg of ammonium sulfate anhydrous are heated under reflux, in nitrogen atmosphere, during 4 hours. Subsequent distillation of non-reacted HMDS and its more volatile by-products yield the corresponding N,O-bistrimethylsilyloxyctosine with NMR purity, which is used without any further purification (it precipitates on cooling).

A suspension of N,O-bistrimethylsilyloxyctosine (15 mmol) and 1-bromohexane (15 mmol) in 30 ml dry acetonitrile, under nitrogen, are heated at 130 °C in PARR® bomb during 36 hours. The resulting mixture is boiled with 50 ml methanol for 30 minutes and stirred overnight at room temperature. The corresponding N$^1$-hexylcytosine hydrobromide appears (50% yield) from the methanol solution after a first precipitation of non-reacted cytosine and white crystals, suitable for X-ray diffraction studies, were obtained after further recrystallisation in methanol. Anal. Found: C, 58.77; H, 8.62; N, 20.80 %. Calc. for C$_{11}$H$_{13}$N$_2$O$_2$·0.25H$_2$O: C, 58.80; H, 8.88; N, 20.57 %. IR (cm$^{-1}$): 552w, 582w, 622m, 669m, 712w, 788m, 1129w, 1204m, 1269m, 1389s, 1488s, 1522m, 1554m, 1578m(br), 1617vs, 1655s, 2855m, 2928m, 2955m, 3030s(br), 3158m(br).

**Synthesis of N$^1$-hexylcytosine hydrobromide (3)**

A suspension of N,O-bistrimethylsilyloxyctosine (15 mmol) and 1-bromohexane (15 mmol) in 30 ml dry acetonitrile, under nitrogen, are heated at 130 °C in PARR® bomb during 36 hours. The resulting mixture is boiled with 50 ml methanol for 30 minutes and stirred overnight at room temperature. The corresponding N$^1$-hexylcytosine hydrobromide appears (50% yield) from the methanol solution after a first precipitation of non-reacted cytosine and white crystals, suitable for X-ray diffraction studies, were obtained after further recrystallisation in methanol. Anal. Found: C, 41.19; H, 6.61; N, 15.17 %. Calc. for C$_{10}$H$_{13}$BrN$_3$O·0.5H$_2$O: C, 42.12; H, 6.72; N, 14.73 %. IR (cm$^{-1}$): 610m, 650w, 729w, 760m, 823m, 989w, 1146m, 1186m, 1263m, 1357s(br), 1419w, 1463w, 1532s, 1574m, 1671vs, 1721vs, 2760m, 2857s, 2927s, 2959s, 3116s, 3315s. $^1$H NMR (DMSO-$d_6$): $\delta$ (ppm) 7.57 [d, 1H, H(6), J = 7.2 Hz], 7.20 [br s, 1H, N(4)=H$_2$], 7.00 [br s, 1H, N(4)=H$_2$], 6.20 [d, 1H, H(5), J = 7.2 Hz], 6.48 [t, 2H, H(7), J = 5.0 Hz], 1.45 [m, 2H, H(8)], 1.23 [br s, 6H, H(9,10,11)], 0.82 [t, 3H, H(12), J = 6.9 Hz]. ESI-HRMS: [M+H]$^+$: m/z, 211.1504; calc., 211.1441.
white solid is obtained after evaporating at dryness the organic fraction. Anal. Found: C, 53.48; H, 8.36; N, 11.79 %. Calc. for C$_{16}$H$_{30}$BrN$_2$O·C: 53.33; H, 8.39; N, 11.66 %. IR (cm$^{-1}$): 421w, 675w, 726vw, 759w, 815m, 1027vw, 1103vw, 1170w, 1227m, 1376m, 1402w, 1456m, 1535m, 1660vs, 1706s, 2856m, 2926s, 2955s, 3002s, 3171m. $^1$H NMR (DMSO-d$_6$): δ (ppm) 9.71 [br s, 1H, (N(4)-H$_2$)], 9.11 [br s, 1H, N(4)-H$_2$], 7.97 [d, 1H, H(6), J = 7.5 Hz], 7.09 [d, 1H, H(5), J = 7.5 Hz], 3.88 [t, 2H, N$_3$-CH$_3$(7), J = 7.5 Hz], 3.77 [t, 2H, N$_3$-CH$_3$(7), J = 7.5 Hz], 1.54 [br m, 4H, H(8)], 1.23 [br s, 12H, H(9,10,11)], 0.83 [br s, 6H, H(12)]. ESI-HRMS: [M+]: exact mass, 391.2826; calc., 391.2821 (for C$_{20}$H$_{35}$N$_2$O$_2$).

**Computational details**

All calculations were carried out using the turbomole package version 6.10$^1$ using the RI-MP2 method,$^2$ which has been widely used to study noncovalent interactions using the crystallographic coordinates optimizing the position of the hydrogen atoms. In this study, aug-cc-pVDZ basis set was used. The basis set superposition error (BSSE) has been corrected using the counterpoise method. Since this is a quite large basis set, the substituent in N$^+$ has been replaced by a methyl group to reduce the size of the model. The RI-MP2 method$^2$ applied to the study of weak interactions involving π-systems is considerably faster than the MP2 and the interaction energies and equilibrium distances are almost identical for both methods.$^3$ We have recently demonstrated that this level of theory gives comparable results to the CCSD(T)/AVTZ//RI-MP2/aug-cc-pVQZ level for anion–π complexes of pyrazine.$^4$ In compound 1, where the interactions are dominated by dispersion effects, we have used the SCS-RI-MP2/aug-cc-pVQZ level of theory. The spin-component-scaled MP2 method is based on the scaling of the standard MP2 amplitudes for parallel and antiparallel spin double excitations. The SCS-MP2 correlation treatment yields structures that are superior to those from standard MP2 calculations, particularly in systems that are dominated by dispersive interactions.$^5$

**More detailed description of 3**

The stepped layers (see main text) are piled, resembling glide planes, where the cytosinium rings are located in a head to tail manner. The bromide atoms are the driving force for the gliding, in order to achieve a saturated environment, formed by the four planar hydrogen bonds and additional interactions with the upper and lower layers.

Having a look to the layers, the cytosinium rings lie in perfectly parallel planes at 3.255 Å while the anion–π interaction distance (between the bromide and the mean plane of the ring) is 3.582 Å. A likely explanation for the compression of the crystal in the piling direction is shown in Figure S1, where a C-H···π interaction (orange dotted lines) established between the aliphatic chain and the ring of adjacent layers is presented.

![Figure S1](image-url)  
*Figure S1.* The presence of C-H···π interactions in compound 3 between bromide and N$^+$ atom from the cytosinium ring (depicted in orange) permits to explain why the distance between mean planes of the cytosinium moieties (green) is shorter than the anion–π interactions (black).

**A more detailed theoretical analysis of 3**

We have also studied the energy associated to the anion–π interaction, using the scheme shown in Figure S2. We have used a theoretical model initially neutral (protonated cytosine interacting with bromide) in order to
estimate the anion–π interaction without the contribution of the purely electrostatic contribution of the ion-pair. The computed interaction energy is –21.7 kcal/mol. The results derived from this theoretical study indicate that the anion–π interaction is important in the crystal packing of compound 3 and gives an explanation to the rare location of the bromide: exactly above one nitrogen atom of the ring.

**Figure S2.** Reaction used to estimate the anion–π interaction in compound 3.

**More detailed theoretical analysis of 4**

**Figure S3.** Equations used to compute the interaction energies of several fragments of compound 4. ΔE=34.7 kcal/mol

**References**