Supplementary material for manuscript:

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

Miquel Barceló-Oliver,*[a] Beatriz A. Baquero,[a] Antonio Bauzá,[a] Angel García-Raso,[a] Angel Terrón,[a] Ignasi Mata[b] Elies Molins[b] and Antonio Frontera,*[a]

Table of contents:

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 [^{1}H-NMR, δ(DMSO = 2.47 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^1-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 ºC 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-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 $C_{11}H_{18}N_2O_2 \cdot 0.25H_2O$: C, 61.51; H, 8.68; N, 13.04 %. IR (cm^{-1}): 549w, 684m, 765m, 894m(br), 946m, 1007w, 1051w, 1102m, 1144m, 1219m, 1238m, 1257m, 1309w, 1361s(br), 1423s, 1478s, 1657vs(br), 1690vs, 2828m, 2857m, 2926s, 2954s, 3030s(br), 3158m(br). ^{1}H NMR (DMSO-d_6): δ (ppm) 11.14 [br s, 1H, N(3)-H], 7.49 [s, 1H, H(6)], 3.57 [t, 2H, H(7), J = 7.5 Hz], 1.71 [s, 3H, CH_3(6)], 1.52 [br m, 2H, H(8)], 1.22 [br s, 6H, H(9,10,11)], 0.82 [t, 3H, H(12), J = 6.6 Hz]. ESI-HRMS: [M+H]: m/z, 211.1504; calc., 211.1441.

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-bistrimethylsilyloxycytosine with NMR purity, which is used without any further purification (it solidifies on cooling).
A suspension of N,O-bistrimethylsilyloxycytosine (15 mmol) and 1-bromohexane (15 mmol) in 30 ml dry acetonitrile, under nitrogen, are heated at 130 ºC 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 (instead of the expected neutral N^1-hexylcytosine) appears from the methanol solution after a first precipitation of non-reacted cytosine. 1 mmol of N^1-hexylcytosine hydrobromide was boiled with 1 mmol Na_2CO_3 in 30 ml methanol. After 1 h reflux, the mixture was filtered to get rid of non-reacted reagents and vacuum distilled. White powder appeared from the concentrated solution and was filtered off (40% yield). Anal. Found: C, 58.77; H, 8.62; N, 20.80 %. Calc. for $C_{10}H_{17}N_3O \cdot 0.5H_2O$: C, 58.80; H, 8.88; N, 20.57 %. IR (cm^{-1}): 552w, 582w, 622m, 669m, 712w, 788m, 1129w, 1204m, 1269m, 1389s, 1488s, 1522m, 1617vs, 1665s, 2855m, 2928m, 2955m, 3104m, 3351s. ^{1}H NMR (DMSO-d_6): δ (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], 5.62 [d, 1H, H(5), J = 7.2 Hz], 3.58 [t, 2H, H(7), J = 7.2 Hz], 1.51 [m, 2H, H(8), J = 6.9 Hz], 1.23 [br s, 6H, H(9,10,11)], 0.82 [t, 3H, H(12), J = 6.9 Hz.

Synthesis of N^1-hexylcytosine hydrobromide (3)

A suspension of N,O-bistrimethylsilyloxycytosine (15 mmol) and 1-bromohexane (15 mmol) in 30 ml dry acetonitrile, under nitrogen, are heated at 130 ºC 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 $C_{10}H_{18}BrN_3O \cdot 0.5H_2O$: 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): δ (ppm) 9.25 [br s, 1H, N(4)-H_2], 8.07 [br s, 1H, N(4)-H_2], 7.97 [d, 1H, H(6), J = 7.5 Hz], 5.97 [d, 1H, H(5), J = 7.5 Hz], 3.72 [t, 2H, H(7), J = 7.2 Hz], 1.57 [br m, 2H, H(8)], 1.24 [br s, 6H, H(9,10,11)], 0.84 [t, 3H, H(12), J = 6.9 Hz]. ESI-HRMS: although the corresponding signal [M+H] is found in the spectrum (m/z, 196.1427; calc., 196.1444 for $C_{10}H_{18}N_3O$), the main product in the mass spectrum is the corresponding to the hemiprotonated form CHC^+, as in compound **4**.

The double substituted by-product N^1,N^3-dihexylcytosinium bromide is also synthesized during the reaction and can be purified from the mother liquor by vacuum evaporation and subsequent $CHCl_3$:H_2O extraction. A

white solid is obtained after evaporating at dryness the organic fraction. Anal. Found: C, 53.48; H, 8.36; N, 11.79 %. Calc. for $C_{16}H_{30}BrN_3O$: 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, 2956s, 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], 6.09 [d, 1H, H(5), J = 7.5 Hz], 3.88 [t, 2H, N^3-CH_2(7), J = 7.5 Hz], 3.77 [t, 2H, N^1-CH_2(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, 280.2396; calc., 280.2389 (for $C_{16}H_{30}N_3O$).

Synthesis of [(N^1-hexylcytosinium)·(N^1-hexylcytosine)]$_2$ ·[$Cl_2Hg(\mu\text{-}Cl)_2HgCl_2$] (4)

1 mmol of **3** was solved in methanol (25 ml) and 0.5 mmol of mercuric chloride in methanol (10 ml) were added drop by drop (with stirring) to the first solution. The resulting clear solution was filtered and let to evaporate slowly. After 15 days, very unstable crystals are formed. Intents of recrystallization were unsuccessful and it was not possible to obtain samples with analytical purity. The resulting unstable crystals were covered with Infineum V8512 oil to prevent the lost of crystallinity and then picked out and mounted directly on the X-ray device.

However, it was possible to obtain a pure sample of CHC^+Br^-, the hemiprotonated form with bromide as counterion, by water recrystallization of raw **1**. Anal. Found: C, 49.03; H, 7.31; N, 16.85 %. Calc. for $C_{20}H_{35}BrN_6O_2\cdot H_2O$: C, 49.08; H, 7.62; N, 17.17 %. IR (cm^{-1}): 494vw, 618m, 782m, 965vw, 1077vw, 1192w, 1273w, 1379m, 1400m, 1442m, 1465m, 1491m, 1531m, 1609m, 1668s, 1722vs, 1898w, 2855s, 2925s, 2955s, 3179s, 3345s. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.18 [br s, 1H, N(4)-H_2], 7.75 [d, 1H, H(6), J = 7.5 Hz], 7.50 [br s, 1H, N(4)-H_2], 5.77 [d, 1H, H(5), J = 7.5 Hz], 3.64 [t, 2H, H(7), J = 7.2 Hz], 1.53 [br m, 2H, H(8)], 1.22 [br s, 6H, H(9,10,11)], 0.82 [t, 3H, H(12), J = 6.6 Hz]. ESI-HRMS: [M+]: exact mass, 391.2826; calc., 391.2821 (for CHC^+: $C_{20}H_{35}N_6O_2$).

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^1 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-pVDZ 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.

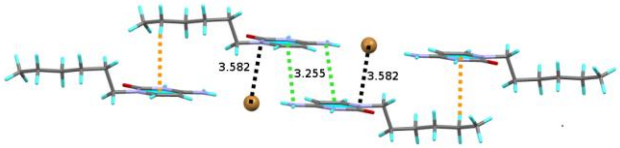

Figure S1. The presence of C-H···π interactions in compound **3** between bromide and N^1 atom from the cytosinium ring (depicted in orange) permits to explain why the distance between mean planes of the cytosinium moieties (green) is shorter than the anion-π interactions (black).

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.

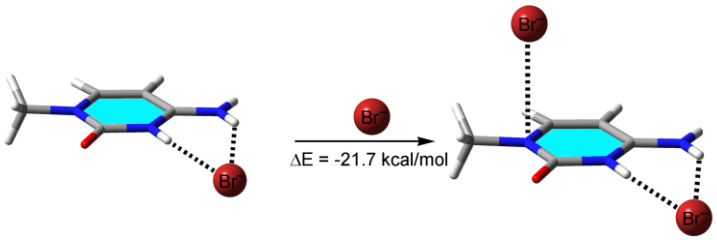

Figure S2. Reaction used to estimate the anion–π interaction in compound **3**.

More detailed theoretical analysis of 4

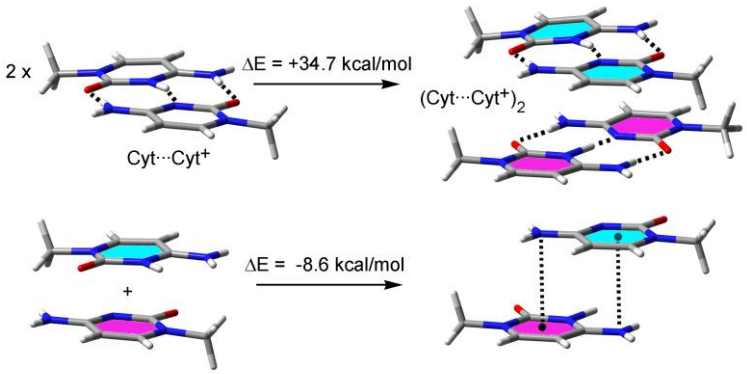

Figure S3. Equations used to compute the interaction energies of several fragments of compound **4**. ΔE=34.7 kcal/mol

References

1 R. Ahlrichs, M. Bär, M. Hacer, H. Horn and C. Kömel, *Chem. Phys. Lett.* 1989, **162**, 165.
2 M.W. Feyereisen, G. Fitzgerald and A. Komornicki, *Chem. Phys. Lett*. 1993, **208**, 359; O. Vahtras, J. Almlof and M.W. Feyereisen, *Chem. Phys. Lett*. 1993, **213**, 514.
3 A. Frontera, D. Quiñonero, C. Garau, P. Ballester, A. Costa and P.M. Deyà, J. Phys. Chem. A 109 (2005) 4632; D. Quiñonero, C. Garau, A. Frontera, P. Ballester, A. Costa and P.M. Deyà, *J. Phys. Chem. A* 2006, **110**, 5144.
4 D. Quiñonero, C. Estarellas, A. Frontera and P.M. Deyà, *Chem. Phys. Lett*. 2011, **508**, 144.
5 M. Gerenkamp and S. Grimme, *Chem. Phys. Lett*. 2004, **392**, 229.