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Supporting Information

Are Two Riboses Better Than One? The Case of the Recognition and Activation of Adenosine Receptors

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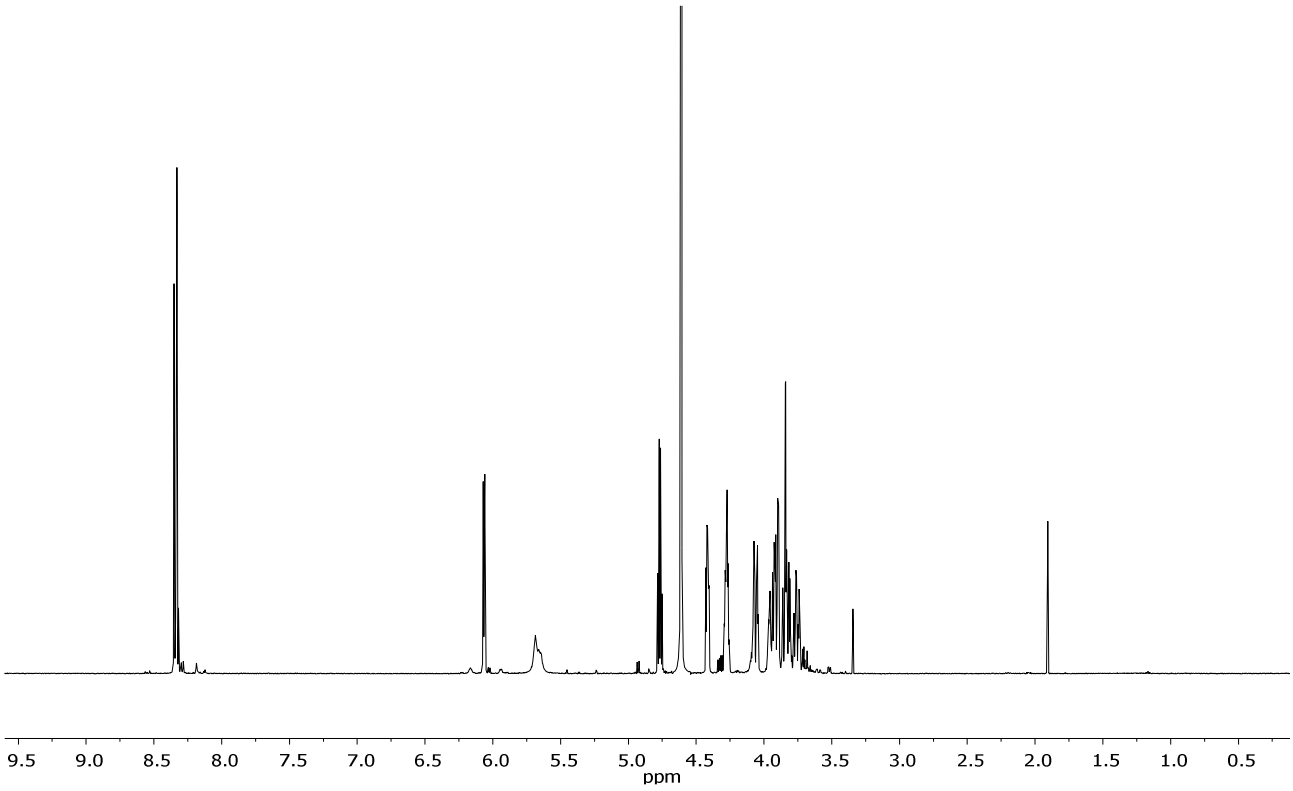

Figure S1: ^{1}H NMR spectrum of N^{6}-(D-ribos-1-yl)-adenosine in D_2O at T = 40 °C.

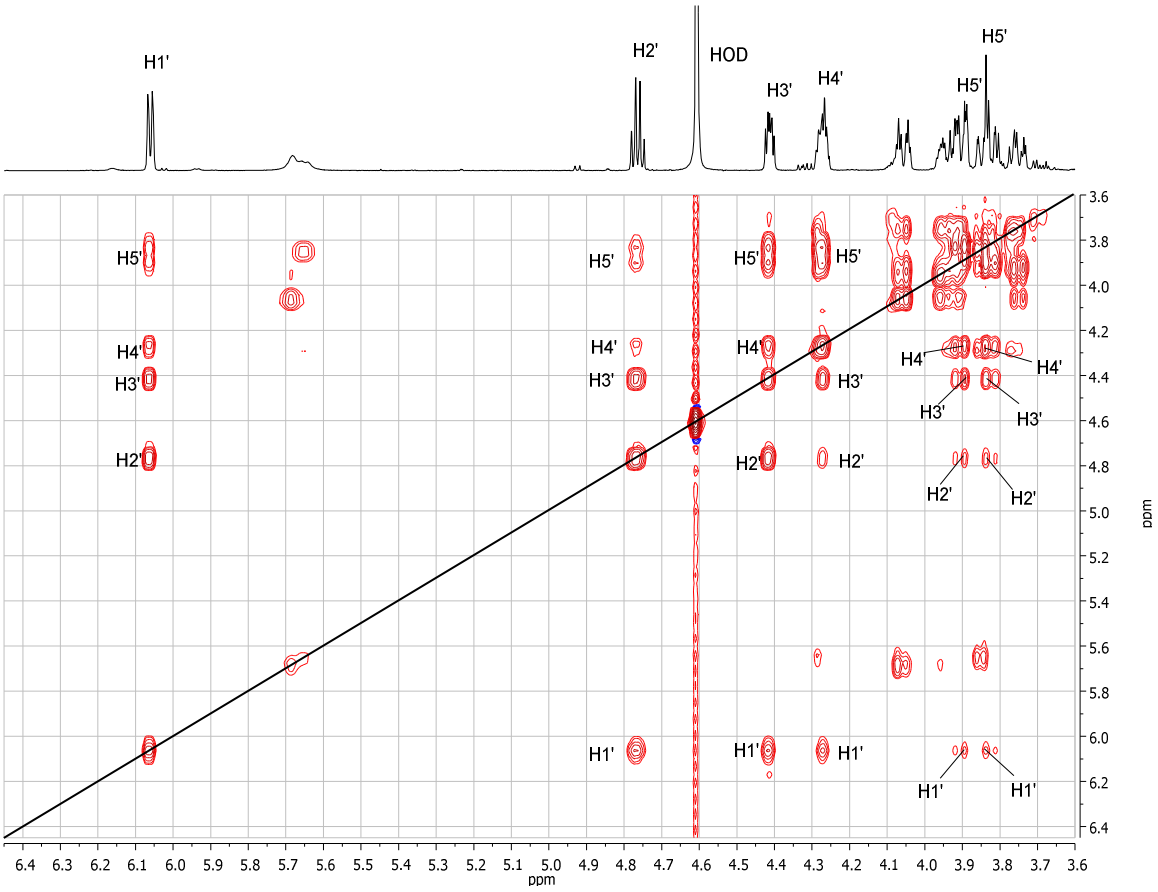

Figure S2:TOCSY NMR spectrum N^6-(D-ribos-1-yl)-adenosine in D_2O at T = 40 °C in the aliphatic region. See Figure 1 for the numbering scheme.

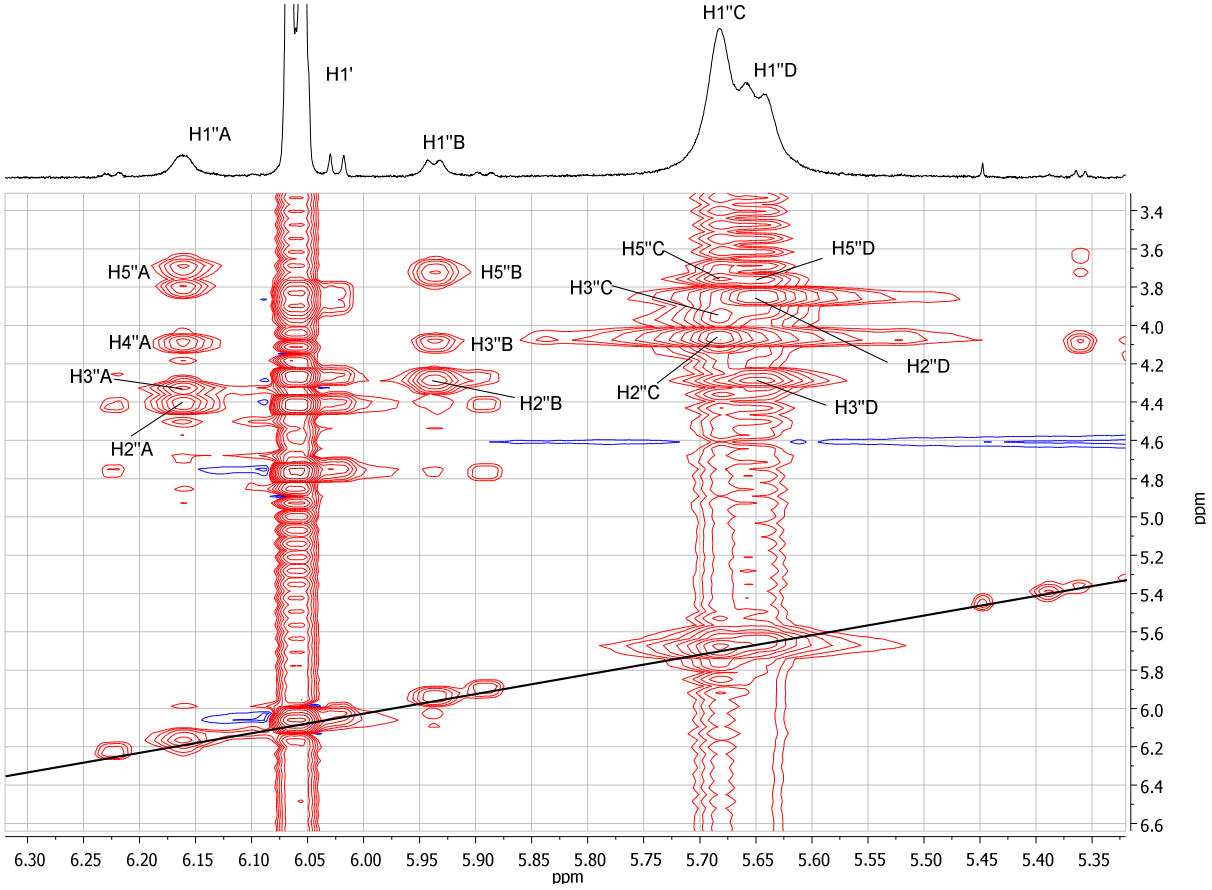

Figure S3:TOCSY NMR spectrumofH1"protonsofN^6-(D-ribos-1-yl)-adenosine in D_2O at T = 40 °C. See Figure 1 for the numbering scheme.

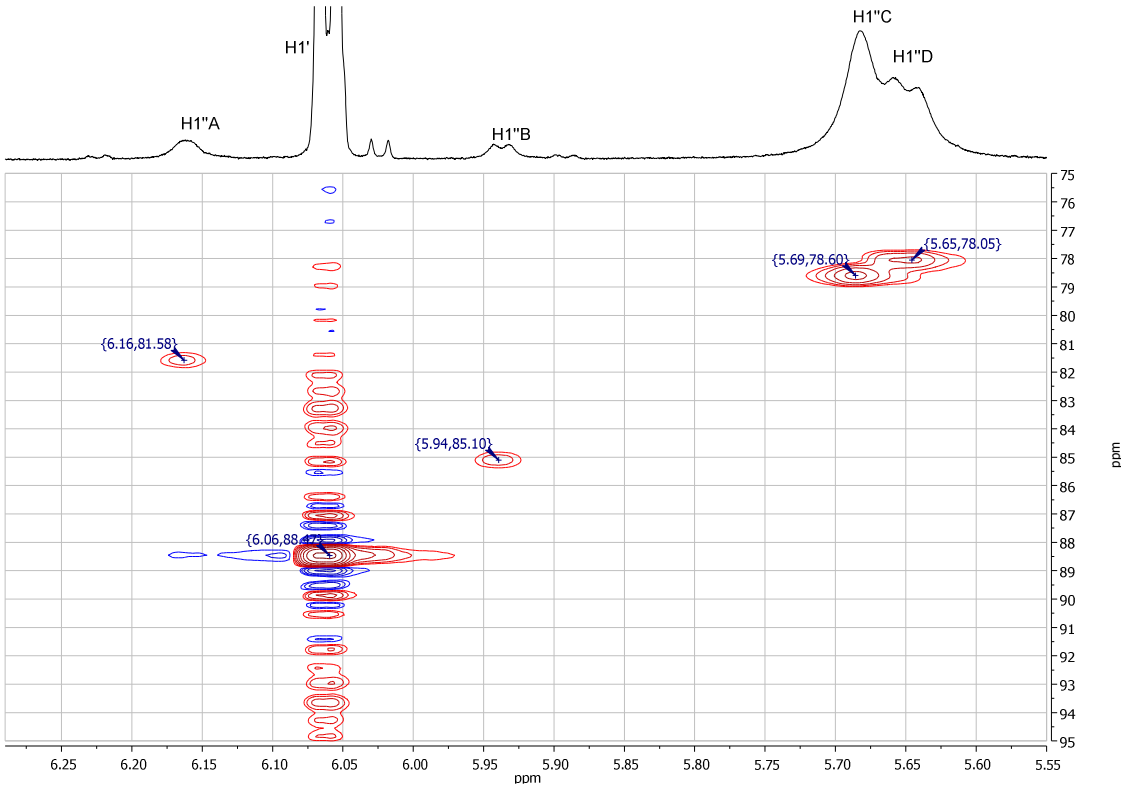

Figure S4: HSQC spectrum in the region of the anomeric resonances of N^6-(D-ribos-1-yl)-adenosine in D_2O at T = 40 °C.

NMR chemical shifts and correlations for of N^6-(D-ribos-1-yl)-adenosine in D_2O at T = 40 °C

Table S1: adenine

Proton	^{1}H (δ)	HSQC (δ)
H2	8.33 s 8.32 s 8.30 s 8.28	CH 152.6 CH 152.5 CH-152.4 -
H8	8.35 s 8.35 s 8.34 s -	CH 141.3 - CH 141.2 -

Table S2: N^9-β-D-ribose

Proton	^{1}H (δ, *J* in Hz)	H-H COSY	HSQC (δ)	TOCSY
H1'	6.06 dd 1H (5.9, 1.3)	4.76 H2'	CH 88.5	4.76 H2' 4.41 H3' 4.27 H4' 3.90 H5'a 3.83 H5'b
H2'	4.76 q 1H (5.7)	4.41 H3'	CH 73.8	6.06 H1' 4.41 H3' 4.27 H4' 3.90 H5'a 3.83 H5'b
H3'	4.41 m 1H	4.27 H4'	CH 70.6	6.06 H1' 4.76 H2' 4.27 H4' 3.90 H5'a 3.83 H5'b
H4'	4.27 m 1H	3.86 H5'	CH 85.8	6.06 H1' 4.76 H2' 4.41 H3' 3.90 H5'a 3.83 H5'b
H5'a	3.90 d 1H	4.27-3.91 4.27-3.89 H4'	CH_2 61.6 61.6	6.06 H1' 4.76 H2' 4.41 H3' 4.27 H4'
H5'b	3.82 d 1H	4.27-3.83 4.27-3.81 H4'	CH_2 61.6 61.6	6.06 H1' 4.76 H2' 4.41 H3' 4.27 H4'

Table S3:N^6-ribose (A, B, C, and D isomers)

Proton	^{1}H (δ, *J* in Hz)	H-H COSY	HSQC δ	TOCSY
H1''A	6.16 br s 0.07H	4.41 H2"A	CH 81.6 consistent with a 5 member ring	4.41 H2"A 4.33 H3"A 4.09 H4"A 3.76 H5"A
H1''B	5.94 br d 0.05H (5Hz)	4.30 H2"B	CH 85.1 consistent with a 5 member ring	4.29 H2"B 4.09 H3"B 3.73 H5"B
H1''C	5.68 br s	4.05 H2"C	CH 78.6 consistent with a 6 member ring	4.05 H2"C 3.96 H3"C 3.76 H5"C
H1''D	5.65 br d (8 Hz)	3.85 H2"D	CH 78.1 consistent with a 6 member ring	4.29 H3"D 3.85 H2"D 3.73 H5"D
H2''A	4.41		CH 73.9	
H2''B	4.29		CH 74.0	5.65 H1"B
H2''C	4.05		CH 69.1	5.68 H1"C 3.94 H3"C 3.75 H5"C
H2''D	3.85		CH 69.4	5.65 H1"D 4.29 H3"D
H3''A	4.33	4.09 H4"A	CH 70.8	6.16 H1"A 4.40 H2"A 4.09 H4"A 3.80 H5"A 3.69 H5"A
H3''B	4.09			
H3''C	3.96		CH 67.4	5.68 H1"C 4.05 H2"C 3.75 H5"C
H3''D	4.29		CH 70.5	
H4''A	4.09	4.31 H3"A	CH 81.9 (or 83.7)	4.31 H3"A
H4''B	4.09		CH 83.7 (or 81.9)	4.31 H3"B
H4''C	4.07		CH 69.3	5.68 H1"C 3.94 H3"C 3.75 H5"C
H4''D	3.86	4.27 H3"D	CH 69.4	5.65 H1"D 4.29 H2"D
H5''A	3.70 a (3.71,3.68)		CH_2 61.7	6.16 H1"A 4.40 H2"A 4.31 H3"A 4.08 H4"A
	3.76 b (3.77,3.74)			
H5''B	3.91 a (3.92,3.89)		CH_2 63.0	
	3.83 b (3.82,3.84)			
H5''C	3.93 a (3.95,3.91)		CH_2 64.3	5.68 H1"C 4.05 H2"C
	3.75 b (3.76,3.74)			5.68 H1"C 4.05 H2"C
H5''D	3.85 a (3.84,3.82)		CH_2 63.6	5.65 H1"D
	3.76 b (3.77,3.74)			

LC/MS analysis of N^6-(D-ribos-1-yl)-adenosine

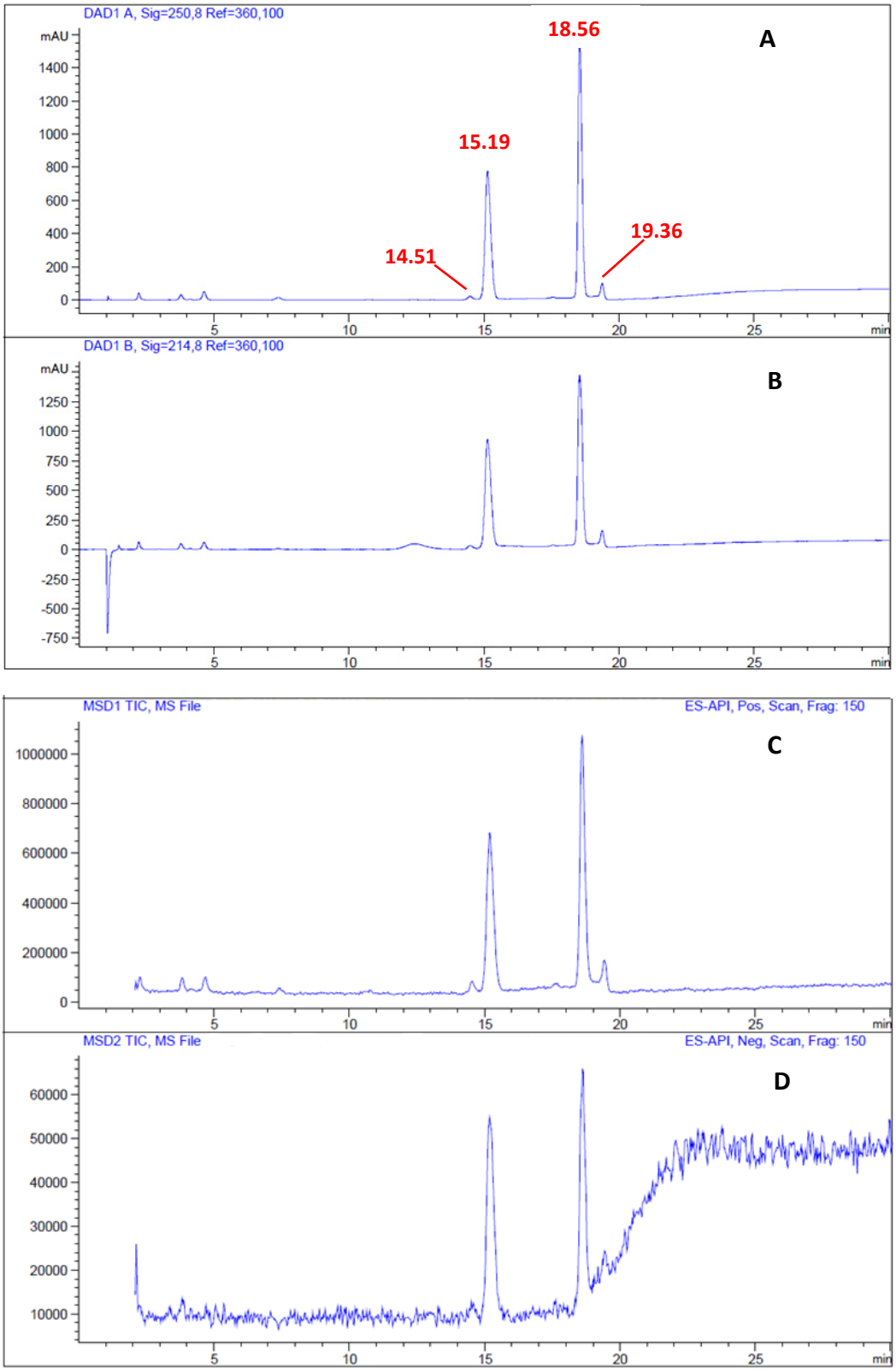

Figure S5: LC/MS analysis: **A**) chromatogram atλ 250.8 nm; **B**) chromatogram atλ 214.8 nm; **C**) chromatogram TIC, positive mode; **D**) chromatogram TIC, negative mode.

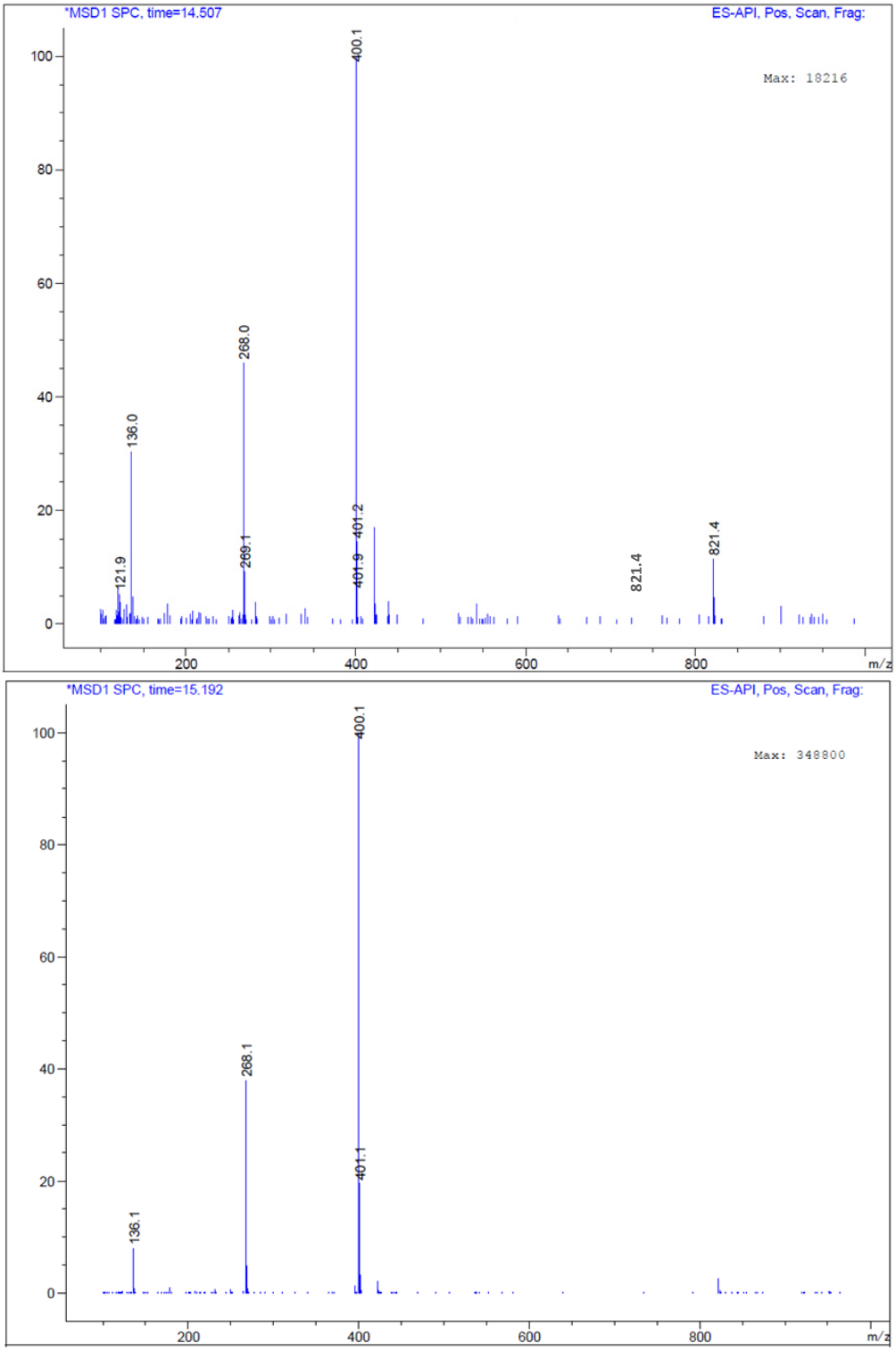

Figure S6:ESI-MS of peaks at R_t 14.51 e 15.19 min, positive mode. m/z 400.1 MH^+, 821.4 [2M+Na^+], 268.0 MH^+ adenosine, 136.0 MH^+ adenine.

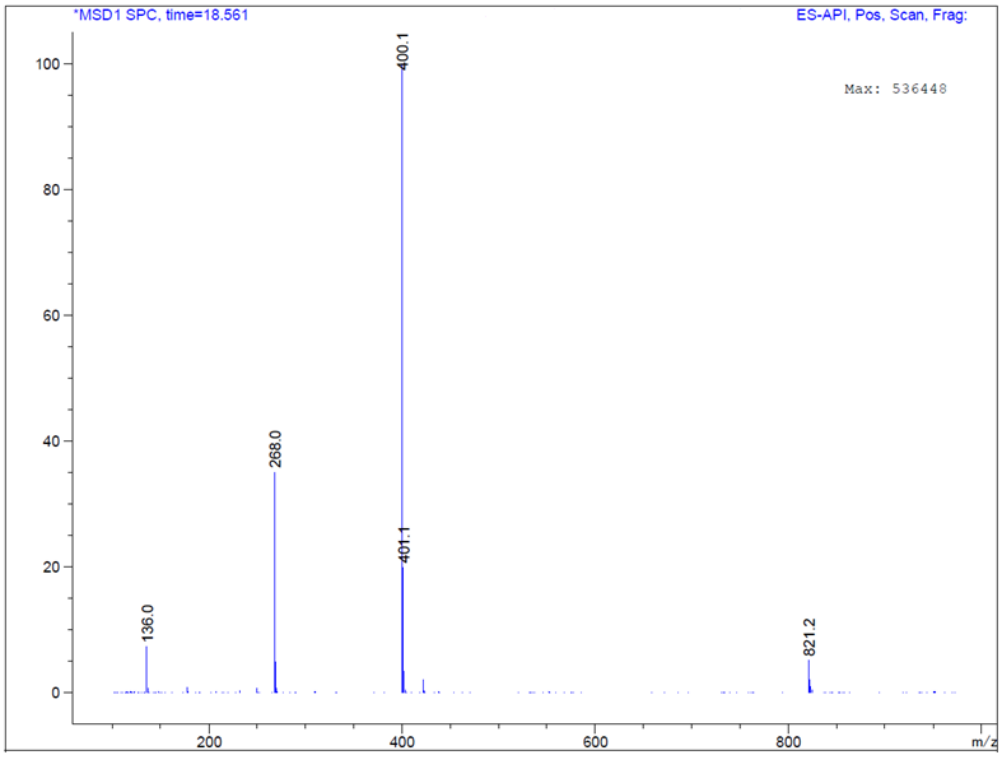

Figure S7: ESI-MS of peaks at R_t 18.56 e 19.36 min, positive mode. m/z 400.1 and 400.0 MH^+, 821.2 $2M+Na^+$, 268.0 MH^+ adenosine, 136.0 e 135.9 MH^+ adenine.

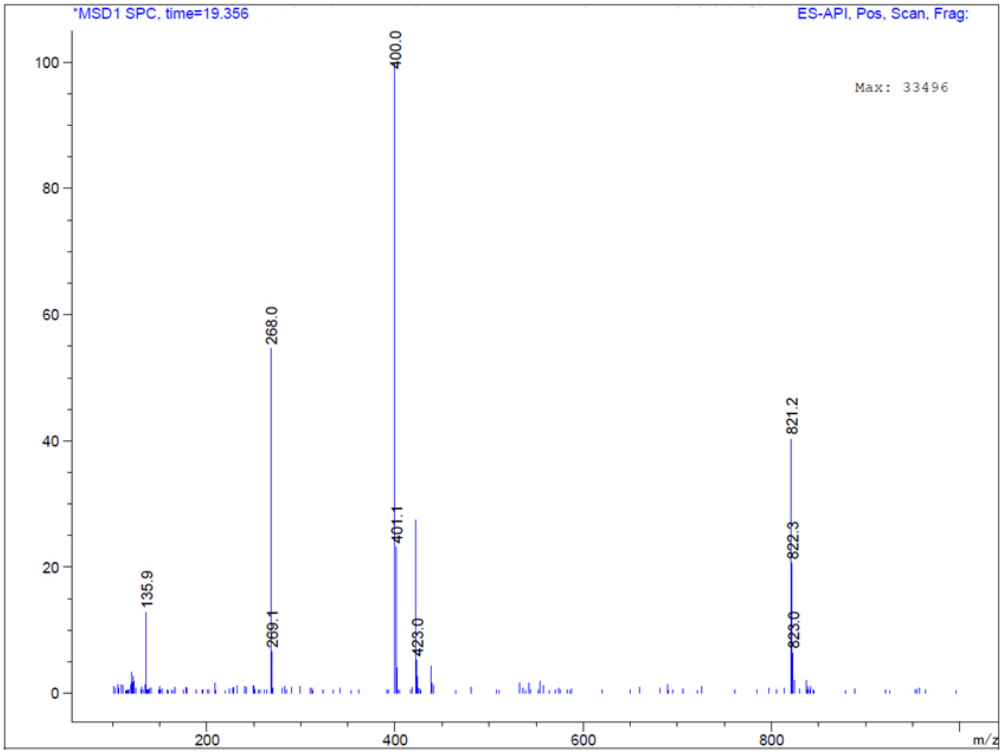

Figure S8: ESI-MS spectrum: m/z 400.2 MH^+; m/z 422.1 $M+Na^+$.

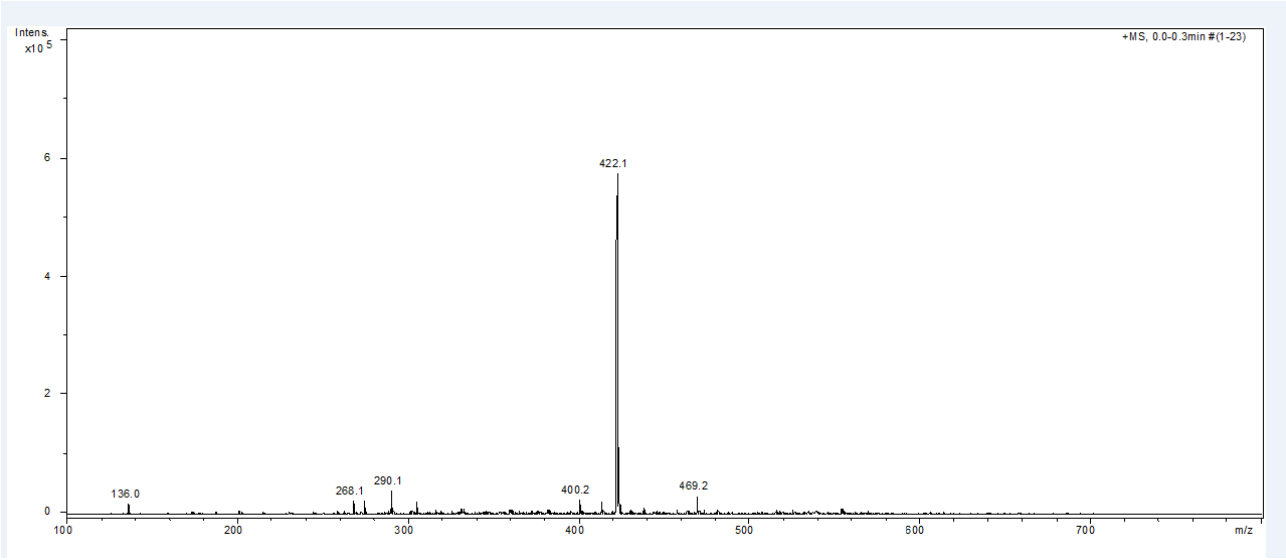

Figure S9: ESI-TOF spectrumexperimental (A) and calculated (B): m/z 422.1281 M+Na^+(calc. 422.1282); m/z 423.1310 M+Na^++1 (calc. 423.1310); m/z 424.1356 M+Na^++2 (calc. 424.1331).

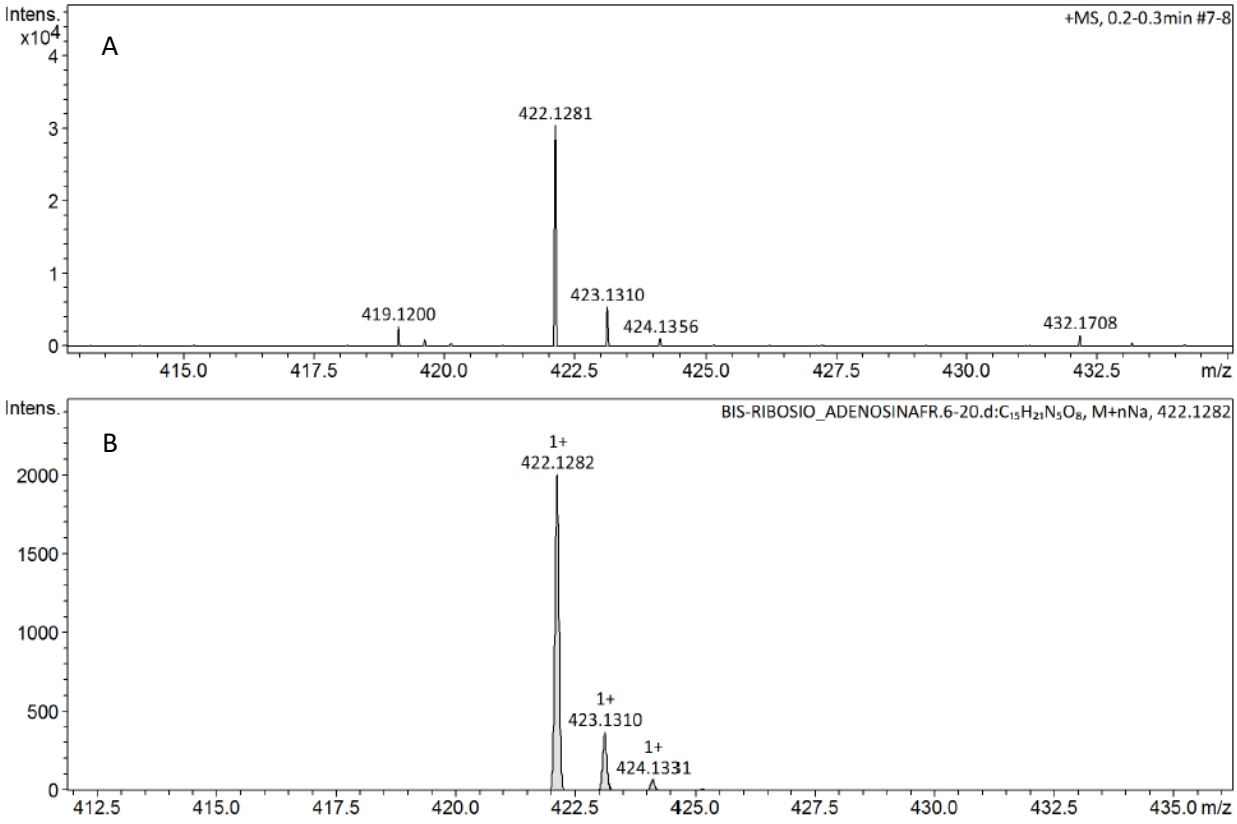

Figure S10. Per-residue interaction energy heatmap encompassing the recognition features of the best docking pose for each of the four solution species of N6-(D-ribos-1-yl)-adenosine and for the crystal adenosine within the orthosteric binding site of adenosine receptor A2A (PDB ID: 2YDO). The vertical axis reports the compound name, while the horizontal axis reports the protein residue name. The upper plot depicts a per-residue decomposition of the electrostatic interaction energy, with colors ranging from blue (negative, thus attractive, interaction energy value) to red (positive, therefore repulsive, interaction energy value). The lower plot, instead, illustrates a per-residue decomposition of the hydrophobic interaction contribution to the total interaction energy, ranging from white (low intensity) to dark green (high intensity).

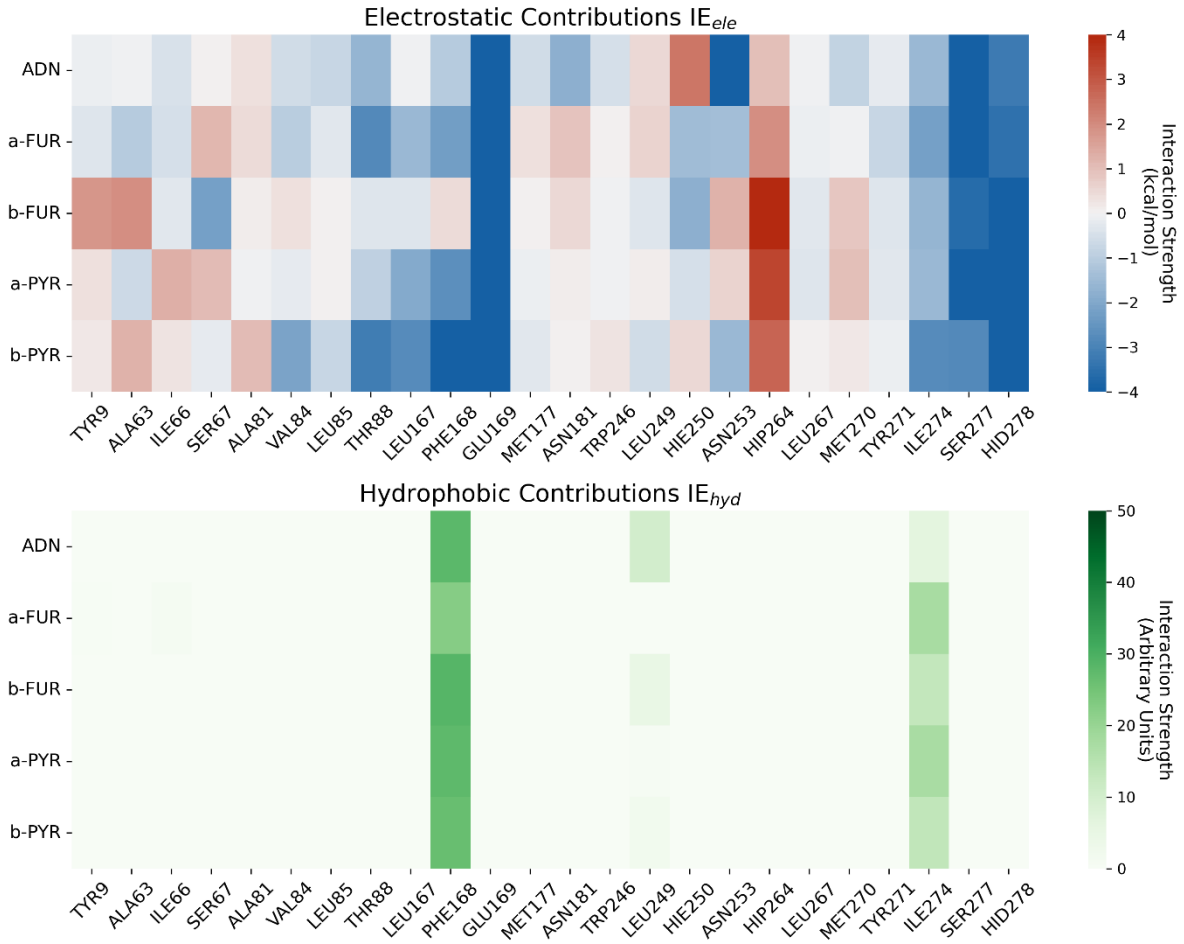

Figure S11. Per-residue interaction energy heatmap encompassing the recognition features of the best docking pose for each of the four solution species of N6-(D-ribos-1-yl)-adenosine and the reference binding pose for adenosine within the orthosteric binding site of adenosine receptor A3 (homology model). The vertical axis reports the compound name, while the horizontal axis reports the protein residue name. The upper plot depicts a per-residue decomposition of the electrostatic interaction energy, with colors ranging from blue (negative, thus attractive, interaction energy value) to red (positive, therefore repulsive, interaction energy value). The lower plot, instead, illustrates a per-residue decomposition of the hydrophobic interaction contribution to the total interaction energy, ranging from white (low intensity) to dark green (high intensity).

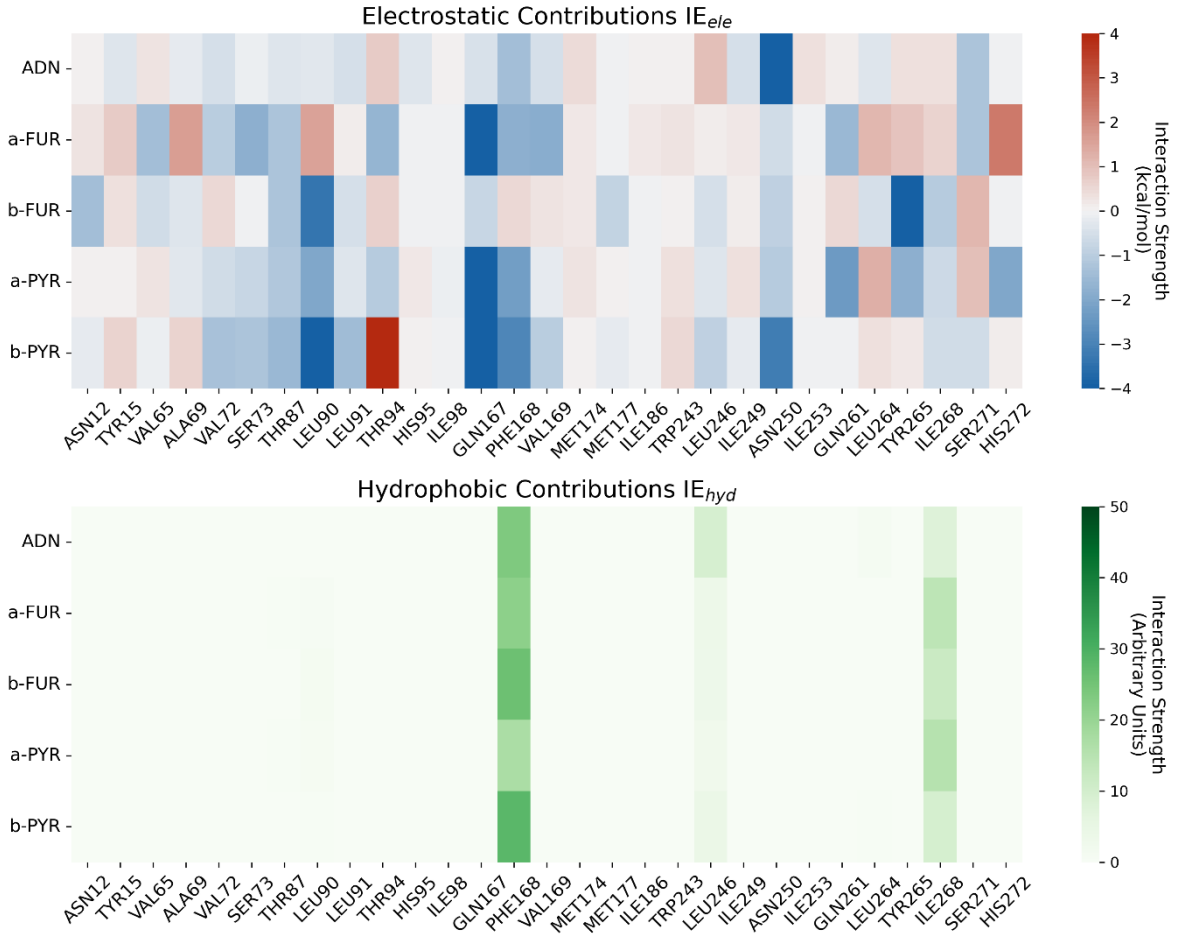

Figure S12. This panel reports the superposition of the best docking pose within the orthosteric binding site of the A2B adenosine receptor for each of the four solution species of N6-(D-ribos-1-yl)-adenosine (magenta) and the reference binding pose of adenosine (grey). A) α-FUR. B) β-FUR. C) α-PYR. D) β-PYR.

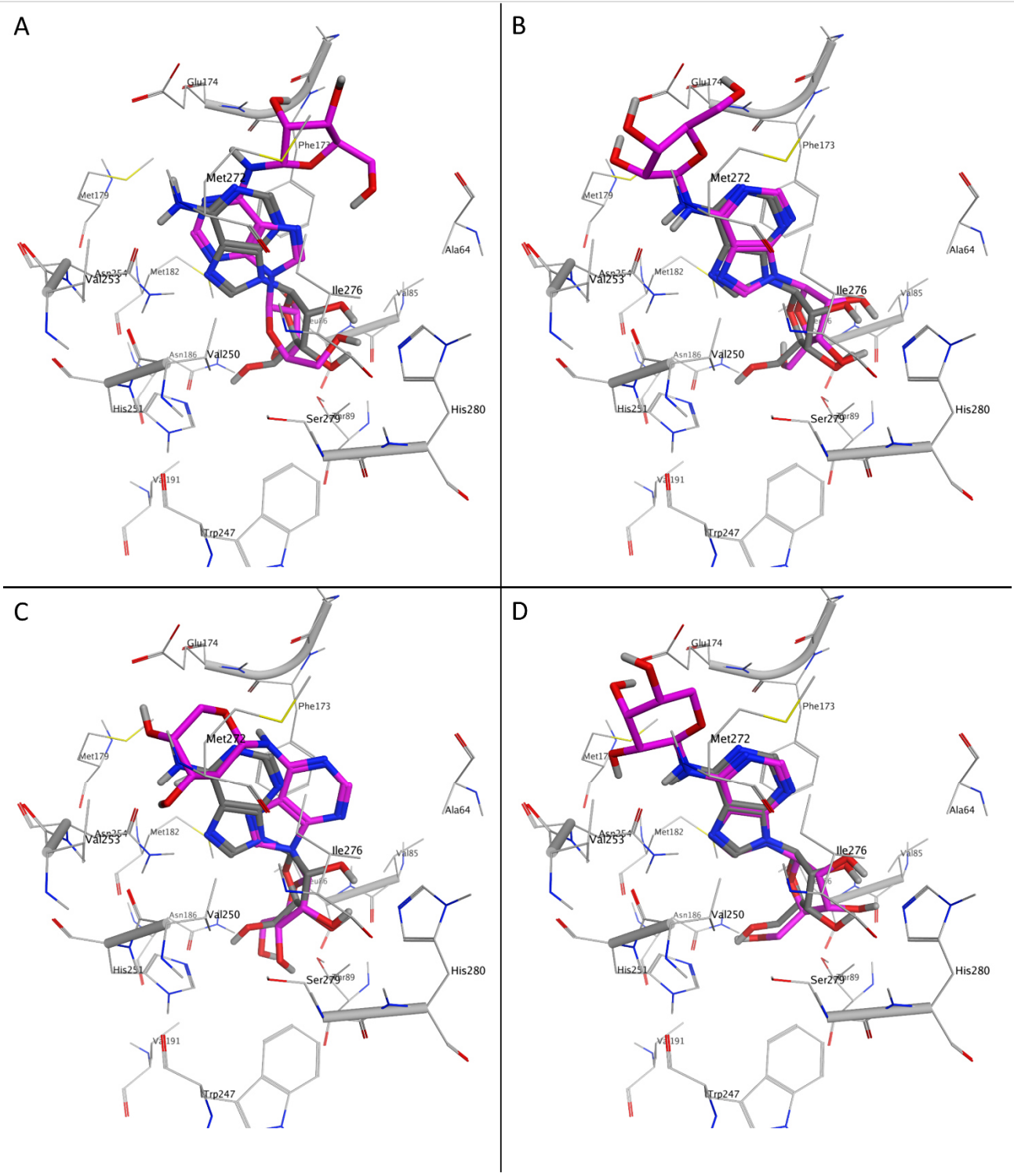

Figure S13. Per-residue interaction energy heatmap encompassing the recognition features of the best docking pose for each of the four solution species of N6-(D-ribos-1-yl)-adenosine and for the crystal adenosine within the orthosteric binding site of adenosine receptor A2B (PDB ID: 8HDP). The vertical axis reports the compound name, while the horizontal axis reports the protein residue name. The upper plot depicts a per-residue decomposition of the electrostatic interaction energy, with colors ranging from blue (negative, thus attractive, interaction energy value) to red (positive, therefore repulsive, interaction energy value). The lower plot, instead, illustrates a per-residue decomposition of the hydrophobic interaction contribution to the total interaction energy, ranging from white (low intensity) to dark green (high intensity).