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Bis(6-carboxypurine)-Cu^{2+}: A Possibly Primitive Metal-Mediated Nucleobase Pair

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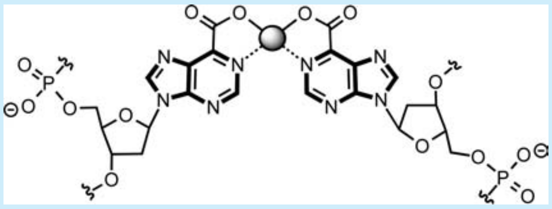
Supporting Information

ABSTRACT: A metal-mediated self-pair is described that emulates Watson−Crick base pair properties in a DNA double helix.



Although the prebiotic origins of nucleic acids have been studied for more than half a century, it remains unclear whether nucleotides are more easily derived from serial condensations of integral subcomponents or from organic processes.[1] Oro's discovery that hydrogen cyanide oligomerizes over the course of days to generate adenine was an early testament to the abiotic accessibility of purine nucleobase constituents.[2] While meteoritic amino acids have been long established,[3] unequivocal evidence for extraterrestrial nucleobases is recent.[4] In particular, the detection of terrestrially rare nucleobases in a wide range of carbonaceous meteorites notably expands the inventory of molecules that would have been available on the early Earth and elsewhere during periods of heavy bombardment.[4,5] Within this expanded inventory, nucleobases capable of association by hydrophobic or metal-mediated interactions are anticipated, in addition to hydrogen bonding.

Metal-ion-mediated base pairing was reported initially for nucleosides[6] but rapidly expanded to the formation of double helices by oligonucleotides. A large number of metal-mediated base pairs now exist where purine and pyrimidine nucleobases are replaced by various ligands.[7−9] We have sought to explore metal-mediated base pairs that retain a core purine or pyrimidine. Our efforts have resulted in several noncanonical nucleobase pair motifs that coordinate to different types of metal ions.[8] Herein we characterize one of the simplest noncanonical motifs with a natural nucleobase core to date, the 6-carboxypurine (Pur^C) self-pair with Cu^{2+} (Figure 1a).

The synthesis of 6-carboxypurine-2′-deoxyriboside phosphoramidite (**9**) in a protected form suitable for solid-phase DNA synthesis is shown in Scheme 1. Synthesis commenced with 2′-dA, from which 6-iodoadenine deoxyriboside **2** was prepared employing the photoinduced diazotization—deamination of Nair and Richardson.[10] Introduction of a carboxylic acid group at the 6-position was accomplished by way of a Negishi cross-coupling between (benzoyloxymethyl)zinc iodide and **2** according to the general method of Silhar et al.,[11] followed by debenzoylation and oxidation[12] of the resultant 6-hydroxymethyl group. Methyl esterification of carboxylic acid **5** followed by desilylation gave 6-carboxymethylpurine nucleoside **7**, which could be converted into phosphoramidite **9** for use in DNA synthesis.

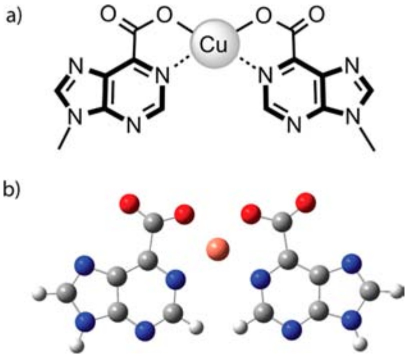


Figure 1. (a) Pur^C self-pair with Cu^{2+} studied in this work. (b) BP86/def-SV(P) geometry of $Pur^C \cdot Cu^{2+} \cdot Pur^C$ (Turbomole, version 5.9).

Two mutually complementary oligonucleotides containing Pur^C, d-5′-CACATTAPur^{C}TGTTGTA-3′ (**10**) and 5′-d-TACAACA-Pur^{C}TAATGTG (**11**), were prepared on a PE Biosystems, Expedite 8909 DNA synthesizer using phosphoramidite **9**. Deprotection of the oligonucleotides was performed with 0.1 N NaOH to effect saponification of the Pur^C methyl ester to a carboxylate, in addition to removal of the other standard protecting groups, followed by polyacrylamide gel electophoretic purification. MALDI-TOF mass spectrometry was used to confirm the identity of oligomers.

To assess if metallo base pairs of the form Pur^C-M^{n+}-Pur^C might exist, Pur^C-containing oligonucleotides **10** and **11** were thermally denatured in the presence of different metal ions. The duplex T_m values derived from these experiments are depicted in Table 1. Eleven different metal ions were explored. However, only a single metal ion, Cu^{2+}, led to appreciable helix stabilization. In this case, 1 equiv of Cu^{2+} was found to

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Scheme 1. Synthesis of 6-Carboxymethylpurine Phosphoramidite 9

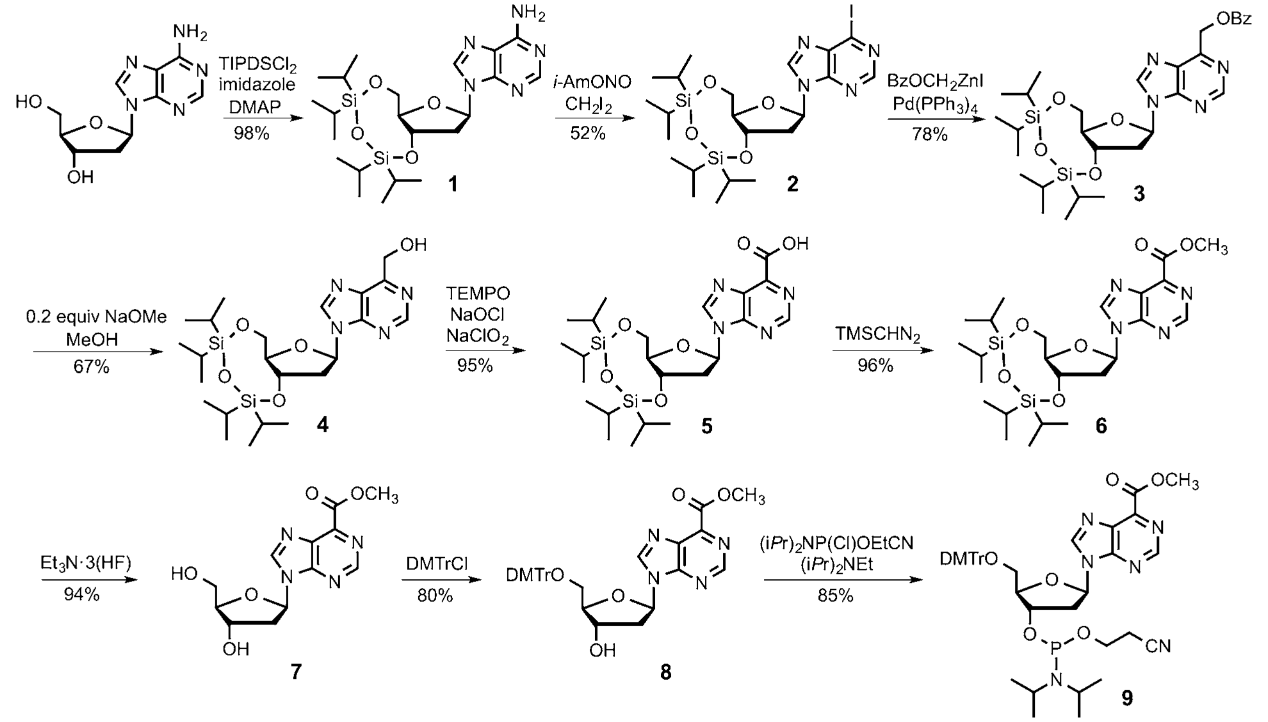

Table 1. DNA Duplex (2.5 μM) Melting Temperatures Measured in 50 mM $NaNO_3$, 10 mM HEPES Buffer (pH 7) and with or without the Indicated Metal Ions

5′-d-CAC ATT A**X**T GTT GTA-3′
3′-d-GTG TAA T**Y**A CAA CAT-5′

entry	X·Y	M^{n+} (equiv)	T_m, °C[a]	ΔT_m[b]
1	Pur^C·Pur^C	—	25.3	−17.3
2	Pur^C·Pur^C	Cu^{2+} (1)	48.0	+5.4
3	Pur^C·Pur^C	Cu^{2+} (2)	46.5	+3.9
4	Pur^C·Pur^C	Cu^{2+} (5)	46.0	+3.4
5	Pur^C·Pur^C	Ni^{2+} (1)	33.6	−9.0
6	Pur^C·Pur^C	Zn^{2+} (1)	32.1	−10.5
7	Pur^C·Pur^C	Co^{2+} (1)	31.1	−11.5
8	Pur^C·Pur^C	Cd^{2+} (1)	27.7	−14.9
9	Pur^C·Pur^C	Ag^{+} (1)	26.5	−16.1
10	Pur^C·Pur^C	Mn^{2+} (1)	22.1	−20.5
11	Pur^C·Pur^C	Fe^{2+} (1)	32.1	−10.5
12	Pur^C·Pur^C	Fe^{3+} (1)	32.0	−10.6
13	Pur^C·Pur^C	Hg^{2+} (1)	31.0	−11.6
14	Pur^C·Pur^C	Mg^{2+} (1)	30.5	−12.1
15	A·T	—	41.0	−1.6
16	A·T	Cu^{2+} (1)[c]	42.6	0.0
17	Pur^C·T	Cu^{2+} (1)	22.6	−20.0
18	A·Pur^C	Cu^{2+} (1)	24.1	−18.5

[a] Estimated error ±1 °C. [b] Difference in T_m relative to that of the natural dA·dT counterpart in the presence of 1 equiv of Cu^{2+} (entry 16). [c] 1 equiv Cu^{2+} was added as a control.

increase the stability of the **10**/**11** duplex by 23 °C relative to the absence of any di- or trivalent ions (entries 2 vs 1) and 7 °C relative to the fully natural duplex containing A/T (entries 2 vs 15). Further, in a control experiment, the natural A/T duplex was found to have effectively the same stability in the presence or absence of 1 equiv of Cu^{2+} (entries 16 vs 15). Finally, duplexes containing mispairs of Pur^C with A or T were highly destabilized relative to the matched duplexes containing A/T or Pur^C-Cu^{2+}-Pur^C (entries 16 and 2 vs 17 and 18). These data support Pur^C-Cu^{2+}-Pur^C base pair formation.

The structure of Pur^C-Cu^{2+}-Pur^C is congruent with a DNA double helix of the natural nucleobases given the greater thermal stability of **10**/**11** (Cu^{2+}) in comparison to the A/T duplex. Indeed, the 5.4 °C stability enhancement for Pur^C-Cu^{2+}-Pur^C relative to A/T is nearly identical to the 5.9 °C stabilization reported for a neutral, bidentate, Cu^{2+} base pair with hydroxypyridone.[7d] To probe further, the structure and energy of Pur^C-Cu^{2+}-Pur^C in a Watson−Crick-like geometry, resulting from an *anti-anti* orientation of the bases, was calculated with density functional theory. The result is depicted in Figure 1b. The N9−N9′ distance in this structure was found to be 10.69 Å, which may be compared to the corresponding 9.05 Å distance found for natural base pairs in B-DNA. Structures and energies were additionally determined for two alternative Hoogsteen geometries that derive from *anti-syn* and *syn-syn* orientations of Pur^C (Supplementary Figure S-1). As these alternative Hoogsteen geometries were, respectively, 6.5 and 14.9 kcal higher in energy than the Watson−Crick geometry (Figure 1b), they are considered relatively unlikely candidates for the structure of Pur^C-Cu^{2+}-Pur^C. We have noted in prior work that Hoogsteen coordination modes presenting donor atoms in a 1,5-relationship to the metal ion can be suboptimal.[8a] Although the Watson−Crick geometry of Pur^C-Cu^{2+}-Pur^C is predicted to be wider than the canonical geometry, the DNA double helix is known to readily accommodate single occurrences of expanded base pairs.[13]

In summary, we have characterized the propensity of Pur^C to form metal-mediated base pairs. The similar stability found for Pur^C-Cu^{2+}-Pur^C and canonical Watson−Crick base pairs in a helix suggests that these motifs are mutually compatible. As the identity of primitive molecules on extraterrestrial bodies widens with future experiments, Pur^C may be identified among them. Independent of such findings, we suggest that the structure of

Pur^{C} is fundamental and warrants further exploration of its properties.

ASSOCIATED CONTENT

Supporting Information

Spectra of all synthetic compounds and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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