Coordination site discrimination in substituted bioessential purine ligands

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The synthesis and crystallographic investigations of N6 and/or N9 substituted adenine analogues with coinage metal ions are described. While N6 disubstitution in an N9-unsubstituted adenine unit results in N3,N9-bidentate coordination mode resulting in a dinuclear motif, further substitution at N9 position directs the incoming metal to get coordinated at N3 position. However, N6,N9-monosubstituted adenine derivative, which relieves some steric hindrance at N6, allows for N7 coordination of copper ions.

Keywords: Coordination chemistry, Metal-nucleobase frameworks, Adenine analogues, Purine ligands, Transition metals, Coinage metals, Silver, Copper

Active interest in investigating metal binding properties of nucleic acids is primarily driven by the interaction of metal ions with heterocyclic nucleobases, which provide suitably positioned centers for metal ion coordination.1 Adenine, a purine nucleobase, allows its exocyclic amino group and four ring nitrogens for coordination, with N1/N7 being the preferred positions. Coordination to N3 is relatively less observed perhaps due to the steric crowding engendered by N9 substituents.2 On the other hand, N9-unsubstituted adenines not only allow for N3 coordination, but also offer N9 as an additional interaction site, invariably leading to dimeric motifs.3 Thus, subtle variation in adenine substitution pattern could serve as metal ion director, affording interesting coordination patterns.

We have been investigating adenine coordination with transition metal ions particularly silver and copper, for the generation of metal-nucleobase frameworks in order to use them as catalysts and as fluorescent sensors.4 We have recently undertaken a systematic study of N9 substituent variation on the coordination behavior of metal ions, thereby affording different structural patterns.5 In the present study, we have investigated crystallographic manifestations of coinage metal interaction with three variably N6-substituted modified adenine analogues, namely, diethyl-(9H-purin-6-yl)-amine (A), propyl-(9-propyl-9H-purin-6-yl)-amine (B), diethyl-(9-propyl-9H-purin-6-yl)-amine (C) (Scheme 1).

Materials and Methods

Synthesis of adenine analogue ligands

Synthesis of diethyl-(9H-purin-6-yl)-amine (A)
Hypoxanthine (0.4 g, 4.57 mmol) was taken in a round-bottom flask followed by the addition of POCl3 (7.5 mL) and triethyl amine (2 mL) and stirred for 15 min. The reaction mixture was then refluxed for 5 h and then POCl3 was removed via distillation. Then the reaction mixture was poured in cold water, while maintaining the pH at 9 to obtain the needle-like crystalline solid (0.500 g, yield 40%). HRMS: (M+H)+ = 192.1249, found 192.1247. M. pt. > 200 °C.

1H NMR (500 MHz, DMSO-d6, 25 ºC, TMS): δ (ppm) 1.14-1.17 (t, 6H), 3.90 (b, 4H), 8.04 (s, 1H), 8.13 (s, 1H), 12.81 (b, 1H).

13C NMR (100 MHz, DMSO-d6, 25 ºC, TMS); δ (ppm) 14.03, 42.94, 118.59, 139.05, 151.27, 152.24, 153.16.
Synthesis of 9-propyl 6-chloropurine

Experimental details for the synthesis of 9-propyl 6-chloropurine have been reported earlier. A similar protocol was followed and the purity of product so obtained was confirmed by spectroscopic analysis.

Synthesis of diethyl-(9-propyl-9H-purin-6-yl)-amine (B)

Diethyl-(9H-purin-6-yl)-amine (0.4 g, 2.09 mmol) was dissolved in DMF (10 mL), followed by the addition of NaH (0.16 g as 60% in paraffin, 2.51 mmol) and stirred under nitrogen atmosphere for 30 min. After this, n-propyl bromide (0.198 mL, 2.09 mmol) was added and stirred overnight under nitrogen atmosphere. Then DMF was evaporated under high vacuum and the compound purified by column chromatography (0.25 g of the liquid compound was obtained). FABMS: (M+H)^+ = 234.1719; found 234.1476. 1H NMR (400 MHz, CDCl_3, 25 °C, TMS): δ (ppm) 0.95-0.97 (t, 3H), 1.27-1.30 (t, 6H), 1.88-1.92 (m, 2H), 3.99 (b, 2H), 4.07-4.11 (t, 3H) 7.68 (s, 1H), 8.17 (s, 1H). 13C NMR (100 MHz, CDCl_3, 25 °C, TMS): δ (ppm) 11.15, 13.46, 23.35, 25.74, 43.07, 57.32, 123.71, 143.16, 156.38, 157.28, 158.31.

Synthesis of propyl-(9-propyl-9H-purin-6-yl)-amine (C)

9-Propyl-6-chloropurine (0.5 g, 2.09 mmol) was dissolved in n-butanol (5 mL), followed by addition of excess n-propylamine and stirring for 15 min. The reaction mixture was refluxed for another 2 h, after which n-butanol was removed by distillation. The compound separated out from water-ethyl acetate solvent as a yellowish liquid (0.360 g, yield 40%). HRMS: [M+H]^+ = 220.1562, found 220.1561. 1H NMR (400 MHz, CDCl_3, 25 °C, TMS): δ (ppm) 0.90-0.94 (t, 6H), 1.20-1.27 (m, 2H), 1.83-1.88 (m, 2H), 3.96 (bs, 2H), 4.07-4.10 (t, 2H), 7.67 (s, 1H), 8.31 (s, 1H). 13C NMR (100 MHz, CDCl_3, 25 °C, TMS): δ (ppm) 11.15, 13.46, 23.35, 25.74, 43.07, 45.28, 119.55, 138.29, 150.40, 152.18, 153.58. Anal. (%): Caled for C_{12}H_{22}N_{2}; C, 61.77; H, 8.21; N, 30.02; found C, 61.69; H, 8.24; N, 30.07.

Synthesis of complexes (1)-(4)

Synthesis of (1)

In a 25 mL round-bottom flask wrapped with aluminium foil, diethyl-(9H-purin-6-yl)-amine (50 mg) was dissolved in methanol (3 mL) and 50 µL of nitric acid was finally added to completely dissolve the compound. To this, saturated silver nitrate solution (3 mL) was added dropwise with stirring, facilitating precipitation of the complex. Stirring was continued further for another one hour and then the precipitate was filtered carefully to avoid interaction with light and washed with water (4×5 mL) and methanol (4×5 mL), to remove traces of unreacted metal salt and ligand. The product so obtained was dried under high vacuum. HRMS [L+Ag]^+ = 596.0444; found 596.0476. M. pt. > 200 °C. 1H NMR: (500 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm) 1.15-1.18 (t, 6H), 3.69 (b, 4H), 8.21 (s, 1H), 8.42 (s, 1H). 13C NMR (100 MHz, DMSO-d_6, 25 °C, TMS); δ (ppm) 18.77, 47.45, 123.71, 143.16, 156.38, 157.28, 158.31.

Synthesis of (2)

In a 25 mL round bottom flask wrapped with aluminium foil, diethyl-(9H-purin-6-yl)-amine (50 mg) was dissolved in methanol (3 mL) and to this, saturated silver nitrate solution (3 mL) was added dropwise with stirring. Although the complex started precipitating out with the addition of silver salt solution, the stirring was continued for another hour. The precipitate was filtered carefully to avoid direct light and washed with water (4×5 mL) and methanol (4×5 mL) to remove any traces of unreacted metal salt and ligand. The product so obtained was dried under high vacuum. HRMS [2L+2Ag]^+ = 596.0444; found 596.0476. M. pt. > 200 °C. 1H NMR: (500 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm) 1.15-1.18 (t, 6H), 3.69 (b, 4H), 8.21 (s, 1H), 8.42 (s, 1H). 13C NMR (100 MHz, DMSO-d_6, 25 °C, TMS); δ (ppm) 18.77, 47.45, 123.71, 143.16, 156.38, 157.28, 158.31.

Synthesis of (3)

Diethyl-(9-propyl-9H-purin-6-yl)-amine (100 mg) was dissolved in methanol (3 mL), followed by addition of saturated copper chloride (in excess) and stirred for 30 min. The solvent was then evaporated and the brownish solid thus obtained was washed with water (4×5 mL) and methanol (4×5 mL) to remove any traces of unreacted metal salt and ligand. The product so obtained was dried under high vacuum. HRMS [2L+2Ag]^+ = 596.0444; found 596.0476. M. pt. > 200 °C. 1H NMR: (500 MHz, DMSO-d_6, 25 °C, TMS) δ (ppm) 0.79-0.83 (t, 3H), 1.16-1.18 (t, 6H), 1.73-1.80 (m, 2H), 3.91 (b, 4H), 4.09-4.12 (t, 4H), 7.82 (s, 1H), 8.17 (s, 1H). 13C NMR (100 MHz, DMSO-d_6, 25 °C, TMS); δ (ppm) 11.30, 13.84, 24.11, 43.80, 45.71, 120.53, 140.45, 150.54, 153.38, 154.55.

Synthesis of (4)

Complex (4) was obtained by the above method using propyl-(9-propyl-9H-purin-6-yl)-amine. HRMS [2M+Cu+H]^+ = 501.2442, found 501.2261;
Crystal structure determination and refinement

X-ray crystal data was collected on a Bruker SMART CCD4 X-ray diffraction instrument using graphite monochromated Mo-Kα radiation (λ = 0.71073Å) at 100 K. The crystal was solved by Direct methods using SIR92 program and refined by using full-matrix least squares on F2 (SHELX97). The structure was expanded using Fourier technique. The propyl group in (4) is disordered and was solved by using various geometrical restraints like SIMU, DELU, SADI, DFIX and refined isotropically. All other non-hydrogen atoms were refined anisotropically. Hydrogen atoms are placed at geometrically idealized positions. Crystal structure refinement parameters are given as Table 1, while Table S1-S4 (Supplementary Data) give H–bonding parameters for complexes (1)-(4).

Results and Discussion

Crystal structure analysis of (1)

Crystals suitable for X-ray diffraction of (1) were grown from its methanolic solution acidified with a little HNO₃. Further refinement of crystal data suggested that it belonged to monoclinic space group P2₁/n. Acidic conditions lead to protonation of N1 and N7 nitrogens and it is likely that prevailing steric effects at N6 substituent prevents the possibility of silver coordination at N1 and N7. Instead, a bidentate coordination mode is observed via N3 and N9 nitrogens resulting in the formation of a dinuclear motif, with an Ag…Ag bond distance of 3.00 Å, which suggests an argentophilic interaction. Each silver ion in the dimeric unit exhibits N3-Ag-N9 coordination, while other valencies are satisfied by two nitrate anions affording a distorted trigonal pyramidal geometry (Fig. 1a). Eventually, charge neutrality of the complex is achieved through...
the presence of five nitrate counteranion and a hydronium ion.

An interesting aspect of this crystal lattice is the formation of a 2D polymeric chain via nitrate bridges bringing two dimeric units together (Fig. 1b). Hydrogen bonding interactions between C8-H of adenine with a nitrate group of adjacent polymeric chain results in the formation of extended lattice, while weak stacking interactions are also observed between two adjacent adenine moieties further stabilizing the crystal lattice (Fig. 1c).

Crystal structure analysis of (2) and (3)

Formation of complex (2) was achieved by mixing methanolic solution of ligand B with saturated methenolic solution of silver nitrate, which resulted in a white precipitate. It was dissolved in acetonitrile and crystals suitable for X-ray diffraction were obtained after a period of one week, under slow evaporation. Careful inspection of the crystal data revealed that the crystal belonged to monoclinic space group $C2/c$. Similarly, green colored good quality single crystals for complex (3) were obtained by slow evaporation of saturated methanolic solution of ligand B and CuCl$_2$. Further refinement of the crystal data suggested that these crystals belonged to monoclinic space group $P21/c$. Considering the coordination mode exhibited in (1), we surmised that N9-alkylation would probably direct the incoming metal ion to N3 position, a site exposed in the minor groove of double helical DNA. While small molecule minor groove binders have greater affinity for N3 of adenine nucleobase, the corresponding metal coordination at this site is relatively unusual.$^6$ Prior investigation with relevant model systems suggests that steric bulk of the substituents in the purine ring$^7$ or chelating groups could facilitate N3 coordination.$^8$ As expected, the solid state structures of silver and copper complexes of B (2 and 3, respectively) reveals N3-M-N3 coordination affording discrete mononuclear dimeric complexes (Fig. 2).

The asymmetric unit of (2) comprised a silver ion coordinated to N3 nitrogen atoms of two modified purine ligands and two nitrate groups, thereby acquiring a distorted tetrahedral geometry around the silver ion (Fig. 2a). The positioning of adenine moieties and the nitrate group prevents adjacent adenine moieties to come in close proximity, thereby prohibiting possibility of stacking interactions. This fact is also supported by larger dihedral angles between the plane passing through the N1, N3, N7 and N9 nitrogens of the ligands (~81.81°). Hydrogen bonding interactions between the oxygen atom of nitrate group and C8-H of adjacent adenine moiety could also stabilize the crystal lattice (Fig. 2c).

![Fig. 1](image1.png)

![Fig. 2](image2.png)
Similar coordination mode was observed for the copper complex (3), where N3 nitrogen atoms of two different ligands coordinate to copper ions, while the other two coordination sites are occupied by the chloride counteranions. This arrangement results in the formation of mononuclear dimeric unit, leading to a square planar geometry around copper ion with the two adenine moieties facing trans to each other (Fig. 2b). Interestingly, when the crystal lattice is viewed along the a-axis, an extensive network of intermolecular π-π interaction is observed, where a six member ring of adenine moiety stacks with the five member ring of an adjacent adenine moiety with a separation of 4.37Å between the two ring centroids (Fig. 2d). It is likely that geometric requirements of the crystal lattice to accommodate N6 and N9 substituted alkyl chain weakens the stacking interaction.

Crystal structure analysis of (4)

Single crystals of (4) suitable for X-ray diffraction were grown by following the same procedure as for (3). Evaluation of the crystal data revealed triclinic dimeric space group P-1. Preferential N7 coordination mode for adenine is evident in (4) where mono substituent at N6 position adopts an anti orientation, with respect to N7 site, thereby resulting in a discrete mononuclear complex where copper is coordinated to four modified adenine ligands. The asymmetric unit consists of a copper ion coordinated to N7 nitrogen atom of four adenine moieties and one chloride ion, thereby leading to a square pyramidal geometry around copper. Four N7 nitrogen atoms occupy equatorial positions, while the apical position is occupied by the chloride ion (Fig. 3a). Geometrical constraint on the copper center to afford a pentacoordinated mode precludes the possibility of intramolecular base interaction. This is evident from the larger angle formed between the planes passing through N1, N3, N7 and N9 nitrogens of the two adenine moieties present in cis-arrangement in the asymmetric unit (61.18°).

Interestingly, N6-hydrogen atoms are found to be involved in the intramolecular interaction with an axial chloride ion further stabilizing the square pyramidal geometry (Fig. 3a). The bond distance and bond angle for N6H…Cl interaction are in the ranges of 2.36–2.61 Å and 130.97–157.49°, respectively. Finally, in-space hydrogen bonding interaction between solvent molecules, chloride anion and C2H, N1 and C8H of the different adenine moieties results in lattice formation (Fig. 3b).

Conclusions

In conclusion, synthesis and crystallographic investigations of N6 and/or N9 substituted adenine analogues with coinage metal ions are reported. While N6 disubstitution in an N9-unsubstituted adenine unit results in bidentate coordination mode forming dinuclear dimeric entity, further substitution at N9 position of adenine moiety directs the incoming metal to rare coordination at N3 position. However, N6-monosubstitution in 9-substituted adenine reveals preferred N7 coordination for copper ion. Thus, it appears that subtle variation in adenine substitution could dictate coordination preference for metal ions, which could be harnessed for creating interesting architectures.

Supplementary Data

CCDC 767286 (complex 1), 767287 (complex 2), 733871 (complex 3) and 733872 (complex 4) contain the supplementary crystallographic data for this article. Copies of this information can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK. [Fax: +44-1223/336-033; Email: deposit@ccdc.cam.ac.uk]. Other supplementary data associated with this article, viz., crystal structure refinement details, selected hydrogen bond distances and angles and X-ray crystallographic data in CIF format are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_52A(8-9)1041-1046_SupplData.pdf.

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