Formation of Bifunctional Cross-Linked Products Due To Reaction of NAMI-A With DNA Bases – A DFT Study

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Research Article

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Abstract

It is reported that NAMI-A and other Ru-anticancer complexes preferably bind with the N7 site of guanine and can also form DNA inter-strand cross-links. Therefore, in order to understand the DNA cross-link formation capability of NAMI-A, we have investigated here the structure and energetics of the reactions of the G_{N7}NAMI-A (a monofunctional adduct of NAMI-A with the N7 site of guanine) with the N3, N7 and O6 sites of guanine, the N1, N3 and N7 sites adenine, the O2 and N3 sites of cytosine and the O2 and O4 sites of thymine, using the M06-2X functional of density functional theory. It is found that the G_{N7}NAMI-A can form stable cross-linked products at all the sites studied here except at the N3 site of cytosine and O2 site of thymine. The calculated reaction free energies and reaction enthalpies indicate that the N3 site of adenine (A_{N3}) and N7 site of guanine (G_{N7}) are most exothermic among all the studied reactions. This study shows that NAMI-A would favorably form the cross-linked products involving the N7 site of guanine at one side and the N7 site of guanine or the N3 site of adenine at the other side.

1. Introduction

The initiation and development of cancer which is known to be a leading cause of death worldwide is linked with DNA damage caused by a myriad of endogenous and exogenous agents including free radicals, reactive oxygen species (ROS), reactive nitrogen oxide species (RNOS), alkylating agents and ionizing radiation [1–5]. It is reported that these agents react with DNA bases and yield a plethora of modified DNA bases which can result the formation of mispairing of bases, abasic sites, strand-breaks, inter- and intra-strand DNA cross-links and DNA-protein cross-links [1, 4, 6, 5]. In addition to cancer, these DNA lesions, if not repaired, can cause several other lethal consequences including mutation, aging and neurodegenerative diseases [5, 2–4, 6]. Remarkably, anticancer drugs such as cisplatin, temozolamide, mechlorethamine etc. which are used to treat cancer also act by interacting with DNA bases [7–10]. Metal-based anticancer drugs and their interaction and reaction with DNA have gathered a lot of attention of researchers over the past few decades [10, 9, 11–14]. Ruthenium (Ru)-complexes are considered to be one of the most promising alternatives to the prevalent platinum (Pt)-based drugs due to their favourable toxicity, drug resistance and various remarkable properties [12, 15–17]. Therefore, a large number of Ru-complexes have been synthesised and tested for their anticancer activity to date [12, 18]. It is reported that two Ru-complexes, namely, NAMI-A [ImH[trans-RuCl_4(DMSO-S) imidazole(Im)] and KP1019 [InH[trans-RuCl_4(Indazole)_2]] have entered the clinical trials [18, 16, 19]. Ru-based drugs are, in general, believed to act via two mechanisms: activation-by-reduction and hydrolysis. Previous studies [20–28] on the hydrolysis of NAMI-A show that the first hydrolysis where one chloride (Cl^-) ligand of NAMI-A is replaced by a water molecule is faster than its second hydrolysis. The hydrolyzed products of Ru-based drugs such as NAMI-A, ICR and KP1019 are reported to be more reactive towards biological molecules than their parent complexes [20–24, 29–31]. DNA is considered to be the prime pharmacological target for ruthenium and other metal-based anticancer drugs [9–12, 15, 16, 19, 20, 29, 31, 30]. The Ru-based drugs bind favorably to the N7 site of
guanine [30, 29, 16, 19, 20]. Moreover, Ru-based drugs can also bind to other DNA bases such as adenine and cytosine [16, 17, 19, 29, 32]. Novakova et al. [33] showed that Ru-complex, mer-[Ru(III)(terpy)Cl₃], formed DNA inter-strand cross-linking by coordinating specially with isolated guanine residues. P.M. van Vliet et al. [34] showed that the mer-[Ru(III)(terpy)Cl₃] complex formed DNA inter-strand cross-linking in vitro via binding with two guanine bases in trans-configuration. Malina et al. [35] demonstrated that Ru-based drugs such as NAMI, KP1019 and ICR yielded bifunctional inter-strand crosslinks on double helical DNA with guanine as the prevalent binding site. It is noted that NAMI can produce bifunctional adducts affecting the conformation of DNA more efficiently as compared to KP1019 and ICR [35]. Extensive researches have been carried out both experimentally and theoretically to understand the mechanisms of action of Ru-based drugs but its therapeutic effect and specific pharmacological targets are not completely understood [36, 32, 30, 31, 29, 20, 19, 12].

In order to understand the DNA inter-strand cross-link formation capability of NAMI-A, we herein perform the density functional theoretic (DFT) calculations to investigate theoretically the structure and energetics of the formation of cross-linked DNA bases due to the reactions of mono-functional adduct of NAMI-A formed at the N7 site of guanine (hereafter denoted as G₇-NAMI-A) and each of the four DNA bases (as shown in Scheme 1). In view of the fact that Ru-based drugs bind predominantly with the N7 site of guanine and are hydrolyzed before reacting with any target, the G₇-NAMI-A (Fig. 1a) has been chosen as a reactant for the present study. The mechanisms of the formation of mono-functional adduct of NAMI-A at the N7 site of guanine can be found in our previous work [36].

2. Computational Details

The DFT calculations were performed by modelling the Ru(III)-anticancer NAMI-A drug as the \([trans-RuCl₄(DMSO-S)(Imidazole)]^−\) anion. The geometry optimization followed by frequency calculations for all the species involved in Scheme 1 were carried out using the hybrid meta-GGA functional M06-2X [37] and the hybrid basis set (LanL2DZ+6-31G**) in gas and aqueous phases. The effective core potential basis set (LanL2DZ) was used for Ru-atom whereas the Pople’s standard basis set (6-31G**) was used for all other atoms. Each gas (aqueous) phase geometry optimization calculation was further followed by the single-point energy calculation at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas (aqueous) phase. The solvent effect was estimated using the conductor-like polarisable continuum model (CPCM) [38, 39]. The genuineness of the optimized species being total energy minima was confirmed by all positive vibrational frequencies. The gas (aqueous) phase thermal energy corrections determined at 298.15 K were also applied to the total energies obtained by single-point energy calculations to obtain the Gibbs free energies at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas (aqueous) phase. All DFT calculations and visualizations of structure and vibrational modes were done using the Gaussian09 quantum chemistry package [40] and the GaussView [41] program, respectively.

3. Results And Discussion
The optimized geometries of G\textsubscript{N7}-NAMI-A and DNA bases (adenine (A), guanine (G), cytosine (C) and thymine (T)) along with the reaction sites considered for the present study are displayed in Fig. 1. The optimized geometries of the cross-linked products formed by the reaction of G\textsubscript{N7}-NAMI-A at the N1, N3 and N7 sites of adenine as well as the N3, O6 and N7 sites of guanine are displayed in Fig. 2 whereas those formed at the O2 and N3 sites of cytosine and the O2 and O4 sites of thymine are displayed in Fig. 3. The certain optimized bond distances are also displayed in Figs. 2 and 3. The reaction free energies (Δ\textsubscript{G\textit{f}}) and reaction enthalpies (Δ\textsubscript{H\textit{f}}) of reactions of Scheme 1 obtained at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas phase (aqueous media) are presented in Table 1. The Δ\textsubscript{G\textit{f}} (Δ\textsubscript{H\textit{f}}) of a reaction was calculated as the sum of the Gibbs free energies (enthalpies) of the products minus the sum of the Gibbs free energies (enthalpies) of the reactants. The net NPA charges \[42, 43\] (in the unit of magnitude of electronic charge, |e|) on important atoms of cross-linked products calculated at the M06-2X/(LanL2DZ+6-311+G**) level of theory in gas phase (aqueous media) are presented in Table 2. For reactions of G\textsubscript{N7}-NAMI-A with adenine, we note that Ru−Cl bond distances are appreciably larger (by ~0.20−0.33 Å) than the Ru−A\textsubscript{N1/N3/N7} bond distances, the Ru−A\textsubscript{N1}, Ru−A\textsubscript{N3} and A\textsubscript{N7} bond distances in gas phase (aqueous media) being 2.12 (2.13), 2.09 (2.09) and 2.17 (2.16) Å, respectively (Fig. 2). This indicates that as compared with the chloride ligands, the adenine base is more strongly bonded with the Ru-atom in the cross-linked products. Similarly, it is found that all other Ru−DNA base bonds like Ru−G\textsubscript{N3/N7/O6}, Ru−C\textsubscript{O2/O6} or Ru−T\textsubscript{O2/O4} are also stronger than the Ru−Cl bonds as their bond distances are shorter than those of Ru−Cl bonds (Figs. 2 and 3). In order to understand why the Ru−A\textsubscript{N1/N3/N7} bonds are stronger than the Ru−Cl bonds in the products, we examined the net NPA charges on the atoms concerned. It is found that the charges on the reacting sites of DNA bases are significantly more negative than those of Cl atoms in all products (Table 2). For instance, the NPA charges on N1/N3/N7 atoms of adenine are more negative than those on Cl1/Cl2 atoms by ~0.15−0.2 |e| (Table 2). This shows that the Ru−DNA base bonds are stronger due to the greater negative charge on the reactive sites of DNA bases. Based on above discussion, we can conclude that Ru−DNA base bonds formed in the cross-linked products are relatively stable.

The feasibility of formation of cross-linked products can be determined by examining the Δ\textsubscript{H\textit{f}} and Δ\textsubscript{G\textit{f}} values of the reactions. It is apparent from Table 1 that all the studied reactions are exothermic as their Δ\textsubscript{H\textit{f}} values are negative in both gas phase and aqueous media, their values lie in the range from -19.37 to -6.79 (-11.16 to -2.43) kcal/mol in gas phase (aqueous media). Further, the Δ\textsubscript{G\textit{f}} values are also found to be negative for all the reactions in both gas phase and aqueous media, excepting the reaction at the N3 site of cytosine (C\textsubscript{N3}) for which Δ\textsubscript{G\textit{f}} is somewhat positive (0.12 kcal/mol) in aqueous media (Table 1). Although negative, Δ\textsubscript{G\textit{f}} is also very low (-0.31 kcal/mol) for reaction at the O2 site of thymine (T\textsubscript{O2}) in aqueous media. Thus, our calculations indicate that G\textsubscript{N7}-NAMI-A would not form stable products at the N3 site of cytosine and the O2 site of thymine in biological media. However, it can form stable cross-linked products at the A\textsubscript{N1/N3/N7}, G\textsubscript{N3/N7/O6}, C\textsubscript{O2} and T\textsubscript{O4} sites in both gas phase and aqueous media. Considering the aqueous media results more relevant for biological systems, both the Δ\textsubscript{H\textit{f}} and Δ\textsubscript{G\textit{f}} values show that the N3 site of adenine (A\textsubscript{N3}) and N7 site of guanine (G\textsubscript{N7}) are most exothermic among all the
studied reactions (Table 1). The $\Delta G_f (\Delta H_f)$ for reactions at the $A_{N3}$ and $G_{N7}$ are -8.39 (-11.16) and -8.38 (-10.21) kcal/mol, respectively (Table 1). According to the Bell-Evans-Polanyi principle, the activation energies for reactions at the $A_{N3}$ and $G_{N7}$ sites would be very less as compared to the other reaction sites. This shows that NAMI-A would favorably form the cross-linked products involving the N7 site of guanine at one side and the N7 site of guanine or the N3 site of adenine at the other side. This is in agreement with experimental observation that Ru-based drugs such as NAMI, KP1019 and ICR form bifunctional inter-strand crosslinks on double helical DNA with guanine as the prevalent binding site [35]. Based on our present study, we propose the following mechanism (as shown in Fig. 4) for the formation of the cross-linked products from the reaction of NAMI-A with DNA bases.

4. Conclusions

The DNA cross-link formation capability of the Ru-anticancer drug NAMI-A has been evaluated by studying the structure and energetics of the reactions of the $G_{N7}$-NAMI-A (monofunctional adduct of NAMI-A at the N7 site of guanine) at the N1, N3 and N7 sites of adenine; N3, N7 and O6 sites of guanine; O2 and N3 sites of cytosine and O2 and O4 sites of thymine, using density functional theory calculations. It is found that the $G_{N7}$-NAMI-A can form stable cross-linked products at all the sites studied here except at the N3 site of cytosine and O2 site of thymine. The calculated reaction free energies ($\Delta G_f$) and reaction enthalpies ($\Delta H_f$) indicate that the N3 site of adenine ($A_{N3}$) and N7 site of guanine ($G_{N7}$) are most exothermic among all the studied reactions. In aqueous media, the $\Delta G_f (\Delta H_f)$ for reactions at the $A_{N3}$ and $G_{N7}$ sites are -8.39 (-11.16) and -8.38 (-10.21) kcal/mol, respectively. Thus, this study shows that NAMI-A would favorably form the cross-linked products involving the N7 site of guanine at one side and the N7 site of guanine or the N3 site of adenine at the other side, which is in agreement with experimental observation that NAMI-A produce bifunctional cross-linked products with guanine as the prevalent binding site. Based on our present study, we propose that the formation of the cross-linked products from the reaction of NAMI-A with DNA bases would follow the Scheme 2.

Declarations

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References

1. Mishina Y, Duguid EM, He C (2006) Direct reversal of DNA alkylation damage. Chem Rev 106(2):215–232
2. Gates KS (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. Chem Res Toxicol 22(11):1747–1760
3. Cadet J, Douki T, Ravanat J-L (2010) Oxidatively generated base damage to cellular DNA. Free Radic Biol Med 49(1):9–21
4. Meikrantz W, Bergom MA, Memisoglu A, Samson L (1998) O6-alkylguanine DNA lesions trigger apoptosis. Carcinogenesis 19(2):369–372
5. Shukla P, Jena N, Mishra P (2011) Quantum theoretical study of molecular mechanisms of mutation and cancer-a review. Proceedings of the National Academy of Sciences, India-Section A 81 (Part 2):79-98
6. David SS, O’Shea VL, Kundu S (2007) Base-excision repair of oxidative DNA damage. Nature 447(7147):941–950
7. Luo M, Tan Y, Chen W, Hu B, Wang Z, Zhu D, Jiao H, Duan C, Zhu Y, Wang H (2021) Clinical Efficacy of Temozolomide and Its Predictors in Aggressive Pituitary Tumors and Pituitary Carcinomas: A Systematic Review and Meta-Analysis. Front Neurol 12:959
8. Sochacka-Ćwikła A, Mączyński M, Regiec A (2022) FDA-Approved Drugs for Hematological Malignancies—The. Last Decade Review Cancers 14(1):87
9. Galanski M (2006) Recent developments in the field of anticancer platinum complexes. Recent Pat Anti-cancer Drug Discov 1(2):285–295
10. Galanski M, Jakupec MA, Keppler BK (2005) Update of the preclinical situation of anticancer platinum complexes: novel design strategies and innovative analytical approaches. Curr Med Chem 12(18):2075–2094
11. Pages BJ, Ang DL, Wright EP, Aldrich-Wright JR (2015) Metal complex interactions with DNA. Dalton Trans 44(8):3505–3526
12. Allardyce CS, Dyson PJ (2016) Metal-based drugs that break the rules. Dalton Trans 45(8):3201–3209
13. Shah PK, Shukla P (2020) Effect of axial ligands on the mechanisms of action of Ru (III) complexes structurally similar to NAMI-A: a DFT study. Struct Chem 31(2):679–689
14. Shah PK, Shukla P (2020) A DFT study of reactions of Ru (III) anticancer drug KP1019 with 8-oxoguanine and 8-oxoadenine. Struct Chem 31(5):2087–2092
15. Kostova I (2006) Ruthenium complexes as anticancer agents. Curr Med Chem 13(9):1085–1107
16. Antonarakis ES, Emadi A (2010) Ruthenium-based chemotherapeutics: are they ready for prime time? Cancer Chemother Pharmacol 66(1):1–9
17. Bergamo A, Sava G (2011) Ruthenium anticancer compounds: myths and realities of the emerging metal-based drugs. Dalton Trans 40(31):7817–7823
18. Bergamo A, Gaiddon C, Schellens J, Beijnen J, Sava G (2012) Approaching tumour therapy beyond platinum drugs: status of the art and perspectives of ruthenium drug candidates. J Inorg Biochem 106(1):90–99
19. Alessio E, Messori L (2019) NAMI-A and KP1019/1339, two iconic ruthenium anticancer drug candidates face-to-face: a case story in medicinal inorganic chemistry. Molecules 24(10):1995
20. Bacac M, Hotze AC, van der Schilden K, Haasnoot JG, Pacor S, Alessio E, Sava G, Reedijk J (2004) The hydrolysis of the anti-cancer ruthenium complex NAMI-A affects its DNA binding and antimetastatic activity: an NMR evaluation. J Inorg Biochem 98(2):402–412
21. Bouma M, Nuijen B, Jansen MT, Sava G, Flaibani A, Bult A, Beijnen JH (2002) A kinetic study of the chemical stability of the antimetastatic ruthenium complex NAMI-A. Int J Pharm 248(1–2):239–246
22. Chatlas J, Van Eldik R, Keppler B (1995) Spontaneous aquation reactions of a promising tumor inhibitor trans-imidazolium-tetrachlorobis (imidazole) ruthenium (III), trans-Hlm [RuCl4 (Im) 2]. Inorg Chim Acta 233(1–2):59–63
23. Sava G, Bergamo A, Zorzet S, Gava B, Casarsa C, Cocchietto M, Furlani A, Scarcia V, Serli B, Iengo E (2002) Influence of chemical stability on the activity of the antimetastasis ruthenium compound NAMI-A. Eur J Cancer 38(3):427–435
24. Cebrian-Losantos B, Reisner E, Kowol CR, Roller A, Shova S, Arion VB, Keppler BK (2008) Synthesis and reactivity of the aquation product of the antitumor complex trans-[RuIIICl4 (indazole) 2]−. Inorg Chem 47(14):6513–6523
25. Bešker N, Coletti C, Marrone A, Re N (2008) Aquation of the ruthenium-based anticancer drug NAMI-A: A density functional study. J Phys Chem B 112(13):3871–3875
26. Chen J, Chen L, Liao S, Zheng K, Ji L (2007) A theoretical study on the hydrolysis process of the antimetastatic ruthenium (III) complex NAMI-A. J Phys Chem B 111(27):7862–7869
27. Chen J, Chen L, Liao S, Zheng K, Ji L (2007) The hydrolysis process of the anticancer complex [ImH] [trans-RuCl 4 (Im) 2]: a theoretical study. Dalton Transactions(32):3507–3515
28. Vargiu AV, Robertazzi A, Magistrato A, Ruggerone P, Carloni P (2008) The hydrolysis mechanism of the anticancer ruthenium drugs NAMI-A and ICR investigated by DFT–PCM calculations. J Phys Chem B 112(14):4401–4409
29. Brabec V, Nováková O (2006) DNA binding mode of ruthenium complexes and relationship to tumor cell toxicity. Drug Resist Updates 9(3):111–122
30. Pluim D, van Waardenburg RC, Beijnen JH, Schellens JH (2004) Cytotoxicity of the organic ruthenium anticancer drug Nami-A is correlated with DNA binding in four different human tumor cell lines. Cancer Chemother Pharmacol 54(1):71–78
31. Groessl M, Tsybin YO, Hartinger CG, Keppler BK, Dyson PJ (2010) Ruthenium versus platinum: interactions of anticancer metallo-drugs with duplex oligonucleotides characterised by electrospray ionisation mass spectrometry. J Biol Inorg Chem 15(5):677–688
32. Ambrosek D, Loos P-F, Assfeld X, Daniel C (2010) A theoretical study of Ru (II) polypyridyl DNA intercalators: structure and electronic absorption spectroscopy of [Ru (phen) 2 (dppz)] 2+ and [Ru (tap) 2 (dppz)] 2+ complexes intercalated in guanine–cytosine base pairs. J Inorg Biochem 104(9):893–901
33. Novaková O, Kasparkova J, Vrana O, van Vliet PM, Reedijk J, Brabec V (1995) Correlation between cytotoxicity and DNA binding of polypyridyl ruthenium complexes. Biochemistry 34(38):12369–12378
34. van Vliet PM, Toekimin SM, Haasnoot JG, Reedijk J, Nováková O, Vrána O, Brabec V (1995) mer-[Ru (terpy) CI3](terpy= 2, 2′: 6′, 2 ″-terpyridine) shows biological activity, forms interstrand cross-links in DNA and binds two guanine derivatives in a trans configuration. Inorg Chim Acta 231(1–2):57–64
35. Malina J, Novakova O, Keppler BK, Alessio E, Brabec V (2001) Biophysical analysis of natural, double-helical DNA modified by anticancer heterocyclic complexes of ruthenium (III) in cell-free media. J Biol Inorg Chem 6(4):435–445
36. Shah PK, Bhattacharjee K, Shukla PK (2016) Mechanisms of reactions of Ru (iii)-based drug NAMI-A and its aquated products with DNA purine bases: a DFT study. RSC Adv 6(114):113620–113629
37. Zhao Y, Truhlar DG (2008) The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. Theor Chem Acc 120(1):215–241
38. Barone V, Cossi M (1998) Quantum calculation of molecular energies and energy gradients in solution by a conductor solvent model. The Journal of Physical Chemistry A 102(11):1995–2001
39. Cossi M, Rega N, Scalmani G, Barone V (2003) Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model. J Comput Chem 24(6):669–681
40. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich AV, Bloino J, Janesko BG, Gomperts R,
Table 1: Reaction free energies ($\Delta G_f$) and reaction enthalpies ($\Delta H_f$) of reactions of Scheme 1 obtained at the M06-2X/(LanL2DZ+6-311+G**) level of theory in Gas Phase (Aqueous Media)

| Reaction Site | $\Delta G_f$ | $\Delta H_f$ |
|---------------|--------------|--------------|
| Gn7-NAMI-A_AN1 | -7.64 (-4.93) | -12.33 (-8.13) |
| Gn7-NAMI-A_AN3 | -11.55 (-8.39) | -15.28 (-11.16) |
| Gn7-NAMI-A_AN7 | -2.08 (-1.22) | -6.79 (-4.74) |
| Gn7-NAMI-A_GN3 | -5.52 (-4.64) | -9.49 (-7.19) |
| Gn7-NAMI-A_GO6 | -14.28 (-4.77) | -17.06 (-6.95) |
| Gn7-NAMI-A_GN7 | -16.52 (-8.38) | -19.37 (-10.21) |
| Gn7-NAMI-A_C02 | -9.97 (-1.65) | -13.64 (-5.37) |
| Gn7-NAMI-A_CN3 | -9.87 (0.12) | -13.77 (-3.64) |
| Gn7-NAMI-A_TO2 | -4.01 (-0.31) | -7.43 (-2.43) |
| Gn7-NAMI-A_TO4 | -5.36 (-2.28) | -7.18 (-4.13) |

Table 2: The net NPA Charges (in the unit of magnitude of electronic charge) of some important atoms calculated at M06-2X/(LanL2DZ+6-311+G**) level of theory in Gas phase (Aqueous media)
| Reaction site | Ru    | Cl1   | Cl2   | N     | S     | G_{N7} | DNA bases |
|--------------|-------|-------|-------|-------|-------|--------|-----------|
| G_{N7}-NAMI-A_{A_345} | 0.094 (0.129) | -0.318 (-0.355) | -0.361 (-0.397) | -0.448 (-0.458) | 1.456 (1.438) | -0.452 (-0.464) | -0.520 (-0.530) |
| G_{N7}-NAMI-A_{A_345} | 0.089 (0.123) | -0.327 (-0.358) | -0.364 (-0.401) | -0.452 (-0.462) | 1.462 (1.446) | -0.456 (-0.465) | -0.498 (-0.501) |
| G_{N7}-NAMI-A_{A_347} | 0.107 (0.141) | -0.315 (-0.339) | -0.323 (-0.379) | -0.447 (-0.461) | 1.450 (1.431) | -0.452 (-0.460) | -0.460 (-0.468) |
| G_{N7}-NAMI-A_{G_33} | 0.109 (0.163) | -0.265 (-0.325) | -0.402 (-0.398) | -0.443 (-0.445) | 1.434 (1.407) | -0.442 (-0.453) | -0.570 (-0.572) |
| G_{N7}-NAMI-A_{G_310} | 0.192 (0.209) | -0.349 (-0.359) | -0.336 (-0.387) | -0.421 (-0.431) | 1.415 (1.416) | -0.428 (-0.439) | -0.625 (-0.648) |
| G_{N7}-NAMI-A_{G_310} | 0.118 (0.172) | -0.337 (-0.386) | -0.336 (-0.378) | -0.432 (-0.427) | 1.438 (1.420) | -0.439 (-0.455) | -0.440 (-0.448) |
| G_{N7}-NAMI-A_{C_36} | 0.236 (0.273) | -0.307 (-0.382) | -0.393 (-0.427) | -0.428 (-0.427) | 1.422 (1.406) | -0.442 (-0.457) | -0.607 (-0.633) |
| G_{N7}-NAMI-A_{C_36} | 0.137 (0.169) | -0.277 (-0.346) | -0.419 (-0.428) | -0.440 (-0.433) | 1.438 (1.425) | -0.443 (-0.462) | -0.592 (-0.594) |
| G_{N7}-NAMI-A_{T_38} | 0.165 (0.195) | -0.298 (-0.354) | -0.392 (-0.419) | -0.435 (-0.439) | 1.432 (1.420) | -0.423 (-0.431) | -0.618 (-0.628) |
| G_{N7}-NAMI-A_{T_38} | 0.172 (0.195) | -0.359 (-0.385) | -0.338 (-0.394) | -0.435 (-0.440) | 1.446 (1.434) | -0.426 (-0.430) | -0.613 (-0.682) |

**Schemes**

Schemes 1 and 2 are available in the Supplemental Files section.

**Figures**

The optimized geometries of G_{N7}-NAMI-A and DNA bases (adenine (A), guanine (G), cytosine (C) and thymine (T)) along with the reaction sites studied here, as obtained at M06-2X/(LanL2DZ+6-31G**) level of theory in gas phase.
Figure 2

The optimized geometries of the cross-linked products formed by the reaction of G_N7-NAMI-A at the N1, N3 and N7 sites of adenine as well as at the N3, O6 and N7 sites of guanine along with their certain interatomic distances (Å), as obtained at the M06-2X/(LanL2DZ+6-31G**) level of theory in gas phase (aqueous media)
Figure 3

The optimized geometries of the cross-linked products formed by the reaction of \( G_{N7} \)-NAMI-A at the O2 and N3 sites of cytosine as well as at the O2 and O4 sites of thymine along with their certain interatomic distances (Å), as obtained at M06-2X/(LanL2DZ+6-31G**) level of theory in gas phase (Aqueous media)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.png
- Scheme2.png