Pergamon

Tetrahedron Letters 41 (2000) 10035–10039

TETRAHEDRON LETTERS

Synthesis and duplex stability of oligonucleotides containing 7-vinyl-7-deazaguanine as a strong electron-donating nucleobase

Akimitsu Okamoto, Toshiji Taiji, Kazuo Tanaka and Isao Saito*

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-8501, Japan, and CREST, Japan Science and Technology Corporation

Received 16 October 2000; accepted 18 October 2000

Abstract

We synthesized 7-vinyl-7-deazaguanine (^{vz}G), an N7-modified guanine base, which has a smaller oxidation potential than G and 7-deazaG. The incorporation of ^{vz}G into oligonucleotides was achieved by phosphoramidite chemistry on a DNA synthesizer. The T_m of the duplex containing ^{vz}G was higher than that of the corresponding duplex containing G or 7-deazaG. The stabilization of the duplex containing ^{vz}G is due to the favorable enthalpy change by increased π-stacking between the vinyl group and the 5′-flanking base. © 2000 Published by Elsevier Science Ltd.

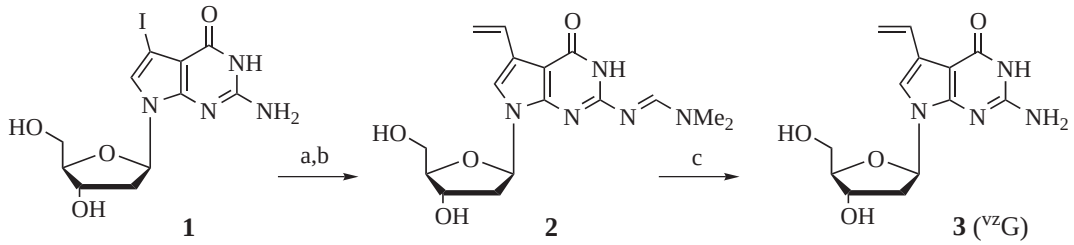
The π-stacking of DNA bases plays an important role in duplex stabilization. Recently, the importance of π-stacking of hydrophobic nucleobases in the stabilization of duplex was noted by Kool[1–3] and Schultz.[4,5] For example, Kool et al. suggested that stacking surface area would strongly affect the duplex stability of oligonucleotides containing hydrophobic dangling bases.[3] Extended and well-overlapped π-stacking is also important in long-range charge transport through DNA duplex.[6–10] Efficiency of charge transport is strongly affected by π-stacking in DNA structure such as A-form DNA,[11] DNA containing bulge sequence[12] and mismatched DNA.[13] Furthermore, the expansion of π-stacking area lowers the ionization potentials of stacked bases.[14] The extension of the π-conjugate system of 7-deazaguanine, which has a lower oxidation potential than that of guanine (G), would result in a further lowering of the oxidation potential. We therefore designed a new base, 7-vinyl-7-deazaguanine (^{vz}G) functionalized at guanine N7 where the vinyl group is extruded into the major groove upon duplex formation. Herein, we report the synthesis of ^{vz}G and the incorporation of ^{vz}G into oligonucleotides. We also measured the oxidation potential of ^{vz}G and examined the stability of ^{vz}G-containing DNA duplex.

* Corresponding author. E-mail: saito@sbchem.kyoto-u.ac.jp

0040-4039/00/$ - see front matter © 2000 Published by Elsevier Science Ltd.
PII: S0040-4039(00)01815-3

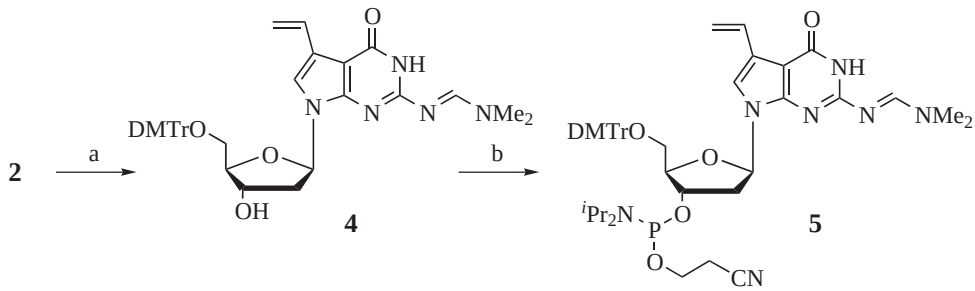
The synthetic route of ^{vz}G is shown in Scheme 1. The nucleoside 7-iodo-7-deaza-2′-deoxyguanosine (**1**) was prepared according to the synthetic protocol previously reported.[15] The exocyclic amino group of **1** was protected with DMF–diethylacetal (83%), and vinyl group was subsequently introduced into C7 by Stille coupling with tributyl(vinyl)tin to afford **2** in 60% yield.[16,17] Nucleoside **2** was deprotected under alkaline conditions to afford ^{vz}G monomer **3** (83%). The UV absorption band for **3** showed a pronounced bathochromic shift by the substitution of the vinyl group at the 7-position (**3**, $\lambda_{max}=264$ nm, $\varepsilon=2820$; $\varepsilon_{320}=550$; 7-deazaG, $\lambda_{max}=259$ nm, $\varepsilon=3760$; $\varepsilon_{320}=\sim 0$ in 10% acetonitrile–10 mM sodium cacodylate, pH 7.0).

In order to estimate the ability of **3** as an electron donating base, the oxidation potential of **3** was compared with those of 7-deazaG and G by means of cyclic voltammetry. The order of oxidation potential was as follows: G (1.07 V)>7-deazaG (0.74 V)>**3** (0.67 V) versus SCE, suggesting that **3** can serve as a highly electron-donating nucleobase in DNA duplex.



Scheme 1. (a) Dimethylformamide diethylacetal, DMF, 50°C, 2 h (83%); (b) tributyl(vinyl)tin, 5 mol% $Pd(PPh_3)_4$, DMF, 100°C, 5 h (60%); (c) 28% aqueous ammonia, methanol (1:1), rt, 5 h (83%)

For the preparation of phosphoramidite of ^{vz}G, **2** was protected by a 4,4′-dimethoxytrityl group (67%), and then quantitatively converted to cyanoethyl phosphoramidite **5** (Scheme 2). Amidite **5** was employed in solid-phase synthesis of oligonucleotides on an automated DNA synthesizer. The time for the iodine oxidation of phosphite in each step was only 15 seconds, because ^{vz}G incorporated into DNA was readily damaged by long exposure to iodine. Synthesized oligomers were deprotected overnight by aqueous ammonia at 37°C, and then purified by HPLC. The composition of oligomer was proved by MALDI-TOF mass spectrometry[18] and enzymatic digestion by snake venom phosphodiesterase, nuclease P1, and alkaline phosphatase. An example of an HPLC chart after enzymatic digestion is shown in Fig. 1.



Scheme 2. (a) 4,4′-Dimethoxytritylchloride, DMAP, pyridine, rt, 15 h (67%); (b) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, tetrazole, acetonitrile, rt, 1 h (quant.)

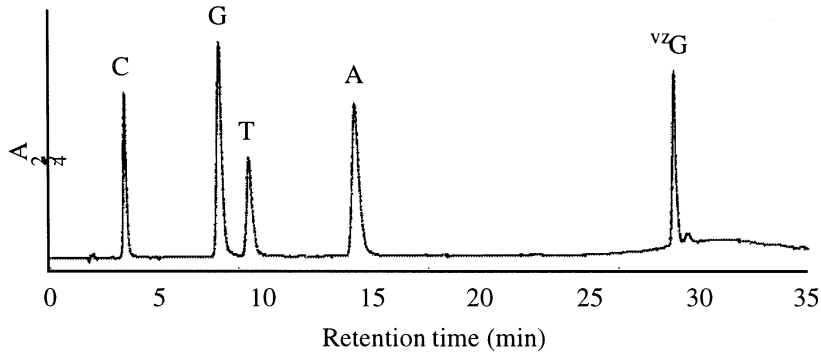


Figure 1. C18 reverse phase HPLC profile of 5′-d(CG^{vz}GAT)-3′ after enzymatic digestion by snake venom phosphodiesterase, nuclease P1 and alkaline phosphatase (100 mM triethylammonium acetate, pH 7.0, 3–8% acetonitrile over 20 min, and then 8–35% over another 15 min)

The stability of the ^{vz}G-containing duplex 5′-d(TTTGGTT^{vz}GTTT)-3′ (ODN1)/5′-d(AAA-CAACCAAA)-3′ was examined by monitoring the melting temperature (T_m). The T_m of the ^{vz}G-containing duplex was 2.1°C higher than that of the corresponding duplex containing G and 7-deazaG. Thermodynamic parameters for the duplex formation, ΔH, ΔS and ΔG, were obtained from van't Hoff plots. As shown in Table 1, the stabilization of ^{vz}G-containing duplex was due to the favorable enthalpy change in comparison with the duplexes replaced by G and 7-deazaG. This enthalpy change seems to be due to the increase of π-stacking between the vinyl group of ^{vz}G and the flanking base. The large favorable enthalpy change by the substitution of the vinyl group at the 7-position would compensate unfavorable entropy changes induced by the exclusion of water molecules in the major groove. B-form structures of the duplexes examined in T_m measurements (ODN1, 2 and 3) were confirmed by means of circular dichroism.

Table 1
T_m values and thermodynamic data of oligonucleotide duplexes

5′-TTTGGTT**X**TTT-3′
3′-AAACCAACAAA-5′

	X	T_m[a] (°C)	$-\Delta H$[b] (kcal mol^{-1})	$-\Delta S$[b] (kcal mol^{-1} K^{-1})	$-\Delta G_{298}$ (kcal mol^{-1})
ODN1	^{vz}G	20.3	279	0.920	4.40
ODN2	G	18.2	232	0.765	3.52
ODN3	7-deazaG	18.2	204	0.670	4.14

[a] Conditions: 1.25 μm duplex, 10 mM sodium cacodylate, pH 7.0.
[b] Thermodynamic data were determined by plotting $(T_m)^{-1}$ versus $\ln(C_t/4)$. C_t (total concentration); 0.30, 0.60, 1.25, and 2.50 μm DNA duplex.

The stacking surface area of ^{vz}G and 5′-flanking thymine in B-form DNA was calculated by the use of molecular modeling. The top views of TG/CA and T^{vz}G/CA where the sugar–phosphate backbone was deleted are shown in Fig. 2. The vinyl group of ^{vz}G overlapped considerably with the pyrimidine ring of 5′-thymine. Stacking surface area was calculated according to the method reported previously by Kool et al.[3,19] The increase of stacking surface area by the incorporation of the 7-vinyl group was 14.9 Å^2 which corresponds to 12% of the total stacking surface area of TG/CA.

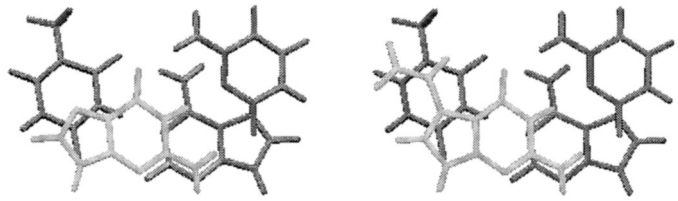


Figure 2. Molecular modeling of stacked geometries in B-form DNA. Models were built using canonical B-form geometry. DNA backbones were removed

In summary, we synthesized DNA duplex containing 7-vinyl-7-deazaguanine (^{vz}G) functionalized at guanine N7 where the vinyl group is extruded into the major groove. The extension of the π-conjugate system resulted in a lowering of the oxidation potential and a stabilization of the duplex by increased π-stacking between the vinyl group of ^{vz}G and the 5′-flanking base. Thus, ^{vz}G would be used as an effective electron-donating nucleobase for DNA-mediated electron-transfer chemistry.

References

1. Kool, E. T. *Biopolymers* **1998**, *48*, 3–17.
2. Martray, T. J.; Kool, E. T. *Nature* **1999**, *399*, 704–708.
3. Guckian, K. M.; Schweitzer, B. A.; Ren, R. X.-F.; Sheils, C. J.; Tahmassebi, D. C.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 2213–2222.
4. McMinn, D. L.; Ogawa, A. K.; Wu, Y.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **1999**, *121*, 11585–11586.
5. Ogawa, A. K.; Wu, Y.; McMinn, D. L.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 3274–3287.
6. Kelley, S. O.; Barton, J. K. *Chem. Biol.* **1998**, *5*, 413–425.
7. Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8353–8358.
8. Giese, B.; Wessely, S.; Spormann, M.; Lindemann, U.; Meggers, E.; Michel-Beyerle, M. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 996–998.
9. Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5893–5894.
10. Lewis, F. D.; Liu, X.; Liu, J.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. R. *Nature* **2000**, *406*, 51–53.
11. Sartor, V.; Henderson, P. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 11027–11033.
12. Hall, D. B.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 5045–5046.
13. Kelley, S. O.; Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 9861–9870.
14. Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063–7068.
15. Ramzaeva, N.; Seela, F. *Helv. Chim. Acta* **1995**, *78*, 1083–1090.

16. For the modification of 7-iodo-7-deazapurine by palladium cross-coupling reaction, see: (a) Ramzaeva, N.; Mittelbach, C.; Seela, F. *Helv. Chim. Acta* **1997**, *80*, 1809–1822; (b) Balow, G.; Mohan, V.; Lesnik, E. A.; Johnston, J. F.; Monia, B. P.; Acevado, O. L. *Nucleic Acids Res.* **1998**, *26*, 3350–3357.
17. Selected data for compound **2**: ^{1}H NMR (400 MHz, CD_3OD) δ 8.65 (s, 1H), 7.23 (s, 1H), 6.87 (dd, $J=11.2$, 6.8 Hz, 1H), 6.56 (t, $J=7.2$ Hz, 1H), 6.03 (dd, $J=16.0$, 1.6 Hz, 1H), 5.09 (dd, $J=9.2$, 2.0 Hz, 1H), 4.49–4.45 (m, 1H), 3.96–3.91 (m, 1H), 3.73 (dd, $J=7.2$, 4.0 Hz, 1H), 3.71 (dd, $J=7.2$, 4.0 Hz, 1H), 3.19 (s, 3H), 3.11 (s, 3H), 2.58–2.51 (m, 1H), 2.30–2.23 (m, 1H); HRMS (FAB) calcd for $C_{16}H_{22}N_5O_4$ [$(M+H)^+$] 348.3839, found 348.1667.
18. MALDI-TOF MS for 5′-GC^{vz}GAT-3′: calcd for $C_{52}H_{65}N_{19}O_{28}P_4$ [$(M-H)^-$] 1527.32, found 1527.12 and 5′-TTTGGTT^{vz}GTTT-3′: calcd for $C_{113}H_{143}N_{30}O_{72}P_{10}$ [$(M-H)^-$] 3383.28, found 3383.60.
19. Surface areas were calculated with molecular mechanics simulations using the MM2* force field as implemented in MacroModel 5.0.