Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1069–1073
Bioorganic & Medicinal Chemistry Letters

Synthesis and incorporation into DNA fragments of the artificial nucleobase, 2-amino-8-oxopurine

Claudio Cadena-Amaro,[a] Muriel Delepierre[b] and Sylvie Pochet[a,*]

[a]*Unité de Chimie Organique, URA CNRS 2128, Institut Pasteur, 28, rue du Docteur Roux, 75724 Paris cedex 15, France*
[b]*Unité de Résonance Magnétique Nucléaire des Biomolécules, URA CNRS 2185, Institut Pasteur, 28, rue du Docteur Roux, 75724 Paris cedex 15, France*

Received 12 November 2004; revised 7 December 2004; accepted 9 December 2004
Available online 30 December 2004

Abstract—The nucleoside 2-amino-9-(2-deoxy-β-D-ribofuranosyl)-7,8-dihydro-8-oxo-purine (dJ) was obtained in eight steps from 2′-deoxyguanosine. The appropriate protected phosphoramidite was synthesized and incorporated into DNA oligonucleotides. The thermal stability of heteroduplexes containing 2-amino-8-oxopurine (J) was investigated by UV-thermal denaturation experiments. The results obtained can be interpreted by the base pairing schemes involving the two edges of dJ depending on the *anti* and *syn* orientations.

Faithful transmission of hereditary messages by nucleic acids depends upon the pairing between a large bicyclic purine and a smaller monocyclic pyrimidine both in the *anti* conformation according to a scheme known as the Watson–Crick geometry.[1] However there is no reason to assume that the requirements for duplex stability and replication must limit the genetic alphabet to only two base pairs. Efforts to expand the genetic alphabet to a third base pair have resulted during the last decades in the development of several candidates. Works with modified nucleobases have explored not only hydrogen-bonded pairs[2–5] but also nonpolar pairs[6,7] and metal-mediated pairs.[8] In addition to heteropairs, several promising self-pairs have been identified, among them, purine–purine pairs have been designed.[9,10] More recently, new base pairing motifs in which building blocks are size-expanded into tricyclic bases have been reported.[11,12]

Purine nucleosides are inherently ambivalent, capable of flipping into the *syn* glycosidic conformation in addition to the *anti* conformation and thus of displaying either of two edges for hydrogen bonding with other bases.[13] Certain purine pairs are indeed genetically encoded, such as the conserved A(*syn*):G(*anti*) pairs present in helices of ribosomal and transfer RNA secondary structures.[14] The formation of other purine pairs is actively avoided by organisms, the most documented case being the repair process for correcting 8-oxodG(*syn*):dA(*anti*) pairs formed during erroneous replication involving 8-oxodGMP.[15] The mutagenic properties of 8-oxodG[16] reflect the stability of the lesion when paired in the *syn* conformation with dA(*anti*). Starting from the 8-oxoguanine(*syn*):adenine(*anti*) mispairing scheme (Fig. 1a), we designed an artificial nucleobase, 2-amino-8-oxopurine (noted J), potentially able to form base pairs in the *syn* or *anti* conformation.

As the 8-keto form predominates at physiological pH, dJ possess a hydrogen bond donor at the 7 position and a hydrogen bond acceptor at the 8 position, coupled with the hydrogen bond pattern of 2-amino-purine on the Watson–Crick side. Thus, J in the *anti* conformation presents its 2-aminopurine edge for pairing and can form two hydrogen bonds with base T according to Watson–Crick geometry (Fig. 1b). Other pairings with C and A involve the formation of less favored protonated Watson–Crick or neutral Wobble pyr–pur and large Wobble pur–pur base pairs. Such hydrogen bonds between 2-aminopurine and A or C have been supported by NMR,[17,18] however only AP:C base pair is involved in its mutagenic profile.

Keywords: Nucleobase; Nucleoside; Conformation; Base pairing; DNA.
* Corresponding author. Tel.: +33 01 40 61 33 28; fax: +33 01 45 68 84 04; e-mail: spochet@pasteur.fr

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.12.021

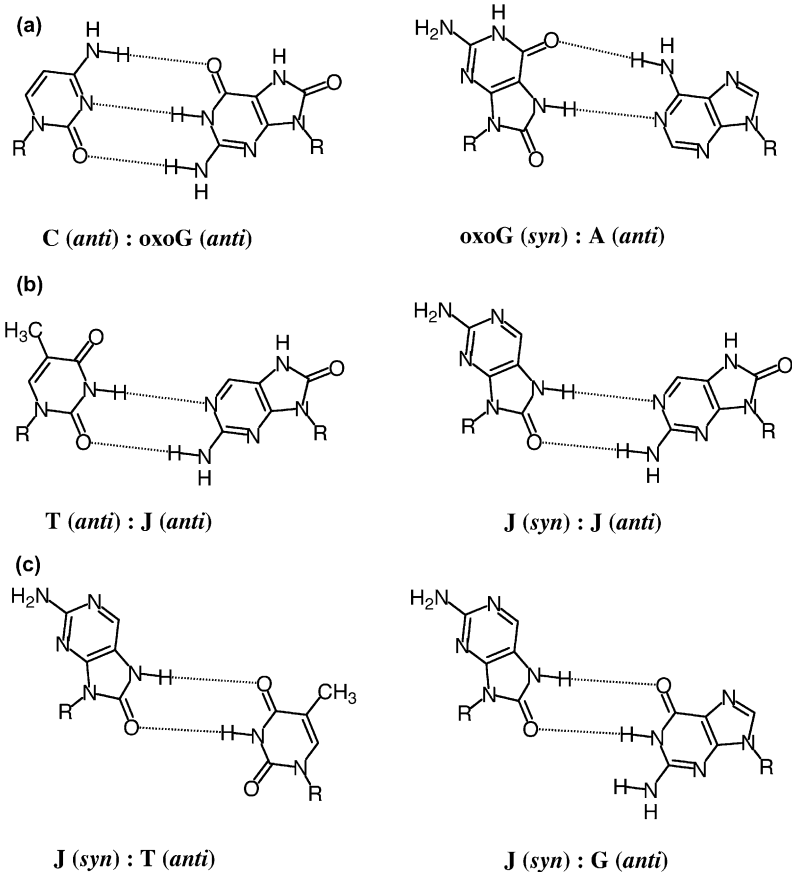


Figure 1. Proposed structures of hydrogen-bonded base pairs involving 2-amino-8-oxopurine (J) compared to those of 8-oxoguanine (oxoG).

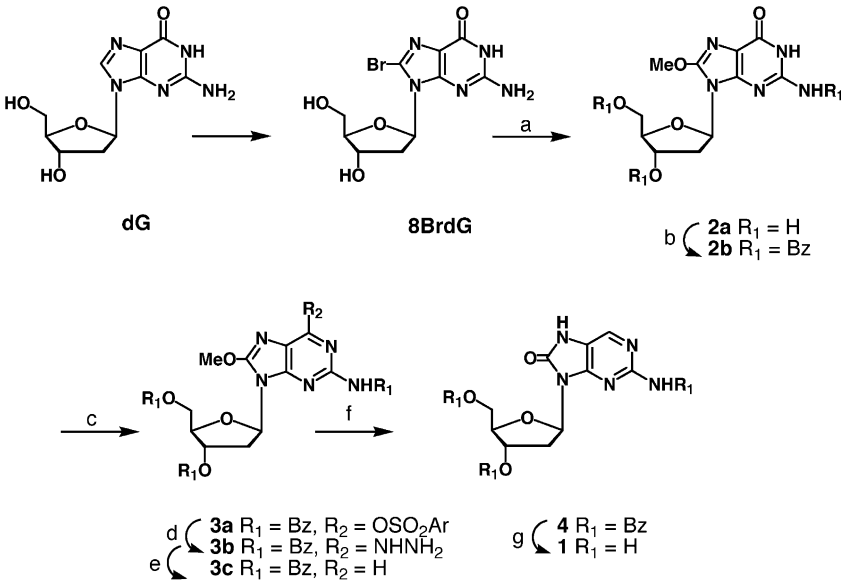
The absence of any donor or acceptor character at the 6 position changes the pairing of J in the *syn* conformation compared to oxoG(*syn*). However, as the result of the 7-NH donor and 8-oxo acceptor characters, J(*syn*) can pair with itself in the *anti* configuration with a shape similar to the oxoG(*syn*):A(*anti*) pair (Fig. 1b). Hydrogen bonded pairs with T and G result in a displacement of the purine J in the *syn* conformation compared to J(*anti*):J(*syn*) pair (Fig. 1c).

Nucleoside **1** was synthesized in eight steps from 2′-deoxyguanosine (dG) according to a previous report with slight modifications.[19] We have selected the benzoyl group for the protection of hydroxyls and exocyclic amine to improve purification and global yield. The main steps are outlined in Scheme 1 and nucleoside **1** was isolated in 6% global yield. Nucleoside **1** was fully characterized by elemental analysis, NMR and mass spectrometries.[20] The UV absorbance spectrum of **1** in water (pH 7.2) exhibited a maximum at 240 nm (ε 8970) and at 309 nm (ε 7100), characteristic of 2-aminopurine derivatives (fluorescence).

The conformational analysis of the furanose puckering of **1** was determined by means of the sums of proton–proton coupling constants.[21] The fraction *S*-type conformer (p*S*) of **1** determined by ^{1}H NMR at 400 MHz was 65%. This preference indicates a rapid dynamic equilibrium between *N* and *S* states in solution.

The orientation of the aromatic base with respect to the sugar moiety is determined by the glycosidic torsion angle χ defined as the O-4′-C-1′-N-9-C-4. A number of methods have been developed to determine the χ values using NMR data. Although quantitative evaluation of interproton distances by mean of NOE measurement is widely used most often, in absence of proton at C8 position the values of χ are determined from the measurement of three-bond carbon–proton scalar couplings $^3J_{CH}$ across the glycosidic bond and DFT analysis using Karplus equations.[22] The $^3J_{C4-H1'}$ and $^3J_{C8-H1'}$ of nucleoside **1** were determined at 30 mM in D_2O (pH 7.2) by gradient selected ^3J-HMBC NMR experiments recorded at 600 MHz. The experimental values of three-bond carbon–proton scalar couplings $^3J_{CH}$ of **1** were 4.29 Hz for $^3J_{C4-H1'}$ and 3.77 Hz for $^3J_{C8-H1'}$. Comparison of these values with the $^3J_{C4-H1'}$ and $^3J_{C8-H1'}$ values reported for purines[22] and taking into account that both $^3J_{C4-H1'} > {}^3J_{C8-H1'}$ and ΔJ ($^3J_{C4-H1'} - {}^3J_{C8-H1'}$) is small[23] allowed us to conclude that the nucleoside **1** adopts in solution a preferential *syn* conformation. As described for adenine and guanine nucleobases, the introduction of a bulky group on C8 of 2-aminopurine moiety results in the increased of the *syn* population and should promote the pairing mode of 2-amino-8-oxopurine (J) in *syn* conformation.

For the preparation of the phosphoramidite derivative **5**, both the 2-amino and 7-imino functions were

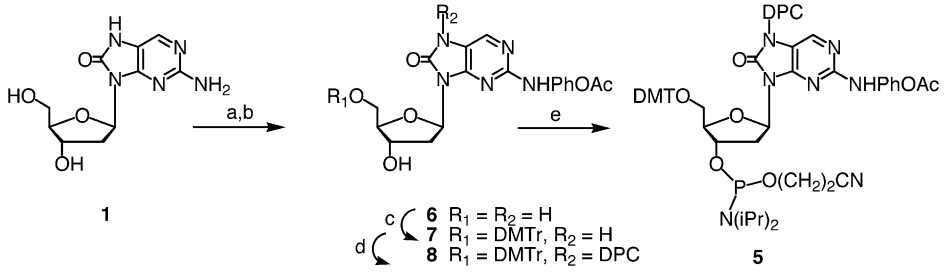


Scheme 1. Reagents and conditions: (a) 3 N sodium methylate, reflux, 72 h, 77%; (b) benzoyl chloride, pyridine, 1 h, 62%; (c) 2,4,6-tri-*iso*-propylbenzenesulfonyl chloride, DIPEA, CH_2Cl_2, 1.5 h, 64%; (d) anhydrous hydrazine, THF, 6 h; (e) Ag_2O, THF-5% H_2O, reflux, 2 h, 38%; (f) thiophenol, triethylamine, DMF, 60 °C, 2.5 h, 80%; (g) 2N NaOH, pyridine/methanol; then 33% aqueous ammonia, 55 °C, 48 h, 63%.

protected with suitable groups. We observed that the complete removal of isobutyryl and benzoyl groups required an extensive heating in aqueous ammonia and was not compatible with the solid-support DNA synthesis. To circumvent this problem, the highly alkali-labile amino protecting group, the phenoxyacetyl group, was chosen.[24] Our synthetic route for the preparation of the phosphoramidite **5** is shown in Scheme 2. Reaction of **1** with an excess of phenoxyacetyl chloride in pyridine followed by treatment with 0.5 N sodium methylate afforded N^2-phenoxyacetyl nucleoside **6** in 62% yield. 5′-*O*-Dimethoxytritylation of **6** gave **7** in 76% yield. Since the presence of the lactam may interfere with the solid-phase DNA synthesis, we chose for *N*-7 protection the diphenylcarbamoyl group previously used for the synthesis of the phosphoramidite derivative of 8-oxodG.[24,25] Treatment of **7** with diphenylcarbamoyl chloride in pyridine in the presence of *N*,*N*-di-*iso*-propylethylamine afforded **8** in 61% yield. Finally, **8** reacted with 2-cyanoethyl-*N*,*N*-di-*iso*-propylchloro-phosphoramidite in CH_2Cl_2 in the presence of *N*,*N*-di-*iso*-propylethylamine to afford the phosphoramidite **5**[26] in 77% yield.

Oligonucleotides (17-mers and 22-mers) containing the modified base J were synthesized on an automated DNA synthesizer according to standard β-cyanoethyl phosphoramidite chemistry. The coupling efficiency of **5** was not different from those of the normal phosphoramidites as estimated by released DMT. The synthesized oligomers were purified by reverse phase HPLC at two stages (DMT on, then after detritylation) and further characterized by HRMS (MALDI-TOF)[27] and nucleoside composition analysis after enzymatic digestion.

The stability of oligonucleotides containing J has been studied by UV thermal-denaturation experiments using a system composed of two complementary heptadecamers. Introducing the artificial base in the center (ninth position) of each strand allowed to measure any pairing preferences of J and test the effects of local nearest-neighbour bases (purines or pyrimidines). Such a design was previously used in our laboratory for studying modified nucleobases.[28,29] The UV thermal denaturating experiments were carried out at 1 μM duplex concentration. In all cases, sharp melting profiles were observed



Scheme 2. Reagents and conditions: (a) phenoxyacetyl chloride, pyridine, 30 min; (b) 0.5 N sodium methylate, 30 min, 62%; (c) DMTCl chloride, pyridine, 2 h, 76%; (d) diphenylcarbamoyl chloride, DIPEA, pyridine, 2 h, 61%; (e) 2-cyanoethyl-*N*,*N*-di-*iso*-propylchlorophosphoramidite, DIPEA, CH_2Cl_2, 30 min, 77%.

indicating co-operativity and reversibility of the denaturation/association process (data not shown).

Results from the melting temperatures data (T_m) (Table 1) indicate that the central replacement of a canonical base by J decreased the duplex thermal stability as compared to natural C:G (entry 1) and A:T (entry 2) base pairs. For example, C:J (entry 3) and G:J (entry 7) pairs lowered the T_m by 10–11 °C as compared to C:G. A similar destabilization is observed when J is placed opposite A (A:J and J:A pairs, entries 4 and 6). No significant context effect is observed. The most favorable T_m terms are found when J is placed opposite T (entries 8 and 10) and opposite itself (entry 11), however the corresponding duplexes are less stable than A:T duplex by 5 °C.

We presumed that J(*anti*) pair with T according to a Watson–Crick mode similar to 2AP:T base pair. As it was reported that the thermodynamic stability of 2AP:T in short heteroduplex is only slightly changed compared to A:T,[30] the lowered thermal stability of J:T and T:J base pairs compared to A:T base pair can be interpreted by the impact of the 8-oxo function. These results are consistent with the finding of several groups studying the influence of oxidative lesion on duplex stability. It is known that 8-oxoG in DNA duplex adopts both *anti* and *syn* conformations depending on the pairing partner (C or A).[31–33] Melting studies have shown that the central 8-oxoG residue reduces duplex thermal stabilities relative to G duplex when C is the cross-strand partner. When A is placed opposite 8-oxoG rather than G, an increase in stability is observed. Thus, despite having one less hydrogen bond, 8-oxoG(*syn*):A base pair is only slightly less stable than 8-oxoG(*anti*):C pair.[34]

Similarly, the relative stability of J:T and J:J duplexes can be explained by the formation of two hydrogen bonds base pairs, J(*anti*):T base pair (according to Watson–Crick mode as 2-AP:T) and J(*syn*):J(*anti*) base pair as illustrated on Figure 1b.

In summary, we described a synthetic scheme for the preparation of nucleoside dJ and its incorporation into oligonucleotides using the appropriate phosphoramidite derivative. Conformational analysis of the free nucleoside concludes that the presence of the 8-oxygen switches the glycosidic orientation preference from *anti* to *syn*. Insertion of J results in a decreased thermal stability of all the duplexes compared to A:T and C:G base pairs (from 5 to 10 °C). However the best T_m terms are found when J is placed opposite T or itself, as compared to the other combinations. Our data are in agreement with the destabilization observed for oxoG(*anti*):C base pairs compared to C:G base pairs due to the C8-oxygen. The results presented here can be interpreted by the base pairing schemes postulated initially involving the two edges of J depending on the *anti* and *syn* orientations.

Previous works on oxoG illustrate the difficulty to expect correlations between the thermodynamics of disruptions of DNA duplexes with interior lesions and the biological consequences (preferentially insertion of nucleotides opposite the lesions). Recognition of the new base J by DNA polymerases is under investigation and will be the subject of a separate paper.

Table 1. Melting temperatures (T_m)[a] of heteroduplexes containing canonical bases and 2-amino-8-oxopurine (noted J)

5′-ACTTGGCC**X**CCATTTTG-3′
3′-TGAACCGG**Y**GGTAAAAC-5′

Entry	X:Y	T_m (°C)	Entry	X:Y	T_m (°C)
1	C:G	56	2	A:T	55
3	C:J	45	4	A:J	47
5	J:C	46	6	J:A	46
7	G:J	48	8	T:J	50
9	J:G	46	10	J:T	50
11	J:J	50			

[a] Conditions: 10 μM sodium cacodylate, 0.1 M NaCl at pH 7.2, 1 μM of each strand.

Acknowledgments

CONACYT (Mexico) is gratefully acknowledged for a doctoral scholarship to C.R.C-A. We would like to thank Professor J. S. Sun (M.N.H.N., Paris) for providing facility for denaturation experiments.

References and notes

1. Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737.
2. Piccirilli, J. A.; Krauch, T.; Moroney, S. E.; Benner, S. A. *Nature* **1990**, *343*, 33.
3. Lutz, M. J.; Held, H. A.; Hottiger, M.; Hubscher, U.; Benner, S. A. *Nucleic Acids Res.* **1996**, *24*, 1308.
4. Ohtsuki, T.; Kimoto, M.; Ishikawa, M.; Mitsui, T.; Hirao, I.; Yokoyama, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4922.
5. Loakes, D. *Nucleic Acids Res.* **2001**, *29*, 2437.
6. Ogawa, A. K.; Wu, Y.; McMinn, D. L.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 3274.
7. Kool, E. T.; Morales, J. C.; Guckian, K. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 991.
8. Wagenknecht, H. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 3204.
9. Henry, A. A.; Romesberg, F. E. *Curr. Opin. Chem. Biol.* **2003**, *7*, 727.
10. Henry, A. A.; Olsen, A. G.; Matsuda, S.; Yu, C.; Geierstanger, B. H.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 6923.
11. Minakawa, N.; Kojima, N.; Hikishima, S.; Ssaki, T.; Kiyosue, A.; Atsumi, N.; Ueno, Y.; Matsuda, A. *J. Am. Chem. Soc.* **2003**, *125*, 9970.
12. Liu, H. B.; Gao, J. M.; Maynard, L.; Saito, Y. D.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 1102.
13. Donohue, J.; Trueblood, K. N. *J. Mol. Biol.* **1960**, *2*, 363.
14. Traub, W.; Sussman, J. L. *Nucleic Acids Res.* **1982**, *19*, 2701.
15. Shibutani, S.; Takeshita, M.; Grollman, A. P. *Nature* **1991**, 349.
16. Kamiya, H. *Nucleic Acids Res.* **2003**, *31*, 517.
17. Fagan, P. A.; Fabrega, C.; Eritja, R.; Goodman, M. F.; Wemmer, D. E. *Biochemistry* **1996**, *35*, 4026.
18. Sowers, L. C.; Boulard, Y.; Fazakerley, G. V. *Biochemistry* **2000**, *39*, 7613.
19. Pochet, S.; Marlière, P. C. R. *Acad. Sci. Life Sci.* **1996**, *319*, 1.

20. Compound (**1**): ^{1}H NMR (DMSO-d_6): δ 2.00 (m, 1H, H2′), 3.05 (m, 1H, H2″), 3.46 (m, 1H, H5′), 3.60 (m, 1H, H5″), 3.78 (m, 1H, H4′), 4.38 (m, 1H, H3′), 4.78 (br s, 1H, OH5′), 5.16 (br s, 1H, OH3′), 6.13 (br s, 3H, H1′ and NH_2), 7.78 (s, 1H, H6), 10.82 (br s, 1H, N^7H). ^{13}C NMR (DMSO-d_6): δ 35.73 (C2′), 62.69 (C5′), 71.65 (C3′), 81.21 (C1′), 87.77 (C4′), 113.58 (C5), 135.43 (C6), 151.02 (C4), 152.71 (C8), 159.09 (C2). HRMS (ESI-TOF): *m/z* calculated for $C_{10}H_{13}N_5O_4$+Na: 290.0865, found: 290.0872. Anal. Calcd for $C_{10}H_{13}N_5O_4$+1/4 H_2O: C, 44.20; H, 4.91; N, 25.77; found: C, 44.32; H, 5.04; N, 25.37. UV (H_2O/pH 7.6) λ_{max} 240 nm (ε 8973) and λ_{max} 309 nm (ε 7104).
21. Rinkel, L. J.; Altona, C. *J. Biomol. Struct. Dyn.* **1987**, *4*, 621.
22. Munzarova, M. L.; Sklenar, V. *J. Am. Chem. Soc.* **2003**, *125*, 3649.
23. Davies, D. B. *Prog. NMR Spectrosc.* **1978**, *12*, 135.
24. Varaprasad, C. V.; Bulychev, N.; Grollman, A. P.; Johnson, F. *Tetrahedron Lett.* **1996**, *37*, 9.
25. Roelen, H.; Saris, C.; Brugghe, H.; van den Elst, H.; Westra, J.; van der Marel, G.; van Boom, J. *Nucleic Acids Res.* **1991**, *19*, 4361.
26. Compound (**5**): ^{1}H NMR (DMSO-d_6): δ 1.05–1.18 (m, 12H, CH_3–*i*-Pr), 2.13–2.27 (m, 1H, H2′), 2.55–2.60 (m, 1H, CH_2CN), 2.67–2.71 (m, 1H, CH_2CN), 2.89 (m, 1H, H2″), 3.11 (m, 1H, H5′), 3.24 (m, 1H, H5″), 3.42–3.56 (m, 3H, 2 × CH–*i*-Pr and CH_2O), 3.64–3.68 (m, 1H, CH_2O), 3.69 and 3.70 (2s, 6H, OCH_3), 3.93 (m, 1H, H4′), 4.53–4.66 (m, 3H, H arom. PhOAc), 7,14–7,35 (m, 21H, H arom. PhOAc, DMT and DPC), 8.57 and 8.58 (2s, 1H, H6–R, 1H, H3′), 4.75–4.88 (m, 2H, CH_2–PhOAc), 6.02–6.10 (m, 1H, H1′), 6.70–6.78 (m, 4H, H arom. DMT), 6.91–6.98 (m, H6–S), 10.61 and 10.62 (2s, 1H, NH–R, NH–S). ^{31}P NMR (DMSO-d_6): δ 148.56 and 149.03. HRMS (ESI-TOF): *m/z* calculated for $C_{61}H_{63}N_8O_{10}$+Na 1121.4302; found: 1121.4344.
27. HRMS(MALDI-TOF) of synthesized oligonucleotides: 5′-ACTTGGCCJCCATTTTG-3′ *m/z* calculated for $C_{165}H_{211}N_{57}O_{104}P_{16}$: 5148.86981, found: 5148.84666. 5′-CAAAATGGJGGCCAAGT-3′ *m/z* calculated for $C_{167}H_{207}N_{73}O_{96}P_{16}$: 5264.92838, found: 5264.90345. 5′-GCATJGTCATAGCTGTTTCCTG-3′ *m/z* calculated for $C_{215}H_{273}N_{76}O_{135}P_{21}$: 6731.4057, found: 6731.3960. 5′-TGACJGTCATAGCTGTTTCCTG-3′ *m/z* calculated for $C_{215}H_{273}N_{76}O_{135}P_{21}$: 6731.4057, found: 6731.4161.
28. Pochet, S.; Dugué, L. *Nucl. & Nucl.* **1995**, *14*, 1195.
29. Beaussire, J.-J.; Pochet, S. *Tetrahedron* **1998**, *54*, 13547.
30. Law, S. M.; Eritja, R.; Goodman, M. F.; Breslauer, K. J. *Biochemistry* **1996**, *35*, 12329.
31. Kouchakdjian, M.; Bodepudi, V.; Shibutani, S.; Eisenberg, M.; Johnson, F.; Grollman, A. P.; Patel, D. J. *Biochemistry* **1991**, *30*, 1403.
32. Oda, Y.; Uesugi, S.; Ikehara, M.; Nishimura, S.; Kawase, Y.; Ishikawa, H.; Inoue, H.; Ohtsuka, E. *Nucleic Acids Res.* **1991**, *19*, 1407.
33. McAuley-Hecht, K. E.; Leonard, G. A.; Gibson, N. J.; Thomson, J. B.; Watson, W. P.; Hunte, r. W. N.; Brown, T. *Biochemistry* **1994**, *33*, 10266.
34. Plum, G. E.; Grollman, A. P.; Johnson, F.; Breslauer, K. J. *Biochemistry* **1995**, *34*, 16148.