Bioorganic & Medicinal Chemistry Letters 22 (2012) 6957–6961

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of 6-amino-2-vinylpurine derivatives for cross-linking and evaluation of the reactivity

Shuhei Kusano, Tomoya Sakuraba, Shinya Hagihara, Fumi Nagatsugi *

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai-shi, Miyagi 980-8577, Japan

ARTICLE INFO

Article history:
Received 5 April 2012
Revised 25 July 2012
Accepted 30 August 2012
Available online 21 September 2012

Keywords:
Cross-linking
Antisense
Purine derivatives
miRNA
Oligonucleotides

ABSTRACT

Oligodeoxynucleotides (ODNs) have been widely used for inhibiting the gene expression in antisense or antigene methods, and the interstrand cross-linking (ICL) forming ODNs have been expected to ensure the inhibition by these methods. Previously, we reported a highly efficient and selective ICL reaction toward cytosine using the 2-amino-6-vinylpurine derivative under acidic conditions. In this Letter, we report the synthesis of ODN containing 6-amino-2-vinylpurine derivatives and evaluation of the cross-linking reactivity.

© 2012 Elsevier Ltd. All rights reserved.

Synthetic oligonucleotides (ONs) are attractive tools in biology, and they have been developed to control the gene expression both in vitro and in vivo. These applications are based on the ability of the ONs to form highly sequence-specific complexes with nucleic acid targets of interest. The antisense ONs inhibit the translation of mRNA by induction of the RNase H endonuclease activity to cleave the RNA–DNA heteroduplex.[1] Alternatively, ONs hybridize with the target RNA to act through steric hindrance (RNase H independent mechanism).[2] A variety of chemically modified ONs has been designed and synthesized to overcome the drawbacks of a natural ON. Some ONs modified in either the carbohydrate part or the phosphate backbone do not elicit RNase H activity. Recently, such ONs have been applied to regulation of the gene expression by a different strategy from a conventional method for inhibiting the translation.[3] 2′-*O*-Methyl RNA ONs and phosphorodiamidate morpholino ONs (PMO) are employed to block splicing signals, resulting in induced exon skipping.[4] In addition, 2′-modified ONs[5], PMO[6] and a locked nucleic acid (LNA)[7] have been used to study the micro RNA (miRNA), which endogenously expresses small regulatory non-coding RNAs that play key roles in diverse cellular processes.[8] Interstrand cross-linking reactions (ICL) are expected to improve the efficiency for regulation of the expression by irreversibly binding to the target RNA through steric blocking. There are numerous of reports on ICL forming ODNs using triggering signals such as photo irradiation[9] or chemical reactions.[10] Recently, photo-cross-linked reactions with cytosine in RNA have demonstrated the ability to promote RNA editing and to possibly manipulate the RNA function.[11]

We have demonstrated that oligonucleotides bearing 2-amino-6-vinylpurine (2-AVP:**1**) achieve a highly selective cross-linking to the cytosine base in DNA under acidic conditions.[12] The high selectivity of **1** might be due to the proximity effect by the formation of a complex between **1** and the cytosine base with two hydrogen bondings under acidic conditions. Based on these results, we have designed 6-amino-2-vinylpurine (6-AVP) derivatives (**2**), expected to form a complex with the thymine base under neutral conditions (Fig. 1A). It is known that the 2-aminopurine-deoxythymine (dT) pair decreases the duplex stability compared to the deoxyadenine (dA)-dT pair[13] (Fig. 1B), and we expect that 6-AVP (**2**) could form a more stable duplex than 2-AVP (**1**).

During the synthesis of **2a**, it turned out that the vinyl group with **2a** exhibited a very low reactivity with a nucleophile. Thus, the carboxyl methyl substitution at the vinyl group was added to increase the reactivity, because introducing an electron-withdrawing substituent on the vinyl group increases its reactivity.[14] In this Letter, we report the synthesis and evaluation of the cross-linking reactivity with the 6-AVP derivatives (**2**).

The synthesis of the oligodeoxynucleotide (ODN) incorporating 6-AVP derivatives (**2a**) is summarized in Scheme 1. The 6-amino-2-idodo-purine derivative (**3**) was synthesized according to a previously reported procedure.[15] The palladium-catalyzed cross-coupling reaction of **3** with *n*-$Bu_3SnCHCH_2$ produced the vinyl derivative which was, without purification, reacted with sodium methane thiol to afford **4** (73% in 2 steps; Scheme 1). It was found that the addition of the thiol to the vinyl group proceeded very

* Corresponding author. Tel./fax: +81 22 217 5633.
E-mail address: nagatugi@tagen.tohoku.ac.jp (F. Nagatsugi).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.122

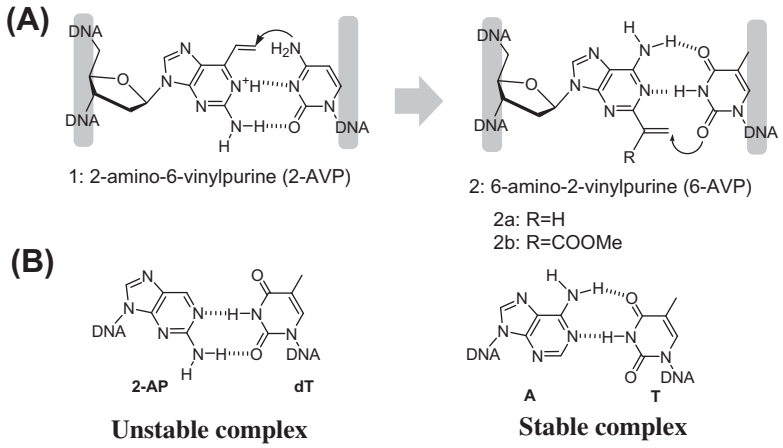
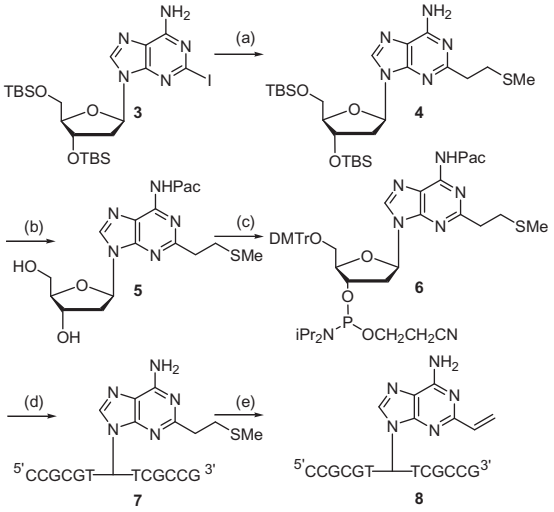
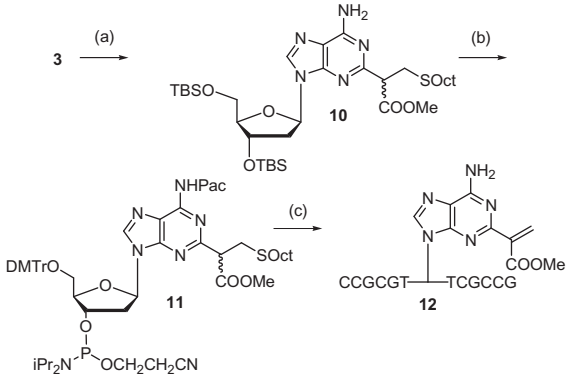


Figure 1. Design of the novel cross-linking agents.



Scheme 1. Reagents and conditions: (a) (1) n-$Bu_3SnCH{=}CH_2$, $Pd(CH_3CN)_2Cl_2$, CH_3CN, 60 °C, (2) CH_3SNa, CH_3CN, H_2O 73% for two steps; (b) (1) $PhOCH_2COCl$, 1-HOBT, CH_3CN, Pyridine, 93%, (2) n-Bu_4NF, THF, 82%; (c) (1) DMTrCl, Py, 92%, (2) $(i\text{-}Pr)_2NP(Cl)OC_2H_4CN$, DIPEA, CH_2Cl_2 0 °C, 29%; (d) (1) synthesis using an automated DNA synthesizer, (2) 28% aqueous NH_3, (3)10% CH_3COOH; (e) (1) 2 equiv MMPP, (2) aqueous NaOH 3 days.



Scheme 2. Reagents and conditions: (a) (1) n-$Bu_3Sn(COOMe)C{=}CH_2$ (**9**), $Pd(PPh_3)_4$, CuI, 60 °C, DMF 71%, (2) 1-octanethiol, iPrOH, 49%; (b) (1) $(PhOCH_2CO)_2O$, Pyridine, quant, (2) n-Bu_4NF, THF, (3) DMTrCl, Py, 22% in two steps, (4) $(i\text{-}Pr)_2NP(Cl)OC_2H_4CN$, DIPEA, CH_2Cl_2 0 °C, 48%; (c) (1) synthesis using an automated DNA synthesizer, (2) 28% aqueous NH_3, (3)10% CH_3COOH.

slowly (3 days), and this suggested that the reactivity of the vinyl group with **2a** is very low compared to **1**, which reacted with the thiol group within 2 h. The 6-amino group of the nucleoside derivative (**4**) was protected with phenoxyacetyl, and subsequent deprotection of the TBS group with TBAF afforded the diol product (**5**). Two subsequent steps following the standard method were carried out to prepare the phosphoramidite precursor (**6**) that is the building block for incorporation into ODN by automated DNA synthesis. The sulfide-protected ODN (**7**) was obtained in good yield by subjecting **6** to an automated DNA synthesizer after purification with RP-HPLC. The ODN (**7**) was then converted to ODN (**8**) by oxidation with magnesium monoperphthalate, following elimination of the sulfoxide group under alkaline conditions.

The synthesis of ODN (**2b**) incorporating a carboxyl-substituted vinyl derivative is summarized in Scheme 2. The vinylstannane reagent (**9**) was prepared by regioselective hydrostannation of the terminal alkyne.[16] The cross-coupling reaction between **3** and **9** was performed with $Pd(PPh_3)_4$ and CuI in DMF to produce the vinyl derivative in a good yield. The vinyl derivative was dissolved in 2-propanol (0.01 M) and the solution was slowly added to excess 1-octanethiol to produce the octanethio derivative (**10**).

Attempts to protect the 6-amino group of the nucleoside derivative (**10**) using phenoxyacetyl chloride resulted in producing a complex mixture. The protection of the amino group of **10** was performed with phenoxyacetic anhydride to give the desired product in good yields. After deprotection of the TBS group, the conventional method produced the phosphoramidite precursor (**11**).

The methyl acrylate-bearing ODN (**12**) was synthesized on an automated synthesizer using **11** and cleaved from the support with 28% ammonia, which had eliminated the sulfide group.

The purified ODN was (**12**) treated with 10% CH_3COOH to give the deprotected ODN. The structures of all the ODNs were determined by MALDI-TOF measurements.

The cross-linking reactions were conducted under acidic and neutral conditions between the functionalized ODNs (**8** and **12**) and target DNAs (**13a**) (N = dG, dA, dC, dT) or RNAs (**13b**) (N = G, A, C, U) labeled with fluorescein at the 5′ end and analyzed by gel electrophoresis with 20% denaturing gel (Fig. 2A).

The formation of the slower mobility band indicates the cross-linked adduct, and the yields of the adduct can be obtained by quantification of both the lower and higher mobility bands. The ODN (**8**) did not give the adduct at all, as anticipated from the reactivity of the vinyl derivative (**2a**) with the thiol. In contrast, the ODN (**12**) incorporating the methyl acrylate nucleoside produced adducts with the target DNA and RNA. Figure 2 illustrates the reactivity of the ODN (**12**) toward the different target site (N = A, G, C, T or U) in DNA or RNA. Under acidic conditions, the

12 : $^{5'}$CCGCGT **X** TCGCCG$^{3'}$ (**X**: 6-AVP)
13a: $^{3'}$d (GGCGCA **N** AGCGGC)-FAM$^{5'}$
13b: $^{3'}$r (GGCGCA **N** AGCGGC)-FAM$^{5'}$

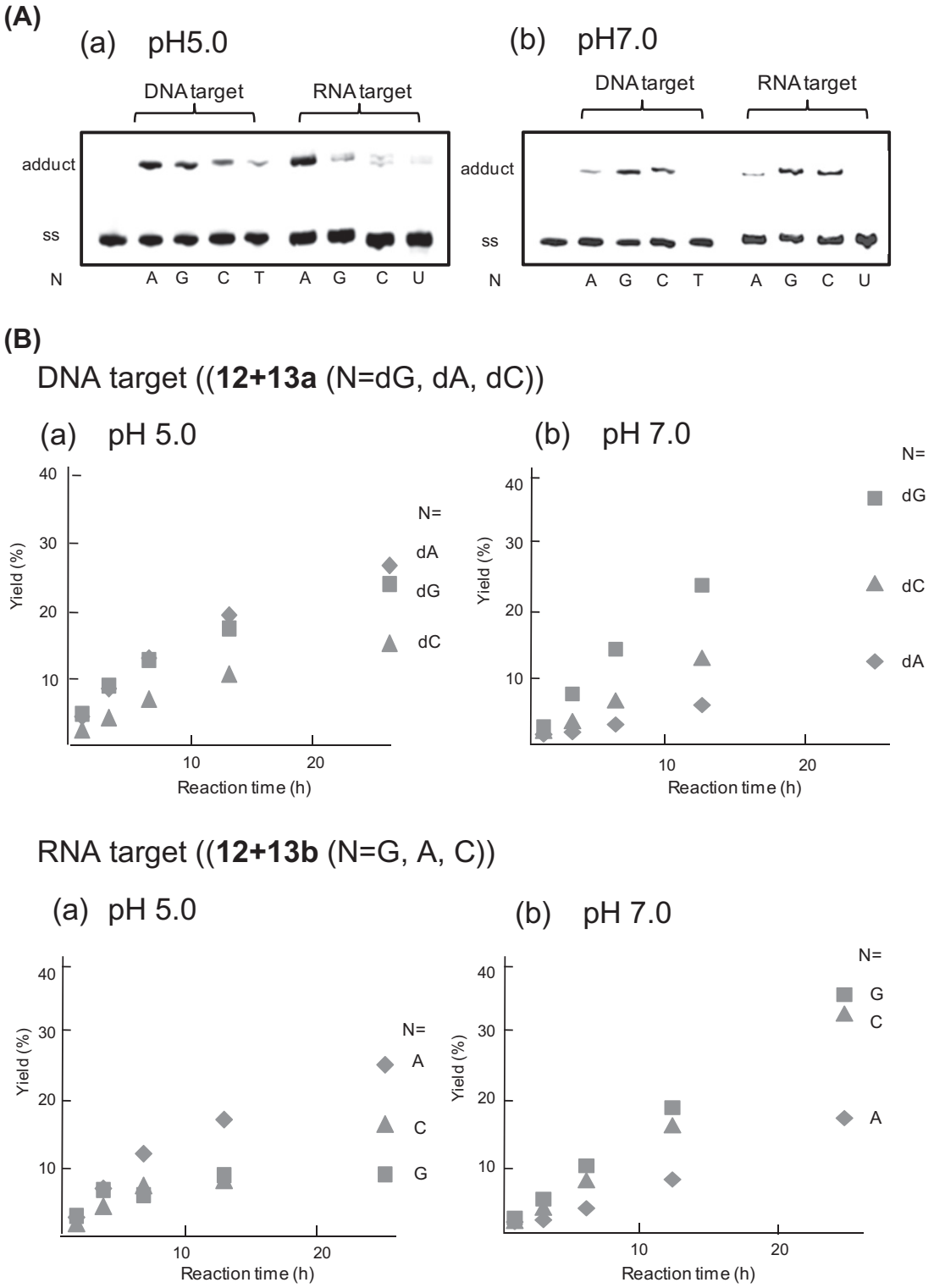


Figure 2. Analysis of cross-linking reactions by gel electrophoresis. (A) Gel electrophoresis of the cross-linking reaction after 24 h (B) Comparison of the reaction yields calculated from the gel electrophoresis analysis of the cross-linking using **12** and target DNA (**13a)** (N = dG, dA, dC) or RNA (**13b)** (N = G, A, C). The reaction was performed with 10 μM ODN (**12**) and 5 μM target ODN (**13**) in 0.1 M NaCl, 50 mM MES, pH 5.0 (a) or pH 7.0 (b), at 30 °C.

reactivity decreased in the order of A ≈ G > C ≫ T to DNA and A > G > C > U to RNA. On the other hand, the order of reactivity changed to G > C > A ≫ T, U under neutral conditions. Contrary to our design, ODN (**12**) produced the adducts to thymine or uracil

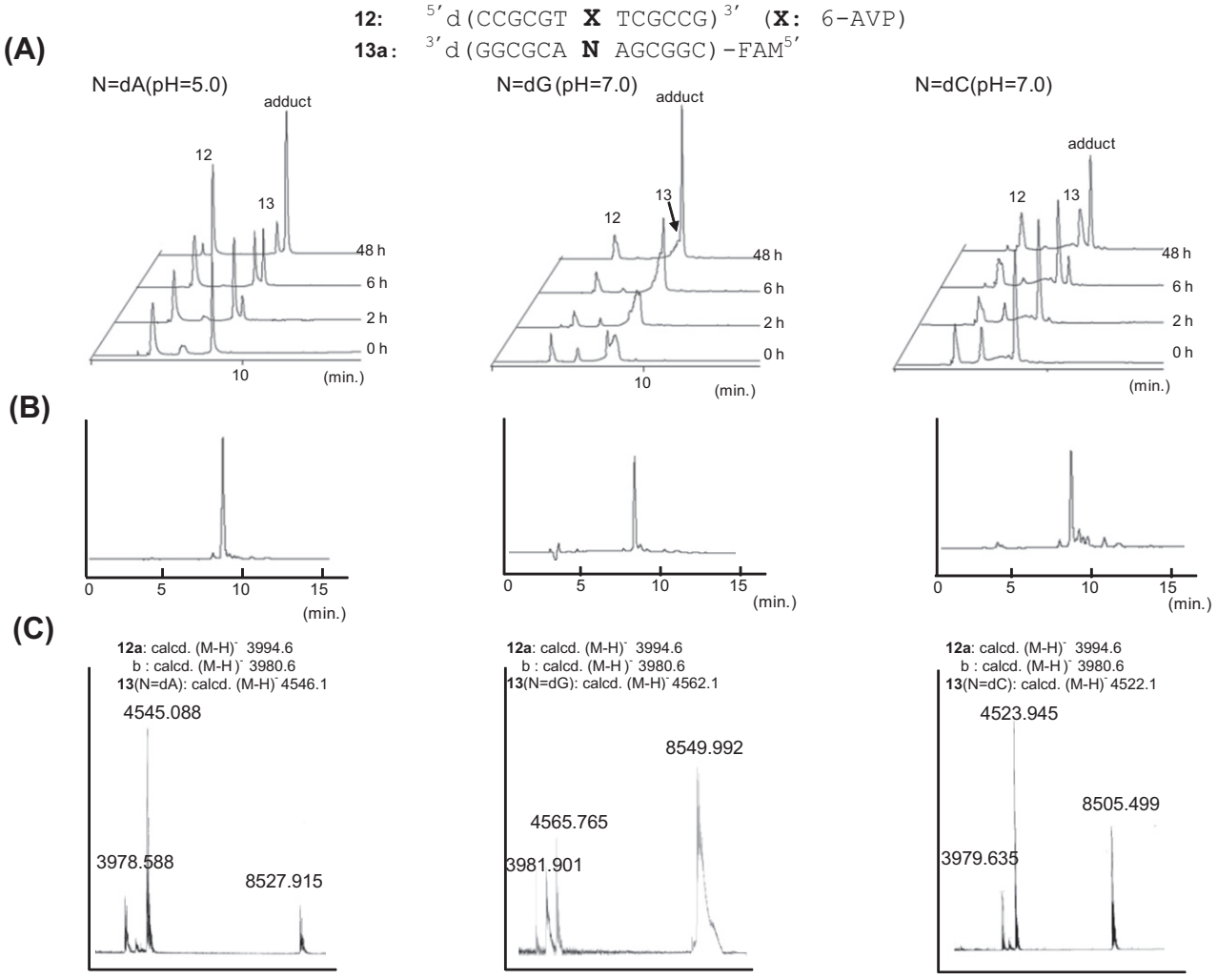


Figure 3. Analysis of the cross-linking reactions with **12** and target DNA (**13a**) (N = dG, dA, dC) by HPLC. The reaction was performed with 50 μM ODN (**12**) and 25 μM target ODN (**13**) in 0.1 M NaCl, 50 mM MES, pH 5.0 or pH 7.0 at 30 °C. (A) Time course of cross-linking reactions analyzed by reversed-phase HPLC. (B) HPLC charts of purified adducts. (C) MALDI-TOF Mass spectra of adducts.

in low yields. The time course of the cross-linking to the target DNA and RNA of ODN (**12**) is shown in Figure 2B. The cross-linked adducts were formed in the highest yields by the reaction with adenine in DNA and RNA under acidic conditions. On the other hand, under neutral conditions, the ODN (**12**) produced adducts with guanine in DNA and RNA in the highest yields. It is interesting that the change in the pH conditions causes a drastic change in the base selectivity. The thermal stability of the duplex was estimated by measuring the melting temperature (Tm) of the duplex formed between the stable ODN (**7**) or ODN (**8**) and DNA targets under neutral conditions (Supplementary data). The melting profile of ODN (**7**) or (**8**) suggested that the stable duplex would be formed with the target DNAs under the reaction conditions. The most favorable Tm values were found when the 6-AVP derivative is placed opposite to dT, however, the corresponding duplexes are less stable than the A:T duplex by 4 °C. A comparison of the Tm values between the 2-AVP derivative and 6-AVP derivative indicates that the destabilizing effect of the 6-AVP derivatives is lower than that of the 2-AVP derivative. These results have suggested that 6-AVP (**2**) could form a more stable duplex than the 2-amino-6-vinylpurine (2-AVP) (**1**). However, the reactivity of 2-AVP (**1**) with the target base is higher than that of 6-AVP (**2**).[12] The differences in the thermal stability could not explain the reaction efficiency and base selectivity of the cross-linking reactions.

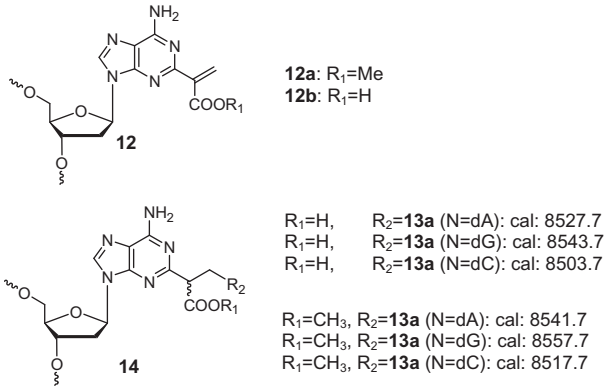


Figure 4. Suggested structure of adducts from molecular weight.

The cross-linking reactions between ODN (**12**) and the target DNA bearing G, A or C were also analyzed by HPLC, and the chromatograms are summarized in Figure 3.

The mixing of **12a** and **13a** in the buffer produced three or four peaks, which might be attributed to the duplex or some complexes, that were observed in the HPLC chart. After 48 h, these peaks

essentially decreased and converged into a main peak with the retention time of around 10 min in each reaction. Each new peak was isolated and its MALDI-TOF mass spectra measured. The observed molecular weight of the cross-linked adducts was in accord with the general structure (**14**) containing the target strand (**13a**) and reactive ODN (**12b**) with a carboxylic acid (R_1=H) (Fig. 4). The unreacted ODNs (**12**) after 48 h in each reaction displayed a molecular weight corresponding to **12** with a carboxylic acid and methyl ester (R_1=CH_3). The 6-AVP with carboxylic acid (**12b**) was isolated by HPLC and the cross-linking reactions with target DNA (**13**) were examined, but no cross-linked products were observed. These results suggested that the ODN (**12a**) with a methyl ester reacted with target DNA and that the cross-linked products might be hydrolyzed under the reaction conditions.

Each purified cross-linked product was cleaved by a hydroxyl radical for confirmation that the cross-linking reactions of **12** took place at the opposing base in the target strand. However, the cross-linked products were unstable under the hydroxyl radical cleavage conditions and decomposed into each strand. To obtain more insight into the cross-linking reaction, the purified cross-linked product was digested by enzymatic hydrolysis, but the cross-linked product was not observed in the hydrolysates. Thus, it is difficult to precisely determine each structure.

In conclusion, we have synthesized the 6-AVP derivatives, expected to cross-link with thymine, and evaluated the cross-linking reactivity. The ODN containing the non-substituted 6-AVP derivative (**2a**) can form a stable duplex with the target DNA under the stated reaction conditions but did not produce any adducts to the target DNA or RNA, due to the low reactivity of the vinyl group. On the other hand, introduction of a carboxyl group onto the vinyl group of the 6-AVP derivative (**2b**) increased the cross-linking reactivity to produce the adducts except for the thymine target base. These results have provided useful information for the design of new cross-linking agents.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (B) and (S) from Japan Society for the Promotion of Science (JSPS), CREST from Japan Science and Technology Agency.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.122.

References and notes

1. Prakash, T. P. *Chem. Biodivers.* **2011**, *8*, 1616.
2. (a) Bennett, C. F.; Swayze, E. E. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 259; (b) Aboul-Fadl, T. *Curr. Med. Chem.* **2005**, *12*, 2193.
3. Lennox, K. A.; Behlke, M. A. *Gene Ther.* **2011**, *18*, 1111.
4. (a) Yokota, T.; Takeda, S.; Lu, Q. L.; Partridge, T. A.; Nakamura, A.; Hoffman, E. P. *Arch. Neurol.* **2009**, *66*, 32; (b) Aartsma-Rus, A. *RNA Biol.* **2010**, *7*, 453.
5. (a) Esau, C.; Kang, X. L.; Peralta, E.; Hanson, E.; Marcusson, E. G.; Ravichandran, L. V.; Sun, Y. Q.; Koo, S.; Perera, R. J.; Jain, R.; Dean, N. M.; Freier, S. M.; Bennett, C. F.; Lollo, B.; Griffey, R. *J. Biol. Chem.* **2004**, *279*, 52361; (b) Krutzfeldt, J.; Rajewsky, N.; Braich, R.; Rajeev, K. G.; Tuschl, T.; Manoharan, M.; Stoffel, M. *Nature* **2005**, *438*, 685; (c) Davis, S.; Lollo, B.; Freier, S.; Esau, C. *Nucleic Acids Res.* **2006**, *34*, 2294; (d) Hutvagner, G.; Simard, M. J.; Mello, C. C.; Zamore, P. D. *Plos Biol.* **2004**, *2*, 465.
6. Flynt, A. S.; Li, N.; Thatcher, E. J.; Solnica-Krezel, L.; Patton, J. G. *Nat. Genet.* **2007**, *39*, 259.
7. (a) Chan, J. A.; Krichevsky, A. M.; Kosik, K. S. *Cancer Res.* **2005**, *65*, 6029; (b) Orom, U. A.; Kauppinen, S.; Lund, A. H. *Gene Ther.* **2006**, *372*, 137.
8. (a) Berezikov, E. *Nat. Rev. Genet.* **2011**, *12*, 846; (b) McDermott, A. M.; Heneghan, H. M.; Miller, N.; Kerin, M. J. *Pharm. Res.* **2011**, *28*, 3016.
9. (a) Xu, Y.; Ito, K.; Suzuki, Y.; Komiyama, M. *J. Am. Chem. Soc.* **2010**, *132*, 631; (b) Higuchi, M.; Kobori, A.; Yamayoshi, A.; Murakami, A. *Bioorg. Med. Chem.* **2009**, *17*, 475; (c) Qiu, Z.; Lu, L.; Jian, X.; He, C. *J. Am. Chem. Soc.* **2008**, *130*, 14398; (d) Peng, X. H.; Hong, I. S.; Li, H.; Seidman, M. M.; Greenberg, M. M. *J. Am. Chem. Soc.* **2008**, *130*, 10299; (e) Yoshimura, Y.; Fujimoto, K. *Org. Lett.* **2008**, *10*, 3237.
10. Op de Beeck, M.; Madder, A. *J. Am. Chem. Soc.* **2011**, *133*, 796; (b) Stevens, K.; Madder, A. *Nucleic Acids Res.* **2009**, *37*, 1555; (c) Weinert, E. E.; Dondi, R.; Colloredo-Melz, S.; Frankenfield, K. N.; Mitchell, C. H.; Freccero, M.; Rokita, S. E. *J. Am. Chem. Soc.* **2006**, *128*, 11940.
11. Fujimoto, K.; Konishi-Hiratsuka, K.; Sakamoto, T.; Yoshimura, Y. *Chem. Commun.* **2010**, *46*, 7545.
12. Kawasaki, T.; Nagatsugi, F.; Ali, M. M.; Maeda, M.; Sugiyama, K.; Hori, K.; Sasaki, S. *J. Org. Chem.* **2005**, *70*, 14.
13. McLaughlin, L. W.; Leong, T.; Benseler, F.; Piel, N. *Nucleic Acids Res.* **1988**, *16*, 5631.
14. Nagatsugi, F.; Tokuda, N.; Maeda, M.; Sasaki, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2577.
15. Nair, V.; Purdy, D. F. *Tetrahedron* **1991**, *47*, 365.
16. Darwish, A.; Lang, A.; Kim, T.; Chong, J. M. *Org. Lett.* **2008**, *10*, 861.