Pergamon

Tetrahedron Letters 42 (2001) 677–680

TETRAHEDRON LETTERS

Post-synthetic functionalization of oligodeoxyribonucleotides at the 2′-position

Hiroaki Ozaki, Shingo Momiyama, Kazuyuki Yokotsuka and Hiroaki Sawai*

Department of Chemistry, Faculty of Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan

Received 20 September 2000; accepted 27 October 2000

Abstract—Oligodeoxyribonucleotides bearing 2′-methoxycarbonylmethylthio-2′-deoxyuridine were synthesized. This reactive substituent at the 2′-position could be used for post-synthetic functionalization.

There has been growing interest recently in 2′-modified oligodeoxyribonucleotides (ODN) as the second-generation antisense molecules. The 2′-position in a duplex is placed in a minor groove, which is a binding site of some nucleic acid-binding proteins. For example, RNase H, which cleaves the RNA strand of RNA/DNA hybrids, is thought to bind to the minor groove.[1] Therefore, the 2′-position is interesting in the study of nucleic acid–protein interaction. An ODN bearing a lanthanide complex at the 2′-position of a thymidine residue, which was designed to attack the bulged nucleotide across the minor groove in the ODN/RNA duplex, was shown to cleave the RNA more efficiently than an ODN bearing the lanthanide complex at the C5-position of the thymidine residue, which was designed to act through the major groove.[2] From this point of view, the syntheses of ODNs bearing an acid–base catalyst at the 2′-position of cytidine or 2′-amino-2′-deoxyuridine were reported recently.[3]

We have been developing a post-synthetic functionalization method as a convenient way to introduce several functional groups into ODN. Previously, we reported incorporation of reactive groups, such as methoxycarbonylmethyl and cyanomethoxycarbonylmethyl groups, into the C5-position of 2′-deoxyuridine and the introduction of a functional group by post-synthetic functionalization.[4] In this paper, the synthesis of 2′-methoxycarbonylmethylthio-2′-deoxyuridine, its incorporation into ODN, and functionalization of the ODN by a post-synthetic method are reported.

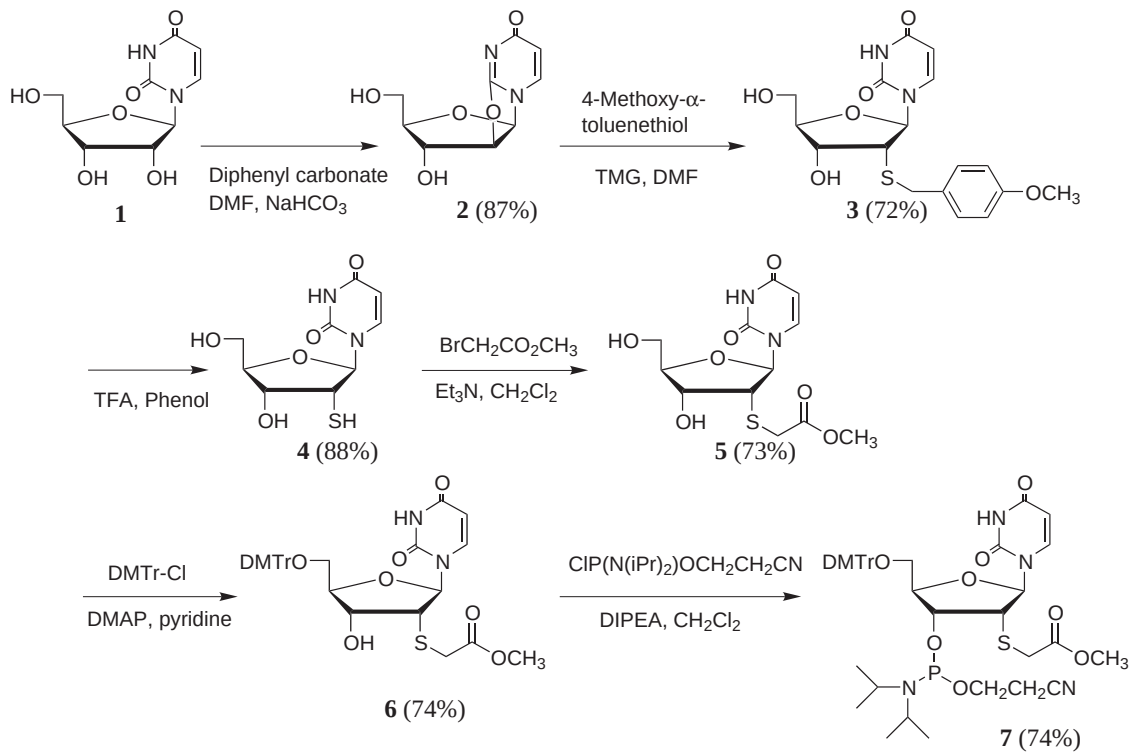
2′-Methoxycarbonylmethylthio-2′-deoxyuridine (**5**) was synthesized from uridine (**1**) as shown in Scheme 1. 2,2′-Anhydrouridine[5] (**2**), 2′-(4-methoxybenzylthio)-2′-deoxyuridine[6] (**3**), and 2′-mercapto-2′-deoxyuridine[7] (**4**) were prepared by the method described previously. Compound **4** was allowed to react with methyl bromoacetate in dichloromethane containing triethylamine, giving 2′-methoxycrbonylmethylthio-2′-deoxyuridine (**5**).[8] In this reaction, slight alkylation of N^3 in the uracil moiety was observed, and the product was purified by silica gel column chromatography. Compound **5** was subjected to 5′-dimethoxytritylation giving compound **6** and the subsequent 3′-phosphitylation (**7**) for the chemical synthesis of ODNs.

To deduce the reactivity of the methoxycarbonylthio group with an amine, a dimer (dU*pT) containing 2′-methoxycarbonylmethylthio-2′-deoxyuridine (dU*) was prepared. The coupling yield of compound **7** on a DNA synthesizer was ca. 60–70% even for 360 s as the coupling time. This may be due to steric hindrance of the substituent at the 2′-position. The dimer attached to the CPG was treated with each amine (Scheme 2; 50% solution of tris(2-aminoethyl)amine, ethylenediamine, or heptylamine in dry ethanol, or methanolic ammonia overnight at rt). The crude products were analyzed on reversed-phase HPLC as shown in Fig. 1. Each chromatogram has two main peaks. One peak at $T_R \approx 12$ min was thymidine, which was a failure product that could not react with the modified nucleoside phosphoramidite, and the other peak was the desired product. The main products (**8**–**10**) of each reaction were purified by HPLC and analyzed by ESI-MS spectroscopy,[9] except for the sample treated with methanolic ammonia. The product (**11**) obtained by treatment

Keywords: modified nucleoside; DNA; post-synthetic functionalization.

* Corresponding author. Fax: 81-277-30-1224; e-mail: sawai@chem.gunma-u.ac.jp

0040-4039/01/$ - see front matter
PII: S0040-4039(00)01926-2

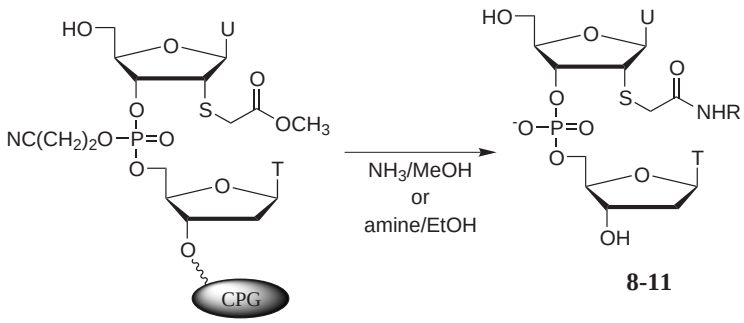


Scheme 1.

with methanolic ammonia was analyzed by HPLC after nuclease digestion. The ratio of thymidine and 2′-carbamoylmethylthio-2′-deoxyuridine[10] was 1:1. This result suggests that the reactions of the 2′-methoxycarbonyl group with the amines used in this study proceeded quantitatively.

Finally, we applied this post-synthetic strategy to 15mer ODN (d5′(CGC TTC TXC CTG CCA)3′, X=**5**) to prepare ODNs bearing a carbamoylmethylthio or bis(2-aminoethyl)aminoethylcarbamoylmethylthio group as a functional group at the 2′-position of the 2′-deoxyuridine residue. HPLC profiles of the crude ODN bearing dimethoxytrityl group at the 5′-end are shown in Fig. 2(a) and (b). The prepared 15mer ODNs were characterized by nuclease digestion. Fig. 2(c) and (d) shows the HPLC profiles of the digested products by nuclease P1 and alkaline phosphatase. The modified deoxynucleosides were confirmed by comparing the retention times with those of authentic samples synthesized from 2′-methoxycarbonylmethylthio-2′-deoxyuridine and the corresponding amines.[11] The composition of the normal and modified deoxynucleosides corresponded to the desired sequence.

In conclusion, we have prepared ODNs bearing a reactive group at the 2′-position of a 2′-deoxyuridine residue and successfully incorporated a variety of functional groups into the ODNs by post-synthetic functionalization. Since this functional group at the 2′-position will be placed in the minor groove, it is expected that it will act as a catalyst such as RNase. Further study on the properties of the modified ODNs and its application to RNA cleavage is in progress.



R=$CH_2CH_2N(CH_2CH_2NH_2)_2$ (**8**), $CH_2CH_2NH_2$ (**9**), $(CH_2)_6CH_3$ (**10**), H (**11**)

Scheme 2.

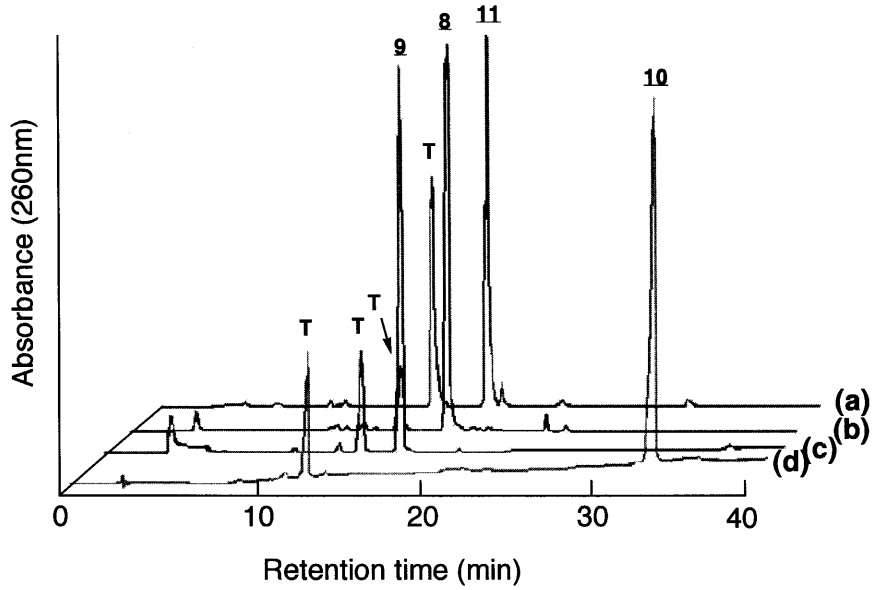


Figure 1. HPLC profiles of crude-modified dimers. The dimers treated with (a) ammonia, (b) tris(2-aminoethyl)amine, (c) ethylenediamine, and (d) heptylamine. HPLC conditions: column, Wakosil 5C18 (4×25 cm); eluent, a linear gradient of acetonitrile (2.1 to 37.1% in 35 min) in 50 mM triethylammonium acetate (pH 7.2); flow rate, 1 mL/min; detection, UV at 260 nm.

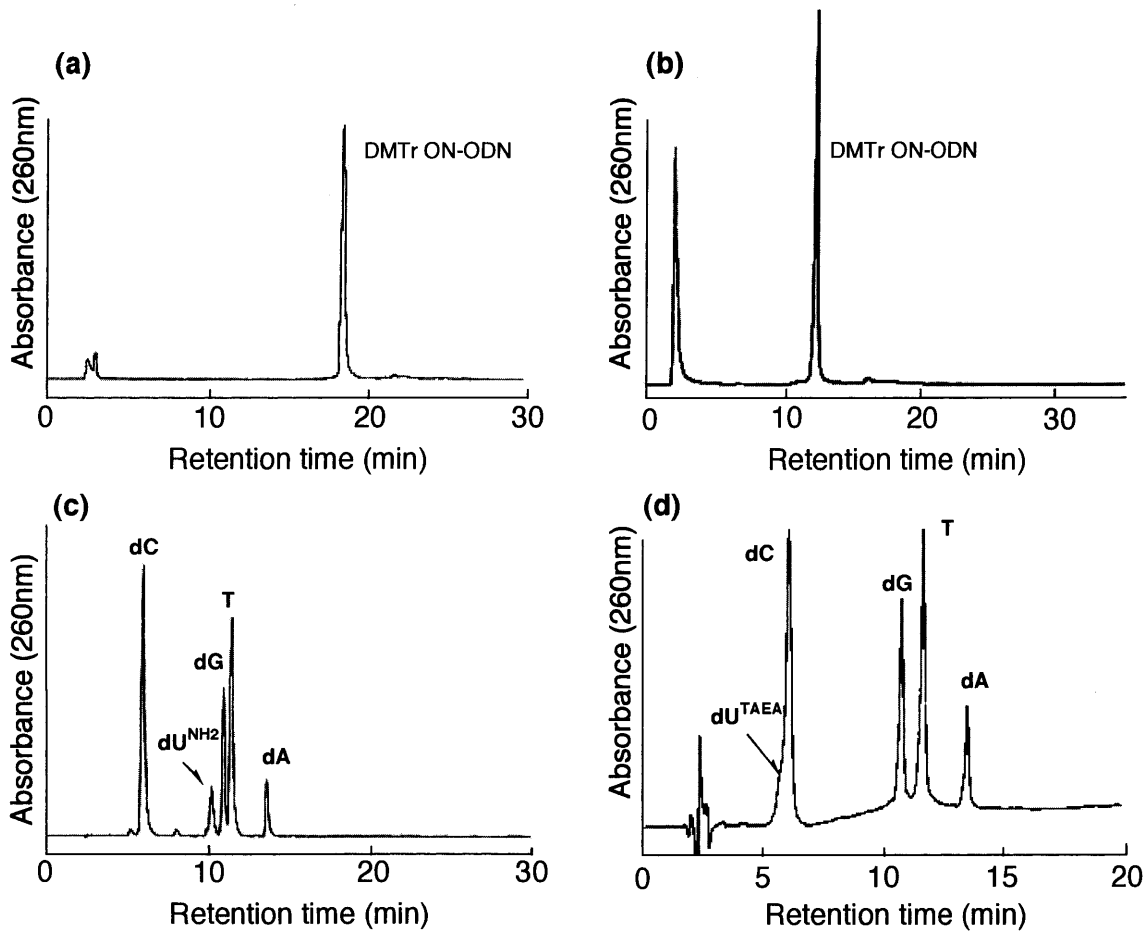


Figure 2. HPLC profiles of the crude 15mer ODNs (a and b) and the digested 15mer ODNs (c and d) by nuclease P1 and alkaline phosphatase. The 15mer ODNs treated with (a and c) ammonia and (b and d) tris(2-aminoethyl)amine. dU^{NH2} and dU^{TAEA} in the chromatograms were 2′-carbamoylmethyl-2′-deoxyuridine and 2′-[bis(2-aminoethyl)-aminoethylcarbamoylmethylthio]-2′-deoxyuridine, respectively. The HPLC conditions for (c) and (d) were the same as in Fig. 1. The HPLC conditions for (a) and (b) were the same as in Fig. 1 except for the eluent. Eluent: A 100 mM triethylammonium acetate, B acetonitrile; gradient, (a) 15 to 40% B in 30 min; (b) 20 to 30% B in 10 min and then 30% B in 10 min.

Acknowledgements

This research was supported by Grant No. 11780414 from Japanese Society for the Promotion of Science.

References

1. (a) Nakamura, H.; Oda, Y.; Iwai, S.; Inoue, H.; Ohtsuka, E.; Kanaya, S.; Kimura, S.; Katsud, C.; Katayanagi, K.; Morikawa, K.; Miyashiro, H.; Ikehara, M. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11535–11539. (b) Daniher, A. T.; Xie, J.; Mathur, S.; Bashkin, J. K. *Bioorg. Med. Chem.* **1997**, *5*, 1037–1042.
2. Hall, J.; Hüsken, D.; Häner, R. *Nucleic Acids Res.* **1996**, *24*, 3522–3526.
3. (a) Wu, X.; Pitsch, S. *Helv. Chem. Acta* **2000**, *83*, 1127–1144. (b) Beban, M.; Miller, P. S. *Bioconjugate Chem.* **2000**, *11*, 599–603.
4. (a) Kohgo, S.; Shinozuka, K.; Ozaki, H.; Sawai, H. *Tetrahedron Lett.* **1998**, *39*, 4067–4070. (b) Shinozuka, K.; Kohgo, S.; Ozaki, H.; Sawai, H. *Chem. Commun.* **2000**, 59–60.
5. Ogilvie, K. K.; Iwacha, D. *Can. J. Chem.* **1969**, *47*, 495–497.
6. Divaker, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1625–1628.
7. Divaker, K. J.; Mottoh, A.; Reese, C. B.; Sanghvi, Y. S. *J. Chem. Soc., Perkin Trans. 1* **1990**, 969–974.
8. 2′-Methoxycarbonylmethylthio-2′-deoxyuridine (**5**): ^{1}H NMR ($CDCl_3$) δ 3.38 (2H, d, CH_2), 3.76 (3H, s, OCH_3), 3.90 (3H, m, H2′ and H5′), 4.27 (1H, m, H4′), 4.34 (1H, dd, H3′), 5.62 (1H, d, H1′), 5.79 (1H, dd, H5), 7.53 (1H, d, H6), 8.47 (1H, s, NH). MS 333.2 (MH^+, 333.1 calcd for $C_{12}H_{16}N_2O_7S$).
9. Compound **8** (tris(2-aminoethyl)amine); MS 751.4 ([M+H]$^+$, 751.25, calcd for $C_{27}H_{44}N_8O_{13}PS$): **9** (ethylenediamine); MS 663.2 ([M−H]$^-$, 663.15, calcd for $C_{23}H_{32}N_6O_{13}PS$): **10** (heptylamine); MS 718.4 ([M−H]$^-$, 718.22, calcd for $C_{28}H_{41}N_5O_{13}PS$).
10. This compound was prepared by treatment of compound **5** with methanolic ammonia. ^{1}H NMR (D_2O) δ 3.15 (2H, t, CH_2), 3.49 (1H, dd, H2′), 3.63 (2H, dd, H5′), 3.98 (1H, q, H4′), 4.26 (1H, dd, H3′), 5.75 (1H, d, H5), 5.92 (1H, d, H1′), 7.67 (1H, d, H6).
11. For 2′-carbamoylmethylthio-2′-deoxyuridine, see Ref. 9. 2′-[Bis(2-aminoethyl)aminoethylcarbamoylmethylthio]-2′-deoxyuridine was prepared by treatment of compound **5** with tris(2-aminoethyl)amine. ^{1}H NMR (D_2O) δ 2.25–2.65 (12H, CH_2), 3.11 (2H, s, CH_2), 3.42 (1H, dd, H2′), 3.56 (2H, dd, H5′), 3.89 (1H, m, H4′), 4.26 (1H, dd, H3′), 5.61 (1H, d, H5), 5.89 (1H, d, H1′), 7.44 (1H, d, H6).