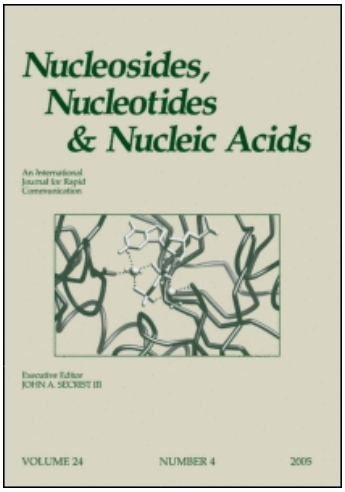

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Nucleosides and Nucleotides. 165. Chemical Ligation of Oligodeoxynucleotides Having a Mercapto Group at the 5-Position Of 2'-Deoxyuridine *via* a Disulfide Bond

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NUCLEOSIDES AND NUCLEOTIDES. 165. CHEMICAL LIGATION OF OLIGODEOXYNUCLEOTIDES HAVING A MERCAPTO GROUP AT THE 5-POSITION OF 2'-DEOXYURIDINE *VIA* A DISULFIDE BOND#,[1]

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ABSTRACT: We describe the nonenzymatic ligation of oligodeoxynucleotides (ODNs) containing a mercapto group at the 5-position of 2'-deoxyuridine *via* a disulfide bond. Two ODNs containing different sequences were efficiently ligated in the presence of a template by this method.

Procedures for linking oligodeoxynucleotides (ODNs) by a nonenzymatic approach are becoming increasingly important. While an enzymatic approach using a ligase such as T_4 DNA ligase is capable of catalyzing the assembly of natural DNA duplexes exclusively, a nonenzymatic approach using chemical coupling agents (chemical ligation) becomes useful when the ODNs are modified. Several methods have been reported for linking ODNs in an aqueous media.[2-13] Shabarova *et al.* reported a chemical ligation using cyanogen bromide as a coupling agent.[2] Dervan *et al.* reported a chemical ligation of double-helical DNA employing a single strand template to align two duplex strand termini in a triple-helix.[6,7]

Recently, we have developed a new and convenient post-synthetic modification method for the synthesis of ODNs with various amino-linkers at the 5-position of 2'-deoxyuridine residues using 5-methoxycarbonyl-2'-deoxyuridine (**1**) or 5-trifluoroethoxycarbonyl-2'-deoxyuridine (**2**) as a convertible nucleoside (FIG. 1).[1,14,15] By treating ODNs containing **1** or **2** at specific positions with several diaminoalkanes, the

#This paper is dedicated to the late Professor Tsujiaki Hata.

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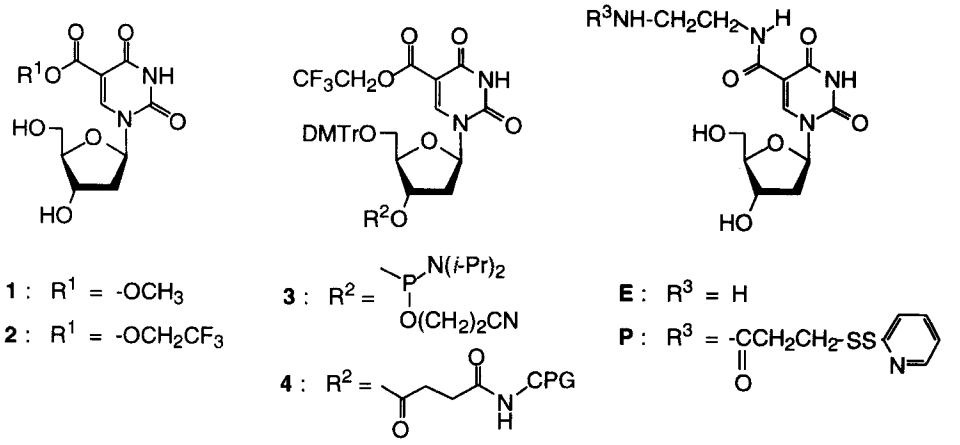
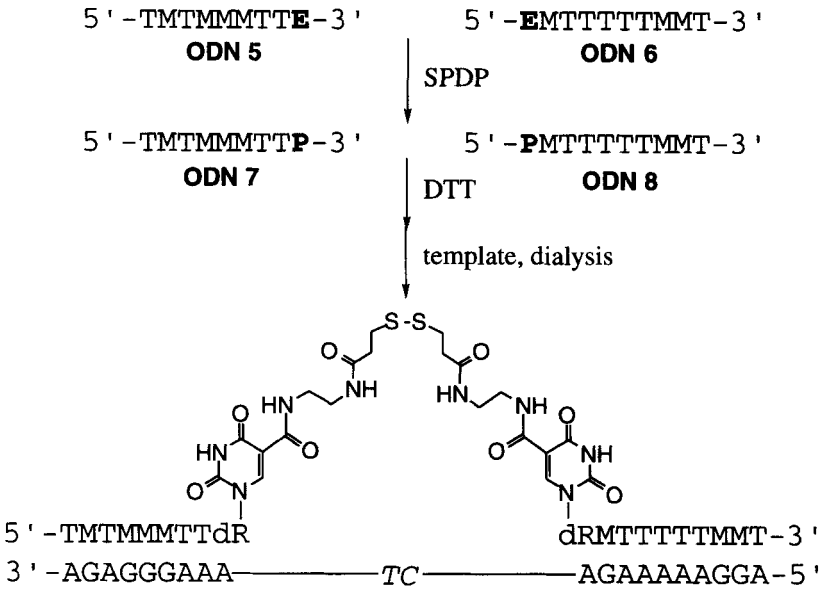


FIG. 1

desired ODNs containing 5-(*N*-aminoalkyl)carbamoyl-2'-deoxyuridines can be readily obtained. Terminal amino groups of the linkers can be further derivatized by several functional groups.[1] Indeed, by converting the amino linker to a mercapto linker, we have successfully synthesized 5'-5'-linked ODNs with potential for triple-helix formation.[16] In this paper, as a continuation of the progress of our post-synthetic modification method, we report the chemical ligation of ODNs having a mercapto group at the 5-position of 2'-deoxyuridine *via* a disulfide bond.

The modified 2'-deoxyuridine analogues **3** and **4** were synthesized starting from commercially available 5-iodo-2'-deoxyuridine as previously described.[1] Compound **3** or **4** was incorporated into 10 mer [5'-**2**M^{a}TTTTTM^{a}M^{a}T-3'-CPG, where T is thymidine, M^a is N^4-acetyl-5-methyl-2'-deoxycytidine, and CPG is a controlled-pore glass], or 9 mer [5'-TM^{a}TM^{a}M^{a}M^{a}TT**2**-3'-CPG] using the phosphoramidite method on a DNA synthesizer.[17] Treatment of the fully protected ODN linked to the solid support with a large excess of diaminoethane in MeOH at 55 °C for 16 h, followed by C-18 column chromatography, and de-tritylation gave ODN **5** or **6** (SCHEME 1).[1,14,15] It was then treated with *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) in 50 mM $NaHCO_3$-Na_2CO_3 buffer (pH 10.8) to give ODN **7** or **8**.[16] To confirm the presence of 5-[3-(2-pyridyldithio)propionylaminoethylcarbamoyl]-2'-deoxyuridine (**P**), ODN **7** and **8** were hydrolyzed by a mixture of snake venom phosphodiesterase and calf intestine alkaline phosphatase to the corresponding nucleosides, and **P** was confirmed by co-elution with an authentic compound by C18-HPLC (data not shown).



SCHEME 1

Thermal stability of the duplexes of ODNs **5-8** with a complementary DNA strand (template) was examined prior to chemical ligation. The sequence of the template, which contains two extra bases (5'-CT-3') between two binding domains, is shown in SCHEME 1. Each profile of thermal denaturation of the duplexes showed a single transition corresponding to a Helix-to-Coil transition, and Tms of the transitions are listed in TABLE 1. Although Tms of the duplexes containing **P** were slightly higher than that of the corresponding duplexes containing 5-(*N*-aminoethyl)carbamoyl-2'-deoxyuridine (**E**), the Tms of all four duplexes were around 38 °C in 0.01 M sodium cacodylate buffer (pH 7.0) containing 0.1 M NaCl. Therefore, chemical ligation reactions were done at 4 °C in the buffer containing 0.1 M NaCl.

5'-^{32}P-Labelled ODN **7** was treated with 0.1 M dithiothreitol (DTT) in 0.01 M sodium phosphate buffer (pH 7.0) containing 0.1 M NaCl for 3 h at 4 °C. The reaction mixture was then dialyzed against a large excess amount of 1 mM sodium phosphate buffer (pH 7.0) for 30 min at 4 °C. After concentration in a speed-vac concentrator, the product was analyzed by electrophoresis on a 20% polyacrylamide gel (20% PAGE) containing 8 M urea.[18] As shown in FIG. 2, a band of ODN having a mobility corresponding to 18 mer (lane 1), which would be formed by spontaneous self-condensation of ODN **7**, was observed. In a similar manner, when 5'-^{32}P-labelled ODN

TABLE 1. Thermal Denaturation[a]

	ODN 5	ODN 6	ODN 7	ODN 8
Tm (°C)	38	37	39	38

[a]Experimental conditions are described under Experimental Section.

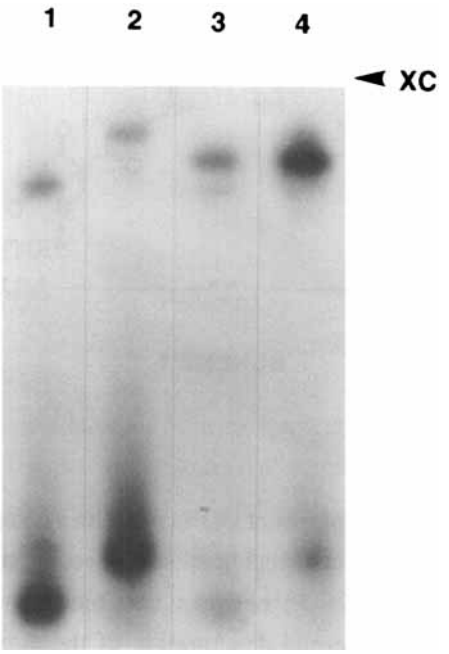


FIG. 2. Polyacrylamide gel electrophoresis of reaction mixture after treatment with DTT and then dialysis: lane 1, 5'-[^{32}P]-ODN **7**; lane 2, 5'-[^{32}P]-ODN **8**; lane 3, a mixture of 5'-[^{32}P]-ODN **7**, ODN **8**, and template; lane 4, a mixture of ODN **7**, 5'-[^{32}P]-ODN **8**, and template. The marker is xylene cyanol (XC). See Experimental Section for the conditions.

8 was treated with the same conditions, a band of ODN having a mobility corresponding to 20 mer (lane 2) was observed. On the other hand, when a mixture of 5'-labelled ODN **7** and ODN **8** in the presence of the template was treated with the same conditions, a band of ODN having a mobility corresponding to 19 mer (lane 3), which would be formed by condensation of ODN **7** and ODN **8**, was mainly observed, along with a faint band of ODN corresponding to 18 mer. In a similar manner, when a mixture of ODN **7** and 5'-labelled ODN **8** in the presence of the template was treated with the same conditions, a

main band of ODN corresponding to 19 mer and a faint band of ODN corresponding to 20 mer (lane 4) were observed. In both cases, the starting ODNs, ODN **7** and ODN **8**, were almost consumed (lanes 3 and 4). From these results, it was found that two ODNs consisting of different sequences could be efficiently ligated in the presence of a template by this method.

In conclusion, we have demonstrated the chemical ligation of ODNs having a mercapto group at the 5-position of 2'-deoxyuridine *via* a disulfide bond. We have shown that two ODNs consisting of different sequences can be efficiently ligated in the presence of a template by this method. Recently, several 5'-5' or 3'-3' linked ODNs have been used for stable triple-helix formations.[16,19-24] Our method has the merit that the length of linker groups can be changed easily. Therefore, our method would be useful for the synthesis of modified ODNs such as the 5'-5' or 3'-3' linked ODNs, as well as the 5'-3' linked ODNs described here.

EXPERIMENTAL

General Methods. 5'-*O*-Dimethoxytrityl-5-trifluoroethoxycarbonyl-3'-*O*-[(2-cyanoethyl)(*N,N*-diisopropylamino)phosphinyl]-2'-deoxyuridine was prepared as previously described.[1] The other cyanoethyl phosphoramidites were purchased from Glen Research. *N*-Succinimidyl-3-(2-pyridyldithio)propionate was purchased from Sigma. 5'-Labelling was performed using [γ-^{32}P]ATP plus polynucleotide kinase (Takara Shuzo Co., Ltd.) and labelled products were desalted by YMC Dispo SPE (YMC Co.). Dialysis tubing (Spectrum) 1000 m.w. cutoff was treated with acetic anhydride for 1 h and then washed with 2% $NaHCO_3$ and 1 mM EDTA before using.

Synthesis of ODNs. ODNs were synthesized on a DNA synthesizer (Applied Biosystem Model 381A) by the phosphoramidite method.[17] Each ODN (1 μmol) linked to the resin was treated with diaminoethane (1 ml) in MeOH (2 ml) for 16 h at 55 °C, then the released ODN was chromatographed over a C-18 silica gel column (1 x 10 cm, Waters) with a linear gradient of CH_3CN in 0.1 M TEAA buffer (pH 7.0). Fractions were concentrated and the residue was treated with 80% AcOH for 20 min at room temperature. The solution was concentrated, and the residue was coevaporated with H_2O. The residue was dissolved in H_2O and the solution was washed with Et_2O, then the H_2O layer was concentrated to give a deprotected ODN bearing an aminolinker. To a solution of the ODN (4.5-5.0 OD_{260} units) in 0.1 M sodium phosphate buffer (pH 10.8) (50 μl) was added a solution of *N*-succinimidyl-3-(2-pyridyldithio)propionate (1 mg) in CH_3CN (50 μl) at room temperature. After 30 min, the mixture was subjected to a C-18 HPLC column (YMC-J'sphere-M80) to give a pyridyldithiopropionylated ODN.

Thermal denaturation. A solution containing each ODN and a template was heated at 80 °C for 3 min, cooled gradually to an appropriate temperature, and used for

the thermal denaturation study. Thermal-induced transitions of each mixture of ODNs were monitored at 260 nm on a Perkin Elmer Lambda2S. Sample temperature was increased one degree per one min. Each sample contained appropriate ODNs (3 μM) and a template (3 μM) in 0.01 M sodium cacodylate buffer (pH 7.0) containing 0.1 M NaCl.

Chemical ligation reactions. A mixture of 5'-labelled ODN **7** (20 pmol), ODN **8** (20 pmol), and a template (20 pmol), or ODN **7** (20 pmol), 5'-labelled ODN **8** (20 pmol), and a template (20 pmol) was treated with 0.1 M dithiothreitol (DTT) in 0.01 M sodium phosphate buffer (pH 7.0) (20 μl) containing 0.1 M NaCl for 3 h at 4 °C. The reaction mixture was then dialyzed against 1 mM sodium Phosphate buffer (pH 7.0) (500 ml) for 30 min at 4 °C. After concentration in a speed-vac concentrator, the products were analyzed by electrophoresis on 20% polyacrylamide gel containing 8 M urea.[18]

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REFERENCES

(1) Part 164: Nomura, Y.; Ueno, Y; Matsuda, A. *Nucleic Acids Res.* in press.
(2) Sokolova, N. I.; Ashirbekova, D. T.; Dolinnaya, N. G.; Shabarova, Z. A. *FEBS Lett.* **1988**, *232*, 153-155.
(3) Chu, B. C. F.; Orgel, L. E. *Nucleic Acids Res.* **1988**, *16*, 3671-3691.
(4) Kool, E. T. *J. Am. Chem. Soc.* **1991**, *113*, 6265-6266.
(5) Ashley, G. W.; Kushlan, D. M. *Biochemistry* **1991**, *30*, 2927-2933.
(6) Luebke, K. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1991**, *113*, 7447-7448.
(7) Luebke, K. J.; Dervan, P. B. *Nucleic Acids Res.* **1992**, *20*, 3005-3009.
(8) Prakask, G.; Kool, E. T. *J. Am. Chem. Soc.* **1992**, *114*, 3523-3527
(9) Purmal, A. A.; Shabarova, Z. A.; Gumport, R. I. *Nucleic Acids Res.* **1992**, *20*, 3713-3719.
(10) Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1993**, *21*, 1403-1408.
(11) Gryaznov, S. M.; Schultz, R.; Chaturvedi, S. K.; Letsinger, R. L. *Nucleic Acids Res.* **1994**, *22*, 2366-2369.
(12) Herrlein, M. K.; Letsinger, R. L. *Nucleic Acids Res.* **1994**, *22*, 5076-5078.
(13) Bannwarth, W.; Dorn, A.; Iaiza, P.; Pannekouke, X. *Helv. Chim. Acta* **1994**, *77*, 182-193.
(14) Ono, A.; Haginoya, N.; Kiyokawa, M.; Minakawa, N.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 361-366.

(15) Haginoya, N.; Ono, A.; Nomura, Y.; Ueno, Y.; Matsuda, A. *Bioconjugate Chem.* **1997**, *8*, 271-280.

(16) Ueno, Y.; Ogawa, A.; Nakagawa, A.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2817-2822.

(17) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.

(18) Maniatis, T.; Fritsch, E. F.; Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, New York.

(19) Horne, D. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2435-2437.

(20) Ono, A.; Chen, C. N.; Kan, L. S. *Biochemistry* **1991**, *30*, 9914-9921.

(21) Froehler, B. C.; Terhorst, T.; Shaw, J. P.; McCurdy, S. N. *Biochemistry* **1992**, *31*, 1603-1609.

(22) Asseline, U.; Thuong, N. T. *Tetrahedron Lett.* **1993**, *34*, 4173-4176.

(23) Asseline, U.; Thuong, N. T. *Tetrahedron Lett.* **1994**, *35*, 5221-5224.

(24) Zhou, B.-W.; Marchand, C.; Asseline, U.; Thuong, N. T.; Sun, J.-S.; Garestier, T.; Helene, C. *Bioconjugate Chem.* **1995**. *6*. 516-523.