Tetrahedron Letters, Vol. 34, No. 25, pp. 4015-4018, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

A Convenient Synthesis of Oligonucleotides with a 3'-Phosphoglycolate and 3'-Phosphoglycaldehyde Terminus

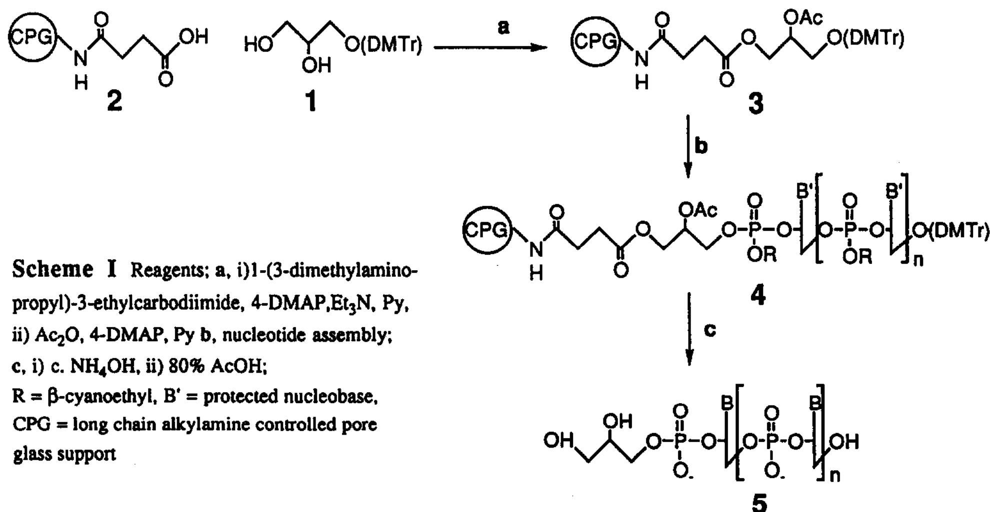
Hidehito Urata and Masao Akagi*

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

Key Words: Oligonucleotide; 3'-Phosphoglycolate; 3'-Phosphoglycaldehyde; Bleomycin

Abstract: *A simple and rapid method for solid phase synthesis of oligonucleotides carrying a 3'-phosphoglycerol terminus was developed. The modified oligonucleotide was readily oxidized by $NaIO_4$ to a 3'-phosphoglycaldehyde. The treatment of this aldehyde with $NaClO_2$ afforded a 3'-phosphoglycolate.*

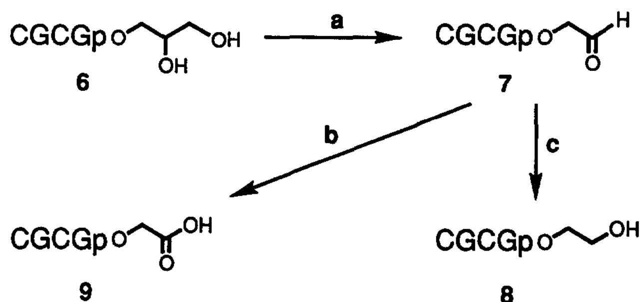
Chemically synthesized oligonucleotides have been widely used in molecular biology and physicochemical studies of nucleic acids due to the recent rapid advance in the solid phase synthesis of oligonucleotides[1]. Synthetic oligonucleotides that can be obtained with defined sequences are also useful for DNA-drug interaction study. Some DNA-targeting molecules such as bleomycin[2], the enediyne antibiotics[3,4] and transition metal complexes[5] cleave DNA strands in a radical process that includes hydrogen abstraction from the deoxyribose moieties of DNA. Identification of these DNA fragments produced by the drugs is



Scheme I Reagents; a, i) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, 4-DMAP, Et_3N, Py, ii) Ac_2O, 4-DMAP, Py b, nucleotide assembly; c, i) c. NH_4OH, ii) 80% AcOH; R = β-cyanoethyl, B' = protected nucleobase, CPG = long chain alkylamine controlled pore glass support

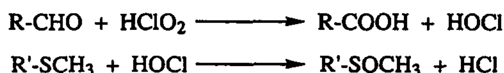
important and useful for the characterization not only of sequence specificity but of regiospecificity for hydrogen abstraction from the deoxyribose moiety as well[6]. Now we report a convenient method for the synthesis of oligonucleotides carrying 3'-phosphoglycolate[3] and 3'-phosphoglycaldehyde[6,7] terminus formed by abstraction of 4'- and 3'-hydrogen, respectively on deoxyribose moieties.

Mono-(dimethoxytrityl) glycerol(**1**) readily obtained from glycerol by treatment with dimethoxytrityl chloride/pyridine was condensed to the succinylated long chain alkylamine-controlled pore glass support (succinylated LCAA-CPG, **2**), synthesized according to the literature procedure[8], in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. The resulting resin was acetylated with acetic anhydride/4-dimethylaminopyridine/pyridine to protect the 2-hydroxy group of the glycerol moieties and unreacted amino groups on the LCAA-CPG resin. The unreacted carboxyl groups on the resin were capped according to the literature procedure[8]. The common coupling cycle was repeated on the glycerol resin (**3**) by the β-cyano-ethylphosphoramidite method until the desired oligonucleotide carrying a 3'-phosphoglycerol terminus (**4**) was obtained. Following conventional deblocking and purification, the chromatographically homogeneous tetranucleotide, d(CGCG) modified at a 3'-terminus (**6**) was quantitatively oxidized with $NaIO_4$ (final concentration; 5 mM)[9] for 1.5 h at 0°C to give a 3'-phosphoglycaldehyde (**7**) as shown in Figure 1A and 1B. After the addition of L-methionine to the mixture to quench excess of $NaIO_4$[10], the product was readily reduced to the corresponding alcohol (**8**) with $NaBH_4$ (Figure 1C). This confirms the structure of the aldehyde. Subsequent oxidation of the aldehyde with a $NaClO_2$ system[11] afforded 3'-phosphoglycolate (**9**)[12]



Scheme II Reagents; a, $NaIO_4$; b, $NaClO_2$; c, $NaBH_4$

as shown in Figure 1D and 1E. L-Methionine and its sulfoxide produced by quenching $NaIO_4$ could also serve as scavengers of HOCl generated by this oxidation reaction.



$$\text{R-CHO} + HClO_2 \longrightarrow \text{R-COOH} + \text{HOCl}$$
$$\text{R'-SCH}_3 + \text{HOCl} \longrightarrow \text{R'-SOCH}_3 + \text{HCl}$$

The tetranucleotide, d(CGCG) having the 3'-phosphoglycolate terminus (**9**) synthesized in this way was cochromatographed with a major bleomycin-mediated degradation product[13] of d(CGCGCG) produced *via* H4' abstraction at the C(5) residue (Figure 1F).

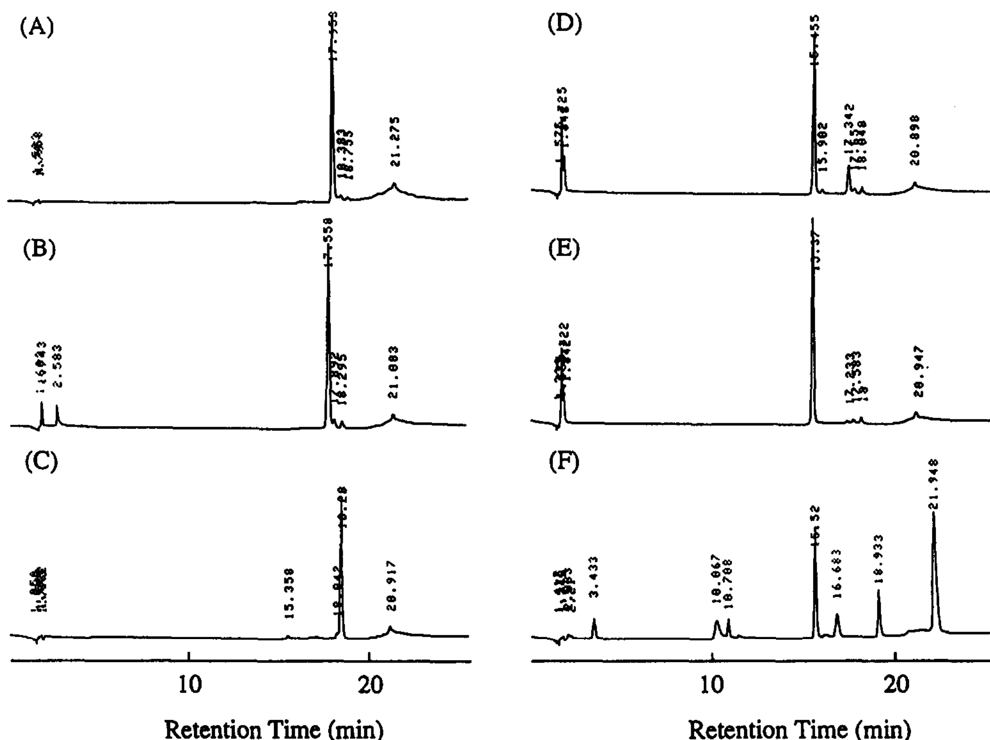


Figure 1. HPLC analysis of 6 and its conversion to 7, 8, and 9, and that of bleomycin-mediated degradation products of d(CGCGCG). 3'-Phosphoglycerol tetranucleotide 6 (A) was converted to 3'-phosphoglycaldehyde 7 by treating with $NaIO_4$ at 0°C for 1.5 h (B). The treatment of 7 with $NaBH_4$ afforded the corresponding alcohol 8 (C). The aldehyde 7 was oxidized with $NaClO_2$ at 25°C for 2 h (D) and 5 h (E). The resulting 3'-phosphoglycolate was comigrated with the major product of the bleomycin-mediated strand cleavage reaction[13] of d(CGCGCG)(F). The peak at 21.9 min in Fig. 1F corresponds to unreacted d(CGCGCG). Analysis was carried out on a μBondasphere C18 100Å column (3.9 x 150 mm); elution was performed with a linear gradient of acetonitrile 0-10% for 20 min containing 50 mM ammonium formate at a flow rate of 0.8 mL/min with 260 nm detection.

This method thus makes possible the direct identification of DNA degradation products produced by DNA damaging agents *via* H4' and H3' abstraction. Oligonucleotides with the 3'-phosphoglycaldehyde terminus should be useful for immobilization and attachment of oligonucleotides to polymer supports, proteins and fluorescent dyes *via* the glycaldehyde terminus.

Acknowledgment: We thank Dr. T. Takita (Nippon Kayaku, Co., LTD.) for providing bleomycin B_2.

References and Notes

1. Ikehara, M.; Ohtsuka, E.; Uesugi, S.; Tanaka, T. *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B.; Ed.; Prenum Press: New York, **1988**; pp. 283-367.
2. Kuwahara, J.; Sugiura, Y. *Proc Natl. Acad. Sci.* USA **1988**, 85, 2459-2463. Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *Biochemistry* **1988**, 27, 58-67. Hiroaki, H.; Nakayama, T.; Ikehara, M.; Uesugi, S. *Chem. Pharm. Bull.* **1991**, 39, 2780-2786. Rabow, L.; Stubbe, J.; Kozarich, J. W.; Gerlt, J. A. *J. Am. Chem. Soc.* **1986**, 108, 7130-7131.
3. Sugiyama, H.; Kawabata, H.; Fujiwara, T.; Dannoue, Y.; Saito, I. *J. Am. Chem. Soc.* **1990**, 112, 5252-5257. Saito, I.; Kawabata, H.; Fujiwara, T.; Sugiyama, H.; Matsuura, T. *J. Am. Chem. Soc.* **1989**, 111, 8302-8303. Sugiyama, H.; Fujiwara, T.; Kawabata, H.; Yoda, N.; Hirayama, N.; Saito, I. *J. Am. Chem. Soc.* **1992**, 114, 5573-5578.
4. Sugiura, Y.; Uesawa, Y.; Takahashi, Y.; Kuwahara, J.; Golik, J.; Doyle, T. W. *Proc. Natl. Acad. Sci. USA* **1989**, 86, 7672-7676. Hangeland, J. J.; De Voss, J. J.; Heath, J. A.; Townsend, C. A.; Ding, W.-d.; Ashcroft, J. S.; Ellestad, G. A. *J. Am. Chem. Soc.* **1992**, 114, 9200-9202.
5. Mei, H.-Y.; Barton, J. K. *Proc. Natl. Acad. Sci. USA* **1988**, 85, 1339-1343. Pyle, A. M.; Long, E. C.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, 111, 4520-4522.
6. Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, 87, 1107-1136.
7. Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, 114, 2303-2312.
8. Damha, M. J.; Giannaris, P. A.; Zabarylo, S. V. *Nucleic Acids Res.* **1990**, 18, 3813-3821.
9. Nadeau, J. G.; Singleton, C. K.; Kelly, G. B.; Weith, L.; Gough, G. R. *Biochemistry*, **1984**, 23, 6153-6159.
10. The aldehyde was stable in this solution at least for a week at 4°C.
11. Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, 51, 567-569.
12. Typically, an aqueous solution of a 3'-phosphoglycerol oligonucleotide was treated with 0.2 M $NaIO_4$ (5 mM final concentration) and the solution was kept at 0°C for 1.5 h. After the addition of 0.2 M L-methionine (7.5 mM final concentration), the mixture was kept at 0°C for additional 30 min. To this solution were added potassium phosphate buffer pH 4.0 (20 mM final concentration) and $NaClO_2$ (400 μM final concentration). The mixture was kept at 25°C. The reaction was complete within 5 h, and excess of $NaClO_2$ was quenched by Na_2SO_3.
13. The reaction mixture contained 200 μM d(CGCGCG), 200 μM bleomycin and 50 mM sodium phosphate pH 7.0. The reaction was initiated by the addition of $Fe(II)(NH_4)_2(SO_4)_2$ (final concentration 200 μM), incubated at 0°C for 30 min and then analyzed by HPLC.

(Received in Japan 3 February 1993)