CHEMISTRY LETTERS, pp. 1799-1802, 1989. © 1989 The Chemical Society of Japan

Synthesis of Oligoribonucleotides by the Hydroxybenzotriazole-Activated Phosphotriester/Dicyclohexylcarbodiimide System

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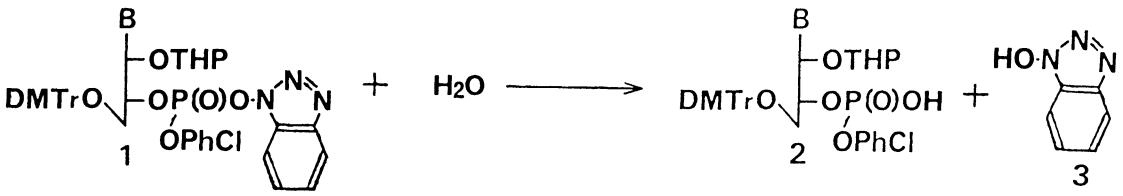
Addition of dicyclohexylcarbodiimide stabilized the benzotriazole-activated phosphotriester intermediates in which were used oligoribonucleotide synthesis. By this system, two oligoribonucleotides, r(CGAAAGC) and r(GCGAAAGC), were synthesized for structural comparison with d(GCGAAAGC) whose structure was unusually stable. Electrophoretic experiments showed r(GCGAAAGC) to behave differently from d(GCGAAAGC), although each had the same base sequence.

We previously reported the synthesis of oligoribonucleotides by a 1-hydroxybenzotriazole-activated phosphotriester approach on a polymer support.[1,2] This approach has firstly been studied by van Boom et al.[3] for oligonucleotide synthesis and was applied to the solid phase synthesis of oligoribonucleotides by us.[1,2] However, the activated phosphotriester intermediates (1) were so labile in acetonitrile that they readily hydrolyzed by a slight amount of moisture during the oligonucleotide synthesis (see Scheme 1). Moreover, the resulting 1-hydroxybenzotriazole (3) in the hydrolysis causes elimination of 5'-dimethoxytrityl groups from the intermediates.

To avoid this problem, dicyclohexylcarbodiimide (DCC)[4] was added to the system as a dehydration agent. 5'-O-Dimethoxytrityl-2'-O-tetrahydropyranyluridine (1.1 mol equiv.)(1d) as a model intermediate was phosphorylated with 2-chlorophenyl-O,O-bis(1-benzotriazolyl) phosphate[3] (1.0 mol equiv.) in dioxane for



DMTr: 4,4'-dimethoxytrityl
THP : tetrahydropyranyl
PhCl: 2-chlorophenyl

B: N^6-benzoyladenine (1a)
N^4-anisoylcytosine (1b)
N^2-isobutyrylguanine (1c)
uracil (1d)

Scheme 1.

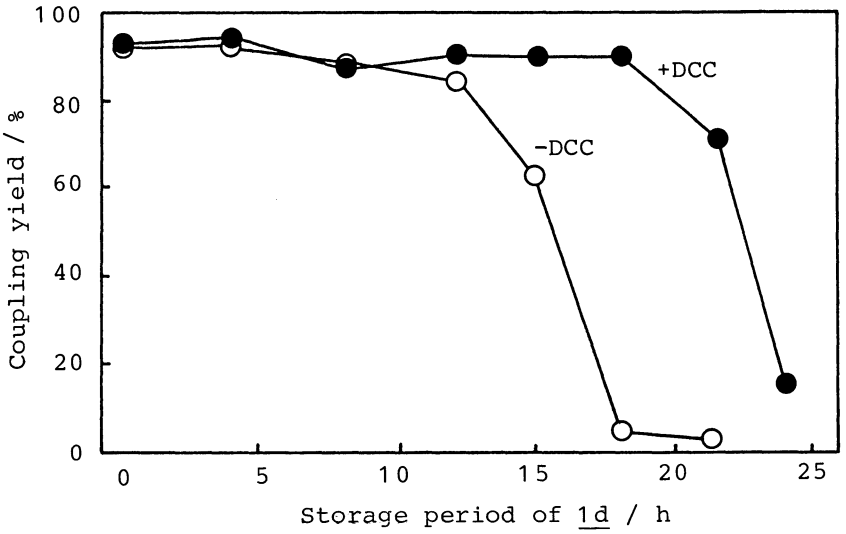


Fig. 1. Coupling yield plotted against the storage period of 1d.

30 min at room temperature. A small amount of pyridine was subsequently added to the mixture followed by evapolation of the solvent. The residue and DCC were dissolved in acetonitrile and this solution was directly used for the dimer synthesis. A condensation reaction was carried out at regular time intervals to mix with the uridine intermediate (1d) adding 5 mol equiv. of DCC, N^6-benzoyl-5'-O-dimethoxytrityl-2'-O-tetrahydropyranyladenosine bound to controlled pore glass (CPG) and a catalytic amount of 1-methylimidazole at 40 °C for 15 min. As shown in Fig. 1, the addition of DCC made it possible to maintain the stability of the uridine intermediate for 18 h. No side reaction during the condensation appeared to occur even with DCC according to the results of product analysis by high performance liquid chromatography (HPLC)(data not shown) comparing with the condensation without DCC.

Consequently, we conducted the synthesis of two oligoribonucleotides, r(GCGAAAGC) and r(CGAAAGC) using a ZEON Genet DNA manual synthesizer. The average condensation yields were rather low (87-88% yields), due to a large amount of nucleoside loaded onto the CPG resin.[2] A fully protected oligomer was treated at 37 °C for 21 h with 0.5 M tetramethylguanidinium 2-pyridinealdoxamate and then filtered to remove the CPG resin. The filtrate was heated with concd aqueous ammonia at 55 °C for 7.5 h. The 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl oligomer was purified by reversed phase C-18 HPLC (Fig. 2a) and treated with 0.01 M^+ HCl (pH 2.0) for 46 h at room temperature. Following deprotection, the crude oligomer was re-purified by C-18 HPLC (Figs. 2b and 2c). From the 2.47-micromole of starting nucleoside, purified r(GCGAAAGC) and r(CGAAAGC) were obtained in the amounts of 24 and 22 OD_{260} units, respectively. ($^+$1 M=1 mol dm^{-3})

The corresponding oligodeoxyribonucleotide, d(GCGAAAGC), had a mobility greater than those of other oligodeoxyribonucleotides with the same chain length in gel electrophoresis in the presence of 7 M urea at neutral pH that the mobility of an oligonucleotide reflects its chain length[5] and takes on a stable hairpin structure possessing two terminal G-C pairs.[6] For example, the ^{32}P-labeled deoxy-oligomer, d(GCGAAAGC)(8mer), showed a higher mobility than the corresponding

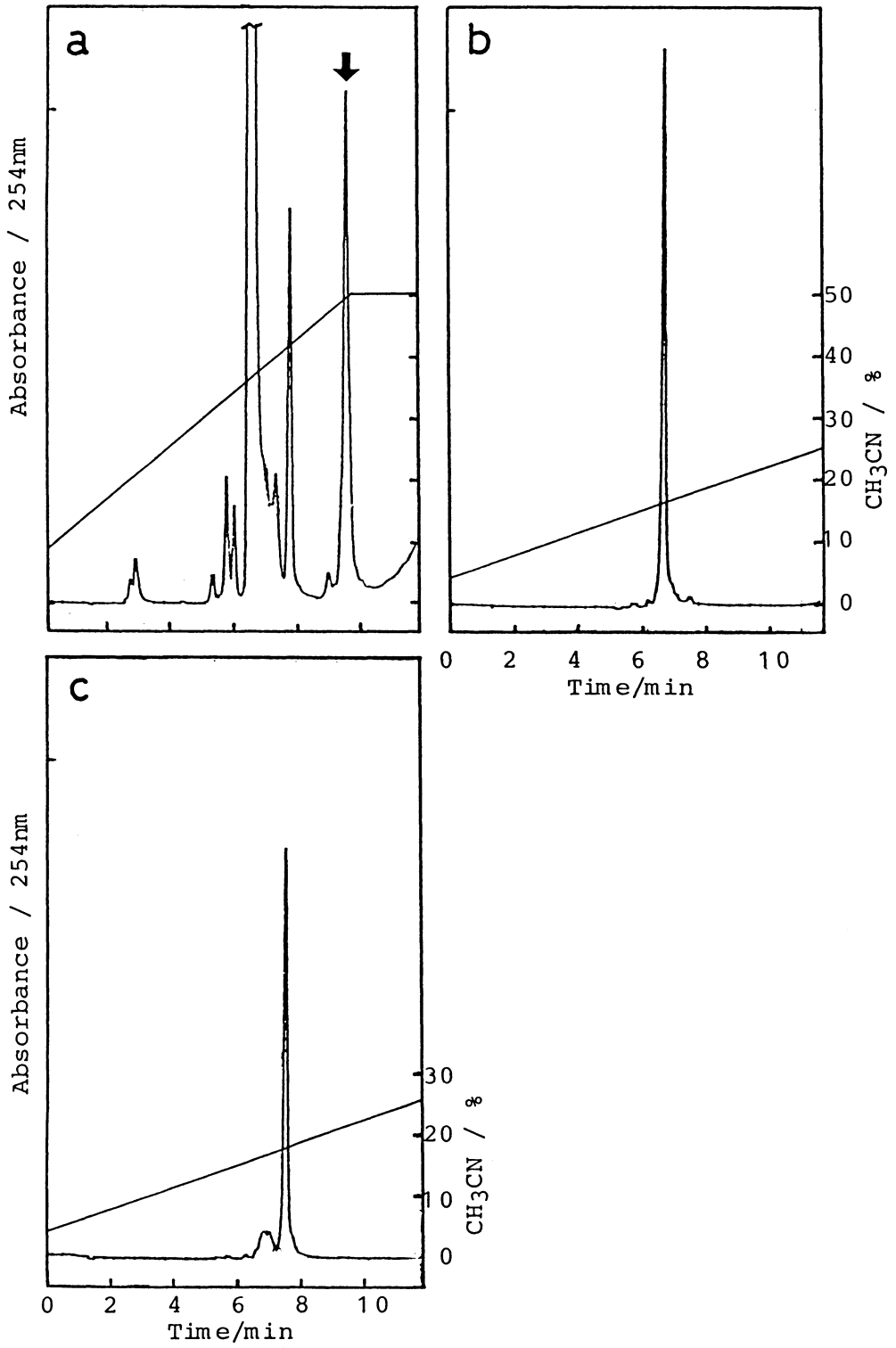


Fig. 2. HPLC profiles for the purification of 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl r(CGAAAGC)(a), crude r(CGAAAGC) (b) and crude r(GCGAAAGC)(c) with a C-18 column (M&S pack C18, 4.6 mm ID x 15 cm). The elution buffer was a mixture of CH_3CN and 0.1 M triethylammonium acetate (pH 7.0).

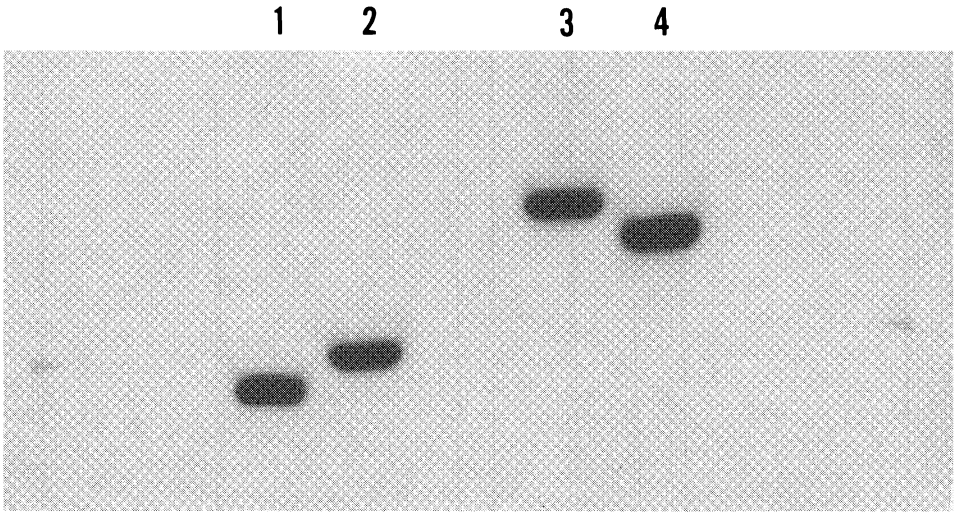


Fig. 3. 20% Polyacrylamide - 7 M urea gel electrophoresis of ^{32}P-labeled d(GCGAAAGC)(lane 1), d(CGAAAGC)(lane 2), r(GCGAAAGC)(lane 3), and r(CGAAAGC)(lane 4).

7mer, d(CGAAAGC) in 20% polyacrylamide - 7 M urea gel electrophoresis (see Fig. 3). ^{32}P-Labeled synthetic r(GCGAAAGC) and r(CGAAAGC) were analyzed by electrophoresis under the same conditions. The octamer r(GCGAAAGC) did not, however, show the unusual mobility comparing with the mobility of r(CGAAAGC) (Fig. 3). This is because the stable hairpin structure of d(GCGAAAGC) is characteristic of oligodeoxynucleotides whose sugar moiety has the C2'-endo conformation.[6)]

Mr. Masahide Ishikawa and Mr. Masahiro Harada of this laboratory are gratefully acknowledged for their technical assistance. We wish also to thank Prof. Kimitsuna Watanabe of the Tokyo Institute of Technology for his helpful comments. The present work was supported in part by a Grant-in-Aid for Scientific Research (No. 01780293) from the Ministry of Education, Science and Culture.

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(Received June 29, 1989)