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A NEW SOLID-PHASE SYNTHESIS OF RIBOOLIGONUCLEOTIDES USING THE 3'-PHOSPHORO-p-ANISIDATE PROTECTING GROUP FOR STEPWISE SYNTHESIS IN THE 3'-DIRECTION

Shigenori Iwai, Miyuki Asaka, Hideo Inoue and Eiko Ohtsuka*
Faculty of Pharmaceutical Sciences, Hokkaido University,
Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan

A nonaribonucleotide CAGGUAAGU has been synthesized by the phosphotriester solid-phase method. The 5'-linked 2'-O-tetrahydrofuranyl-N-benzoylcytidine 3'-O-(o-chlorophenyl) phosphate was used as the starting material and the chain was elongated in the 3'-direction by removal of the phosphoro-p-anisidate group using isoamyl nitrite under neutral conditions.

KEYWORDS — nonaribooligonucleotide; splice site RNA; phosphoro-p-anisidate method; isoamyl nitrite

Solid-phase procedures for the synthesis of deoxyribooligonucleotides have been facilitated by improvements in the phosphodiester activation method and the introduction of the phosphoramidite method.[1] In the ribo-series, the phosphotriester solid-phase synthesis has been investigated only to a limited extent[2] due to difficulties encountered when using combinations of protecting groups for the 2'- and 5'-hydroxyl groups. We have previously reported a synthesis of ribooligonucleotides on a polystyrene support in the 5'-direction by selective removal of the 5'-dimethoxytrityl group with zinc bromide[3] in the presence of a 2'-tetrahydrofuranyl protecting group.[2c] In this communication we describe a solid-phase synthesis of ribooligonucleotides involving elongation of a chain in the 3'-direction by selective removal of the 3'-phosphoro-p-anisidate protecting group with isoamyl nitrite under neutral conditions. In this approach, the dedimethoxytritylation to yield the starting material (**1**) with zinc bromide can be performed in solution and **1** can be purified even if the reaction with zinc bromide does not go to completion. As shown in Chart 1, the 3'-phosphodiester end of the growing chain was activated with 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT).[4] The key intermediates N-protected-2'-O-tetrahydrofuranyl-nucleoside 3'-O-(p-chlorophenyl) phosphoro-p-anisidates(**1**) were prepared by phosphorylation of N-protected 5'-O-dimethoxytrityl-2'-O-tetrahydrofuranylnucleosides[5] with o-chlorophenyl N-p-methoxyphenylchlorophosphoramidate[6] followed by treatment with zinc bromide.[7] The starting nucleotide (**1b**) was joined to 1% cross-linked aminomethylene polystyrene via the activated succinyl derivative of 2'-O-

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tetrahydrofuranyl-N-benzoylcytidine 3'-(o-chlorophenyl) phosphoro-p-anisidate (**2b**) using conditions described for the reaction with N-benzoyldeoxyadenosine 3'-O-(o-chlorophenyl) phosphoro-p-anisidate.[8] The unchanged amino groups on the support were blocked by acetylation with acetic anhydride-pyridine(2:3, v/v). The content of the nucleotide was estimated by formation of a picrate [9] and 0.09 mmol/g of the amino groups was found to have reacted in a yield of 83%.

The nucleotide resin (**3b**)(5 μmol) was used for the synthesis of a nonanucleotide CAGGUAAGU. The procedures for removal of the anisidate with isoamyl nitrate to give **4b** and condensation of mononucleotides (20 μmol each) to yield, e.g., **5** are summarized in Table I. MSNT was used as the condensing reagent and unreacted phosphodiesters were blocked by methylation. 2',3'-O-Ethoxymethylideneuridine[10] was used for the last condensation.

Deblocking of the linked nonamer (**6**, n=7) was performed by hydrolysis with 1,1,3,3,-tetramethylguanidinium pyridine-2-aldoximate[4] and by treatment with concentrated ammonia. The tetrahydrofuranyl groups were removed at pH 2.0 using conditions described for the solution-phase synthesis.[7] The completely deprotected nonanucleotide (**7**) was isolated by gel filtration on Sephadex G-25 and chromatography on DEAE-TOYOPEARL 650S. The product eluted in the last peak was fractionated by high pressure liquid chromatography on C-18 silica gel (Nucleosil $5C_{18}$) in a yield of ca. 1%, 3.7 A_{260} units. The purity and sequence were analyzed by high pressure anion-exchange chromatography on TSK gel DEAE-2SW and by mobility shift analysis.[11]

Thus the ribononanucleotide containing the 5'-consensus splice junction[12] was synthesized on a polymer support in the 3'-direction. Analogs of this oligonucleotide can be used for studies of splicing mechanisms in messenger RNAs. The method can be applied to the synthesis of ribooligonucleotide with modifications at the 3'-end by using 3'-substituted nucleosides at the last condensation.

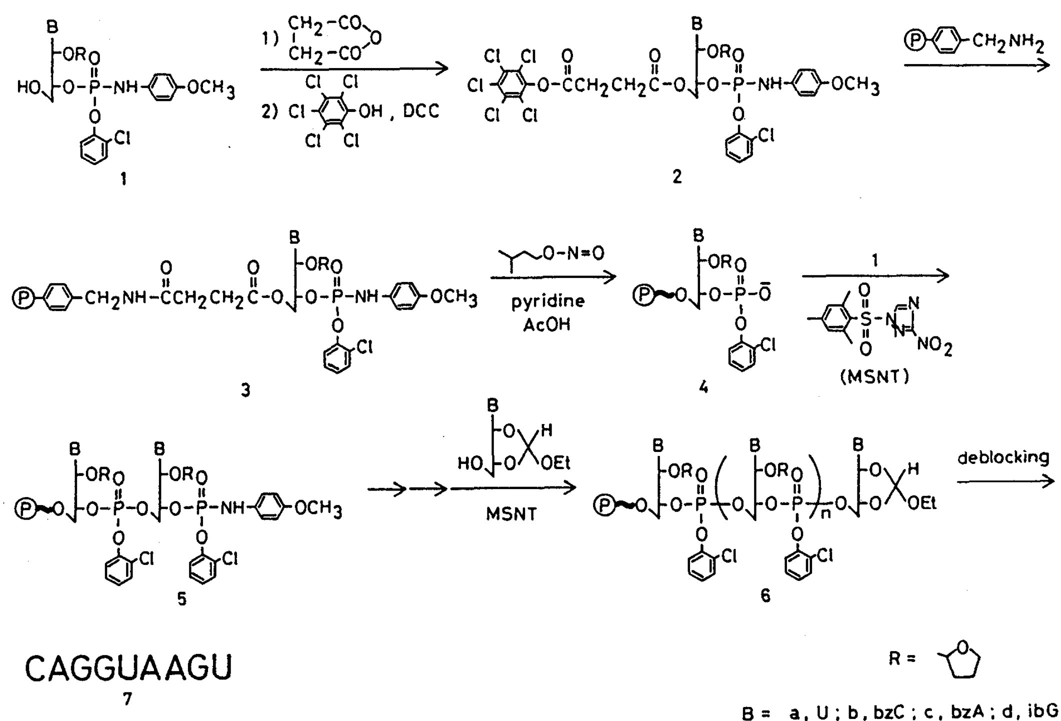

Chart 1

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Table I. Procedure for the Synthesis

Step	Solvent or reagent	Amount (ml)	Operation (time)	Number of operations
1	Pyridine	2	wash	2
2	Isoamyl nitrite	0.5		
	Pyridine-acetic acid (1:1, v/v)	2	2.5h [a]	1
3	Pyridine-acetic acid	2	wash	2
4	0.5 M TAA[b] in DMF	2	wash	3
5	Dichloromethane	2	wash	3
6	Ether	2	wash	3
7	THF	2	wash	3
8	Pyridine	2	wash	3
9	Pyridine	0.5	coevaporation	3
10	Nucleotide in Pyridine	15 mg in 0.5 ml	coevaporation	1
11	MSNT in pyridine	20 mg in 0.5 ml	30 min[a]	1
12	Pyridine	2	wash	1
13	10 % MeOH in pyridine	0.5	10 min [a]	1
	MSNT	20 mg		

a) at 30°C, b) Triethylammonium acetate.

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