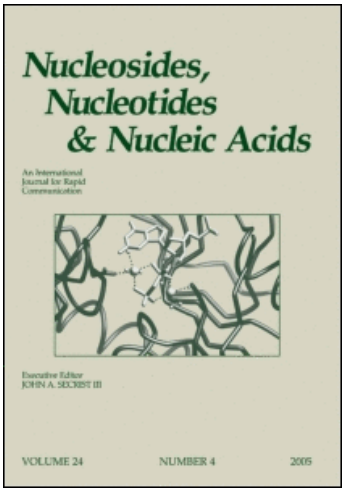

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THE (2-CYANO-1-PHENYL)ETHOXYCARBONYL (CPEOC) GROUP- A NEW PROTECTING GROUP FOR OLIGORIBONUCLEOTIDE SYNTHESIS

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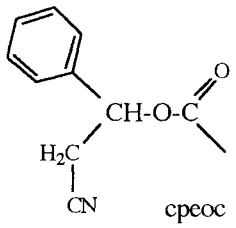
Abstract. - The (2-cyano-1-phenyl)ethoxycarbonyl (cpeoc) group was developed as a new base-labile protecting group for the 5'-OH function in solid-phase synthesis of oligoribonucleotide by the phosphoramidite approach using the 4-methoxytetrahydropyran-4-yl (mthp) group for 2'-protection. The syntheses of the monomeric building blocks and the first oligoribonucleotides obtained by this approach are described.

Introduction. - The development of an adequate protecting group combination for the 2'- and 5'-OH function is still the crucial problem in oligoribonucleotide synthesis. The 2'-protecting group has to be stable under the conditions required for the removal both of the 5'-protecting group during oligonucleotide synthesis and of the phosphate and base protecting groups at the end of synthesis. Therefore the use of an acid-labile 2'-OH protecting group, like the 4-methoxytetrahydropyran-4-yl (mthp) group, calls for the displacement of the traditional trityl blocking groups at the 5'-OH position by using a very base-labile 5'-OH function which can be removed selectively without harming the base and phosphate protecting groups during the building-up of the oligonucleotide chain in a DNA-synthesizer.

We developed the (2-cyano-1-phenyl)ethoxycarbonyl (cpeoc) group for the anticipated purpose since it is very base-labile and is compatible with the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) blocking groups which we use in our npe/npeoc strategy for nucleobase and phosphate protection. The cpeoc group can be cleaved very fast by 0.1 M DBU in acetonitrile whereby the half-lives of the β-elimination process were found in the range of 7-14 sec as determined by HPLC investigations (*Table 1*).

We wish to report the synthesis of the monomeric building blocks and first attempts to synthesize oligoribonucleotides via this new phosphoramidite approach at solid support.



cpeoc

Table 1. *Half-lives of 5'-O-cpeoc-2'-deoxy-nucleosides in 0.1 M DBU*

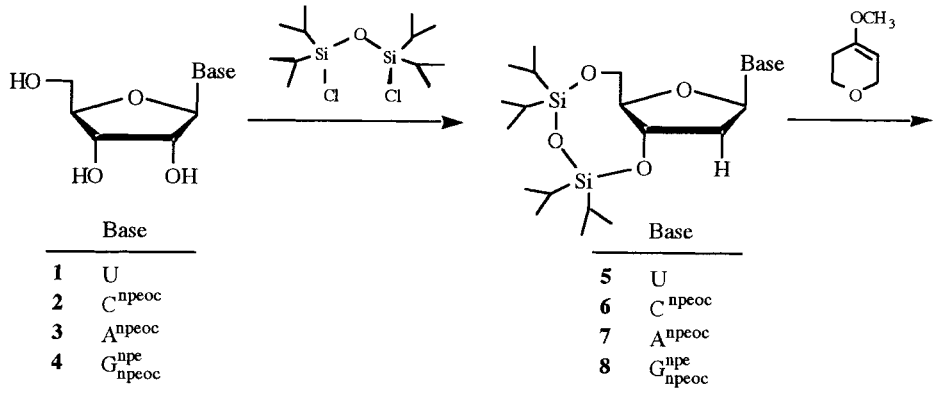
Base	$t_{1/2}$ sec
T	12
C^{npeoc}	12
A^{npeoc}	14
G^{npe}_{npeoc}	7

Syntheses. - At the beginning, the functional groups of the adenosine, cytidine, and guanosine derivatives were protected with the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) protecting groups which we use in our npe/npeoc strategy[1,2].

To achieve a selective introduction of the 2'-mthp protecting group, the 3'- and 5'-OH functions of the nucleosides **1-4** were intermediarily blocked with the bifunctional 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (tipds) group of Markiewicz[3] by reaction with a slight excess of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in abs. pyridine at room temperature[3-5] (*Scheme 1*). The 2'-O-mthp-nucleosides **13-16** were prepared by reaction of the 3',5'-O-tipds-protected nucleosides **5-8** with excess (4-5.5 eq.) of 3,6-dihydro-4-methoxy-2H-pyran[6-8] and a catalytic amount of pyridinium-toluene-4-sulfonate (0.20-0.35 eq.) in CH_2Cl_2 at room temperature[9,10]. Without isolation, the tipds group was removed by treatment with NH_4F in MeOH[11] to give the 2'-O-mthp-nucleosides **13-16**.

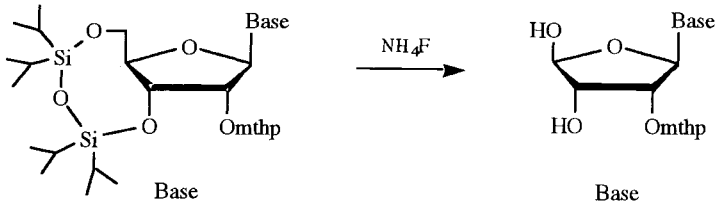
To introduce the cpeoc group into the 5'-O-position, the 2-cyano-1-phenyl-ethoxycarbonylchloride (**18**) was used. This reagent **18** was synthesized by treatment of the 3-hydroxy-3-phenylpropanenitrile[12] (**17**) with 2 eq. trichloromethyl chloroformate and 1 eq. triethylamine in THF within 6 h. The product **18** was obtained in about 70 to 75% yield together with two side-products, which were identified by their NMR-data as the 3-chloro-3-phenylpropanenitrile (**19**, 15%) and the educt **17** (10%) (*Scheme 2*).

It wasn't necessary to separate the 2-cyano-1-phenyl-ethoxycarbonylchloride (**18**) from the side-products **17** and **19** but the whole reaction mixture was applied to introduce the cpeoc group into the 5'-O-position of the npe/npeoc protected 2'-O-mthp-nuclosides **13-16**. Therefore a slight excess (1.3-1.5 eq.) of 2-cyano-1-phenyl-ethoxy-carbonylchloride (**18**) was added at -60°- -40°C to a pyridine solution of the derivatives **13-16** and stirred at -60°- -20°C within 4 to 6 h (*Scheme 3*). After workup and flash chromatography, the desired 5'-O-cpeoc-substituted nucleosides **20**, **22**, **25** and **27** were obtained in 58-83% yield, besides the 3',5'-bis-O-substituted derivatives **21**, **23**, **26** and



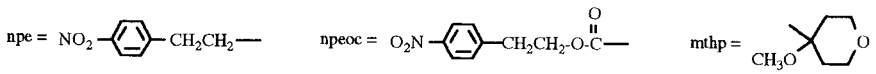
Base	
1	U
2	C^{npeoc}
3	A^{npeoc}
4	G^{npe}_{npeoc}

Base	
5	U
6	C^{npeoc}
7	A^{npeoc}
8	G^{npe}_{npeoc}

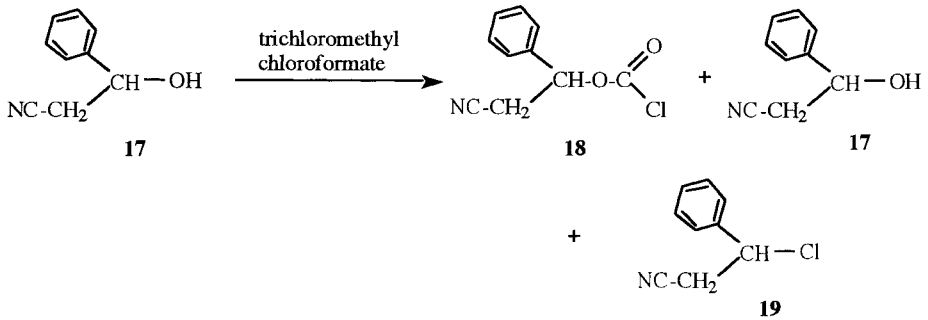


Base	
9	U
10	C^{npeoc}
11	A^{npeoc}
12	G^{npe}_{npeoc}

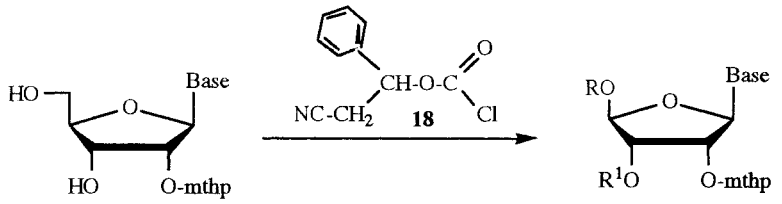
Base	
13	U
14	C^{npeoc}
15	A^{npeoc}
16	G^{npe}_{npeoc}



Scheme 1

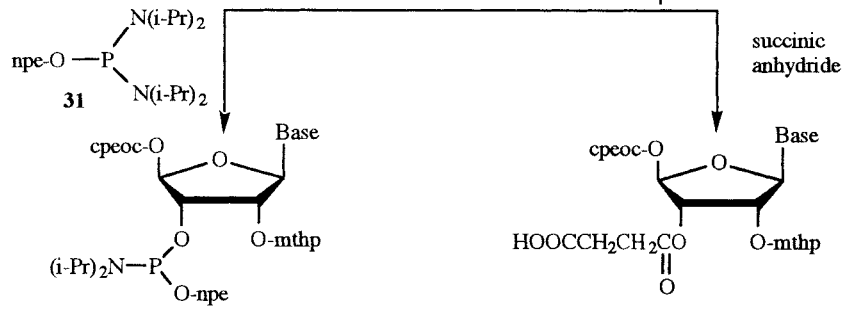


Scheme 2



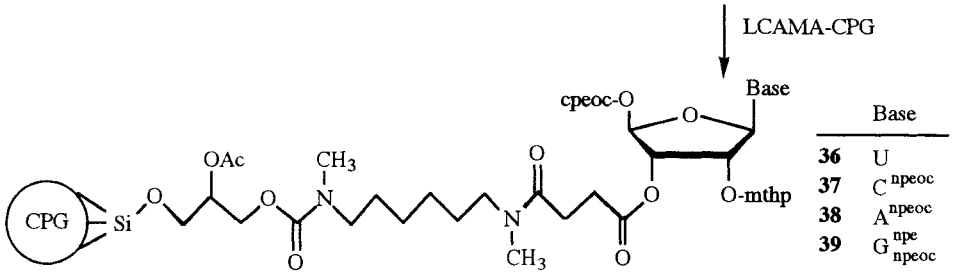
	Base
13	U
14	C^{npeoc}
15	A^{npeoc}
16	G^{npe}_{npeoc}

	Base	R	R^1	Yield %
20	U	cpeoc	H	65
21	U	cpeoc	cpeoc	22
22	C^{npeoc}	cpeoc	H	58
23	C^{npeoc}	cpeoc	cpeoc	20
24	C^{npeoc}	H	cpeoc	3
25	A^{npeoc}	cpeoc	H	72
26	A^{npeoc}	cpeoc	cpeoc	17
27	G^{npe}_{npeoc}	cpeoc	H	83
28	G^{npe}_{npeoc}	cpeoc	cpeoc	10



	Base	Yield %
27	U	75
28	C^{npeoc}	76
29	A^{npeoc}	81
30	G^{npe}_{npeoc}	68

	Base	Yield %
32	U	93
33	C^{npeoc}	94
34	A^{npeoc}	91
35	G^{npe}_{npeoc}	86



	Base
36	U
37	C^{npeoc}
38	A^{npeoc}
39	G^{npe}_{npeoc}

Scheme 3

Table 2. *Synthesized 2'-O-mthp-protected oligonucleotides*

Sequence	Activator	Condensation time sec
AAAA (**40**)	0.5 M tetrazole in CH_3CN	700
AAAA (**40**)	0.5 M pyridinium chloride in CH_3CN	140
AAAA (**40**)	0.6 M 5-ethylthiotetrazole in CH_3CN	300
AAAAAAAA (**41**)	0.5 M tetrazole in CH_3CN	700
AAAAAAAA (**41**)	0.5 M pyridinium chloride in CH_3CN	140
AAAAAAAA (**41**)	0.6 M 5-ethylthiotetrazole in CH_3CN	300
GGGGGGGG (**42**)	0.6 M 5-ethylthiotetrazole in CH_3CN	300
UUUUUUUU (**43**)	0.5 M tetrazole in CH_3CN	700
UUUUUUUU (**43**)	0.6 M 5-ethylthiotetrazole in CH_3CN	300

28 which were isolated as by-products in 10-22% yield. In the case of the cytidine derivate, the 3'-O-monosubstituted product **24** could also be isolated in 3% yield.

The 3'-phosphoramidites **27-30** were synthesized by phosphitylation using bis(diisopropylamino)-[2-(4-nitrophenyl)ethoxy]-phosphane[2] (**31**). The yields after work-up and flash chromatography ranged from 68 to 81%.

As a second series of building blocks for solid-phase synthesis, the 3'-O-succinoylnucleosides **32-35** were synthesized by reaction of the 5'-O-cpeoc-substituted nucleosides **20**, **22**, **25** and **27** with succinic anhydride and N-methylimidazole in CH_2Cl_2 in 86 to 93% yield. The 3'-O-succinoylnucleosides **32-35** were then reacted with LCAMA-CPG[13] (= (long-chain-alkyl)methylamine controlled-pore glass, 500 Å) using the coupling reagent O-{[cyano(ethoxycarbonyl)methylidene]-amino}-1,1,3,3,-tetramethyluronium tetrafluoroborate (TOTU) and N-methylmorpholine in CH_3CN followed by a capping process with acetic anhydride and N-methylimidazole in pyridine to give the solid supports **36** to **39**.

The building-up of oligoribonucleotides was carried out by the solid-phase phosphoramidite method[15-18] and was performed in an *Applied Biosystems 380 B* synthesizer by attachment of a small column filled with the desired starting nucleoside **36-39**. The oligoribonucleotide assembly consists of a programmed repetitive cycle of four chemical steps and intermediate washing steps:

1) deprotection of the terminal cpeoc group with 0.1 M DBU in CH_3CN for 120 sec
2) coupling with 0.1 M phosphoramidite **27-30** and different activators with various condensation times
3) capping with Ac_2O/2,6-dimethylpyridine/1-methylimidazole in THF for 25 sec
4) oxidation with 0.05 M I_2 in THF/pyridine/H_2O for 27 sec

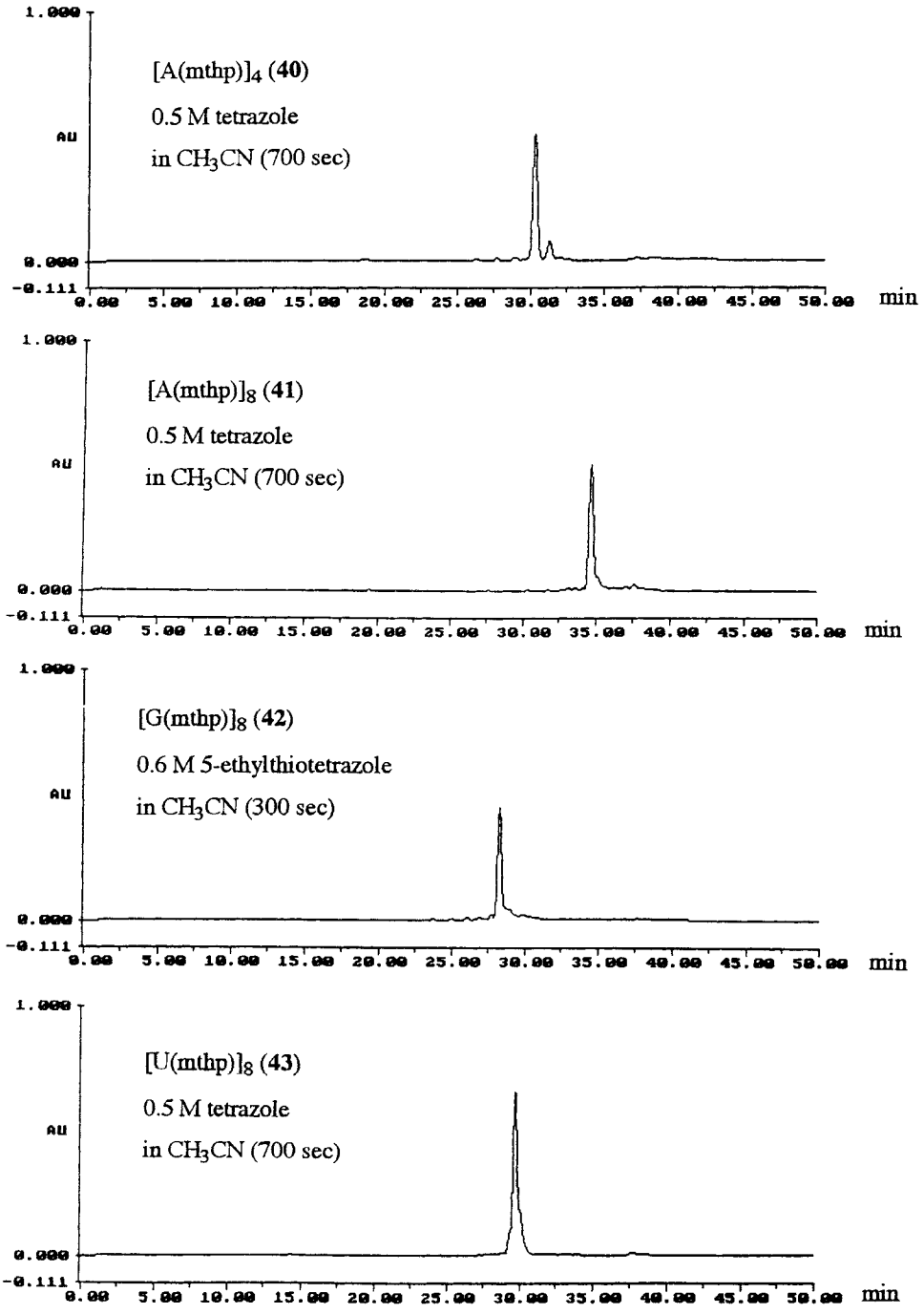


Figure 1. RP-18 *HPLC diagramms of 40, 41, 42 and 43*

After the last synthesis cycle, the support was treated with 2 M DBU in CH_3CN for 10 h to deblock all npe/npeoc protecting groups from the oligonucleotide. Thereafter, the 2'-O-mthp-protected oligoribonucleotide was cleaved from the support by treatment with concentrated NH_3 solution for 2 h. Finally the products were lyophilized in a *Speed-vac* concentrator and the quality of the crude 2'-O-mthp-protected oligoribonucleotide was analyzed by reversed-phase HPLC.

In this way various oligonucleotides have been synthesized, by using different condensation conditions (*Table 2*).

For the tetramer **40** the three condensation activators tetrazole (700 sec), pyridinium chloride[19, 20, 11] (140 sec) and 5-ethylthiotetrazole[21] (300 sec) have been tested getting the best results with the 0.6 M solution of 5-ethylthiotetrazole in CH_3CN. By using tetrazole or pyridinium chloride, the HPLC of product **40** exhibited another peak of a side product towards longer retention times (*Figure 1*). But in the case of the octamer **41** (*Figure 1*), no difference in quality could be observed by varying the condensation conditions (Table 1). The HPLC diagramms of the crude 2'-O-mthp-protected octamers of G **42** and U **43** are shown in *Figure 1*. In the case of the 2'-O-mthp-protected uridine oligomer **43** the RP-18 HPLC gave, independent of the condensation activator (Table 1), a shoulder towards longer retention times of the main peak in accordance to results made by F. Bergmann[10,14] with uridine-rich sequences.

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