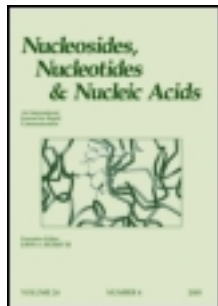


This article was downloaded by: [University of Arizona]

On: 12 January 2013, At: 15:28

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Nucleosides and Nucleotides

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/Incn19>

### The (2-Cyano-1-phenyl)ethoxycarbonyl (CPEOC) Group-A New Protecting Group for Oligoribonucleotide Synthesis

Ursula Münch<sup>a</sup> & Wolfgang Pfeleiderer<sup>a</sup>

<sup>a</sup> Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-78434, Konstanz

Version of record first published: 22 Aug 2006.

To cite this article: Ursula Münch & Wolfgang Pfeleiderer (1997): The (2-Cyano-1-phenyl)ethoxycarbonyl (CPEOC) Group-A New Protecting Group for Oligoribonucleotide Synthesis, *Nucleosides and Nucleotides*, 16:5-6, 801-808

To link to this article: <http://dx.doi.org/10.1080/07328319708002955>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

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

Ursula Münch and Wolfgang Pfeleiderer\*

Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-78434 Konstanz

**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  $\beta$ -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.

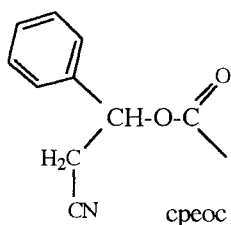


Table 1. Half-lives of 5'-O-cpeoc-2'-deoxy-nucleosides in 0.1 M DBU

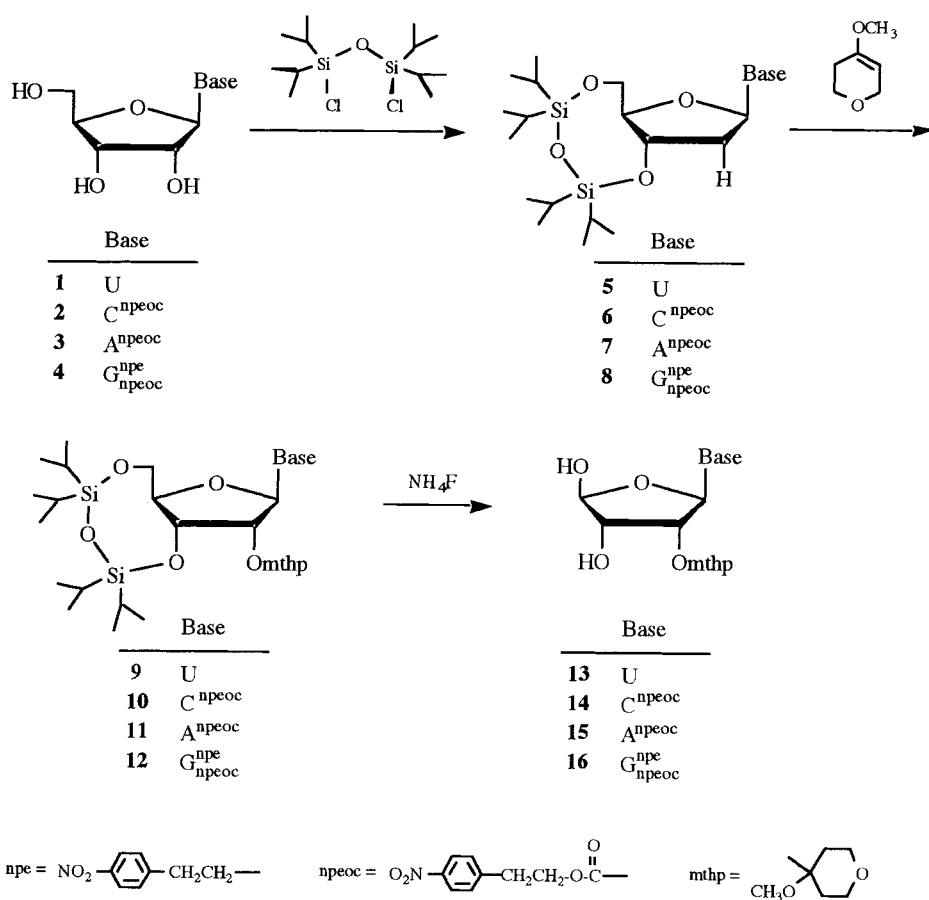
Base	$t_{1/2}$ sec
T	12
C <sup>npeoc</sup>	12
A <sup>npeoc</sup>	14
G <sup>npe</sup> <sub>npeoc</sub>	7

**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<sup>1,2</sup>.

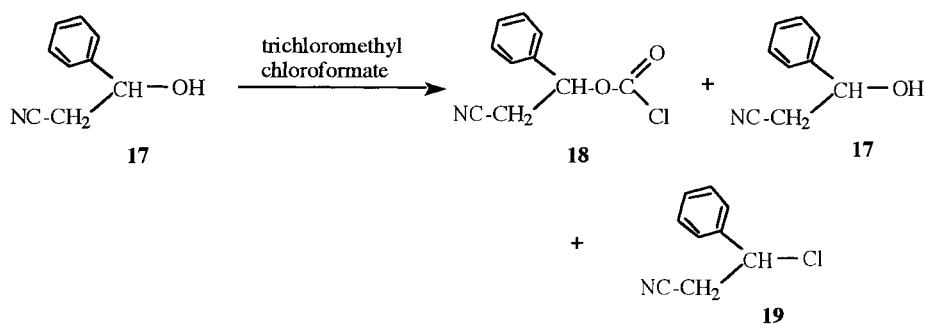
To achieve a selective introduction of the 2'-mthp protecting group, the 3'- and 5'-OH functions of the nucleosides **1-4** were intermediately blocked with the bifunctional 1,1,3,3-tetraisopropylidisiloxane-1,3-diyl (tipds) group of Markiewicz<sup>3</sup> by reaction with a slight excess of 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane in abs. pyridine at room temperature<sup>3-5</sup> (*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<sup>6-8</sup> and a catalytic amount of pyridinium-toluene-4-sulfonate (0.20-0.35 eq.) in CH<sub>2</sub>Cl<sub>2</sub> at room temperature<sup>9,10</sup>. Without isolation, the tipds group was removed by treatment with NH<sub>4</sub>F in MeOH<sup>11</sup> 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<sup>12</sup> (**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*).

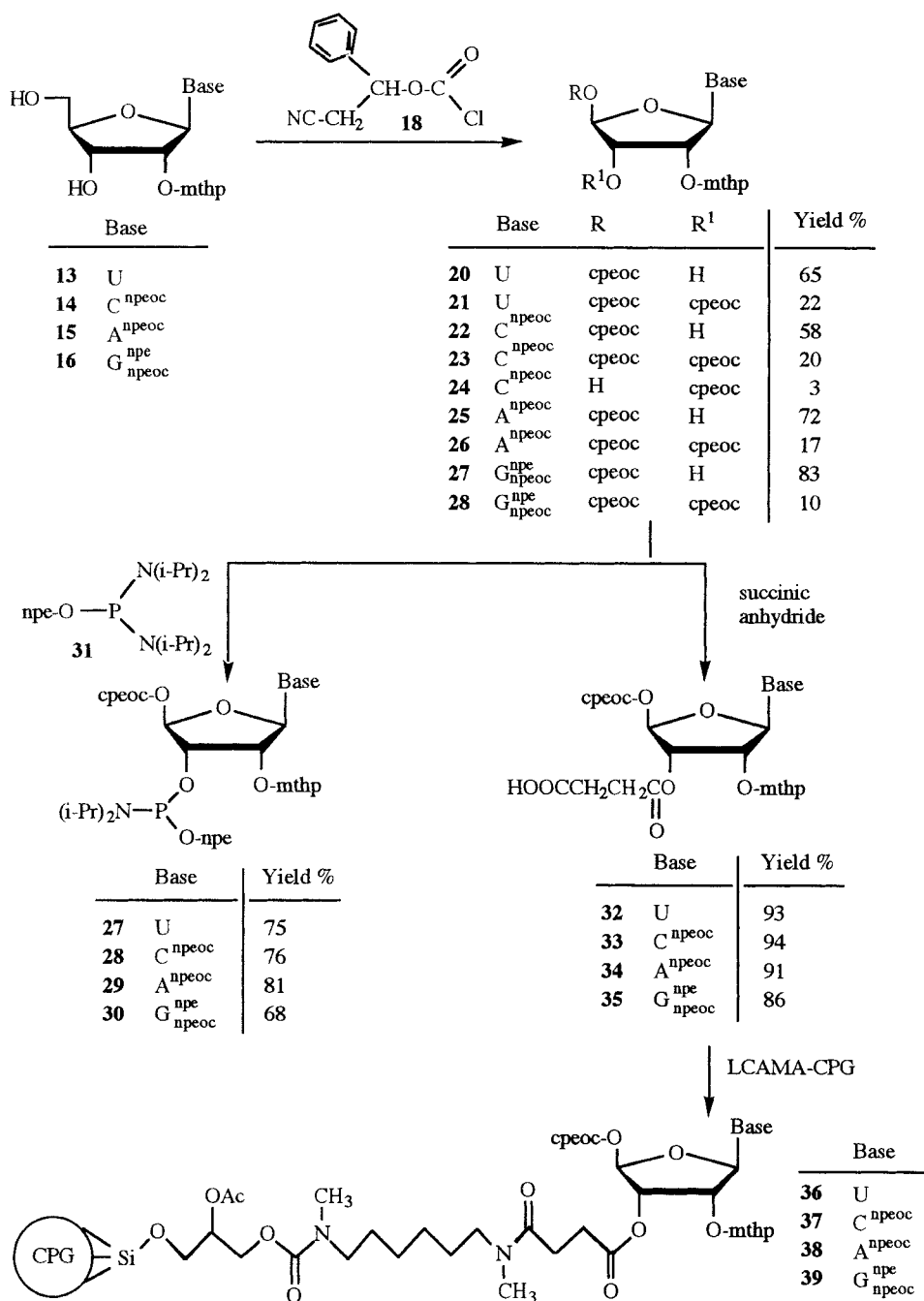
It wasn't necessary to separate the 2-cyano-1-phenyl-ethoxycarbonylchloride (**18**) from the side-products **17** and **19** but the whole reaction mixture was applied to introduce the cpeoc group into the 5'-O-position of the npe/npeoc protected 2'-O-mthp-nucleosides **13-16**. Therefore a slight excess (1.3-1.5 eq.) of 2-cyano-1-phenyl-ethoxycarbonylchloride (**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



Scheme 1



Scheme 2



Scheme 3

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

Sequence	Activator	Condensation time sec
AAAA ( <b>40</b> )	0.5 M tetrazole in CH <sub>3</sub> CN	700
AAAA ( <b>40</b> )	0.5 M pyridinium chloride in CH <sub>3</sub> CN	140
AAAA ( <b>40</b> )	0.6 M 5-ethylthiotetrazole in CH <sub>3</sub> CN	300
AAAAAAAA ( <b>41</b> )	0.5 M tetrazole in CH <sub>3</sub> CN	700
AAAAAAAA ( <b>41</b> )	0.5 M pyridinium chloride in CH <sub>3</sub> CN	140
AAAAAAAA ( <b>41</b> )	0.6 M 5-ethylthiotetrazole in CH <sub>3</sub> CN	300
GGGGGGGG ( <b>42</b> )	0.6 M 5-ethylthiotetrazole in CH <sub>3</sub> CN	300
UUUUUUUU ( <b>43</b> )	0.5 M tetrazole in CH <sub>3</sub> CN	700
UUUUUUUU ( <b>43</b> )	0.6 M 5-ethylthiotetrazole in CH <sub>3</sub> CN	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<sup>2</sup> (**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<sub>2</sub>Cl<sub>2</sub> in 86 to 93% yield. The 3'-O-succinoylnucleosides **32-35** were then reacted with LCAMA-CPG<sup>13</sup> (= (long-chain-alkyl)methylamine controlled-pore glass, 500 Å) using the coupling reagent O-{[cyano(ethoxycarbonyl)methylidene]-amino}-1,1,3,3,-tetra-methyluronium tetrafluoroborate (TOTU) and N-methylmorpholine in CH<sub>3</sub>CN 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<sup>15-18</sup> 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<sub>3</sub>CN for 120 sec
- 2) coupling with 0.1 M phosphoramidite **27-30** and different activators with various condensation times
- 3) capping with Ac<sub>2</sub>O/2,6-dimethylpyridine/1-methylimidazole in THF for 25 sec
- 4) oxidation with 0.05 M I<sub>2</sub> in THF/pyridine/H<sub>2</sub>O for 27 sec

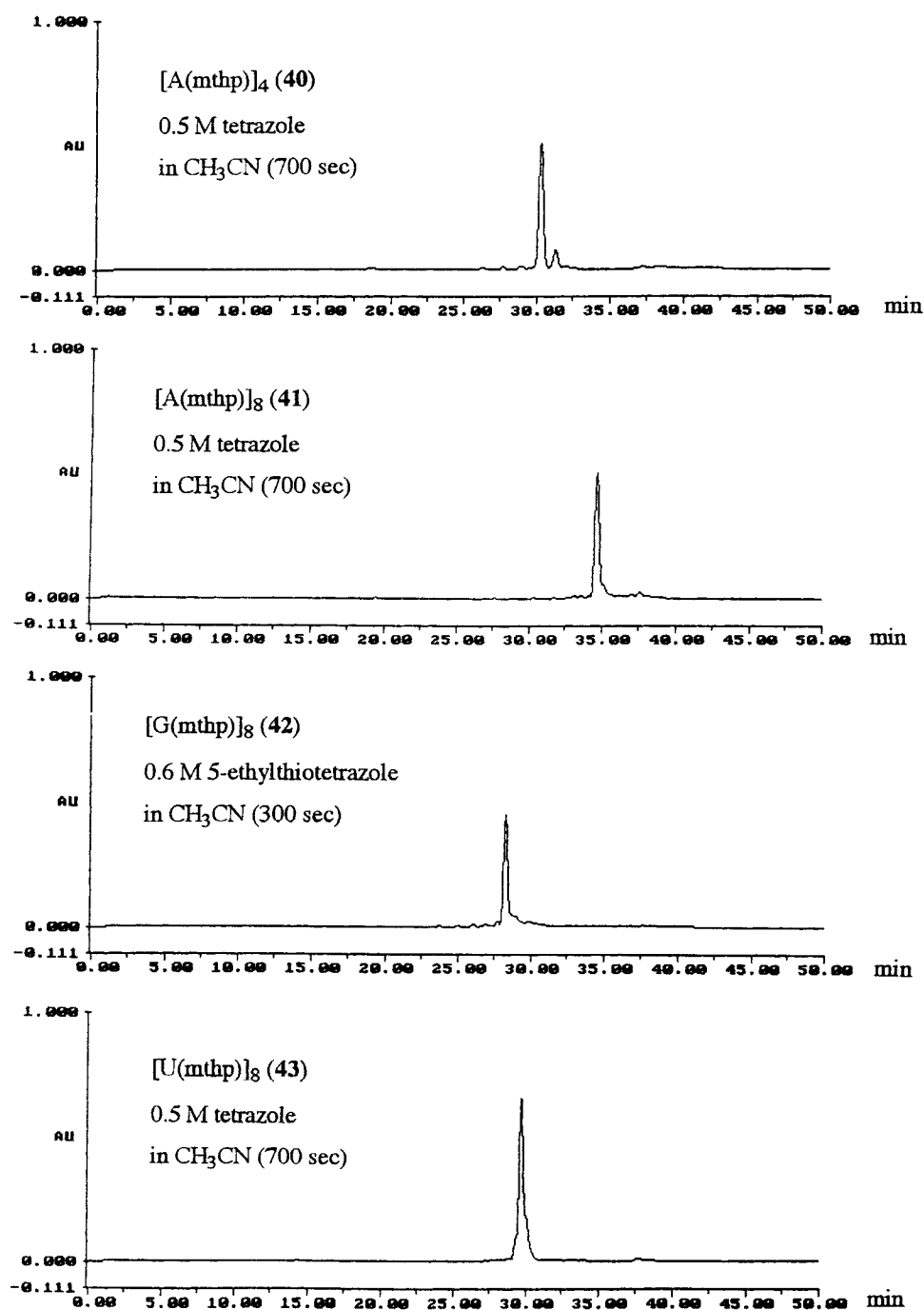


Figure 1. RP-18 HPLC diagrams of 40, 41, 42 and 43

After the last synthesis cycle, the support was treated with 2 M DBU in CH<sub>3</sub>CN 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<sub>3</sub> 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<sup>19, 20, 11</sup> (140 sec) and 5-ethylthiotetrazole<sup>21</sup> (300 sec) have been tested getting the best results with the 0.6 M solution of 5-ethylthiotetrazole in CH<sub>3</sub>CN. 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 diagrams 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<sup>10,14</sup> with uridine-rich sequences.

#### REFERENCES

1. Himmelsbach, F., Ph. D. Thesis, University of Konstanz **1984**
2. Schirmeister, H., Ph. D. Thesis, University of Konstanz **1988**
3. Markiewicz W. T. *J. Chem. Res. (M)* **1979**, 181
4. Schirmeister H., Diploma Thesis, University of Konstanz **1984**
5. Pfister M., Diploma Thesis, University of Konstanz **1986**
6. Owen, G. R.; Reese C. B. *J. Chem. Soc. (C)* **1970**, 2401
7. Arentzen, R.; Yankui, Y. T.; Reese, C. B. *Synthesis* **1975**, 509
8. Reese, C. B.; Saffhill, R.; Sulston, J. E. *Tetrahedron* **1970**, 26, 1023
9. Reese, C. B. *Nucleos. Nucleot.* **1987**, 6, 121
10. Bergmann, F., Ph. D. Thesis, University of Konstanz **1993**
11. Beier, M., Ph. D. Thesis, University of Konstanz **1996**
12. Kaiser, E. M.; Hauscr, C. R. *J. Org. Chem.* **1968**, 33, 3403
13. Stengele, K. P.; Pfeleiderer, W. *Nucleic Acids Res. Sym. Ser.* **1989**, 21, 101
14. Bergmann, F.; Pfeleiderer, W. *Helv. Chem. Acta* **1994**, 77, 988
15. Matteucci, M. D.; Caruthers, M. H. *Tetrahedron Lett.* **1980**, 21, 719



16. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, 22, 1859
17. Dorman, M. A.; Noble, S. A.; McBride, C. J.; Caruthers, M. H. *Tetrahedron* **1984**, 40, 95
18. Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M. D.; Stabiinski, Z.; Tang, J.-T. *Methods Enzymol.* **1987**, 154, 4051
19. Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, 20, 1882
20. Gryaznov, S. M.; Letsinger, R. L. *J. Am. Chem. Soc.* **1991**, 113, 5877
21. Vinayak, R.; Colonna, F.; Tsou, D.; Mullah, B.; Andrus, A.; Sproat, B. *Nucleic Acids Res. Sym. Ser.* **1994**, 31, 165