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# NEW IMPROVEMENTS IN OLIGONUCLEOTIDE SYNTHESIS BY USE OF THE p-NITROPHENYLETHYL PHOSPHATE BLOCKING GROUP AND ITS DEPROTECTION BY DBU OR DBN

EUGEN UHLMANN, WOLFGANG PFLEIDERER
Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-7750 Konstanz/West Germany

Phosphate protection in the phosphotriester approach is improved by the new, versatile p-nitrophenylethyl group due to its stability in the condensation step and its clean removal by DBU and DBN respectively.

Recent investigations in the field of oligonucleotide synthesis [1-4] have revealed that the phosphate blocking group plays the most important role in the phosphotriester approach. According to our present knowledge none of the proposed protecting groups such as phenyl [5], o-chloro-[6] or p-chlorophenyl [7], trichloroethyl [8], tribromoethyl [9], cyanoethyl [10], etc., is perfect due to 1) some degree of instability in the condensation step leading to the formation of the internucleotidic triester, 2) some lability of the phosphotriester group itself during storage or selective deblocking at the 3'- and 5'-end for chain elongation, and 3) wrong or difficult cleavage as well as partial isomerization of the 3' → 5' internucleotide bond on deprotection to the natural phosphodiester function.

We have shown [11] that a new class of phosphate blocking groups based on 2-phenylethanol offers much better properties in every respect including a wide range of reactivities depending on the various phenyl substituents as well as the important advantage of ß-elimination mechanism on deblocking. Especially the latter feature is required in order to guarantee a selective cleavage without harming the internucleotidic bonds.

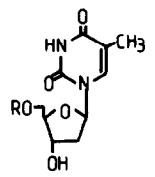
From work with mononucleotide phosphotriesters [11] we recommended the 2-(2-chloro-4-nitrophenyl)ethyl group for phosphate protection because of its intermediary reactivity on deblocking and good stability against various reagents. During the syntheses of oligonucleotides via the triester approach using fully blocked 3'-phosphotriesters as building blocks we noticed, however, that this new group is to a small extent too labile for protection of the internucleotidic linkage in its triester form due to the fact that selec-

tive deblocking of a substituted phenyl group at the fully blocked 3'-phosphotriester function is also associated with a few percent cleavage of the 2-(2-chloro-4-nitrophenyl)ethyl substituent.

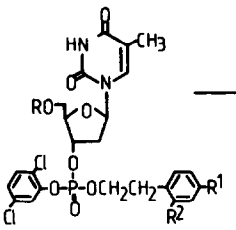
This insufficiency could be overcome by using the much more stable and more easily available 4-nitrophenylethyl group, which in our hands turned out so far to be just perfect for phosphotriester syntheses.
Starting from 5'-O-trityl-(1) and 5'-O-monomethoxytrityl-thymidine (2) phosphorylation was achieved by 2,5-dichlorphenylphosphodichloridate in presence of 1,2,4-triazole and subsequent addition of the substituted 2-phenylethanol as the second alcoholic component yielding the phosphotriesters 3-7. Cleavage of the 2,5-dichlorophenyl blocking group by Reese's method [12] with p-nitrobenzaldoxime and triethylamine works selectively in high yields giving the corresponding phosphodiesters 8-12 in form of their triethylammonium salts. Condensation with thymidine (13) and 3'-O-tert.butyldimethylsilylthymidine (14) respectively was carried out under the activating power of 2,4,6-triisopropylbenzenesulfonyl-nitroimidazolide (TPSNI) [13] leading to the various dinucleoside phosphotriesters 16-20 in variable yields between 47 and 86 %. During these investigations we just recently found that 2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazolide (TPSNT) [14] is an even more powerful condensing agent in this series as seen from the reaction between 9 and 14 forming 18 in almost quantitative yield. Future studies will prove the general usefulness of TPSNT and its superiority over the other known phosphate activators.

Selective deblocking experiments with 16-20 revealed that ß-elimination of the 2-(2-chloro-4-nitrophenyl)ethyl group takes place already with triethylamine in acetonitrile at room temp., whereas the o- and p-nitrophenylethyl groups show very slow cleavage under these conditions. Since also tetramethylguanidine needed at least 24 h for a quantitative deprotection we tried diazabicyclo[5,4,0]undecen (DBU) and diazabicyclo[4,3,0]nonen (DBN) which deblock cleanly the p-nitrophenylethyl group in 16, 17 and 18 to 22-24 within 60 min completely. Interestingly the o-nitrophenylethyl isomer 20 is again more stable and needed 6 h at room temp. to complete the reaction with forming 25 whereas deprotection of 19 to 24 was fastest and finished in about 20 min.

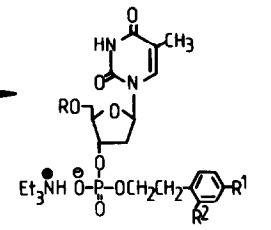
The presented improvements in oligonucleotide synthesis are furthermore seen from the following experiments: on condensation of 9 with thymidine-3'-(2,5-dichlorophenyl)-(4-nitrophenylethyl)-monophosphate (15) the dinucleoside diphosphoditriester 26 is obtained in 54 % yield with TPSNI and in 90 % with TPSNT. Treatment of 26 by the oximate method [12] in the presence of triethylamine as base gave selective deblocking to 27 which on further condensation with 21 led to the fully protected tetranucleoside triphosphotristriester 28 in 82 % yield. ß-Elimination of the phosphate blocking groups by DBU in aceto-



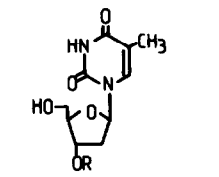
| | R |
|---|---|
| 1 | Tr |
| 2 | MMTr |



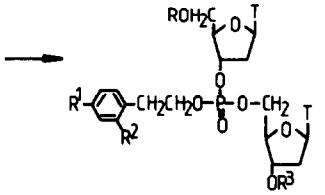
| | R | $R^1$ | $R^2$ | YIELD |
|---|---|---|---|---|
| 3 | Tr | $NO_2$ | H | 70 |
| 4 | MMTr | $NO_2$ | H | 87 |
| 5 | Tr | $NO_2$ | Cl | 60 |
| 6 | MMTr | $NO_2$ | Cl | 72 |
| 7 | Tr | H | $NO_2$ | 86 |



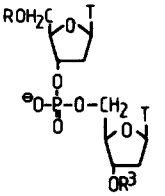
| | R | $R^1$ | $R^2$ | YIELD |
|---|---|---|---|---|
| 8 | Tr | $NO_2$ | H | 95 |
| 9 | MMTr | $NO_2$ | H | 96 |
| 10 | Tr | $NO_2$ | Cl | |
| 11 | MMTr | $NO_2$ | Cl | 90 |
| 12 | Tr | H | $NO_2$ | 91 |



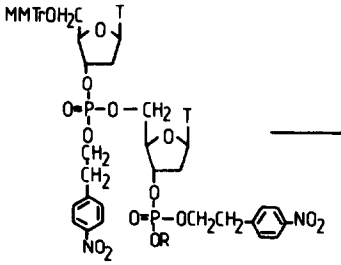
| | R |
|---|---|
| 13 | H |
| 14 | $(CH_3)_2$-Si-C$(CH_3)_3$ |
| 15 |  |



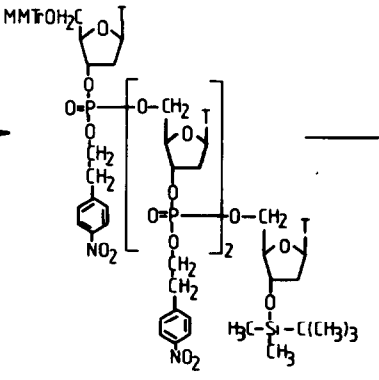
| | R | $R^1$ | $R^2$ | $R^3$ | YIELD |
|---|---|---|---|---|---|
| 16 | Tr | $NO_2$ | H | Si | 86 |
| 17 | MMTr | $NO_2$ | H | H | 47 |
| 18 | MMTr | $NO_2$ | H | Si | 96 |
| 19 | MMTr | $NO_2$ | Cl | Si | 55 |
| 20 | Tr | H | $NO_2$ | Si | 77 |
| 21 | H | $NO_2$ | H | Si | 96 |



| | R | $R^3$ |
|---|---|---|
| 22 | Tr | Si |
| 23 | MMTr | H |
| 24 | MMTr | Si |
| 25 | Tr | Si |



| | R |
|---|---|
| 26 | 2,5-Di-Cl-$C_6H_3$ |
| 27 | $Et_3\overset{\oplus}{N}H$ |



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nitrile or pyridine is completed in 150 min without formation of any detectable side products. Further deprotection was achieved by successive treatment with 80 % acetic acid at 30°C (30 h) and tetrabutylammonium fluoride in pyridine for 1 day giving the tetrameric TpTpTpT 29 after DEAE-Sephadex chromatography with a linear gradient of 0.001 - 0.6 M TEAB puffer in an overall yield of 91 %.

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