Tetrahedron Letters,Vol.23,No.46,pp 4793-4796,1982 0040-4039/82/464793-04$03.00/0
Printed in Great Britain

BIS-(p-NITROPHENYLETHYL)PHOSPHOROMONOCHLORIDATE, A NEW VERSATILE PHOSPHORYLATING AGENT

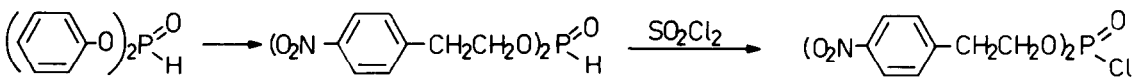
FRANK HIMMELSBACH AND WOLFGANG PFLEIDERER *
Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-7750 Konstanz/West Germany

A new phosphorylating agent, bis-(p-nitrophenylethyl)phosphoromonochloridate, has been prepared and is used for 3'- and/or 5'-phosphorylations of nucleosides. The resulting bis-(p-nitrophenylethyl)phosphotriesters are versatile synthons in oligonucleotide syntheses leading finally to 3'- and/or 5'-terminated oligonucleotides in excellent yields.

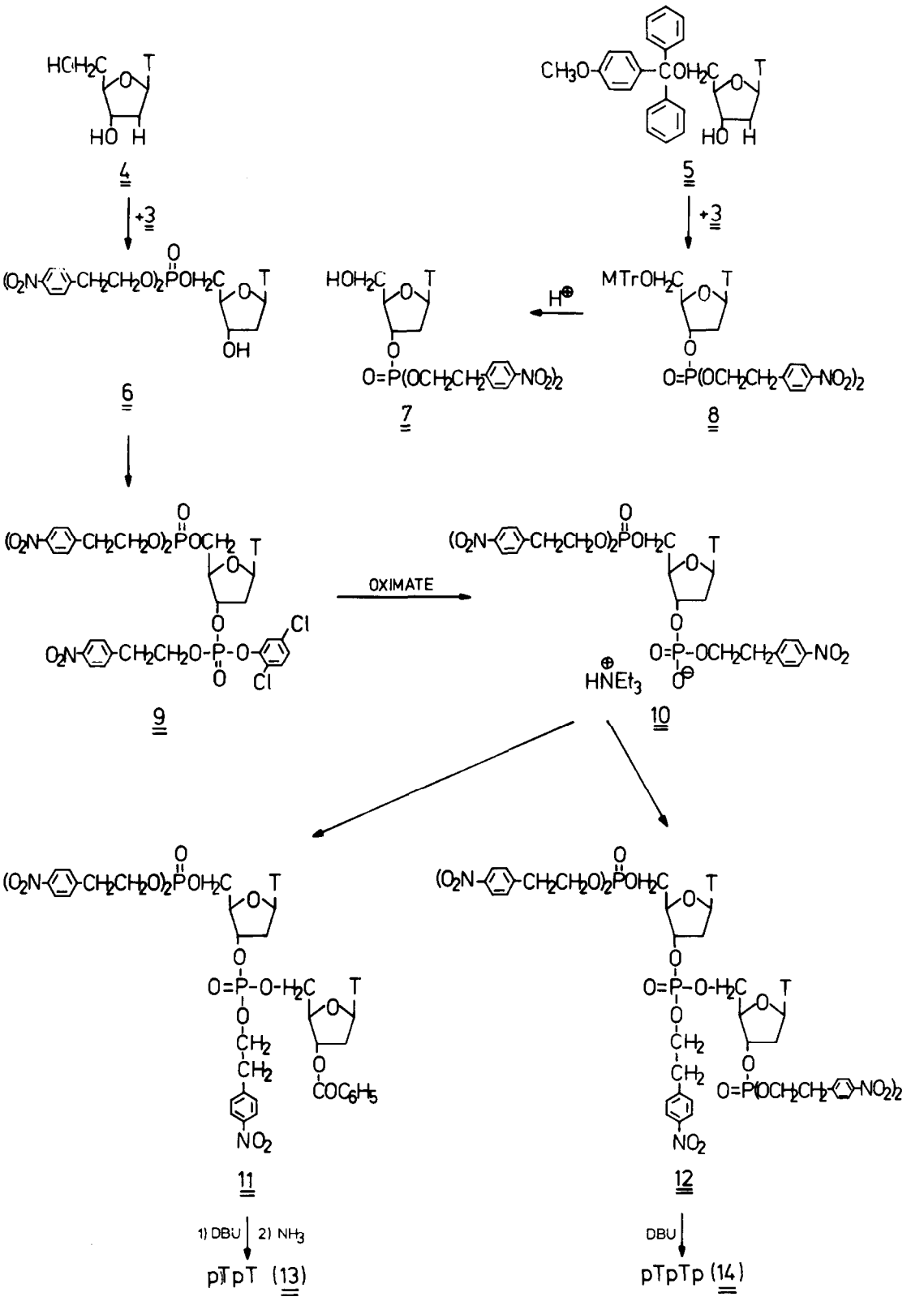
We have recently shown [1,2] that a new class of phosphate blocking groups based on substituted 2-phenylethanols offers some advantages over the commonly used protecting groups such as o-chloro-[3] or p-chlorophenyl [4], trichloroethyl [5] and cyanoethyl [6] due to the relatively high stability under hydrolytic conditions and the easy cleavage in aprotic solvents by a ß-elimination mechanism. We recommended especially the p-nitrophenylethyl group for phosphate protection in the phosphotriester approach [7-10] and could furthermore demonstrate that this blocking group can also be eliminated from a phosphodiester function giving rise to the synthesis of 3'-terminated oligonucleotide 3'-phosphates [11].

In an extension of this strategy we developed now a new phosphorylating agent - bis(p-nitrophenylethyl)phosphoromonochloridate (3) - which exhibits the same structural features of high stability in the corresponding phosphotriesters and can therefore be carried along in 3'- or 5'-position during the condensation steps on built-up of oligonucleotide chains.

The synthesis of 3 was achieved from diphenylphosphite (1) by transesterification with 2 moles of p-nitrophenylethanol to bis(p-nitrophenylethyl)phosphite (2) which was purified by recrystallization and then quantitatively



1 2 3


4
5
+3
+3
6
7
8
H⊕
OXIMATE
9
10
11
12
1) DBU 2) NH3
DBU
pTpT (13)
pTpTp (14)

converted by treatment with 1 equivalent of sulfuryl chloride into bis-(p-nitrophenylethyl)phosphoromonochloridate (3). This new reagent could not be distilled under high vacuum nor could it be obtained in solid form, but it was proven to be pure according to chromatography and NMR-spectra as well as elementary analysis.

The phosphorylating properties of 3 have first been studied with thymidine (4) which reacted in pyridine without any further activation relative selectively at $0^{o}C$ in 73 % yield to thymidine-5'-bis(p-nitrophenylethyl)phosphate (6) and small amounts of the isomeric 3'-mono-(7) and the 3',5'-diphosphotriester. 5'-O-(4-methoxytrityl)thymidine (5) yielded the corresponding 3'-bis(p-nitrophenylethyl)phosphate 8 with 3 in presence of N-methylimidazole at room temp. in 81 % yield and subsequent detritylation with 2 % p-toluenesulfonic acid in methylenchloride/methanol took place in 92 % yield to give thymidine-3'-bis(p-nitrophenylethyl)phosphate (7).

The stability of the bis(p-nitrophenylethyl)phosphotriester function in further phosphorylations was studied by treatment of 6 with 2,5-dichlorophenylphosphorodichloridate and 1,2,4-triazole and subsequent addition of p-nitrophenylethanol to form 5'-O-bis(p-nitrophenylethyl)phosphorylthymidine-3'-(2,5-dichlorphenyl)(p-nitrophenylethyl)phosphate (9). Oximate cleavage of the latter compound led to the corresponding 3'-phosphodiester 10 which was condensed with 3'-O-benzoylthymidine by triisopropylbenzenesulfonylchloride/N-methylimidazole according to Efimov et al. [12] to the fully protected dinucleosidediphosphotriester 11 in 74 % yield. 10 was coupled with 7 in an analogous condensation reaction to yield 5'-O-bis(p-nitrophenylethyl)phosphoryl- thymidylyl-(3'-p-nitrophenylethyl-5')thymidine-3'-bis(p-nitrophenylethyl)phosphate (12).

The deblocking experiments worked just perfectly, whereby in 12 all 5 p-nitrophenylethyl groups were eliminated by 1,5-diazabicyclo[5.4.0]undecene-5 (DBU) in pyridine at room temperature within 24 h to yield 14 and in 11 analogous treatment first with DBU and then with conc. aqueous ammonia to deprotect the benzoyl group led to 13. Purification of 13 and 14 was achieved by DEAE-Sephadex A25 chromatography in TEAB-buffer pH 7.5 using a linear gradient of 0.001-0.6 M to form chromatographically pure material in 94 and 95 % yield respectively. In the enzymatic degradation reactions with spleen and snake venom phosphodiesterases only 13 was cleaved by the latter one to pT as expected, whereas in 14 cleavage of the internucleotidic linkage was prevented by the 3'- and 5'-terminal phosphate groups providing an additional proof of the anticipated structure.

REFERENCES

1) E. Uhlmann, W. Pfleiderer, Tetrahedron Lett. 21, 1181 (1980).

2) E. Uhlmann, W. Pfleiderer, Helv.Chim. Acta 64, 1688 (1981).

3) J.H. van Boom, P.M.J. Burgers, G.R. Owen, C.B. Reese, R. Saffhill, Chem.Commun. 1971, 869.

4) K. Itakura, C.P. Bahl, N. Katagiri, J.J. Michniewicz, R.H. Wightman, S.A. Narang, Can.J.Chem. 51, 3649 (1973).

5) F. Eckstein, Angew.Chem., Int.Ed.Engl. 5, 671 (1966).

6) R.C. Letsinger, K.K. Ogilvie, J.Am.Chem.Soc. 89, 4801 (1967).

7) C.B. Reese, Tetrahedron 34, 3143 (1978).

8) J.H. van Boom, Heterocycles 7, 1197 (1977).

9) V. Amarnath, A.D. Broom, Chem.Rev. 77, 183 (1977).

10) C.B. Reese, Phosphorus and Sulfur 1, 245 (1976).

11) E. Uhlmann, R. Charubala, W. Pfleiderer, Nucleic Acid Res., Symp.Ser. 9, 131 (1981).

12) V.A. Efimov, S.V. Reverdatto, O.G. Chakhmakhcheva, Tetrahedron Lett. 23, 961 (1982).

(Received in Germany 3 August 1982)