Tetrahedron Letters,Vol.23,No.42,pp 4365-4368,1982 0040-4039/82/424365-04$03.00/0
Printed in Great Britain

PHTHALOYL GROUP : A NEW AMINO PROTECTING GROUP OF DEOXYADENOSINE IN OLIGONUCLEOTIDE SYNTHESIS

Akiko Kume, Mitsuo Sekine and Tsujiaki Hata
Department of Life Chemistry, Tokyo Institute of Technology
Nagatsuta, Midoriku, Yokohama, 227 Japan

Summary: A phthaloyl group has been introduced into the N^6-amino group of deoxyadenosine *via* silylation followed by acylation. The phthaloyl group resulted in remarkable retarding effects on depurination, while it could be removed under milder conditions than the benzoyl group. Thus, a tetradeoxyadenylate has been successfully synthesized in high yield.

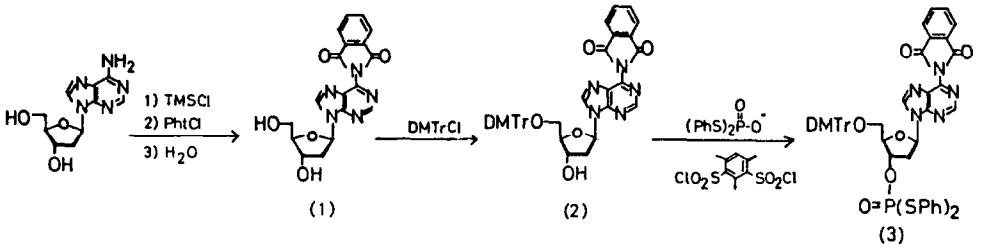
Despite recent advances in oligodeoxyribonucleotide synthesis, there still remains the important problem of how to prevent depurination of N^6-benzoyldeoxyadenosine.[1] In order to overcome this problem, many workers have searched for several conditions to remove a dimethoxytrityl function selectively[2] or have explored new protecting groups for 5'-hydroxyl functions in place of the dimethoxytrityl group.[3] In the case of the solid-phase synthesis, the synthesis of oligonucleotides containing a 3'-terminal deoxyadenosine has been avoided owing to the unnegligible depurination.[4]

In this paper, we wish to report a new method for the synthesis of oligodeoxyadenylates utilizing a N^6-phthaloyldeoxyadenosine unit (3) where the phthaloyl group has a function to prevent the depurination.

The phthaloyl group was introduced onto the N^6-amino group of deoxyadenosine by a modification of the procedure recently reported by Jones[5]: To a solution of deoxyadenosine (0.50 g, 2 mmol) in dry pyridine (20 ml) was added trimethylchlorosilane (0.6 ml, 5 mmol) at room temperature. After 15 min, phthaloyl chloride (0.4 ml, 2.8 mmol) was added. The reaction mixture was stirred at room temperature for 2 h and then quenched with ice (1 g). After 15 min, the mixture was extracted with ethyl acetate (30 ml) and washed with brine (2 x 30 ml). The washings were combined and further extracted with ethyl acetate (2 x 30 ml). The combined organic layers were concentrated *in vacuo* and coevaporated with toluene (2 x 5 ml) to remove trace amounts of pyridine. The residue was dissolved in CH_2Cl_2 (5 ml) and added dropwise with stirring to n-hexane (100 ml). The precipitate was collected by filteration and dried *in vacuo* to give crude N^6-phthaloyldeoxyadenosine (1)[6] (0.71 g, 93%).

The crude material 1 (311 mg, 2.1 mmol) was tritylated with 4,4'-dimethoxy-

trityl chloride (720 mg, 2.5 mmol) in dry pyridine at room temperature overnight. After the usual workup, chromatography on silica gel (CH_2Cl_2) afforded pure 2[7] (1.02 g, 70%). Compound 2 (254 mg, 0.37 mmol) was allowed to react with cyclohexylammonium S,S-diphenyl phosphorodithioate[8a] (170 mg, 0.45 mmol) in the presence of mesitylenedisulfonyl chloride[8b] (MDS) (119 mg, 0.45 mmol) in dry pyridine (10 ml). After the usual workup, chromatography afforded compound 3[9] (309 mg, 87%).



In order to examine the effect of N^6-protecting groups on the cleavage of the glycoside bond, N^6-phthaloyldeoxyadenosine derivatives and the corresponding N^6-benzoyl derivatives were treated with 80% AcOH at 30°C, and the rates of depurination were estimated by thin layer chromatography. Comparative data are given in Fig. 1. These results suggest that the phthaloyl group is superior to the benzoyl group in terms of depression of the depurination.

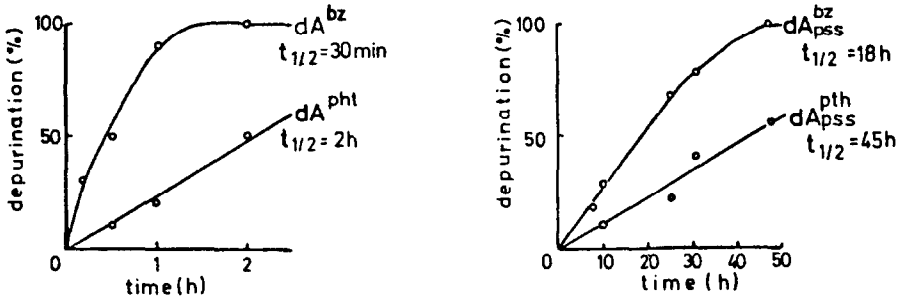
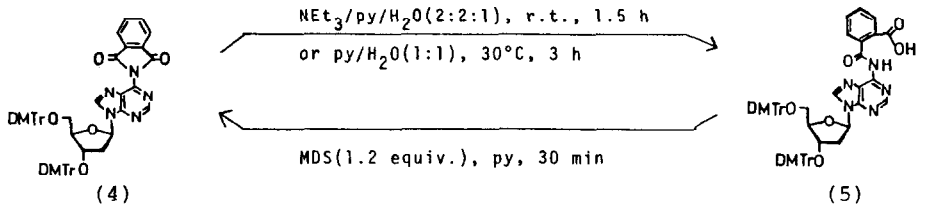


Fig. 1 Depurination of N^6-acylated substrates with 80% AcOH.
In each case, 10 μmol of the substrate was treated with 80% AcOH (2 ml) at 30°C: dA^{bz}; N^6-benzoyldeoxyadenosine, dA^{pht}; N^6-phthaloyldeoxyadenosine, dA^{bz}_{pss}; N^6-benzoyldeoxyadenosine S,S-diphenyl 3'-phosphorodithioate, dA^{pht}_{pss}; N^6-phthaloyldeoxyadenosine S,S-diphenyl 3'-phosphorodithioate.

The phthaloyl group was more base-labile than the benzoyl group and could be readily removed from 1 by treatment with a 0.5 M solution of hydrazine hydrate in pyridine-AcOH (4:1, v/v) at room temperature for 1 min or conc. ammonia-pyridine (9:1, v/v) at room temperature for 30 sec. In contrast to these results, the benzoyl group required 10 h for its complete removal under the latter conditions.

When N^6-phthaloyl derivatives were treated with a weakly basic aqueous solution, for example, pyridine-water, one of the N-C bonds was cleaved by the attack of OH^- to form *o*-carboxylbenzoyl derivatives. Even if the undesirable reaction

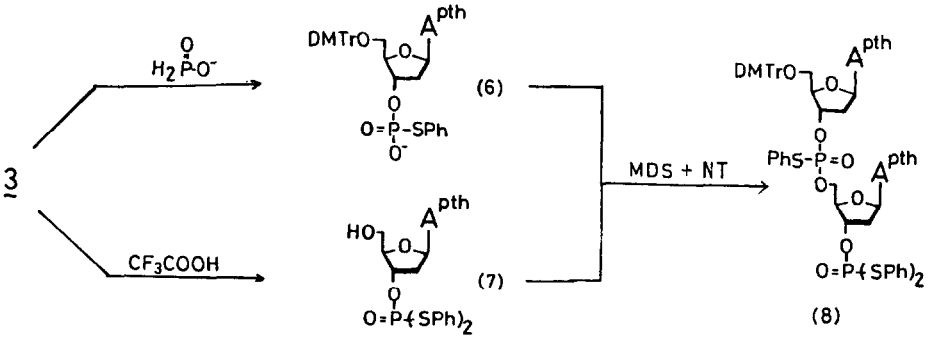
occurs during the operation to synthesize oligonucleotides, the ring opening compounds can be repaired in the presence of condensing agents. In fact, compound 4 was completely converted to 5[10] by treatment with pyridine-Et_3N-H_2O (2:2:1, v/v) at room temperature for 1.5 h or with pyridine-H_2O (1:1, v/v) at 30°C for 3 h. However, when the reaction mixture containing 5 was evaporated to dryness and treated with 1.2 equiv. of MDS in dry pyridine for 30 min, the usual workup followed by chromatography afforded 4 (86%).[11]



(4) (5)

In previous papers we reported the mild and selective removal of one phenylthio group from appropriately protected nucleoside S,S-diphenyl phosphorodithioates by use of pyridinium hypophosphonate (PHP) in dry pyridine.[8] The phthaloyl group was found to be stable under these conditions. Thus, treatment of 3 with 2 M PHP (50 equiv.) at 25°C for 2.5 h gave the diester (6) as triethylammonium salt, which was used in the next coupling reaction without further purification.

Removal of the dimethoxytrityl group from 3 was performed by treatment with 1% CF_3COOH in $CHCl_3$ (0.01 mmol of 3 / ml) at room temperature for 5 min. The mixture was quenched with pyridine, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated in vacuo. The residue was chromatographed on silica gel to give the detritylated product (7) in 96% yield. During these operations any products resulting from depurination were not observed.



The condensation of 7 (282 mg, 0.46 mmol) with 6, prepared from 3 (569 mg, 0.6 mmol), was performed by using MDS (957 mg, 1.8 mmol) and NT[12] (205 mg, 1.8 mmol). The reaction was completed in 30 min and the usual workup[13] gave the desired dimer (8) in 89% yield.

According to the methods described previously, the phenylthio and dimethoxy-

trityl groups were removed from 8 to give 9 and 10, and both the components were coupled by using 3 equiv. each of MDS and NT for 3 h. A fully protected tetradeoxyadenylate (11) was obtained by chromatography on silica gel in 76% yield.

Deprotection of all the protecting groups from 11 was performed rapidly as follows: 1) To a solution of 11 (12.5 mg, 5 μmol) in pyridine-H_2O (1 ml, 2:1, v/v) was added AgOAc (209 mg, 1.25 mmol).[8d] The mixture was vigorously stirred at room temperature for 3 days and then H_2S gas was bubbled into the mixture for 20 min. The mixture was centrifuged and the supernatant was concentrated. At this stage all phenlythio groups and a part of phthaloyl groups were removed. 2) To the residue was added conc. ammonia and the mixture was kept at 40°C for 1 h to complete removal of the phthaloyl groups. 3) The solution was concentrated and 80% AcOH was added. After 15 min, the solution was evaporated and applied to a column of Dowex 50 W x 2 (NH_4^+ form). Thus, d-ApApApAp was isolated in 74% (93 OD) yield after chromatographic separation using Whatman 3 MM papers with iPrOH-conc. ammonia-H_2O (6:1:3, v/v). The tetramer was completely degraded by spleen phosphodiesterase to give a single spot of d-Ap.

References and Notes

1) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, J. Am. Chem. Soc., 85, 3821 (1963).
2) a) J. Stawinski, T. Hozumi, S. A. Narang, C. P. Bahl, and R. Wu, Nucleic Acids Res., 4, 353 (1977); b) V. Kohli, H. Blöcker, and H. Köster, Tetrahedron Lett., 21, 2683 (1980); c) M. D. Matteucci, and M. H. Caruthers, Tetrahderon Lett., 21, 3243 (1980); d) H. Köster and N. D. Sinha, Tetrahedron Lett., 23, 2641 (1982); e) T. Tanaka and R. L. Letsinger, Nucleic Acids Res., 10, 3249 (1982).
3) a) J. B. Chattopadhyaya, C. B. Reese, and A. H. Todd, Chem. Commun., 987 (1979); b) J. F. M. de Rooij, G. W. Hazeleger, P. H. van Deursern, J. Serdijn, and J. H. van Boom, Recl. Trav. Chim. Pays-Bas, 98, 537 (1979).
4) K. Miyoshi, T. Huang, and I. Itakura, Nucleic Acids Res., 8, 5491 (1980).
5) G. S. Ti, B. L. Gaffney, and R. A. Jones, J. Am. Chem. Soc., 104, 1316 (1982).
6) Analytically pure sample of 1 was obtained by repeated chromatography on silica gel. ^{1}H nmr($CDCl_3$): 9.12(s, 1H, 8-CH), 8.56(s, 1H, 1-CH), 8.04(md, 4H, CH of phthaloyl group), 6.60(dd, 1H, 1'-CH), 4.88(m, 1H, 3'-CH), 4.27(m, 1H, 4'-CH), 3.96(m, 2H, 5'-CH_2). Anal. Calcd for $C_{18}H_{15}O_5N_5 \cdot 1/2H_2O$: C, 55.38; H, 4.13; N, 17.94%. Found: C, 55.18; H, 4.21; N, 17.47%.
7) Anal. Calcd for $C_{39}H_{33}O_7N_5 \cdot 1/2H_2O$: C, 67.62; H, 4.94; N, 10.11%. Found: C, 67.99; H, 5.01, N, 9.71%.
8) a) M. Sekine, K. Hamaoki, and T. Hata, Bull. Chem. Soc. Jpn., 54, 3815 (1981); b) M. Sekine, J. Matsuzaki, and T. Hata, Tetrahedron Lett., 22, 3209 (1981); c) S. Honda, K. Terada, Y. Sato, M. Sekine, and T. Hata, Chem. Lett., 15 (1982); d) M. Sekine, J. Matsuzaki, and T. Hata, in preparation.
9) Anal. Calcd for $C_{51}H_{42}O_8N_5S_2P_1$; C, 64.61; H, 4.47; N, 7.39; S, 6.76%. Found: C, 64.24; H, 4.77; N, 7.19; S, 6.98%.
10) An attempt to isolate 5 by chromatography on silica gel was unsuccessful and 3',5'-Bis-O-(dimethoxytrityl)adenosine (12) was obtained in 80% yield.
11) Compound 12 was also obtained in 14% yield. The formation of 12 might result from a loss of phthaloyl group during the evaporation of the mixture of 5.
12) C. B. Reese, R. C. Timas, and L. Yan, Tetrahedron Lett., 19, 2727 (1978).
13) In order to avoid the dephthaloylation, the mixture was diluted with CH_2Cl_2 and then washed with brine.

(Received in Japan 12 July 1982)