CHEMISTRY LETTERS, pp. 699-702, 1986.

USE OF 2-(2-PYRIDYL)ETHYL GROUP AS A NEW PROTECTING GROUP OF INTERNUCLEOTIDIC PHOSPHATES IN OLIGONUCLEOTIDE SYNTHESIS

Hiroshi TAKAKU,* Shoji HAMAMOTO, and Testuo WATANABE
Laboratory of Bioorganic Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino, Chiba 275

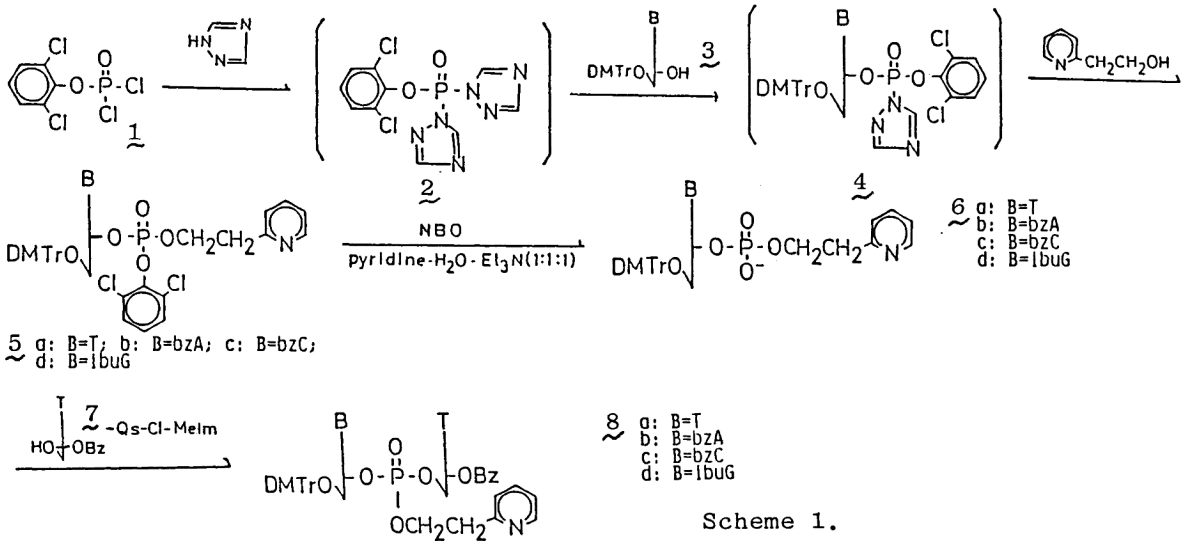
2-(2-Pyridyl)ethyl group is used as a highly selective protecting group of internucleotidic phosphates that is removable under mild conditions by treatment with CH_3I in CH_3CN without a side reaction such as N-alkylation.

In the fild of oligonucleotide synthesis, the phosphate protecting group plays the most important role in the phosphotriester approach.[1] In particular, it seems that the most suitable protecting group in the phosphotriester approach can be removed from the internucleotidic bonds by β-elimination mechanism.[2] Several decades ago, Cramer reported[3] that 2-(2-pyridyl)ethyl group could be used as a 5'-terminal phosphate protecting group in oligodeoxyribonucleotide synthesis by the phosphodiester approach. This group was removed by treatment with sodium methylate in pyridine. We have tried to apply this protecting group to the synthesis of oligodeoxyribonucleotides by the phosphotriester approach. It is now found that 2-(2-pyridyl)ethyl (Pyet) group is stable under standard manipulations required for oligodeoxyribonucleotide synthesis, removal being achieved specifically under mild conditions by treatment with CH_3I in CH_3CN.

The starting 3'-phosphotriester units 5 were prepared as follows: 2,6-Dichlorophenyl phosphorodichloridate (1) (6.0 mmol) was allowed to react with 1,2,4-triazole (12.0 mmol) in dry THF (15 ml) for 1 h. 5'-O-Dimethoxytritylthymidine (3a) (4.0 mmol) was added and the mixture was stirred for 1 h. Then 2-(2-pyridyl)-ethanol (12.0 mmol) and N-methylimidazole (MeIm) (12.0 mmol) were added and the reaction was continued for 1 h. After the usual workup, chromatography afforded the phosphotriester unit 5a[4] (2.87 g, 82%). In a similar manner, other nucleoside 3'-phosphotriester units, 5b, 5c, and 5d were obtained in 88%, 89%, 79% yields, respectively.[4]

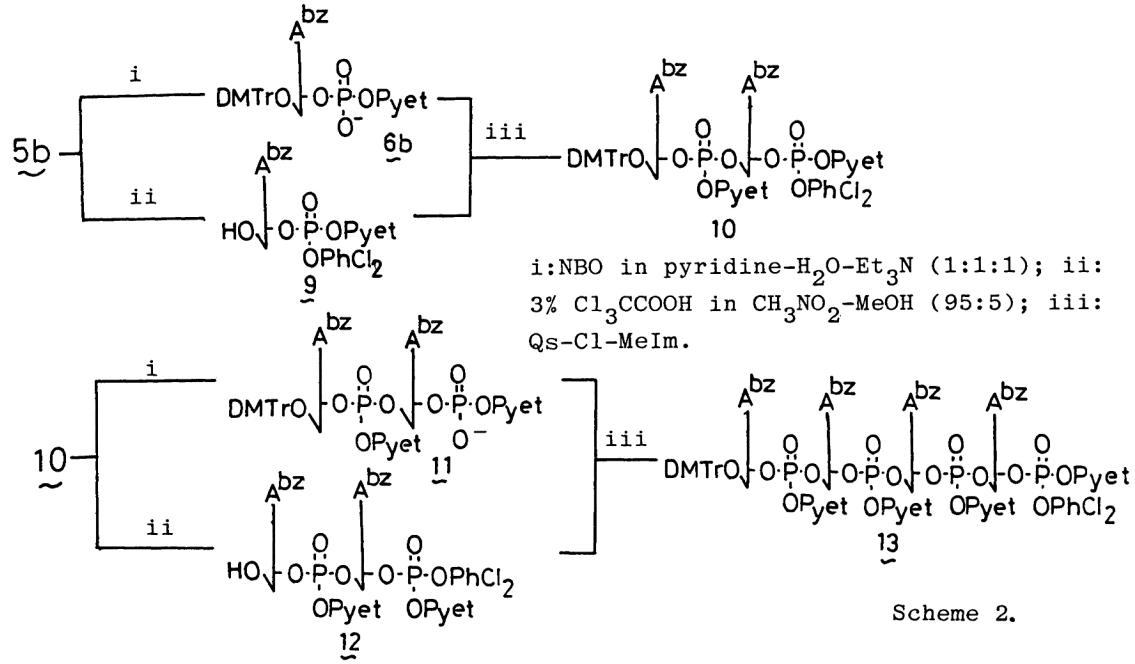
The Pyet group in 5, is stable at 20 °C under both basic [0.1N-NaOH in a mixture of dioxane and MeOH (15:5, v/v), 3h] and acidic [in 80% AcOH, 6h] conditions. Removal of the 2,6-dichlorophenyl group was attemped by our method,[5] with $t\text{-BuNH}_2$ in a mixture of pyridine and H_2O (8:2, v/v), but the corresponding phosphodiester 6a could not be obtained in a satisfactory yield (75%). We now found that 2,6-dichlorophenyl group could be easily removed from 5 with 4-nitrobenzaldoximate (NBO) (10 molar equiv) in a dioxane-H_2O-Et_3N mixture (1:1:1, v/v) within 240 min ($t_{1/2}$=20 min) at room temperature to give the phosphodiester derivative 6 in quantitative yield. The condensation reaction of 6a (1.24 g, 1.5 mmol) and 3'-O-benzoyl-thymidine (7)0.34 g, 1.0 mmol) in the presence of 8-quinolinesulfonyl chloride

(Qs-Cl)[7] (1.02 g, 4.5 mmol) and MeIm[8] (0.36 ml, 4.5 mmol) was carried out in dry pyridine (5 ml). After 3 h, the usual workup followed by chromatography afforded the fully protected dimer (8a) (1.18 g, 75%). Similarly, The fully protected dimer derivatives (8b-d) were synthesized in 77%, 78%, and 70% yields, respectively.



Scheme 1.

Further, we examined the synthesis of tetradeoxyadenylate (13) as shown in Scheme 2. The dimethoxytrityl group was selevtively removed from 5b by treatment with 3% Cl_3CCOOH in a CH_3NO_2-MeOH mixture (95:5, v/v) at room temperature for 5 min[9] to give the corresponding 5'-hydroxyl component (9) in 88% yield. The 3'-phosphodiester component (6b) was then treated with 9 in dry pyridine containing an excess of Qs-Cl and MeIm for 3h. After the usual workup, chromatography afforded the dimer (10) in 79% yield. According to the methods described previously, the 2,6-dichlorophenyl and DMTr groups were removed from 10 to give 11 and 12, respectively, and both the components were reacted by using Qs-Cl and MeIm for 3 h. The

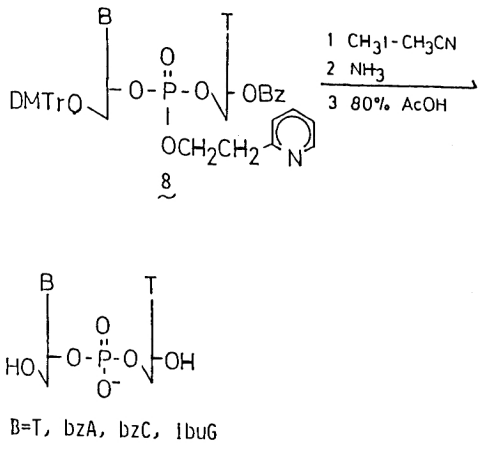


Scheme 2.

fully protected tetradeoxyadenylate was obtained in 71% yield after separation by silica gel column chromatography.

The protection of internucleotidic phosphates with 2-(2-pyridyl)ethyl group would be expected to selectively removable under mild conditions via B-elimination mechanism. The dimer 8a was therefore subject to three tests. First, it was tretaed with 0.2 M-NaOH in a mixture of dioxane and MeOH (15:5, v/v) at room temperature and found to be stable (by TLC) after 3 h. Secondly, 8a was treated with CH_3I (3.0 molar equiv) in CH_3CN at room temperature for 3 h, followed by treatment with 0.2 M-NaOH in a mixture of dioxane and MeOH (15:5, v/v) at room temperature. While 8a was converted to the corresponding DMTrTpTOH in 30% yield after 4 h. Finally, when the solution of 8a in dry CH_3CN was treated with CH_3I (20 molar equiv) at room temperature for 18 h, it was almost quantitatively converted to DMTrTpTOBz.[10] DMTrTpTOBz was deprotected by successive treatment with concentrated ammonia and with 80% AcOH to afford the unprotected dimer, TpT in 95% yield. In a similar manner, other unprotected dimers, d-ApT, d-CpT, and d-GpT were obtained as shown in Table 1. An important observation we made in removal of the Pyet group from 8 is that no alkylation of base residues were characterized by Rf values on paper chromatography, HPLC, and UV spectroscopy. Compared with other protecting groups, it is noted that the "safety form" of the Pyet group is converted with CH_3I in CH_3CN into pyridinium form, which can be easily decomposed via β-elimination reaction without loss of the amino and hydroxyl protecting groups.

Deprotection of 13 was performed by the above methods after removal of the 2,6-dichlorophenyl group by treatment with NBO to afford d-ApApApAp in 73% (181 OD units) yield from 10 μmol of the protected tetramer 13. The tetramer was completely degraded by spleen phosphodiesterase to give a single spot of d-Ap (Fig. 1).



Scheme 3.

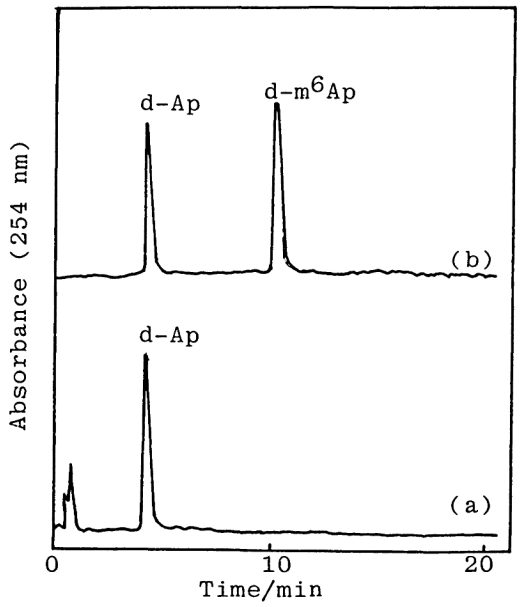


Fig. 1. HPLC Elution Profile on Senshupak (4251-N) Column Using a 5% MeOH in 0.05 M-$NH_4H_2PO_4$ in 25 min.
a) The product obtanied after digestion of d-ApApApAp by spleen phosphodiesterase.
b) Authentic samples=d-Ap, d-m^6Ap.

Table 1. Yields and Enzymic Digestions of Unprotected Dinucleoside Monophosphates.

Compd.	Yield of complete deprotection %	Enzymatic analyses
d-ApT	85	d-A/pT (1.00:1.08)
TpT	93	T/pT (1.00:1.11)
d-CpT	85	d-C/pT (1.00:0.93)
d-GpT	81	d-G/pT (1.00:1.02)

We thank the Saneyoshi Memorial Fundation for generous support of this work. We are grateful to the Central Research Leboratories of Nissan Chemical Ind. Co. for meansurement of ^{31}P-NMR spectra.

References

1) H. Kössel and H. Seliger, Fortschr. Chem. Org. Naturstoffe, 32, 297 (1975); V. Amarnath and A. D. Broom, Chem. Rev., 77, 183 (1977); C. B. Reese, Tetrahedron, 34, 3143 (1978); M. Ikehara, E. Ohtsuka, and A. F. Markham, Adv. Carbohydr. Chem. Biochem., 36, 135 (1979); E. Ohtsuka, M. Ikehara, and D. Söll, Nucleic Acids Res., 10, 6553 (1982); S. A. Narang, Tetrahedron, 39, 3 (1983).
2) R. L. Letsinger and K. K. Ogilvie, J. Am. Chem. Soc., 89, 4801 (1967); F. Eckstein and I. Rizk, Angew. Chem., 79, 939 (1967); E. Uhlman and W. Pfleiderer, Tetrahedron. Lett., 21, 1181 (1980); S. Honda and T. Hata, ibid., 22, 2093 (1981); R. L. Letsinger, E. P. Groody, and T. Tanaka, J. Am. Chem. Soc., 104, 6805 (1982); J. E. Marugg, C. E. Dreef, G. A. van der Marel, and J. H. van Boom, Recl. Trav. Chim. Pays-Bas, 103, 97 (1984).
3) W. Freist, R. Helbig, and F. Cramer, Chem. Ber., 103, 1032 (1970).
4) 5a: mp 90-92 °C; UV λmax (MeOH) 275 (sh) 264, 258, 232 nm. ^{1}H-NMR ($CDCl_3$) δ 9.72 (br s, 1H, NH), 8.55 (m, 1H, α-H), 6.43 (t, 1H, C-1'), 4.61 (t, 2H, CH_2CH_2O), 3.75 (s, 3H, OCH_3), 3.40 (t, 2H, CH_2CH_2O). Anal. Calcd for $C_{44}H_{44}N_3O_{10}PCl_2$: C, 60.42; H, 4.84; N, 4.80. Found: C, 60.57; H,4.90; N, 4.68.
5b: mp 79-81 °C; UV λmax (MeOH) 275. 267 (sh), 258, 231 nm. ^{1}H-NMR ($CDCl_3$) δ 8.69 (s, 1H, C-8), 8.45 (m, 1H, α-H), 8.10 (s, 1H, C-2), 6.48 (q, 1H, C-1'), 4.62 (t, 2H, CH_2CH_2O), 3.75 (s, 3H, OCH_3), 3.40 (m, 2H, C-5'), 3.08 (t, 2H, CH_2CH_2O). Anal. Calcd for $C_{51}H_{46}N_6O_9PCl_2$: C, 61.95; H, 4.69; N, 8.50. Found: C, 61.93; H, 4.77; N, 8.46.
5c: mp 86-88 °C; UV λmax (MeOH) 275 (sh), 258, 232 nm. ^{1}H-NMR ($CDCl_3$) δ 8.50 (m, 1H, α-H), 8.10 (1H, C-6), 6.80 (d, 1H, C-5), 6.31 (q, 1H, C-1'), 4.65 (t, 2H, CH_2CH_2O), 3.78 (s, 6H, OCH_3), 3.48 (br s, 2H, C-5'), 3.20 (t, 2H, CH_2CH_2O). Anal. Calcd for $C_{50}H_{46}N_6O_{10}PCl_2$: C, 62.25; H, 4.81; N, 5.81. Found: C, 62.50; H, 4.77; N, 5.72.
5d: mp 94-97 °C; UV λmax (MeOH) 275 (sh), 258, 250, 232 nm. ^{1}H-NMR ($CDCl_3$) δ 9.25 (br s, 1H, NH), 8.45 (m, 1H, α-H), 7.78 (s, 1H, C-8), 6.10 (t, 1H, C-1'), 4.70 (m, 2H, CH_2CH_2O), 3.80 (s, 6H, OCH_3), 3.50 (br s, 2H, C-5'), 3.30 (m, 2H, CH_2CH_2O). Anal. Calcd for $C_{48}H_{47}N_6O_{10}PCl_2.3/4CH_3OH$: C, 58.91; H, 5.07; N, 8.49. Found: C, 58.77; H, 4.84; N, 8.70.
5) H. Tsuchiya, S. Ueda, H. Kobayashi, and H. Takaku, Chem. Lett., 1985, 347.
6) ^{31}P-NMR spectra of 6a-d are shown below. 6a: δ -1.16 ($CDCl_3$, 85% H_3PO_4), Rf 0.88; 6b: δ -1.21 ($CDCl_3$, 85% H_3PO_4), Rf 0.81; 6c: δ -1.14 ($CDCl_3$, 85% H_3PO_4), Rf 0.78; 6d: δ -1.34 ($CDCl_3$, 85% H_3PO_4), Rf 0.84. RPTLC was performed on plates of silanized silica gel (Merck 60F_{254}) by using an acetone-water mixture (7:3, v/v).
7) H. Takaku, M. Yoshida, M. Kato, and T. Hata, Chem. Lett., 1979, 811.
8) V. A. Efimov, S. V. Reverdatto, and O. G. Chakhmakcheva, Tetrahedron Lett., 23, 961 (1982); idem, Nucleic Acids Res., 10, 6675 (1982).
9) H. Takaku, K. Morita, and T. Sumiuchi, Chem. Lett., 1983, 1661.
10) 2-(2-Pyridyl)ethyl group was removed from internucleotidic phosphates by treatment with 20 molar equiv of CH_3I without further alkali treatment.

(Received February 4, 1986)