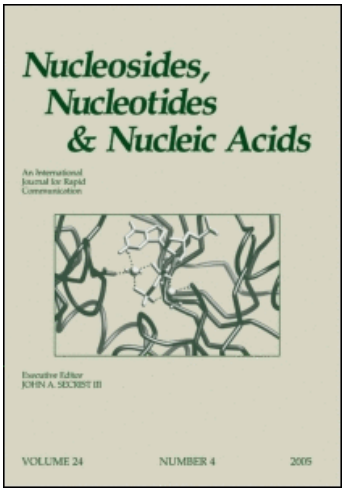

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USE OF THE 2-(2-PYRIDYL)ETHYL PROTECTING GROUP IN THE SYNTHESIS OF DNA FRAGMENTS VIA PHOSPHORAMIDITE INTERMEDIATES

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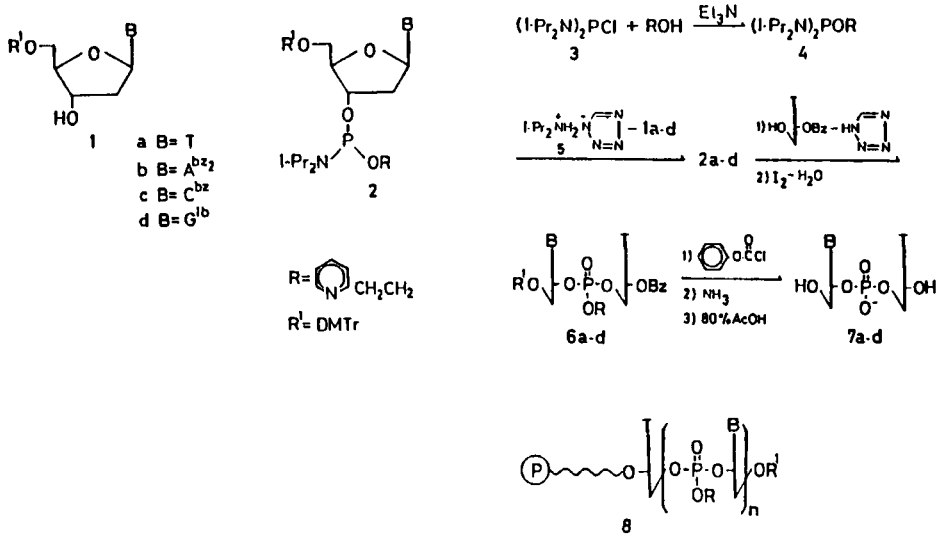
ABSTRACT 2-(2-Pyridyl)ethyl is a new protecting group for the internucleotidic linkages in the synthesis of oligodeoxyribonucleotides by the phosphoramidite method. This group is stable to alkali and acid, and can be removed by two step procedures under mild conditions. Furthermore, we have found that bis-(diisopropylamino)chlorophosphine is much more effective for the preparation of bis-(diisopropylamino)alkoxyphosphine than various dichlorophosphines. The synthesis of oligodeoxyribonucleotides by using 2-(2-pyridyl)ethyl-deoxyribonucleoside-3'-O-N,N-diisopropylamidite units is also described.

Recently, deoxyribonucleoside phosphoramidites have been used for the chemical synthesis of DNA fragments on solid supports.[1] Bis-(dialkylamino)methoxyphosphines[2] are appropriate phosphitylating agents for their synthesis. The amino groups of which can be exchanged under quite different conditions. On the other hand, several workers have also reported[3] new protecting groups for the internucleotidic linkages with better removal properties. However, most of the new phosphate protecting groups are unstable under alkaline condition. In this paper, we wish to report that the phosphoramidite intermediates (2) can be prepared rapidly by a one flask reaction, and the 2-(2-pyridyl)ethyl group (Pyet) of protecting group can be removed from the internucleotidic linkages under mild conditions. We examined a new procedure for the preparation of 4 and have found that bis-(diisopropylamino)-chlorophosphine (3)[4] is much more effective than various phosphorodichloridites. When 3 was treated with 2-(2-pyridyl)-ethanol in the presence of Et_3N, the phosphitylating agent 4 was obtained in an almost quantitive yield. After the removal of triethylammonium hydrochloride, and 2-(2-pyridyl)ethanol[5], the



crude, 4 (1.5 molar equiv.) was treated with nucleoside (1a) (1.0 molar equiv.) in the presence of 5[6] (1.0 molar equiv.) in CH_2Cl_2. After 1 h, the usual work-up followed by flash silica gel chromatography gave 2a (83%). The ^{31}P-NMR of 2a showed two characteristic signals corresponding (δ147.1, δ147.4 ppm) to a 1:1 diastereomeric mixture, and no 3',3'-O-dinucleoside phosphite could be detected. The phosphoramidites (2b-d) were prepared by a similar procedure in 80-88% yields.[7] Further, the reaction of 2 (1.1 molar equiv.) and 3'-O-benzoylthymidine (1.0 molar equiv.) in the presence of 1H-tetrazole in CH_3CN led to the formation of the 3'-5'-O-dinucleoside phosphite triesters. After 15 min and the usual work-up, the residue was treated with I_2 in THF-pyridine-H_2O (40:20:1) to give the dimers (6a-d) in 80-87% yields. The Pyet protecting group was removed from the internucleotidic linkages follows: The Pyet group was treated first with Phenyl-chloroformate (5 molar equiv.) in CH_3CN at 20°C for 6 h and then with NH'OH-pyridine (9:1) at 20°C for 6 h. DMTrTpT was obtained in an almost quantitative yield. The DMTr group was removed by succesive treatment with 80% AcOH to afford TpT in 95% yield.[8]

The phosphoramidite intermediates 2 containing the Pyet group were used to the synthesis of a part of the Dynorphine gene, d-TTATCCTAG.[9] The reaction was carried out on a controlled pore glass[10] (8) (215 mg, 48 μmol/g, n=0, R^1=DMTr, B=T) in a column similar to that previously described.[11] We showed the following elongation cycle to be effective: treatment with (1) 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5)[12], 3 min; (2) CH_3NO_2, 4 min; (3) CH_3CN, 4 min; (4) 2 (25 molar equiv.) and 1H-tetrazole (50 molar equiv.)

in CH_3CN, 10 min; (5) CH_3CN, 2 min; (6) I_2 in THF-pyridine-H_2O (40:20:1), 1 min; (7) CH_3CN, 2 min; (8) Ac_2O-DMAP-collidine-THF (0.5:0.3:0.5:5), 2 min; (9) THF, 1 min; (10) CH_3CN, 2 min. Each average yield was 94% yield (DMTrOH) per cycle for the addition of a phosphoramidite unit.

The synthetic DNA fragment was treated first with phenyl-chloroformate and then with concentrated ammonia and finally with 80% AcOH. Following the evaporation of the supernatant, the purification was accomplished by Toyoperal HW-40 chromatography followed by HPLC. A sample purified by HPLC was completely digested and the ratios of nucleotides was judged from HPLC-analysis[13] by nuclease P1 and snake venom phosphodiesterase. Furthermore, a sample thus obtained was characterized by polyacrylamide gel electrophoresis (a single spot was obtained and it was a 9-mer).

In conclusion, 2-(2-pyridyl)ethyl is a suitable protecting group for internucleotidic phosphate protecting group. This group is easily removed via two step procedures with the concomitant loss of the amino and hydroxyl protecting groups. Since bis-(diisopropylamino)chlorophosphine (3) is more stable than various phosphorodichloridites, the phosphoramidite units 2 described in this paper can be easily synthesized by using bis-(diisopropylamino)-2-(2-pyridyl)ethoxyphosphine (4) which was prepared from 3 and 2-(2-pyridyl)ethanol in a one flask reaction.

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4) 3 was prepared in high yield by a modification of the procedure of King (King, R.B.; Sundaram, P.M. J.Am.Chem.Soc. 1984, 49, 1784.) and it is much more stable than various phosphorodichloridites.
5) 4 could not be distilled as it undergoes thermal decomposition, but it was sufficiently pure (ca. 95%, δ129.3 ppm) for synthetic purposes. In this case, only bis-(diisopropylamino)-chlorophosphine was detected as the only impurity by ^{31}P-NMR (ca. 3%, δ140.0 ppm).
6) Diisopropylammonium tetrazolide, 4,5-dichloroimidazole, collidine hydrochloride, and 1H-tetrazole were tested as catalysts in the preaparation of 2a and the result was obtained with diisopropylammonium tetrazolide.
7) ^{31}P-NMR Spectra data of phosphoramidites. 2b: δ147.5,δ147.2; 2c: δ147.6,δ147.4; 2d: δ147.3,δ146.5.
8) The d-TpT (7a) was completely degraded with Nuclease P1 to d-T and d-pT in ratios of 1.00:1.01. The unprotected dinucleotides 7b-d were isolated in 81-95% yields.
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