CHEMISTRY LETTERS, pp. 111-114, 1983.

A SIMPLE SYNTHETIC METHOD OF DEOXYRIBODINUCLEOTIDE BLOCKS

Hiroshi TAKAKU*, Kazuo KAMAIKE, and Masanori SUETAKE

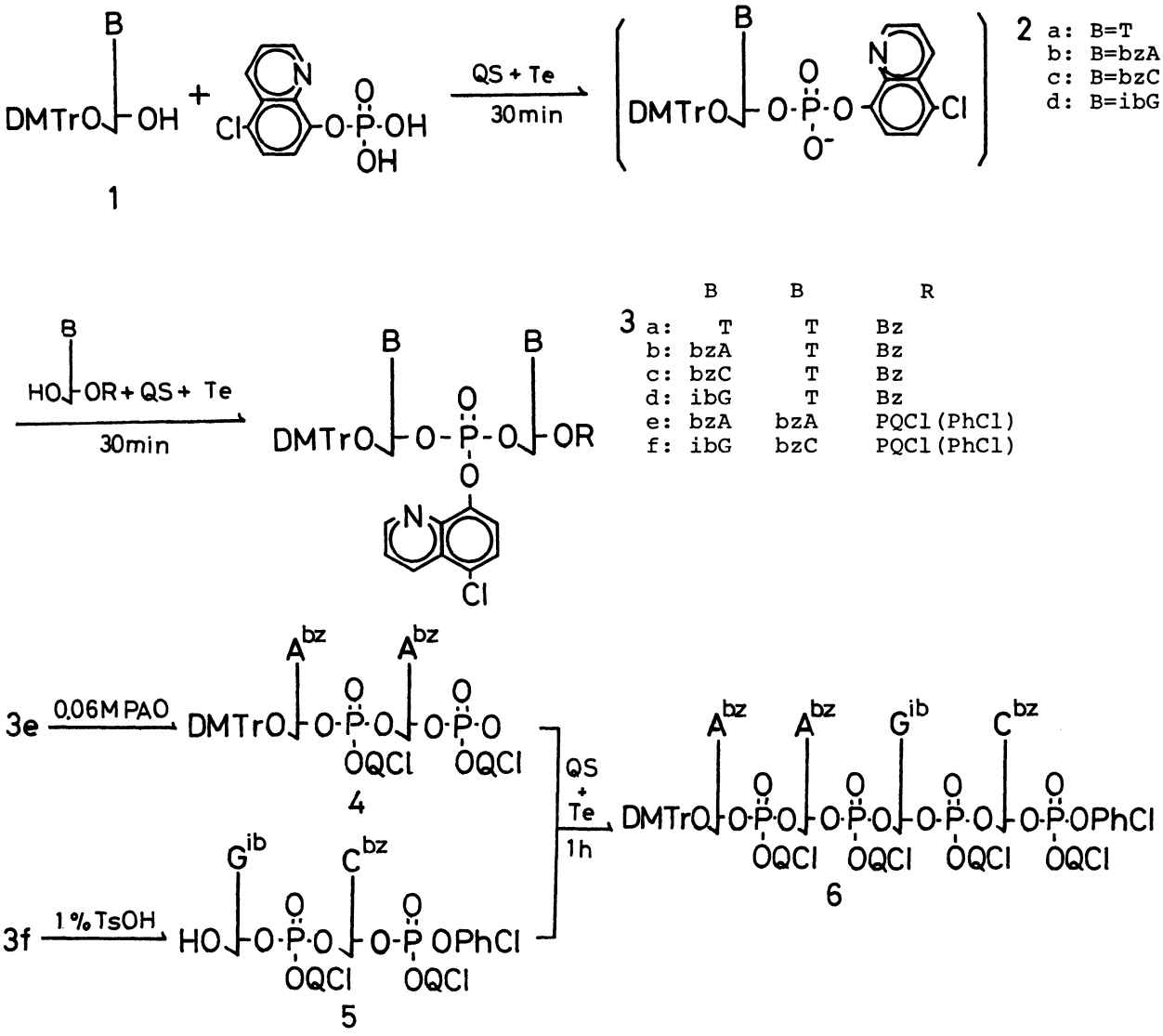
Laboratory of Organic Chemistry Chiba Institute of Technology, Tsudanuma, Narashino-shi, Chiba 275

Deoxyribodinucleotide blocks can be prepared rapidly by combining the phosphorylation and coupling reaction steps in one flask which also contents the synthesis of deoxyribodinucleotide blocks containing the guanosine unit.

The most crucial step in the synthesis of deoxyribooligonucleotides by the phosphotriester approach[1] has involved the synthesis and isolation of phosphodiester derivatives which were condensed with the 5'-hydroxyl group of partially protected nucleoside or nucleotide to form a new internucleotidic bonds. In the synthesis of deoxyribooligonucleotides, the isolation step of phosphodiester derivatives serves to slow down the speed of process.[2]

In this report we wish to describe the rapid and convenient synthetic method for deoxyribodinucleotide blocks by combining the phosphorylation of the 3'-hydroxyl group and condensation with the 5'-hydroxyl group in one flask.

The 5'-O-dimethoxytrityl-N-acylnucleosides were phosphorylated with 5-chloro-8-quinolyl phosphate[3] (1.1 mole equiv.) in the presence of 8-quinolinesulfonyl chloride (QS)[4] (2.2 mole equiv.) for 1.5 h. The phosphodiesters 2 were found to be formed almost quantitavely as judged from tlc-analysis, being uncontaminated with undesired side products[5]. In case of guanosine, however, undesired side products were indicated by ^{31}P-NMR [(pyridine-d_5, 85%H_3PO_4) δ +6.17, +6.40, +6.89] during phosphorylation of 3'-hydroxyl group. In order to overcome this problem, a combination of QS and tetrazole was employed in place of QS as a coupling agent.[6] 5'-O-Dimethoxytrityl-N^2-isobutylylguanosine was treated with 5-chloro-8-quinolyl phosphate (1.1 mole equiv.) in the presence of QS (2.2 mole equiv.) and tetrazole (2.42 mole equiv.) in dry pyridine for 30 min at room temperature. The reaction mixture was quenched with ice-water, followed by extraction with methylene chloride, and the organic layer was washed with 0.1M TEAB solution. The methylene chloride extract was concentrated in vacuo. The residue was dissolved in methylene chloride and added dropwise to hexane. The triethylammonoium salt of phosphodiester 2d (85%) was obtained as stable colourless solid, uncomtaminated [^{31} -NMR (pyridine-d_5, 85%H_3PO_4) δ +6.27] with undesired side products. The phosphorylation of 3'-hydroxyl group of 5'-dimethoxytrityl-N-acylnucleosides was sufficiently fast so that no side reaction occurred when a combination of QS and



QCl=5-chloro-8-quinolyl; PhCl=4-chlorophenyl; QS=8-quinolinesulfonyl chloride; Te=tetrazole

tetrazole was used as a coupling agent.

A typical procedure for the rapid synthesis of deoxyribodinucleotide blocks is as follows: 5'-O-Dimethoxytritylthymidine (408 mg, 0.75 mmole) was treated with 5-chloro-8-quinolyl phosphate (214 mg, 0.82 mmole) in the presence of QS (341 mg, 1.50 mmole) and tetrazole (115 mg, 1.65 mmole) in dry pyridine (11 ml) for 30 min at room temperature. The phosphodiester product 2a was detected by tlc [Rf=0.1, solvent A (CH_2Cl_2/MeOH, 9:1 v/v)] and the reaction mixture was concentrated to 5 ml under reduced pressure at 20°C. To this solution were added 3'-O-benzoylthymidine (173 mg, 0.50 mmole), QS (341 mg, 1.50 mmole), and tetrazole (115 mg, 1.65 mmole), and the reaction mixture was stirred for 30 min. The reaction mixture was quenched with ice-water, followed by extraction with methylene chloride, and the combined organic extracts were washed with 0.1M TEAB (pH 7.5) solution, then with water, dried with anhydrous sodium sulfate and concentrated.

Table. The yields of dideoxyribonucleotide blocks (3).

5'-protectednucleoside mmole	5'-hydroxyl component mmole	product	yield %
(DMTr)dT 0.75	dTOBz 0.5	(DMTr)dTp(QCl)TOBz	91
(DMTr)dbzA 0.75	dTOBz 0.5	(DMTr)dbzAp(QCl)TOBz	89
(DMTr)dbzC 0.75	dTOBz 0.5	(DMTr)dbzCp(QCl)TOBz	86
(DMTr)dibG 0.67	dTOBz 0.45	(DMTr)dibGp(QCl)TOBz	78
(DMTr)dbzA 0.75	dbzApQCl(PhCl) 0.5	(DMTr)dbzAp(QCl)bzAp-QCl(PhCl)	77
(DMTr)dibG 1.5	dbzCpQCl(PhCl) 1.0	(DMTr)dibGp(QCl)bzCp-	76

The residue was dissolved in methylene chloride and chromatographed on a silica gel column. The deoxyribodinucleotide block 3a was isolated in 91% (505 mg) yield by eluting the column with a stepwise gradient of methanol (0-2%) in methylene chloride.

In a similar manner, other deoxyribodinucleotide blocks (3b-f) were obtained in good yields as shown in Table. The deoxyribodinucleotide blocks 3 were produced within 1 h and isolated quite easily by silica gel short column.

Next, we examined the synthesis of tetradeoxyribonucleotide 6 by using the dimer blocks 3e and 3f. The dimer block 3f (558 mg, 0.36 mmole) was treated with 1% p-toluenesulfonic acid in a mixture of methylene chloride and methanol (1:1 v/v) for 15 min at 0°C to give 5.[3] The 5'-hydroxyl deoxyribodinucleotide 3'-phosphotriester 5 was isolated in 97% (435 mg) by precipitation with hexane-ether (10:1, v/v) and used for the next coupling reaction without further purification. On the other hand, 3e (948 mg, 0.60 mmole) was treated with 1M-N^1,N^1,N^3,N^3-tetramethylguanidinium salt of 2-pyridine aldoxime (1.25 ml) in a mixture of dioxane and water (2:1 v/v) (11 ml) for 8 h at room temperature.[7] The mixture was treated with Dowex 50W-X2 (pyridinium form) and the resin was removed by filtration and washed with aqueous pyridine (50%). The filtrate and washings were combined and washed with ether and extracted with methylene chloride. The methylene chloride extract was rendered anhydrous by repeated coevaporation with dry pyridine. The phosphodiester 4 thus obtained was dissolved in dry pyridine (3 ml) and then 5 (373 mg, 0.30 mmole), QS (341 mg, 1.50 mmole), and tetrazole (115 mg, 1.65 mmole) were added. The reaction mixture was stirred for 1 h at room temperature. After removal of 8-quinolinesulfonic acid by filtration, the corresponding tetradeoxyribonucleotide (d-ApApGpCp) 6 was obtained in 73% (593 mg) after separation by silica gel column chromatography.

The tetramer 6 was completely deblocked by treatment with (i) 1M-N^1,N^1,N^3,N^3-tetramethylguanidinium salt of 2-pyridine aldoxime in a mixture of dioxane and water (2:1 v/v) for 8 h; (ii) zinc Chloride in aqueous pyridine for 24 h[8]; (iii) concentrated ammonia for 5 h at 60°C; and finally, 80% acetic acid for 10 min

at room temperature. The deblocked tetranucleotide d-ApApGpCp was obtained in 76% yield after chromatography using Toyo Roshi No. 514 paper (iso-PrOH-conc. NH_4OH-H_2O, 55:10:35 v/v). The purity of d-ApApGpCp was checked by PE and HPLC on Finepak C_{18}[9] as well as hydrolysis with spleen phosphodiesterase to d-Ap, d-Gp, and d-Cp in the ratio 1.97:1.01:1.00.

In conclusion, the combination of phosphorylation and coupling reaction steps in one flask without the phosphodiester isolation has reduced period of times for reaction in the synthesis of deoxyribodinucleotides with much higher yields. Further, the use of QS and tetrazole as a coupling agent has also improved the yield of dinucleotide block synthesis containing guanosine.

We thank Professor Tsujiaki Hata of Tokyo Institute of Technology for measurements of ^{31}PNMR spectra.

References

1) C.B,Reese, Tetrahedron, 34, 3143(1978); M.Ikehara, E.Ohtsuka, and A.F.Markham, Adv.Carbohyd.Chem.&Biochem., 36, 135(1979).
2) P.Cashion, K.Porter, T.Cadger, G.Sathe, T.Tranquilla, H.Notman, and E.Jay, Tetrahedron Lett., 1976, 3769; K.L.Agarwal and F.Riftina, Nucleic Acids Res., 5, 2809(1978); G.van Marel, C.A.A.van Boeckel, Mrs G.Wille, and J.H.van Boom, Tetrahedron Lett., 1981, 3887; K.L.Sadana, F.E.Hruska, and P.C.Lowen, ibid., 1981, 3367.
3) H.Takaku, R.Yamaguchi, T.Nomoto, and T.Hata, Tetrahedron Lett., 1979, 3857.
4) H.Takaku, M.Kato, M.Yoshida, and T.Hata, Chemistry Lett., 1979, 811.
5) The phosphodiesters 2a-c were isolated in almost quantitative yields by precipitation with hexane. The ^{31}PNMR spectra (pyridine-d_5, 85%H_3PO_4) of 2a-c are as follows: 2a: δ +6.06; 2b: δ+6.54; 2c: δ+6.08.
6) A.K.Seth and E.Jay, Nucleic Acids Res., 8, 5445(1980); M.Sekine, J.Matsuzaki, and T.Hata, Tetrahedron Lett., 1981, 3209.
7) H.Takaku and M.Yoshida, J.Org.Chem., 46, 589(1981).
8) H.Takaku, M.Kato, M.Yoshida, and R.Yamaguchi, ibid., 45, 3347(1980).
9) H.Takaku, T.Nomoto, and K.Kamaike, Chemistry Lett., 1981, 543; H.Takaku and K.Kamaike, ibid., 1982, 189.

(Received November 6, 1982)