CHEMISTRY LETTERS, pp. 1597-1600, 1983.

FURTHER IMPROVEMENTS OF OLIGODEOXYRIBONUCLEOTIDES SYNTHESIS: SYNTHESIS OF TETRADEOXYADENYLATE ON A NEW SILICA GEL SUPPORT USING N^6-PHTHALOYLDEOXYADENOSINE

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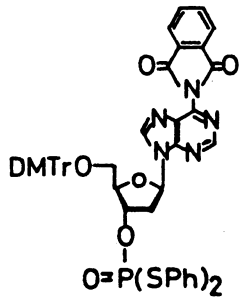
Introduction of a long alkyl chain between the nucleoside and the silica gel support was effective in DNA synthesis *via* the phosphotriester method. dApApApA containing 3'-terminal deoxyadenosine was successfully synthesized on the support by using N^6-phthaloyldeoxyadenosine.

Recentry, synthesis of deoxyribonucleotides on a polymer support has been improved by many research groups.[1-4] Polyacrylamide,[1] polystyrene[2] and silica gel[3] are most commonly used in the phosphotriester approach. Mechanically strong and nonswellable inorganic materials such as silica gel are thought to be the most suitable for automatized synthesis using a column system. In fact silica gel was successfully employed in the phosphite method.[4] In the phosphotriester approach, however, there remained a problem that early coupling yields were low and the successive coupling yields were gradually increased with the chain elongation.[5] This might be due to the variety of the pore size so that the chain elongation of a nucleoside introduced in small pores was sterically difficult. In order to overcome this problem, Gilham[6] and Köster[7] used glass beads of controlled pore size.

Another problem in deoxyribonucleotide synthesis is that undesired depurination of N^6-benzoyldeoxyadenosine takes place during removal of a dimethoxytrityl function in acidic media. Especially when 5'-O-dimethoxytrityl-N^6-benzoyldeoxyadenosine bound to a support was treated with acids, the depurination tended to occur so that the synthesis of oligodeoxyribonucleotides containing a 3'-terminal deoxyadenosine has been avoided. Previously, we reported[10] that the phthaloyl group introduced onto the N^6-amino group of deoxyadenosine derivative (1) had remarkable retarding effects on depurination in acidic media during the liquid phase synthesis of $d(Ap)_4$.



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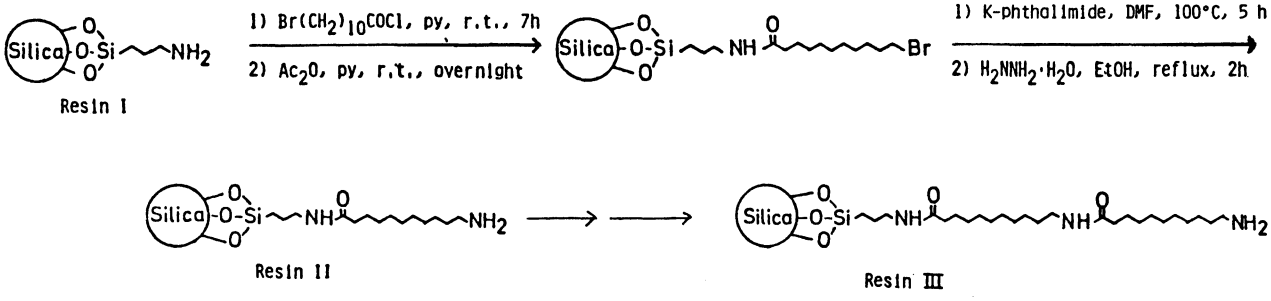
In this paper, we wish to report an application of the phthaloyl group to the synthesis of oligodeoxyribonucleotides in the phosphotriester approach utilizing N^6-phthaloyldeoxyadenosine at the 3'-terminus on a long-alkylated silica gel support.

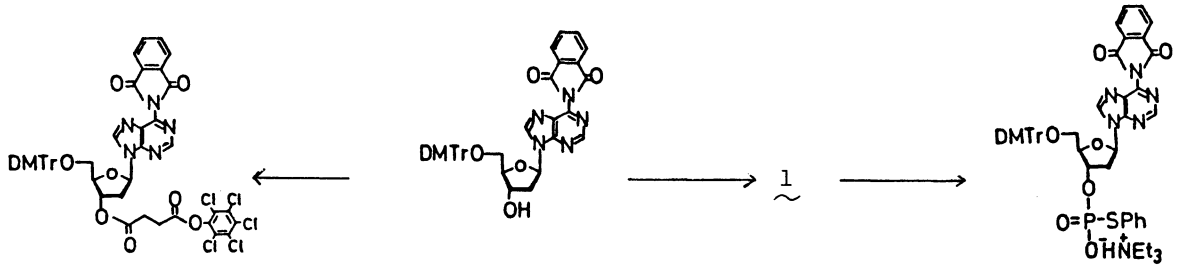
The significant low yields of early coupling reactions due to steric

hindrance of silica gel might be overcome by introduction of a spacer between the 3'-terminal nucleoside and silica gel. On this hypothesis, we prepared three resins which have different length of alkyl chain by a modification of the procedure reported by Tundo.[11] Commercially available silica gel (Merck Kiesel gel 60, 70-230 mesh, pore diameter 60 Å) was functionalized by 3-aminopropyltriethoxysilane (1.05 mequiv. NH_2/g determined by the weight increase). The remaining excess silanol groups were masked by treatment with trimethylsilyl chloride to obtain Resin I. Resin I was treated with 11-bromoundecanoyl chloride and then the remaining free amino groups were capped with Ac_2O/pyridine (1/9, v/v) overnight. Completion of disappearance of the amino residue was confirmed by using the picrate sulfate test.[12] The bromo-functionalized silica gel was converted to amino-functionalized silica gel (Resin II) via the Gabriel reaction (Scheme 1). Resin III was obtained by repeating this reaction twice.



Scheme 1.

As the first step, 5'-O-dimethoxytrityl-N^6-phtaloyldeoxyadenosine[10] (2) was converted to its 3'-O-(pentachlorophenyl succinate) derivative (3) by the method of Itakura.[13] Each Resin (200 mg) was coevaporated with dry pyridine repeatedly and treated with the activated deoxyadenosine derivative 3 (0.3 mmol) in the presence of triethylamine (3 mmol) in dry DMF (1 ml) at r.t. for 2 days. After the resin was washed successively with pyridine, chloroform, ethanol, and ether, unreacted amino functions were capped with Ac_2O/pyridine/4-(dimethylamino)pyridine (DMAP) at r.t. for 3h. The amounts of deoxyadenosine bound to Resin I, II, and III were determined to be 97, 117, and 81 μmol/g, respectively, by detritylation and spectrophotometric measurement of the dimethoxytrityl carbinol.[5]



3 2 1 4

The Resin (10 μmol) functionalized with the adenosine derivative was placed in a column and the void of the column was packed with silanized silica gel which was prepared by trimethylsilylation of Merck Kiesel gel 60. Then, the column was connected with a synthesizer (solid phase synthesizer Model 25A, Genetic Design

Co.).

A mononucleotide block, triethylammonium S-phenyl 5'-O-dimethoxytrityl-N^6-phthaloyldeoxyadenosine 3'-phosphorothioate (4), was prepared from 1 by the procedure previously reported.[10,15] A mixture of the mononucleotide block, isodurenedisulfonyl chloride (DDS),[16] and 3-nitro-1,2,4-triazole (NT) in dry pyridine (0.5 ml) was injected in each coupling. The semi-automatized synthesis of tetradeoxyadenylate was achieved by the synthetic cycle outlined in Table 1. The yields estimated by measuring the dimethoxytrityl carbinol were shown in Table 2.

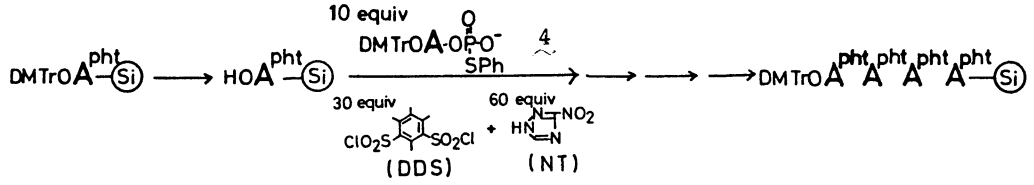


Table 1. Operation Cycle

Step	Solvent or reagent	Time/min
1	Py	5
2	Ac_2O-DMAP-Py	5 X 3
3	Py	10
4	$CHCl_3$	5
5	1% TFA in $CHCl_3$	3 X 2
6	$CHCl_3$	5
7	Py	10
8	N_2	7
9	Coupling reaction	45

Table 2. Synthesis of protected dApApApA

Resin	Coupling yield[a]/% 1 st	2 nd	3 rd
I	63	88	91
II	70	81	92
III	79	82	98

a) The yields were estimated by measuring the amount of DMTrOH released after acid treatment.

The coupling yields increased with the length of the nucleotide chain and reached over 90% at the third coupling reaction in all cases. The subsequent coupling reactions might proceed quantitatively on all the three resins. Among them, Resin III which had the longest alkyl chain gave the best results in the first and third condensations and Resin II was better than Resin I. These results suggested that the less hindered amino functions available for the oligonucleotide synthesis were selectively linked to the 3'-terminal nucleoside as the consequence of introduction of a long alkyl spacer on silica gel. In addition, lipophilicity of Resin III might enhance the efficiency of interaction between the solid and liquid phases. Similarly, in the case of the phosphoramidite method, Adams[17] reported that introduction of a long spacer was effective, especially when a sterically hindered nucleotide phosphoramidite was used.

The deprotection was performed by the following procedure:Resin III (40 mg) containing the fully protected tetradeoxyadenylate was treated with AgOAc[10,18] (100 mg) in pyridine-water (2/1, v/v, 2 ml) at r.t. for 20 h to remove the phenylthio groups and washed successively with pyridine, pyridine-0.5 M phosphate buffer (pH 7) (1/1, v/v), and methanol. As the phenylthio groups were selectively

removed under the neutral conditions, the following operations could be easily performed on the support. After treatment with 80% AcOH (10 ml) for 15 min, the resin was filtered and washed with pyridine and methanol. To the resin was added conc. ammonia-pyridine (5/1, v/v, 6 ml) and the mixture was stirred at r.t. for 48 h to remove the phthaloyl groups and to release the nucleotide from the resin.

The solution was evaporated and applied to Whatman 3 MM papers. Paper chromatography was performed with iPrOH-conc. ammonia-H_2O (6:1:3, v/v/v). Thus, dApApApA was isolated in 51% (53 OD) yield and the tetramer was completely degraded by incubation with spleen phosphodiesterase to give dAp and dA (3.2:1.0).

In conclusion, the phthaloyl group is superior to the benzoyl group for the protection of the amino residue of deoxyadenosine because of its remarkable retarding effects on depurination which enables us to constract any deoxyribonucleotide sequences even containing a 3'-terminal deoxyadenosine in the solid phase synthesis. We believe that this approach might be applicable to the synthesis of deoxyadenosine-rich and longer oligodeoxynucleotides.

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(Received July 30, 1983)