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A NEW CLASS OF CONDENSING REAGENTS FOR RAPID INTERNUCLEOTIDE BOND FORMATION IN THE PHOSPHOTRIESTER APPROACH AND PREPARATION OF N^3-BENZOYLTHYMIDINE AS A KEY INTERMEDIATE IN OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

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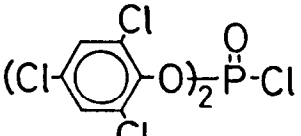
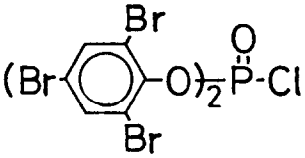
Summary: Rapid internucleotide bond formation in the phosphotriester approach has been achieved in high yield by use of bis(2,4,6-trihalophenyl) phosphorochloridates (TCP and TBP) as new condensing reagents and the benzoyl group as the N^3-imide protecting group of thymidine.

In the rapid oligodeoxyribonucleotide synthesis, the essential rate-determing step is the construction of an internucleotide phosphotriester linkage. One of the exciting methods is the phosphoramidite approach developed by Caruthers[1] and the other research groups.[2] This approach can provide an internucleotide bond rapidly and quantitatively, but has some disadvantages; instability of the phosphoramidite reagents and a necessity of oxidation step after each coupling reaction. On the other hand, the phosphotriester method seems to be yet intriguing since it gives directly a phosphotriester linkage. Only drawback of the latter method is a relatively slow rate of condensation. To accelerate the coupling reaction, catalysts such as tetrazole[3a] and N-methylimidazole[3b] have been employed. Condensations at elevated temperatures have also been reported.[4] However, little attention has been directed to exploration of condensing reagents.

An plausible mechanism of the condensation reaction using arenesulfonyl chlorides or azolides was proposed by Knorre and coworkers:[5] A symmetric pyrophosphate produced from bimolecular phosphodiester components was explained as a valid intermediate that can be activated by azoles to react with the hydroxyl function. Such tetrasubstituted pyrophposphate species can



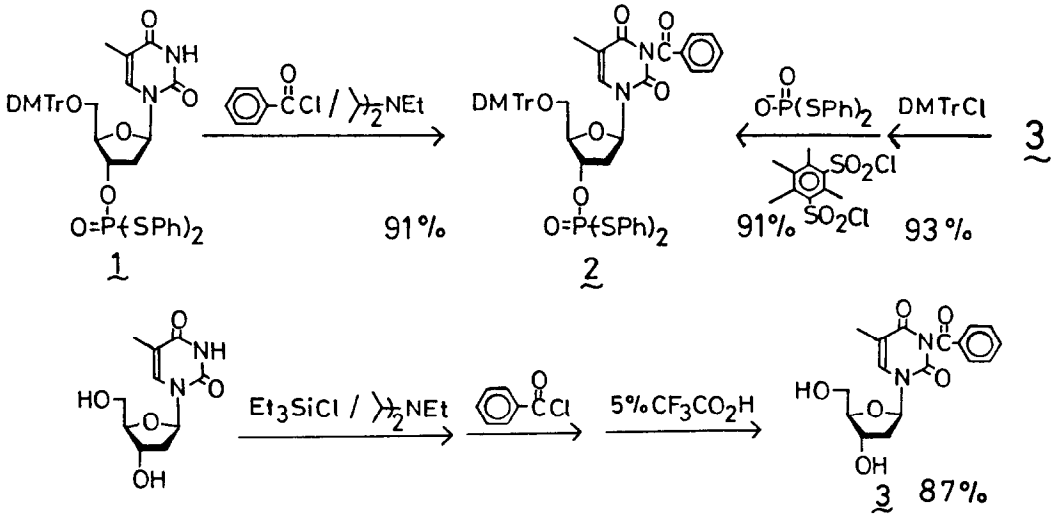
TCP



TBP

be prepared more directly by reactions of the phosphodiester component with monofunctional phosphorylating agents such as dialkyl phosphorochloridates [$(RO)_2P(O)Cl$]. If the R group is substituted with electron-withdrawing groups, the dialkyl phosphate residue [$(RO)_2P(O)O^-$] may be expected to serve as a good leaving group so that condensation occurs selectively on the phosphorus of the phosphodiester component. Therefore, our attention was focussed on sterically hindered diaryl phosphorochloridates[6] as new condensing reagents. These reagents were expected to react with the phosphodiester component in pyridine to produce a more active asymmetric pyrophosphate that could give smoothly the desired triester bond in the presence of an azole. Consequently, bis(2,4,6-trichlorophenyl) phosphorochloridate (TCP)[7] and bis(2,4,6-tribromophenyl) phosphorochloridate (TBP)[8] were found to be very effective for the rapid internucleotide bond formation.

When 1.3 equiv. each of TCP and 3-nitrotriazole (NT) was employed in the condensation between the phosphodiester [DMTrTp(SPh)($O^-HNEt_3^+$)] and the hydroxyl [$Tp(SPh)_2$] components derived from the previously reported thymidine unit 1 [$DMTrT(SPh)_2$][9], the reaction was completed in a short time (within 5 min). However, considerable amounts of some byproducts were observed on TLC and the dimer was obtained in 76% yield. These side reactions seemed to be caused from reactivity of the imide function of thymidine[10] as reported in the case of uridine and guanosine.[11] Therefore, we tried to introduce an acyl group into the imide residue of thymidine as in the case of uridine.[12] Reaction of the thymidine unit 1 with benzoyl chloride (2 equiv.) in the presence of diisopropylethylamine (2 equiv.) in pyridine for 15 h gave 2 in 91% yield. A more usuful synthetic intermediate, N^3-benzoylthymidine 3, could be prepared by a modification of the procedure of Jones:[13] Thymidine



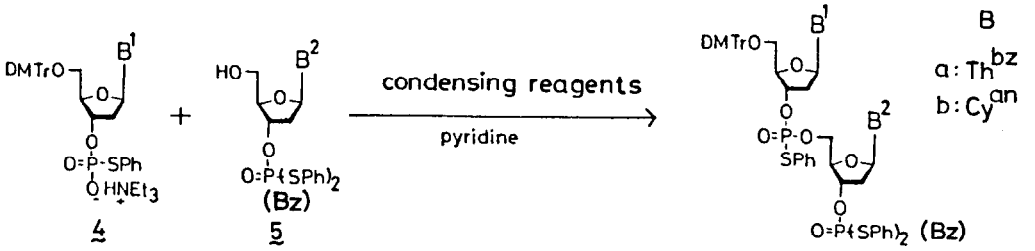


Table I. Results of Coupling Reactions Using TCP or TBP

phosphdiester (equiv.)	hydroxyl component	condensing reagents (equiv.)		time (min)	yield (%)
4a(1.2)	5'a*	TCP(1.3)	NT(1.3)	10	88
4b(1.2)	5a	TBP(1.3)	NT(2.0)	15	88
4a(1.2)	5'a*	TBP(1.3)	NT(1.3)	15	95
4a(1.2)	5a	TBP(1.3)	NT(1.0)	15	87
4b(1.2)	5a	TBP(1.3)	NT(1.0)	10	99

*5'a: 3'-O,N^3-Dibenzoylthymidine

(5 mmol) was treated with trietylsilyl chloride (13 mmol) in pyridine for 30 min and then benzoyl chloride (7.5 mmol) was added. After being stirred for 1 h, the mixture was evaporated and coevaporated with toluene to remove pyridine completely. The residue was further treated with 5% CF_3CO_2H (TFA) in CH_2Cl_2-CH_3OH (7:3, v/v, 50 ml) for 20 min. The usual workup followed by silica gel column chromatography gave 3[14] in 87% yield. The use of trimethylsilyl chloride resulted in a complicated mixture at the acylation stage. The N^3-benzoyl group was stable in acidic (80% AcOH and 2% TFA/$CHCl_3$) and neutral conditions and gradually released by treatment with triethylamine-water-pyridine (2:1:2, v/v/v) at r. t., but yet could be smoothly removed by treatment with conc. NH_4OH at r. t. for 1 h. The usual tritylation of 3 followed by phosphorylation gave 2[15] in 85% overall yield. The selective removal of one phenylthio group from 2 by treatment with 5 M phosphinic acid (pyridine soln.)-triethylamine (2:1, v/v) at 40 °C for 15 min gave the diester 4a as triethylammonium salt in 88% yield. Treatment of 2 in 2% TFA/$CHCl_3$ at 0 °C for 5 min gave the hydroxyl component 5a in 86% yield. These components 4 and 5 were condensed by TCP or TBP in the presence of NT in good yields. A mixture of 4a (0.24 mmol), 5'a (0.2 mmol), and NT (0.26 mmol) were rendered anhydrous by repeated coevaporations with dry pyridine and dissolved in pyridine (2 ml). TBP (0.26 mmol) was added and the reaction was monitored on TLC. A spot of 5'a dissappeared within 5 min, and the reaction was quenched by addition of water. In the usual manner, the fully protected dimer was obtained in 95% yield. The results of the other experiments are summarized in Table I. Only a small excess amount of TBP was enough to shorten the coupling reaction with good yields. Deprotection of all the protective groups from the thymidylate dimer was performed as

follows: 1) 0.2 M NaOH-pyridine (1:1, v/v) at 0 °C for 20 min followed by neutralization with Dowex 50W-X8 (PyH^+ form); 2) conc. NH_4OH at r.t. for 1 h; 3) I_2 (30 equiv.) treatment in pyridine-water (2:1, v/v) at r. t. for 1 h followed by removal of the excess iodine by extraction with benzene; 4) 80% acetic acid at r. t. for 15 min. Finally, purification by DEAE-Sephadex A-25 (HCO_3^- form) gave the pure thymidylate dimer, TpTp, (103 A_{260} O. D. units from 14 mg of the protected dimer).

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14) N^3-Benzoylthymidine 3: ^{1}H NMR ($CDCl_3$-CD_3OD) δ 7.88-7.31(6H, m, ArH and 6-H), 6.22(1H, t, J=6Hz, 1'-H), 4.39(1H, m, 3'-H), 3.91(1H, m, 4'-H), 3.78 (2H, m, 5'-H), 2.32(2H, m, 2'-H), 1.93(3H, s, 5-CH_3). ^{13}C NMR ($CDCl_3$-CD_3OD) δ 169.15(C=O), 163.24(4-C), 149.54(2-C), 136.81(6-C), 135.29(4-C(Bz)), 131.44 (1-C(Bz)), 130.46(2,6-C(Bz)), 129.28(3,5-C(Bz)), 110.91(5-C), 87.34(4'-C), 85.82(1'-C), 70.81(3'-C), 61.77(5'-C), 40.42(2'-C), 12.46(5-CH_3). IR (KBr) ν 1749 (PhC=O), 1700 and 1653 (imide C=O). Anal. calcd for $C_{17}H_{18}O_6N_2$: C, 58.96; H, 5.24; N, 8.09. found C, 58.47; H, 5.40; N, 7.93.
15) N^3-Benzoylated thymidine unit 2: ^{1}H NMR($CDCl_3$) δ 7.22-7.96(25H, m, ArH), 6.84(4H, d, J=8Hz, 4,5-H(DMTr)), 6.36(1H, t, J=6Hz, 1'-H), 5.36(1H, m, 3'-H), 4.10(1H, m, 4'-H), 3.78(3H, s, CH_3O(DMTr)), 3.41(2H, m, 5'-H), 2.42(2H, m, 2'-H), 1.39(3H, s, 5-CH_3). Anal. calcd for $C_{50}H_{45}O_9N_2S_2P$: C, 65.78; H, 4.97; N, 3.07; S, 7.02. found C, 66.03; H, 5.20; N, 3.00; S, 7.13.

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