Use of Isobutyloxycarbonyl as a Blocking Group in Preparation of 3′-O-*p*-Monomethoxytritylthymidine[1,2]

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For a projected synthesis of oligonucleotides it was desirable to have available deoxyribonucleosides protected at the 3′-OH position with an alkali-stable blocking group. We describe in this Note a convenient route to one such compound which utilizes isobutyloxycarbonyl as a blocking group for the 5′-oxygen and *p*-monomethoxytrityl as a blocking group for the 3′-oxygen.

Thymidine is first treated with isobutyl chloroformate in pyridine. This reagent reacts preferentially at the 5′-OH, producing isobutyl thymidine 5′-carbonate (I) which is easily isolated as a crystalline compound in 73% yield. As a consequence of the selectivity for the primary hydroxyl group, isobutyloxycarbonyl is superior to acetyl[3] and comparable with pivalyl[4] as an alkali-labile blocking group for the 5′-OH position. It gives a better yield of the 5′ ester than does *p*-nitrophenyl chloroformate[5] and the derivative is much more stable in aqueous pyridine. Under suitable conditions isobutyl chloroformate will react at the 3′-OH. Thus, isobutyl 5′-O-tritylthymidine 3′-carbonate (IV) is obtained (82%) when 5′-O-tritylthymidine is treated with isobutyl chloroformate in pyridine for 6 days. Hydrolysis with aqueous acetic acid then yields the 3′-O-blocked derivative, isobutyl thymidine 3′-carbonate (V).

On reaction with *p*-monomethoxytrityl chloride, isobutyl thymidine 5′-carbonate is converted to isobutyl 3′-O-*p*-monomethoxytritylthymidine 5′-carbonate (II). Subsequent hydrolysis with 0.4 *M* sodium hydroxide in aqueous dioxane gives the desired 3′-O-*p*-monomethoxytritylthymidine. The over-all yield from thymidine is 64%. As in the case of 5′-O-*p*-monomethoxytritylthymidine,[6] the *p*-monomethoxytrityl group may be removed quantitatively by treatment with hot, 80% aqueous acetic acid.

That the isobutyloxycarbonyl group is suitable as a blocking group in phosphorylation reactions as well as in methoxytritylation was demonstrated by conversion of isobutyl thymidine 5′-carbonate to thymidine 3′-phosphate (VI) by reaction with dicyclohexylcarbodiimide and β-cyanoethyl phosphate followed by hydrolysis. No evidence for the formation of thymidine 5′-phosphate in this reaction was found. (See Scheme I.)

Experimental Section

Isobutyl Thymidine 5′-Carbonate (I).—A solution of 1.0 g (4.1 mmoles) of thymidine and 0.57 ml (4.3 mmoles) of isobutyl chloroformate (Eastman Kodak Co.) in 25 ml of dry pyridine was stirred at room temperature for 3.5 days. The pyridine was removed at reduced pressure, the residue was dissolved in ethyl acetate, and the resulting solution was chromatographed on a silica gel column (45 × 2 cm) with 300 ml of ethyl acetate–chloroform (2:8), 500 ml of ethyl acetate, and 200 ml of tetrahydrofuran. On concentration of the ethyl acetate fractions, 905 mg of isobutyl thymidine 5′-carbonate crystallized out. An additional 125 mg of this product was obtained from the mother liquors, bringing the total yield to 1.03 g (73%). The ester melted at 127° and exhibited bands in the infrared at 2.92, 3.38, 5.76, 5.94 (a triplet), and 7.9 μ.

Anal. Calcd for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52.67; H, 6.64; N, 8.58.

This compound was stable to the conditions used to hydrolyze cyclic carbonates (10 min in refluxing pyridine–water[5]); however, it was hydrolyzed completely to thymidine (R_f 0.07, 0.62, and 0.68 on silica thin layer plates in ethyl acetate, dioxane, and tetrahydrofuran, respectively) when stirred for 30 min at room temperature with 3 ml of dioxane and 3 ml of 0.68 *M* aqueous sodium hydroxide.

As a test of the stability of the carbonate under conditions used for phosphorylation, a sample (0.072 g, 0.21 mmole) of I was mixed with 1.25 mmoles of β-cyanoethyl phosphate (pyridinium salt) and 0.52 g (2.5 mmoles) of dicyclohexylcarbodiimide in 3 ml of pyridine. After stirring for 4.5 days at room temperature in the dark, the mixture was diluted with 3 ml of water and stirred for 1 additional day. The solution was separated from the solids which had precipitated, washed with hexane, and stirred with 10 ml of 1 *M* aqueous sodium hydroxide at room temperature for 1 hr. After neutralization with Dowex-50 (pyridinium form) and concentration, the solution was chromatographed on Whatman 3MM paper (descending). The product was thymidine 3′-phosphate (R_f 0.085 in solvent A, isopropyl alcohol–ammonium hydroxide–water, 7:1:2; R_f 0.22 in solvent B, *n*-butyl alcohol–acetic acid–water, 5:2:3).

Traces of two other components were seen in the chromatogram for solvent B (R_f 0.30, 0.39). For comparison the R_f values for thymidine 3′-phosphate and thymidine 5′-phosphate, respectively, are solvent A, 0.086 and 0.053; solvent B, 0.22 and 0.18.

Isobutyl 3′-O-*p*-Monomethoxytritylthymidine 5′-Carbonate (II).—*p*-Monomethoxytrityl chloride[7] (0.40 g, 1.3 mmoles) and 0.29 g (0.85 mmole) of isobutyl thymidine 5′-carbonate were heated in 5 ml of pyridine for 3 hr on a steam bath; then the solvent was removed at reduced pressure and the residue was taken up in ether and applied to a silica gel column (45 × 2 cm). Elution with ether afforded monomethoxytriphenylcarbinol in the first 75-ml fraction and isobutyl 3′-O-*p*-monomethoxytritylthymidine 5′-carbonate in the next 550-ml portion. This product was recovered by evaporating the solvent and crystallizing the residual gum from benzene–hexane. It melted at 93–95°; the yield was 0.475 g (91%). Principal bands in the infrared (KBr) occurred at 2.9, 3.35, 5.75, 5.93, 7.9, and 14.2 μ.

Anal. Calcd for $C_{35}H_{38}N_2O_8$: C, 68.39; H, 6.23; N, 4.56. Found: C, 68.43; H, 6.30; N, 4.39.

3′-O-*p*-Monomethoxytritylthymidine (III).—Carbonate II (0.35 g) was hydrolyzed by stirring with 5 ml of dioxane and 5 ml of 0.8 *M* aqueous sodium hydroxide at room temperature for 30 min. Following neutralizing by Dowex-50 (pyridinium form) the solution was diluted with water and extracted twice with chloroform. The chloroform solution was dried with sodium sulfate and evaporated and the residue was crystallized from ether–hexane, yielding 0.281 g (96%) of 3′-O-*p*-monomethoxytritylthymidine: mp 126–128°; infrared bands at 2.83, 5.94, and 14.2 μ.

Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.49; H, 6.11; N, 5.34.

Isobutyl 5′-O-Trityl thymidine 3′-Carbonate (IV).—A mixture of 1.12 g (2 mmoles of benzene adduct) of 5′-O-tritylthymidine and 0.3 ml of isobutyl chloroformate in 10 ml of pyridine was

(1) Part VII in series on Nucleotide Chemistry. Part VI: R. L. Letsinger, M. H. Caruthers, and D. M. Jerina, *Biochemistry*, **6**, 1379 (1967).

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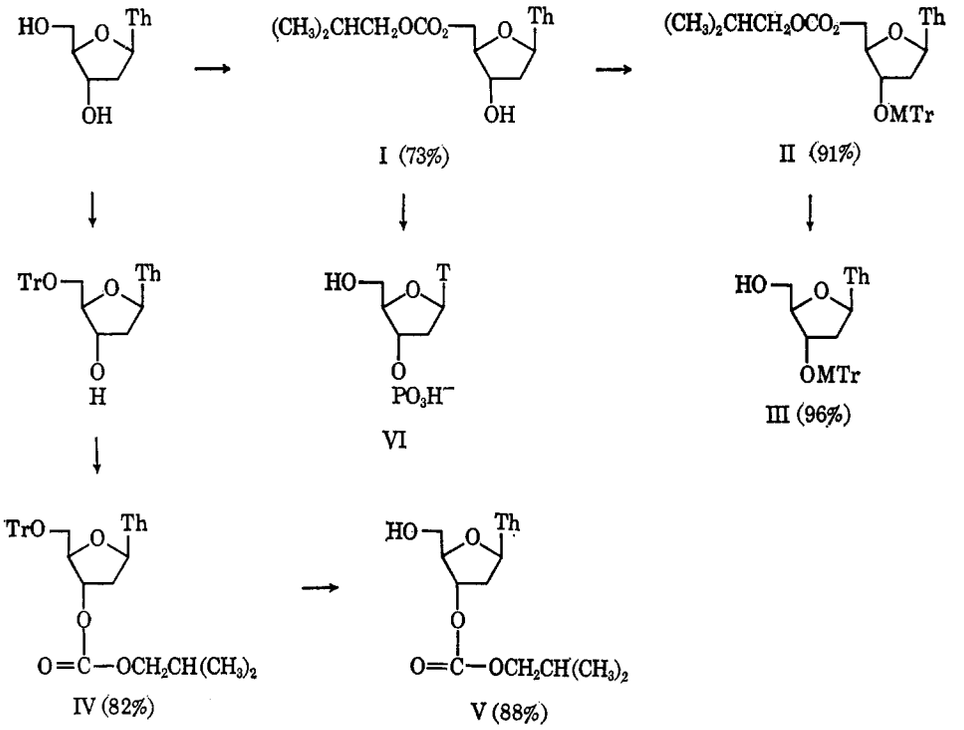
SCHEME I



MTr = monomethoxytrityl, Tr = trityl, Th = thymine ring

stirred at room temperature for 6 days, whereupon the pyridine was evaporated and the residue was taken up in ether and chromatographed in silica, with ether as the eluent. A total of 0.145 g of 5′-O-tritylthymidine and 0.91 g (82% yield, 96.5% yield based on tritylthymidine that had reacted) of isobutyl 5′-O-tritylthymidine 3′-O-carbonate was obtained: mp 178–179°; R_f 0.56 with ether on silica thin layer plates; infrared bands at 2.89, 3.40 (close doublet), 5.77, 5.94, 7.90, and 14.2 μ.

Anal. Calcd for $C_{34}H_{36}N_2O_7$: C, 69.85; H, 6.21; N, 4.79. Found: C, 69.55; H, 6.00; N, 4.97.

Isobutylthymidine 3′-carbonate (V) was prepared by heating 0.350 g of isobutyl 5′-O-tritylthymidine 3′-carbonate in 15 ml of 80% aqueous acetic acid at reflux for 10 min. Concentration, extraction with ethyl acetate, and chromatography on silica (ethyl acetate) afforded after recrystallization from ethyl acetate 0.180 g (88%) of ester V: mp 138–139°; infrared bands at 2.89, 3.40 (doublet), 5.77, 5.83, 5.94, and 7.90 μ.

Anal. Calcd for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52.75; H, 6.52; N, 8.23.

Chromatographic data for the compounds described in this paper are collected in Table I. The data are for silica thin layer plates (Eastman Chromagram Sheet 6060).

TABLE I

Compound	R_f in solvent: Ethyl acetate	R_f in solvent: Ether
3′-O-*p*-Monomethoxytritylthymidine	0.65	0.26
5′-O-*p*-Monomethoxytritylthymidine	0.54	0.22
Isobutyl thymidine 3′-carbonate	0.66	0.25
Isobutyl thymidine 5′-carbonate	0.51	0.16
Isobutyl 3′-O-*p*-monomethoxytritylthymidine 5′-carbonate	0.72	0.55
Isobutyl 5′-O-tritylthymidine 3′-carbonate	0.72	0.56

Registry No.—I, 13084-59-8; II, 13084-60-1; III, 13084-61-2; IV, 13084-64-5; V, 13084-67-8.