Use of Chloroacetic Anhydride for the Protection of Nucleoside Hydroxyl Groups

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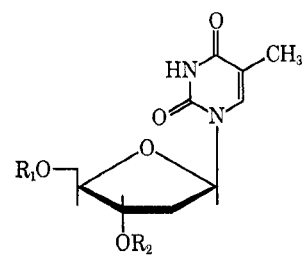
The use of the chloroacetyl group as a protecting group in the nucleoside and nucleotide field has been examined. The reactions of chloroacetic anhydride with the hydroxyl groups of partially protected nucleosides gave good yields of the corresponding chloroacetyl derivatives. Removal of the chloroacetyl group(s) from these compounds was accomplished without loss of other protecting groups. The reagents employed for the removal of chloroacetyl groups included thiourea, 2-mercaptoethylamine, ethylenediamine, and *o*-phenylenediamine. Of these, the most efficient was found to be 2-mercaptoethylamine. The stability of the chloroacetyl group in water, buffered aqueous pyridine, and buffered aqueous 2,6-lutidine has been examined. Chloroacetylation of mononucleotides was readily accomplished, but instability problems prevented the purification of these compounds. Diphenylchloroacetyl chloride reacted selectively with the primary hydroxyl group of thymidine, and a high yield of the 5′ derivative was obtained. Attempts to remove this group with 2-mercaptoethylamine were unsuccessful, although cleavage was effected using thiourea or aqueous ammonia.

Chemical manipulations in the nucleoside and nucleotide fields are often governed by the feasibility of differential protection and removal of the protecting groups employed in a particular synthesis. The value of acid-labile protecting groups is sometimes limited by the lability of the purine–glycosyl bond of nucleosides and nucleotides, particularly when the purine bears an N-acyl group. The extremely acid-sensitive di-O-*p*-methoxytrityl group, for example, cannot be removed from N-benzoyldeoxyadenosine derivatives without a substantial amount of concomitant depurination.[1,2] Acyl groups, on the other hand, are widely used as base-labile groups for the protection of amino and hydroxyl functions. Thus, other protecting groups, which do not require acidic or basic conditions for their removal, and hence are compatible with the N-benzoyldeoxyadenosine moiety, may be useful in the chemical manipulation of these materials. Hydroxyl protecting groups which can be removed under conditions close to neutrality have already been employed in the nucleoside field. The dinitrobenzenesulfenyl moiety has been successfully removed from N-benzoyldeoxyadenosine derivatives using thiophenol or hydrogen sulfide,[3] and β-benzoylpropionyl esters have been cleaved with buffered hydrazine.[4] A dihydrothiophene adduct with 5′-O-acetylthymidine has been removed with silver ion,[5] and β,β,β-tribromoethoxycarbonyl derivatives have been deshielded using a zinc–copper couple.[6] Recent reports on the protection of amino and hydroxyl functions with the chloroacetyl group[7,8] and subsequent deprotection under mild conditions prompted our investigation into the utility of the chloroacetyl group for the protection of nucleoside hydroxyl functions.

While the reactions between nucleosides and chloroacetyl chloride were generally unsatisfactory, giving intractable mixtures, the reactions with chloroacetic anhydride proceeded smoothly at 0° with no major byproducts. The reaction of chloroacetic anhydride with 3′-O-acetylthymidine (**1**) required 2 hr for completion, and the corresponding 5′-chloroacetate **2** was isolated in crystalline form. The presence of the chloroacetate group in the product, as well as in all other chloroacetyl derivatives isolated, was readily detected by nmr spectroscopy; each chloroacetyl group gave rise to a sharp singlet in the region δ 4.0–4.5. For removal of the chloroacetyl group from the **2**, the compound was treated with thiourea in refluxing ethanol.



1, R_1 = H; R_2 = $COCH_3$
2, R_1 = $COCH_2Cl$; R_2 = $COCH_3$
3, R_1 = $C(C_6H_5)_3$; R_2 = H
4, R_1 = $C(C_6H_5)_3$; R_2 = $COCH_2Cl$
5, R_1 = H; R_2 = $COCH_2Cl$
11, R_1 = $COCl(C_6H_5)_2$; R_2 = H
12, R_1 = H; R_2 = $C(C_6H_5)_2C_6H_4OCH_3$

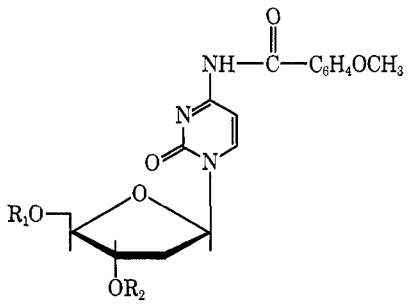
All of the starting material was consumed after 1 hr, and 3′-O-acetylthymidine was isolated from the mixture in 81% yield. As previously described,[7] the removal of chloroacetyl groups probably involves an isothiourea intermediate which undergoes intramolecular amidinolysis with formation of the alcohol and a pseudothiohydantoin.[9]

5′-O-Tritylthymidine (**3**) also afforded a chloroacetyl derivative **4** which was isolated without difficulty. Removal of the chloroacetyl group from **4** using thiourea in ethanol was unsatisfactory since this reaction was accompanied by extensive detritylation. This problem was avoided by the use of pyridine as cosolvent, and 5′-O-tritylthymidine (**3**) was recovered in good yield. Thus, the conditions required for the introduction and removal of the chloroacetyl group are completely compatible with the commonly used trityl and acetyl protecting groups. The trityl group was removed from **4** using 80% acetic acid at 100° for 30 min, and these conditions did not affect the chloroacetyl group; 3′-O-chloroacetylthymidine (**5**) was isolated in good yield.

In the deoxycytidine series, N-anisoyl-5′-O-mono-*p*-methoxytrityldeoxycytidine (**6**)[1] gave a 3′-chloroacetyl

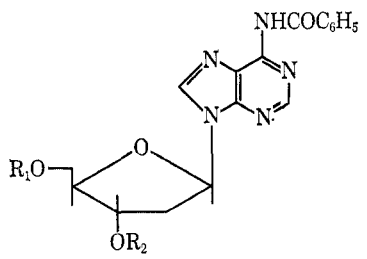
(1) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 3821 (1963).
(2) M. W. Moon, S. Nishimura, and H. G. Khorana, *Biochemistry*, **5**, 937 (1966).
(3) G. W. Grams and R. L. Letsinger, *J. Org. Chem.*, **33**, 2589 (1968).
(4) R. L. Letsinger, M. H. Caruthers, P. S. Miller, and K. K. Ogilvie, *J. Amer. Chem. Soc.*, **89**, 7146 (1967).
(5) L. A. Cohen and J. A. Steele, *J. Org. Chem.*, **31**, 2333 (1966).
(6) A. F. Cook, *ibid.*, **33**, 3589 (1968).
(7) M. Masaki, T. Kitahara, H. Kurita, and M. Ohta, *J. Amer. Chem. Soc.*, **90**, 4508 (1968).
(8) A. Fontana and E. Scoffone, *Gazz. Chim. Ital.*, **98**, 1261 (1968).
(9) Pseudothiohydantoins have been prepared by a similar cyclization procedure: C. Liebermann, *Ann. Chem.*, **207**, 121 (1881).

derivative **7** by reaction with chloroacetic anhydride in pyridine at 0° for 4 hr. Treatment with thiourea in ethanol–pyridine in the usual way removed the chloroacetyl group without loss of the other protecting groups, and **6** was recovered by silica gel column chromatography. Removal of the mono-*p*-methoxytrityl group from **7** gave the 5′-hydroxy compound **8**, a partially protected deoxycytidine derivative which may be of use for subsequent transformations.



6, $R_1 = CH_3OC_6H_4(C_6H_5)_2C$; $R_2 = H$
7, $R_1 = CH_3OC_6H_4(C_6H_5)_2C$; $R_2 = COCH_2Cl$
8, $R_1 = H$; $R_2 = COCH_2Cl$

Experiments were also carried out in the deoxyadenosine series in order to determine whether the chloroacetyl group is compatible with the much more labile N-benzoyldeoxyadenosine moiety. No problems were encountered during the chloroacetylation of N-benzoyldeoxyadenosine (**9**),[1] and its 3′,5′-di-O-chloroacetyl derivative **10** was obtained crystalline in good yield. Attempts to remove the chloroacetyl groups with thiourea in ethanol–pyridine were unsuccessful; depurination rapidly occurred, and N-benzoyladenine was isolated in high yield and identified by comparison with a commercially available sample.[10] Depurination also occurred when the reaction was carried out in the presence of triethylamine although none could be detected in a series of control experiments with N-benzoyldeoxyadenosine.



9, $R_1 = H$; $R_2 = H$
10, $R_1 = COCH_2Cl$; $R_2 = COCH_2Cl$

In order to overcome this problem, other reagents for removing the chloroacetyl group were sought. Of the reagents examined, the most satisfactory was found to be 2-mercaptoethylamine hydrochloride; using this reagent in the presence of triethylamine, dechloroacetylation of **10** was complete within 2 hr at room temperature, and no evidence of depurination was detected. Ethylenediamine was also found to be satisfactory, and only traces of debenzoylation were observed. *o*-Phenylenediamine was also quite suitable for dechloroacetylation of **10**, and a high yield of N-benzoyldeoxyadenosine was isolated. This last reagent has been used for the liberation of amino groups from their chloroacetyl amides during peptide synthesis[11] although these reactions were carried out under strongly alkaline conditions. 1,2-Ethanedithiol was also investigated, but it was not found to be suitable; a number of products were observed. In subsequent dechloroacetylation studies, using **2** as a model compound, 2-mercaptoethylamine was again the preferred reagent.

An indication that dechloroacetylation occurs *via* a cyclic intermediate was obtained by the isolation of 1,4-thiazin-3-one from the reaction of **2** with 2-mercaptoethylamine hydrochloride. Thus the thiol group must initially have acted as the nucleophilic agent in the displacement of the chloride ion with the intermediate undergoing intramolecular aminolysis and liberation of the alcohol. The presence of triethylamine is apparently necessary in order to generate the nucleophilic amino group from its hydrochloride salt, since in the absence of base, no dechloroacetylation could be observed. Excess triethylamine was removed at the end of the reaction by evaporation, thus avoiding the alkaline conditions which would produce indiscriminate deacylation. As expected, the free base ethylenediamine was shown to cause debenzoylation of **10** as well as dechloroacetylation, whereas the former reaction was completely eliminated with the use of a limited amount of its hydrochloride in triethylamine. *o*-Phenylenediamine, on the other hand, must be a sufficiently weak base (pK_B = 9.6) not to produce deacylation under these conditions.

Chloroacetylation of mononucleotides was also studied. Thymidine 5′-phosphate reacted smoothly with chloroacetic anhydride in pyridine, and paper chromatography of the reaction mixture after 2 hr indicated complete absence of starting material. Upon addition of water, dechloroacetylation began to occur, and attempts to isolate pure chloroacetate were not successful.[12] Similar problems were encountered during attempts to isolate the chloroacetyl derivatives of N-anisoyldeoxycytidine 5′-phosphate and N-benzoyldeoxyadenosine 5′-phosphate. Efforts were therefore made to define the reasons for the instability of the chloroacetyl group. In these experiments, 3′-O-chloroacetylthymidine (**5**) was used as a model compound, and the reactions were monitored by thin layer chromatography followed by elution and spectrophotometric assay of the appropriate spots. The chloroacetate group was relatively stable in water and in 0.2 *M* sodium chloroacetate, pH 6.3; in the latter experiment, a half-time of approximately 100 hr was found for the dechloroacetylation reaction. In aqueous pyridine containing pyridinium chloride or chloroacetate, pH 6–7, the reaction was much more rapid, being complete after 20 hr at room temperature with a half-time of 4 hr. When pyridine was replaced by 2,6-lutidine, deacylation was much slower at the same pH value (half-time 24 hr). These experiments clearly implicate pyridine as the deacylating agent, presumably by nucleophilic attack on the carbon

(10) Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

(11) R. W. Holley and A. D. Holley, *J. Amer. Chem. Soc.*, **74**, 3069 (1952).

(12) In contrast, the simpler procedures employed for the isolation of nucleoside chloroacetyl derivatives did not produce significant amounts of dechloroacetylation.

bearing the chlorine atom with the formation of an acylpyridinium intermediate. This latter would be labilized toward hydrolysis as compared with the chloroacetate. 2,6-Lutidine, on the other hand, cannot readily form an acylpyridinium intermediate owing to the steric hindrance of the adjacent methyl groups.

In the reaction of chloroacetic anhydride with thymidine, no substantial degree of specificity was observed. The use of the bulkier diphenylchloroacetyl group was therefore investigated for the protection of primary hydroxyl functions. The reaction of thymidine with diphenylchloroacetyl chloride gave a high yield of the crystalline 5′ derivative **11**, together with a small amount of the 3′,5′-bisdiphenylchloroacetyl compound; no trace of the 3′ isomer was detected. The nmr spectrum of **11** in deuterated dimethyl sulfoxide gave a doublet (J = 4 Hz) for the hydroxyl proton, indicating the presence of a secondary hydroxyl group. Further evidence for the assignment of **11** as the 5′ derivative was obtained by its treatment with mono-*p*-methoxytrityl chloride in pyridine, followed by treatment with ammonia. After column purification a good yield of 3′-O-mono-*p*-methoxytritylthymidine (**12**)[13] was obtained.

The removal of the diphenylchloroacetyl group from **11** could not be accomplished using 2-mercaptoethylamine hydrochloride in the same way as for the chloroacetyl derivative; even at elevated temperatures, only small amounts of thymidine were detected. Thiourea, on the other hand, did produce deacylation, although at a slower rate than that found for chloroacetyl derivatives. A related reaction between ethyl diphenylchloroacetate and thiourea has recently been described.[14] Treatment with aqueous ammonia also cleaved the diphenylchloroacetyl group, and thymidine was readily isolated. This group may therefore be of use as a protecting group for primary hydroxyl functions.

Experimental Section[15]

3′-O-Acetyl-5′-O-chloroacetylthymidine (**2**).—A solution of 3′-O-acetylthymidine (**1**, 1.27 g, 4.5 mmol) and chloroacetic anhydride (2.31 g, 13.5 mmol) in dry pyridine was stored at 0° for 2 hr. Water (5 ml) was added, after 10 min at 0° the solution was evaporated to dryness, and the residue was partitioned between chloroform and water. The chloroform layer was washed twice with water, dried (Na_2SO_4), and evaporated to dryness. Crystallization of the residue from ethanol–hexane gave 1.14 g (71%) of **2**: mp 130–132°; uv max (C_2H_5OH) 265 mμ (ε 9320); ir (KBr) 1740 cm^{-1} (C=O); nmr ($CDCl_3$) δ 4.23 (s, 2, $COCH_2Cl$).

Anal. Calcd for $C_{14}H_{17}ClN_2O_7$: C, 46.61; H, 4.75; Cl, 9.89; N, 7.76. Found: C, 46.50; H, 4.78; Cl, 9.85; N, 7.73.

3′-O-Chloroacetyl-5′-O-tritylthymidine (**4**).—A solution of 5′-O-tritylthymidine (**3**, 1.35 g, 2.4 mmol, benzene adduct) in dry pyridine (25 ml) was treated with chloroacetic anhydride (2.07 g, 12.1 mmol) for 3.5 hr at 0°, and the product was poured dropwise with stirring into ice water (2 l.). After storage overnight at 5°, the precipitate was collected, washed with water, and dissolved in chloroform. The chloroform solution was dried (Na_2SO_4), evaporated, and crystallized from ethanol to give 1.27 g (94%) of **4**: mp 185–185.5°; uv max (C_2H_5OH) 264 mμ (ε 9200); ir (KBr) 1750 cm^{-1} (C=O); nmr ($CDCl_3$) δ 4.07 (s, 2, $COCH_2Cl$).

Anal. Calcd for $C_{31}H_{29}ClN_2O_6$: C, 66.35; H, 5.23; Cl, 6.32; N, 4.99. Found: C, 66.46; H, 5.35; Cl, 6.20; N, 4.93.

3′-O-Chloroacetylthymidine (**5**).—A solution of **4** (1.0 g, 1.85 mmol) in 80% acetic acid (25 ml) was heated at 100° for 30 min. Crystals of triphenylmethyl alcohol were deposited on cooling and were removed by filtration. The filtrate was evaporated to dryness and extracted with ether (three 30-ml portions), and the residue was crystallized from ethanol–hexane to give 413 mg (73%) of **5**: mp 149.5–150°; uv max (2-propanol) 264 mμ (ε 9980); ir (KBr) 1745 cm^{-1} (C=O); nmr (DMSO-d_6) δ 4.40 (s, 2, $COCH_2Cl$).

Anal. Calcd for $C_{12}H_{15}ClN_2O_6$: C, 45.22; H, 4.71; Cl, 11.12; N, 8.75. Found: C, 45.12; H, 4.47; Cl, 11.12; N, 8.82.

Preparation of 7.—A solution of N-anisoyl-5′-O-mono-*p*-methoxytrityldeoxycytidine (**6**, 1.12 g, 1.8 mmol) in dry pyridine (40 ml) was treated with chloroacetic anhydride (0.98 g, 5.75 mmol) for 4 hr at 0°. Ice–water (20 ml) was added and after 15 min the solution was evaporated to dryness. The residue was partitioned between chloroform and water, and the chloroform layer was washed with water (three portions), dried (Na_2SO_4), and evaporated to a syrup. This material was purified by silica gel column (200 g) chromatography, using chloroform–ethyl acetate (1:2) as the solvent. Fractions 60–140 were evaporated to give **7**, 0.97 g (77%) as a foam: uv max (C_2H_5OH) 286 mμ (ε 21,750); ir (KBr) 1740 cm^{-1} (C=O); nmr ($CDCl_3$) δ 4.08 (s, 2, $COCH_2Cl$).

Anal. Calcd for $C_{39}H_{36}ClN_3O_8$: C, 65.96; H, 5.11; Cl, 4.99; N, 5.94. Found: C, 65.64; H, 5.07; Cl, 4.89; N, 5.83.

Removal of the Mono-*p*-methoxytrityl Group from 7.—A solution of **7** (0.86 g, 1.2 mmol) in 80% acetic acid (25 ml) was allowed to stand at room temperature for 1 hr. Since thin layer chromatography of the reaction mixture indicated that substantial amounts of the starting material still remained, the solution was heated at 100° for 15 min. The product was cooled and evaporated to dryness, and the residue was washed with ether (three 20-ml portions). The residue was crystallized from ethanol–hexane to give 360 mg (68%) of **8**: mp 174–178°; uv max (C_2H_5OH) 289 mμ (ε 25,300); ir (KBr) 1760 cm^{-1} (C=O); nmr (DMSO-d_6) δ 4.40 (s, 2, $COCH_2Cl$).

Anal. Calcd for $C_{19}H_{20}ClN_3O_7$: C, 52.12; H, 4.60; Cl, 8.10; N, 9.60. Found: C, 52.29; H, 4.54; Cl, 7.88; N, 9.52.

N-Benzoyl-3′,5′-di-O-chloroacetyldeoxyadenosine (**10**).—A solution of N-benzoyldeoxyadenosine (**9**, 1.10 g, 3.1 mmol) in dry pyridine (15 ml) was treated with chloroacetic anhydride (1.57 g, 9.2 mmol) for 1.5 hr at 0°. Water (15 ml) was added, and after 10 min the mixture was evaporated to dryness. The residue was dissolved in hot ethyl acetate and filtered, and on cooling crystals of **10** 1.04 g (66%) were deposited from the filtrate. Concentration of the mother liquor gave a second crop, 0.19 g (12%): mp 146–147°; uv max (2-propanol) 280 mμ (ε 20,900); ir (KBr) 1770 cm^{-1} (C=O); nmr (DMSO-d_6) δ 4.42, 4.47 (s, 2, $COCH_2Cl$).

Anal. Calcd for $C_{21}H_{19}Cl_2N_5O_6$: C, 49.62; H, 3.77; Cl, 13.93; N, 13.78. Found: C, 49.73; H, 3.60; Cl, 13.96; N, 13.85.

Use of Thiourea for Removal of Chloroacetyl Groups. A. Dechloroacetylation of 2.—A solution of **2** (340 mg, 0.94 mmol) and thiourea (85 mg, 1.1 mmol) in ethanol (20 ml) was heated under reflux for 1 hr, and then evaporated to half-volume. Addition of hexane yielded crystalline 3′-O-acetylthymidine, 218 mg (81%), mp 177° (lit.[16] mp 176°).

B. Dechloroacetylation of 4.—A solution of **4** (952 mg, 1.7 mmol) and thiourea (146 mg, 1.85 mmol) in pyridine (10 ml) and ethanol (2 ml) was heated at 100° for 20 min and then evaporated to dryness. The residue was chromatographed on a silica gel column (150 g) using chloroform–ethyl acetate (1:1) as the solvent. Fractions 57–120 were evaporated and recrystallized from ethanol–hexane to give 572 mg (70%) of 5′-O-tritylthymidine (**3**), mp 125–128° (lit.[16] mp 128°).

C. Removal of the Chloroacetyl Group from 7.—A solution of **7** (505 mg, 0.71 mmol) and thiourea (59 mg, 0.78 mmol) in pyridine (10 ml) and ethanol (1 ml) was heated at 100° for 45 min and then evaporated to dryness. The residue was purified by silica gel column (150 g) chromatography using ethyl acetate–

(13) K. K. Ogilvie and R. L. Letsinger, *J. Org. Chem.*, **32**, 2365 (1967).

(14) A. U. Rahman and H. S. E. Gatica, *Rec. Trav. Chim. Pays-Bas*, **88**, 905 (1969).

(15) Pyridine was dried by storage over Linde Molecular Sieve Type 4A. Merck silica gel (0.05–0.2 mm) was used for silica column chromatography, and fractions of 20 ml were collected. Nuclear magnetic resonance spectra were obtained using a Varian A-60 spectrometer, ultraviolet spectra using a Cary Model 14 instrument, and infrared spectra with a Beckman IR-5 or IR-9. A Thomas–Hoover apparatus was used for melting point determinations.

(16) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 951 (1953).

methanol (40:1) as the eluting solvent. Fractions 35–60 were combined and evaporated to give 318 mg (71%) of **6** as a foam. The ir spectrum (KBr) was identical with that of an authentic sample.[1]

D. Reaction of 10 with Thiourea.—A solution of **10** (496 mg, 0.98 mmol) in pyridine (6 ml) and ethanol (2 ml) was treated with thiourea (78 mg, 0.99 mmol) at 100° for 20 min. The product was evaporated to dryness and the residue was dissolved in hot water. Upon cooling, crystals of N-benzoyladenine, 143 mg (61%), were deposited, mp 237–242° (lit.[17] mp 237–238°).

Dechloroacetylation Using 2-Mercaptoethylamine. A. Dechloroacetylation of 2.—A solution of **2** (1.11 g, 3.1 mmol) and 2-mercaptoethylamine hydrochloride (378 mg, 3.3 mmol) in pyridine (15 ml) and triethylamine (4 ml) was stored at room temperature for 1 hr. The product was evaporated to dryness and purified by silica gel column (200 g) chromatography, using ethyl acetate–acetone (40:1) as the solvent. Fractions 30–150 were evaporated and recrystallized from ethanol–hexane to give 723 mg (83%) of 3′-O-acetylthymidine.

B. Compound 10.—A solution of **10** (0.98 g, 1.9 mmol) and 2-mercaptoethylamine hydrochloride (0.45 g, 4.0 mmol) in pyridine (10 ml), methanol (10 ml), and triethylamine (5 ml) was stored at room temperature for 2 hr and then evaporated to dryness. The residue was applied to a silica gel column (200 g) which was eluted with ethyl acetate–methanol (10:1). Fractions 100–200 were evaporated to give N-benzoyldeoxyadenosine, 647 mg (91%), mp 113–117° (lit.[1] mp 113–115°).

Use of Ethylenediamine for Dechloroacetylation.—A solution of the chloroacetyl compound (2 mmol) in pyridine (30 ml), methanol (20 ml), and triethylamine (5 ml) was treated with ethylenediamine dihydrochloride (2–4 mmol) for 4 hr at room temperature. The product was isolated either by direct crystallization or by partition between chloroform and water and subsequent isolation from the chloroform layer.

***o*-Phenylenediamine as a Dechloroacetylating Agent.**—A solution of the chloroacetyl compound (2 mmol) and *o*-phenylenediamine (2–3 mmol) in pyridine (30 ml) and ethanol (15 ml) was stored overnight at room temperature. The products were isolated as described in the experiments using 2-mercaptoethylamine.

Studies on the Stability of the Chloroacetate Group. A. In Aqueous Pyridine.—A solution of the chloroacetyl derivative **5** (5 mg) in 50% aqueous pyridine, which had been adjusted to pH 6.7 with either hydrochloric or chloroacetic acid, was stored at room temperature. Aliquots (20 μl) were removed at intervals, evaporated to dryness, dissolved in methanol, and chromatographed on a silica thin-layer plate, using ethyl acetate–methanol (10:1) as the developing solvent. The appropriate uv-absorbing spots were removed, extracted with methanol (three 5-ml portions), and measured spectrophotometrically. Dechloroacetylation was complete after 20 hr, with a half-time for the reaction of 4 hr.

B. In Aqueous 2,6-Lutidine.—This experiment was carried out as described in part A. Dechloroacetylation was much slower in this medium with a half-time of 24 hr.

C. In Aqueous Sodium Chloroacetate.—A solution of **5** (5 mg) in aqueous sodium chloroacetate, pH 6.3 (0.2 *M*), was examined at intervals as previously described. After 24 hr, 10% dechloroacetylation was detected.

5′-O-Diphenylchloroacetylthymidine (11).—A 0° solution of thymidine (1.94 g) in dry pyridine (25 ml) was treated with diphenylchloroacetyl chloride (2.33 g) for 4 hr. Methanol (2 ml) was added, and after 2 hr the solution was evaporated to dryness. The residue was dissolved in chloroform (100 ml), extracted with water (three 100-ml portions), dried (Na_2SO_4), and evaporated to dryness. The product was purified by silica gel column (400 g) chromatography using chloroform–ethyl acetate (1:1) as the eluting solvent. Fractions 180–400 were evaporated and recrystallized from ethyl acetate to give 3.3 g (87%) of **11**: mp 177°, uv max (CH_3OH) 265 mμ (ε 9200); ir (KBr) 1740 cm^{-1} (C=O); nmr (DMSO-d_6) δ 5.40 (d, 1, J = 4 Hz, CHOH).

Anal. Calcd for $C_{24}H_{23}ClN_2O_6$: C, 61.22; H, 4.92; Cl, 7.53; N, 5.95. Found: C, 61.42; H, 5.06; Cl, 7.28; N, 5.67.

3′-O-Mono-*p*-methoxytritylthymidine (12).—A solution of **11** (6.55 g) and mono-*p*-methoxytrityl chloride (8.7 g) in pyridine (25 ml) was heated overnight at 100°. Water (10 ml) was added, and after 1 hr the solution was evaporated to dryness and dissolved in methanol (200 ml) and concentrated aqueous ammonia (200 ml). After 66 hr the solution was evaporated to dryness and purified by silica gel column (500 g) chromatography using chloroform–ethyl acetate (2:1) as the solvent. Fractions 140–230 were evaporated to dryness, and the residue was dissolved in chloroform. Addition of hexane precipitated 3′-O-mono-*p*-methoxytritylthymidine, 4.86 g (68%), as white powder, mp 115° (lit.[13] mp 126–128°).

Reaction of 11 with Thiourea.—A solution of **11** (1.46 g, 3.1 mmol) and thiourea (1.40 g, 18.4 mmol) in ethanol (50 ml) was heated under reflux for 48 hr. The product was evaporated to dryness and purified by silica column (250 g) chromatography, using ethyl acetate–methanol (40:1) as the eluting solvent. Fractions 70–240 were evaporated to dryness and recrystallized from ethanol–hexane to give 385 mg (51%) of thymidine, mp 184–186° (lit.[18] mp 185°).

Treatment of 11 with Aqueous Ammonia.—A solution of **11** (1.0 g, 2.1 mmol) in methanol (5 ml) and concentrated aqueous ammonia (5 ml) was stored at room temperature for 48 hr and then evaporated to dryness. The residue was partitioned between chloroform and water, and the aqueous layer was evaporated to dryness and recrystallized from water to give 294 mg (58%) of thymidine, mp 186° (lit.[18] mp 185°).

Registry No.—Chloroacetic anhydride, 541-88-8; **2**, 24299-19-2; **4**, 24299-20-5; **5**, 24343-75-7; **7**, 24299-21-6; **8**, 24343-76-8; **10**, 24299-22-7; **11**, 24299-23-8; **12**, 13084-61-2.

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(17) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **121**, 131 (1937).

(18) P. A. Levene and E. S. London, *ibid.*, **83**, 793 (1929).