**4-Chloro-3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-*c*]pyridine (XII). Method B.**—The procedure was essentially that used for the synthesis of VIII from X. To a solution of XI (obtained by ribosidation, followed by separation; 2.35 g.) in 80 ml. of absolute methanol was added cyclohexylamine (11.6 g.). The solution was kept at room temperature for 2 days and finally refluxed for 1 hr. (until the paper chromatography in $H_2O$, adjusted to pH 10 had a single spot: $R_f$ 0.60). The solution was concentrated to *ca.* 5 ml. To the solution 20 ml. of water was added and the mixture was treated with chloroform (30 ml.). The aqueous layer was separated and concentrated to dryness. The residue was taken in methanol (20 ml.), and methanol was removed *in vacuo*. The process was repeated twice to afford crystalline nucleoside, 942 mg. (84%), m.p. 177–178°. After recrystallization from aqueous ethanol, m.p. 179–180°; $R_f$ (BuOH–$H_2O$, 84:16 v./v.) 0.54; ultraviolet absorption: $\lambda_{max}^{EtOH}$ 275 mμ (ε 5300).

*Anal.* Calcd. for $C_{11}H_{12}ClN_3O_4$: C, 46.22; H, 4.23; N, 14.71. Found: C, 46.40; H, 4.06; N, 14.95.

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# 2,4-Dinitrobenzenesulfenyl as a Blocking Group for Hydroxyl Functions in Nucleosides[1]

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The dinitrobenzenesulfenic esters of butanol and cyclohexanol were found to be relatively stable in acetic acid and in pyridine solutions containing acetic anhydride, *p*-toluenesulfonyl chloride, and dicyclohexylcarbodiimide; however, they reacted readily at room temperature with solutions containing polarizable nucleophiles such as thiosulfate, cyanide, or thiophenol. As a consequence of the stability of the esters in pyridine and the lability in solutions containing active nucleophiles, the 2,4-dinitrobenzenesulfenyl group offers promise as a blocking agent for hydroxyl functions, supplementing acyl groups, which are removed by alkaline treatment, and trityl or tetrahydropyranyl groups, which are removed by acid treatment. The feasibility of using the 2,4-dinitrobenzenesulfenyl group in nucleoside work was explored by preparing derivatives of thymidine and 5′-*O*-tritylthymidine, removing the trityl and dinitrobenzenesulfenyl groups in stepwise fashion, and acetylating one of the dinitrobenzenesulfenyl derivatives.

The groups most frequently used to protect hydroxyl functions in nucleosides and nucleotides are acetyl,[4] benzyl,[5] triarylmethyl,[6–8] benzoyl,[7,8] tetrahydropyranyl,[7] and isopropylidene.[8] Hydroxyl groups protected by esterification are unblocked by alkaline hydrolysis; those protected by ether formation are unblocked by acid hydrolysis. For use in the synthesis of oligonucleotides on polymer supports[9] it was desirable to have available a blocking group which would withstand the conditions employed in forming internucleotide bonds, yet which could be selectively removed under mild conditions in a neutral medium or in a pyridine solution. Ability to remove the blocking group in an aprotic solvent was especially desirable, since in that case both the formation of internucleotide bonds and unblocking of hydroxyl functions could be carried out without changing the nature of the solvent.

With these considerations in mind we selected the 2,4-dinitrobenzenesulfenyl group for study as a potential blocking agent in nucleotide syntheses. Three lines of evidence supported this choice. (a) Kharasch, McQuarrie, and Buess showed that relatively stable esters could be prepared in high yield from the reaction of 2,4-dinitrobenzenesulfenyl chloride with simple aliphatic alcohols.[10] (b) Edwards and Pearson[11] pointed out that nucleophilicity toward divalent sulfur is largely governed by the polarizability of the attacking nucleophile; accordingly, it appeared likely that facile cleavage of the sulfenic esters might be realized with weakly basic but highly polarizable nucleophiles. (c) Foss[12] described an analytical method for determination of closely related compounds, the 2,4-dinitrobenzenesulfenamides, which involved the quantitative conversion of sulfenamides to amines by reaction with a polarizable ion, thiosulfate, in dilute acid at room temperature.

Preliminary experiments with 2,4-dinitrobenzenesulfenic esters of butanol and cyclohexanol revealed that the dinitrobenzenesulfenic esters were sufficiently stable to serve as blocking groups in synthetic work. Thus, the esters were recovered unchanged after standing 48 hr. in pyridine solutions containing either acetic anhydride or *p*-toluenesulfonyl chloride. Furthermore, chromatographic evidence indicated that cyclohexyl 2,4-dinitrobenzenesulfenate was not affected in a solution in which pyridinium thymidine 5′-phosphate was converted to a mixture of oligonucleotides by dicyclohexylcarbodiimide[13]; that is, the sulfenic ester survived in the presence of the active phosphate comprising the phosphorylating agent.

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(2) Abbott Fellow of Northwestern University, 1962.

(3) National Science Foundation Summer Research Participant, 1963.

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(12) O. Foss, *Acta Chem. Scand.*, **1**, 307 (1947).

(13) Preparation of thymidine oligonucleotides by the action of dicyclohexylcarbodiimide in pyridine on pyridinium thymidine 5-phosphate was described by G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *J. Am. Chem. Soc.*, **80**, 6223 (1958).

The feasibility of cleaving simple model esters under mild conditions was then examined. In view of Foss's work on the sulfenamides[12] the action of thiosulfate on the esters was explored first. As shown in Table I, the alkyl 2,4-dinitrobenzenesulfenates reacted readily with thiosulfate, the order of reactivity being *n*-butyl > *sec*-butyl > cyclohexyl. Thiosulfate reacted with the esters somewhat faster in an acidic medium than a neutral one.

TABLE I

REACTION OF ALKYL 2,4-DINITROBENZENESULFENATES WITH THIOSULFATE IN AQUEOUS ALCOHOL

| Alkyl group | Reaction time, min. | % reaction[a] |
|---|---|---|
| *n*-Butyl | 10 | 41.6 |
| | 30 | 67.6 |
| | 60 | 75.0 |
| | 300 | 97.2 |
| *sec*-Butyl | 10 | 16.7 |
| | 30 | 31.4 |
| | 60 | 54.4 |
| Cyclohexyl | 10 | 10.9 |
| *n*-Butyl[b] | 10 | 64.3 |
| *sec*-Butyl[b] | 10 | 29.3 |

[a] Reactions followed titrimetrically. See Experimental section for details. The per cent reaction was calculated on the basis that the stoichiometry was $ROSAr + S_2O_3^{-2} + H_2O = ArSS_2O_3^- + ROH + OH^-$. [b] In these reactions 5 ml. of 10% acetic acid in water was added to the sulfenate solution just prior to addition of thiosulfate. Dilute acid in the absence of thiosulfate did not affect the ester.

The behavior of several other nucleophilic agents was investigated spectroscopically. Pseudo-first-order rate constants for reactions involving sodium thiosulfate, sodium cyanide, sodium sulfide, and thiophenol are presented in Table II. In absence of the added nucleophiles no detectable change occurred in the buffer solutions of the sulfenic esters. Thiophenol was the most striking agent tested. It reacted with cyclohexyl 2,4-dinitrobenzenesulfenate at a rate too fast for accurate measurement under these conditions; the half-life in 0.0024 *M* thiophenol at pH 11.8 was approximately 5 sec. Sodium azide, sodium nitrite, and thiourea (each 0.02 *M*) had no observable effect on the sulfenic ester over the 50-min. period investigated.

TABLE II

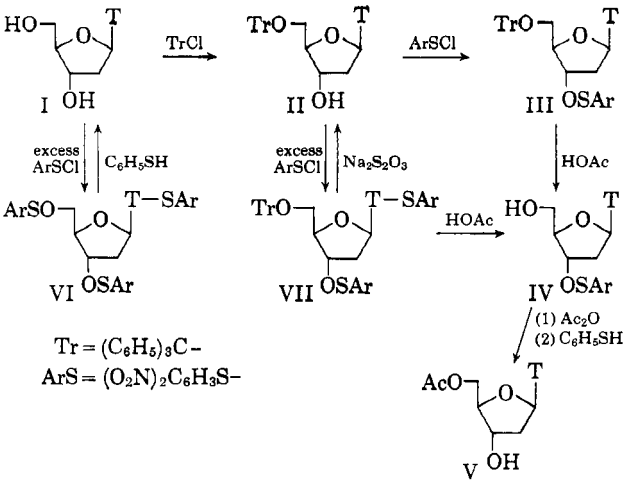
REACTION OF ALKYL 2,4-DINITROBENZENESULFENATES WITH NUCLEOPHILES IN AQUEOUS METHANOL AT 25°[a]

| Alkyl group | Nucleophilic agent added | pH | $k_{obsd}$ min.$^{-1}$ Ester disappearance | $k_{obsd}$ min.$^{-1}$ Product appearance |
|---|---|---|---|---|
| *n*-Butyl | NaCN | 8.9 | 0.16 (342)[b] | 0.022 (442)[b] |
| *n*-Butyl | $Na_2S_2O_3$ | 8.9 | 0.069[c] (340) | 0.078[c] (415) |
| Cyclohexyl | NaCN | 8.9 | 0.17 (342) | 0.022 (429) |
| Cyclohexyl | $Na_2S$ | 6.6 | 0.094 (340) | 0.097 (388) |
| Cyclohexyl | $C_6H_5SH$ | 11.8 | ~7.6 (342) | |

[a] $1 \times 10^{-4}$ *M* in ester; $2.4 \times 10^{-3}$ *M* in nucleophilic agent. [b] Wave length in mμ at which the reaction was followed. [c] This value corresponds to the rate after an "induction period" of a few minutes (see Experimental section).

On the basis of these results an investigation of the chemistry of the 2,4-dinitrobenzenesulfenyl derivatives of a nucleoside, thymidine, was undertaken. The transformations effected are summarized in Chart I.

CHART I




5′-O-Tritylthymidine yielded 2,4-dinitrobenzenesulfenyl-5′-O-tritylthymidine (III) when treated with 2,4-dinitrobenzenesulfenyl chloride in dimethylformamide. Evidence that the sulfenyl group was bound at the 3′-O-position and that it would serve as a blocking group was provided by cleaving the trityl group with acetic acid, acetylating the 2,4-dinitrobenzenesulfenylthymidine (IV) thereby produced, and finally removing the sulfenyl group by treatment with thiophenol in pyridine. 5′-O-Acetylthymidine was the exclusive thymidine derivative obtained as shown by paper chromatography performed under conditions which separate the 5′-O-acetyl-, 3′-O-acetyl-, and 3′-O-5′-O-diacetylthymidine derivatives.

The reaction of thymidine (I) and 5′-O-tritylthymidine (II) with excess 2,4-dinitrobenzenesulfenyl chloride in pyridine furnished products which analyzed as the tris- and the bis-2,4-dinitrobenzenesulfenyl derivative (VI and VII), respectively. Introduction of one more dinitrobenzenesulfenyl group than the number of hydroxyl groups was somewhat surprising since in the acetylation of 5′-O-tritylthymidine with a thirtyfold excess of acetic anhydride in pyridine only a monoacetyl derivative had been obtained.[4,14] The extra arenesulfenyl group is no doubt introduced into the thymine ring. In accord with expectations from the model studies, it was found that the sulfenyl groups could be cleaved from these thymidine derivatives readily. Thus, bis-2,4-dinitrobenzenesulfenyl-5′-O-tritylthymidine was converted to 5′-O-tritylthymidine by sodium thiosulfate in aqueous alcohol and tris-2,4-dinitrobenzenesulfenylthymidine rapidly yielded thymidine when treated with thiophenol in pyridine at room temperature.

The ultraviolet absorption spectra of the dinitrobenzenesulfenylthymidine derivatives showed two strong bands with maxima near 264 and 330 mμ (see Table III). From a comparison with the spectra of thymidine and *n*-butyl 2,4-dinitrobenzenesulfenate it may be seen that the former band arises from absorption by both the thymine ring and the dinitrobenzenesulfenyl group while the latter is associated only with the dinitrobenzenesulfenyl group. The extinction coefficients of the two bands in the spectra of III and IV

(14) On the other hand, benzoylation of the heterocyclic ring in a uridine derivative was observed by Y. Lapidot and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3852 (1963).

TABLE III

ULTRAVIOLET ABSORPTION SPECTRA IN DIOXANE

| Compound | $\lambda_{max}$ | $\epsilon_{max} \times 10^{-4}$ | $\lambda_{max}$ | $\epsilon_{max} \times 10^{-4}$ |
|---|---|---|---|---|
| I | 264 | 0.96 | ... | |
| II | 264 | 1.01 | ... | |
| III | 264 | 2.00 | 330 | 1.10 |
| IV | 264 | 1.80 | 328 | 1.05 |
| VI | 264 | 3.60 | 310 | 2.80 |
| BuOSAr[a] | 256 | 0.98 | 335 | 1.15 |
| I + BuOSAr | 264 | 1.90 | 333 | 1.14 |

[a] BuOSAr = *n*-butyl 2,4-dinitrobenzenesulfenate.

correspond approximately to the sum of the coefficients for thymidine and *n*-butyl dinitrobenzenesulfenate. The maxima were not shifted when the solutions were 0.01 *M* in hydrochloric acid. In 0.1 *N* alkali a reaction of the sulfenic esters occurred as evidenced by the development of a deep reddish brown color.

The carbonyl region of the infrared spectra of 5′-O-tritylthymidine, 3′-O-(2,4-dinitrobenzenesulfenyl)-5′-O-tritylthymidine, and 3′-O-(2,4-dinitrobenzenesulfenyl)thymidine was characterized by a broad, strong band with the maximum at 5.92 mμ. In contrast, the materials which contained an arenesulfenyl group in the thymine ring (VI and VII) exhibited two bands, a weak, sharp one at 5.80 and a much more intense band at 5.92 mμ. Thymidine gave two bands of about equal intensity at 5.85 and 5.97 mμ.

We conclude from these studies of dinitrobenzenesulfenic esters that the 2,4-dinitrobenzenesulfenyl group merits consideration, along with more conventional groups such as trityl, tetrahydropyranyl, and acetyl, as a blocking agent for hydroxyl group. It has the special feature that it can be removed selectively in neutral aqueous–alcoholic solution or in pyridine solution by reaction with nucleophilic agents such as thiosulfate or thiophenol.

## Experimental

The infrared spectra were determined with a Baird recording spectrometer with the sample in potassium bromide, and ultraviolet spectra were determined on a Cary Model 11 recording spectrophotometer. Melting points were taken on a Fisher-Johns apparatus. Microanalyses were performed by the Micro-Tech Laboratories, Skokie, Ill.

2,4-Dinitrobenzenesulfenic esters of 1-butanol, 2-butanol, and cyclohexanol were prepared by the method of Kharasch, McQuarrie, and Buess.[10]

**Kinetics.**—The reactions reported in Table I were carried out by mixing 25 ml. of an ethanol solution 0.0133 *M* in the 2,4-dinitrobenzenesulfenic ester with 25 ml. of 0.01 *N* sodium thiosulfate in water at room teperature. After the indicated time interval (Table I) the solutions were diluted with 40 ml. of water and the excess thiosulfate was titrated with 0.01 *N* iodine solution.

For the reactions reported in Table II, solutions were prepared by diluting 25 ml. of an aqueous solution 0.01 *M* in nucleophile and 0.01 *M* in potassium acid phthalate to 100 ml. with methanol and adjusting the pH by addition of aqueous sodium hydroxide. At zero time 0.10 ml. of a methanol solution of the sulfenic ester ($2.6 \times 10^{-3}$ *M*) was added to 2.50 ml. of the nucleophile solution in a cuvette. Spectral changes were then observed with a Cary spectrophotometer. Ester disappearance was measured by the absorbance decrease near 340 mμ and product appearance by the absorbance increase in the 415–440-mμ region (measured at 387.5 in the case of the sulfide reaction). The thiosulfate reaction was complicated in that the rate was relatively very slow for the first few minutes; thereafter plots of log ($A_\infty - A$) *vs.* time for the absorbance decrease at 340 mμ (ester decrease) and the increase at 415 mμ (product appearance) were linear. Scans of the spectrum from 500 to 270 mμ indicated two consecutive reactions when cyanide was the nucleophile. These reactions could be distinguished by the spectral changes at 342 mμ, where the final products and the intermediate formed in the first reaction had the same absorbance, and above 420 mμ, where the absorbance change was largely due to conversion of intermediate to final product. Both the rapid reaction observed at 342 mμ and the slower reaction observed at the longer wave length gave first-order plots. The reaction involving sodium sulfide exhibited a single isosbestic point (367.5 mμ) and good first-order kinetics, corresponding to a simple displacement on sulfur in the ester with formation of a stable product.

**Nucleotide Synthesis in Presence of Cyclohexyl 2,4-Dinitrobenzenesulfenate.**—As a test of the stability of a sulfenic ester under the conditions of polynucleotide synthesis, a mixture containing dry pyridinium thymidine 5′-phosphate (prepared by passing 34 mg. of calcium thymidine 5′-phosphate over Rexyn RG 50 H resin in the pyridinium form), 150 mg. of dicyclohexylcarbodiimide, and 30 mg. of cyclohexyl 2,4-dinitrobenzenesulfenate in 3 ml. of pyridine was allowed to stand in a dry nitrogen atmosphere in a sealed flask for 4 days at room temperature. The solution was then filtered to remove dicyclohexylurea and a small portion was chromatographed on Whatmann 3MM paper in an ascending manner with 1-butanol–ethanol–water (5:2:5 v./v.). Aromatic products were detected by their fluorescence in ultraviolet light. The chromatogram consisted of a series of oligonucleotides at $R_f$ 0.02, 0.09, 0.19, and 0.29 and sulfenic ester (which appeared as a single yellow spot) at $R_f$ 0.93. The $R_f$ values were the same as those obtained when chromatograms were run (a) on an oligonucleotide mixture prepared in the absence of the sulfenic ester, (b) on cyclohexyl 2,4-dinitrobenzenesulfenate, and (c) on a mixture of the solutions used in a and b.

**3′-O-(2,4-Dinitrobenzenesulfenyl)-5′-O-tritylthymidine (III).**—2,4-Dinitrobenzenesulfenyl chloride (0.160 g., 0.68 mmole) in 0.3 ml. of pyridine was added to 0.240 g. (0.43 mmole) of the monobenzene adduct of 5′-O-tritylthymidine[15] in 4 ml. of dry dimethylformamide. After 40 min. the mixture was poured into ice–water and stirred one hr. The resulting yellow precipitate was separated by filtration, washed with water, and extracted with hot methanol. After filtration of the extract to remove suspended disulfide, the solution was evaporated to give the crude dinitrobenzenesulfenyltritylthymidine in essentially quantitative amount (0.30 g.). This product was used directly for preparation of dinitrobenzenesulfenylthymidine described later. On the melting point block it softened to a viscous yellow liquid at 109–125° and decomposed rapidly, liberating gas and turning black at 208°. By chromatography on Whatman 1 paper in *n*-butyl alcohol–acetic acid–water (5:2:3) it moved as a single spot ($R_f$ 0.96); under these conditions the $R_f$ for thymidine was 0.68. When a dioxane soution was spotted directly on DEAE paper some decomposition to thymidine occurred as evidenced by formation of a brown spot. Chromatography with the butyl alcohol–acetic acid solution revealed three spots: a brown spot at the origin, a fluorescent spot at $R_f$ 0.69 corresponding to II, and a yellow spot at $R_f$ 0.96 corresponding to undecomposed III. If the solution of III was acidified prior to application to the DEAE paper no decomposition occurred during chromatography with butyl alcohol–acetic acid–water, a single spot being obtained at $R_f$ 0.93. For analysis and the ultraviolet spectral determination the dinitrobenzenesulfenyltritylthymidine was recrystallized from methanol. It then softened in the range 125–130° and decomposed at 208°.

*Anal.* Calcd. for $C_{35}H_{30}N_4O_9S$: C, 61.57; H, 4.43; N, 8.21; S, 4.70. Found: C, 60.36; H, 4.70; N, 8.31; S, 4.86; residue, 1.81.

**Bis-2,4-dinitrobenzenesulfenyl-5′-O-tritylthymidine (VII).**—5′-O-Tritylthymidine benzene adduct (0.237 g., 0.422 mmole) and excess 2,4-dinitrobenzenesulfenyl chloride (0.353 g., 1.5 mmoles) were dissolved in 8 ml. of anhydrous pyridine and allowed to stand for 40 min., whereupon the solution was poured onto ice. The resulting yellow precipitate was collected by filtration and extracted with portions of hot chloroform until the chloroform extracts were colorless (this operation separated the ester derivative from 2,4-dinitrophenyl disulfide, which is insoluble in chloroform). On concentration of the combined chloroform extracts to a small volume and addition of pentane

(15) Prepared by the method of G. Weimann and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 419 (1962).

0.301 g. (82% yield calculated as the bis derivative) of yellow precipitate was obtained. It softened at 145–165° and decomposed at 200–201°. The behavior on heating was not altered on recrystallizing the product from methanol.

*Anal.* Calcd. for $C_{41}H_{32}N_6O_{13}S_2$: C, 55.90; H, 3.66; N, 9.54; S, 7.28. Found: C, 56.30; H, 3.91; N, 9.76; S, 7.01; residue (from C, H analysis), 1.81.

**Tris-2,4-dinitrobenzenesulfenylthymidine (VI).**—A solution of thymidine (0.4844 g., 2 mmoles) and 2,4-dinitrobenzenesulfenyl chloride (1.877 g., 8 mmoles) in 15 ml. of dry pyridine was allowed to stand for 40 min.; then it was poured onto ice and worked up as in the reaction involving 5′-O-tritylthymidine and 2,4-dinitrobenzenesulfenyl chloride. The product from the chloroform extracts (90% calculated as the tris derivative) was recrystallized from chloroform. On the melting point block it changed from yellow to dark brown, without melting or softening, at 165–167°. On continued heating the sample turned black.

*Anal.* Calcd. for tris-2,4-dinitrobenzenesulfenylthymidine $C_{28}H_{20}N_8O_{17}S$: C, 40.19; H, 2.41; N, 13.39; S, 11.50. Found: C, 40.20; H, 2.78; N, 13.39; S, 10.85; residue 0.59.

**3′-O-(2,4-Dinitrobenzenesulfenyl)thymidine (IV). A. From VII.**—A mixture of 0.30 g. (0.346 mmole) of bis(2,4-dinitrobenzenesulfenyl)-5′-O-tritylthymidine and 5 ml. of acetic acid was heated at the boiling point for 5 min.; then 1 ml. of water in 1 ml. of acetic acid was added slowly and the mixture was heated for 15 min. on a steam bath. The resulting heterogeneous mixture was extracted with 20-ml. portions of hot heptane to remove triphenylcarbinol. The remaining solid product was recovered by cooling the mixture and filtering. After successive washing with heptane, methanol, and ether and drying it weighed 0.0610 g. (42%) and decomposed vigorously at 189–191° without prior softening. An analytical sample was obtained by recrystallization from ethyl acetate and decomposed sharply within a 1° range. With very slow heating the temperature at which decomposition set in was 188°; with rapid heating decomposition set in at a somewhat higher temperature (up to 193°). Major bands in the infrared spectrum (KBr disk) occurred at 2.87, 5.80, 5.92, 6.22, 6.53, 7.41, and 11.97 $\mu$.

*Anal.* Calcd. for $C_{16}H_{16}N_4O_9S$: C, 43.64; H, 3.66; N, 12.72; S, 7.28. Found: C, 43.23; H, 3.61; N, 12.52; S, 7.33; residue 0.79.

**B. From III.**—Compound III (0.200 g., 0.294 mmole) was treated with 2 ml. of acetic acid on the steam bath for 20 min.; then 5 ml. of 50% acetic acid in water was added dropwise and the mixture was heated an additional 10 min. After dilution with 100 ml. of cold water the yellow precipitate was collected, washed with hot heptane, and recrystallized from ethyl acetate. The yield of purified dinitrobenzenesulfenylthymidine was 100 mg. (65%). The decomposition temperature and the infrared spectrum were the same as those obtained with the compound prepared from VII. On chromatography on Whatman 1 paper with *n*-butyl alcohol–acetic acid–water (5:2:3) the substance moved as a single spot ($R_f$ 0.92).

*Anal.* Calcd. for $C_{16}H_{16}N_4O_9S$: See above. Found: C, 43.28; H, 3.53; N, 12.06; S, 7.32; residue 0.95.

**Conversion of 3′-O-(2,4-Dinitrobenzenesulfenyl)thymidine to 5′-O-Acetylthymidine.**—A solution of 0.050 g. of compound IV and 0.3 ml. of acetic anhydride in 1 ml. of pyridine was allowed to stand overnight; then it was diluted with 25 ml. of water, allowed to stand 1 hr., and evaporated to a gum. This material was dissolved in 2 ml. of pyridine and treated with 0.5 ml. of thiophenol. After 3 hr. at room temperature the solution was concentrated; a portion was chromatographed on Whatman 1 paper in *n*-butyl alcohol–acetic acid–water (5:2:3). Two products were found. One, the sulfur-containing fragment, appeared at the solvent front, and the other, which was colorless and fluoresced blue in ultraviolet light, appeared at $R_f$ 0.76. The latter was identified as 5′-O-acetylthymine by comparison with chromatograms of 5′-O-acetylthymidine, 3′-O-acetylthymidine, and the diacetyl derivative. $R_f$ values in *n*-butyl alcohol–acetic acid–water were thymidine, 0.68; 5′-O-acetylthymidine, 0.76; 3′-O-acetylthymidine, 0.83; and 3′-O-5′-O-diacetylthymidine, 0.85. Mixtures containing the 5′-O-acetyl derivative with the 3′-O-acetyl isomer or the 3′-O-5′-O-diacetyl derivative were readily resolved. Similar results were obtained with isopropyl alcohol–ammonia–water (7:1:2) as solvent for the chromatogram ($R_f$ of product from reaction 0.72, thymidine 0.66, 5′ derivative 0.72, 3′ derivative 0.76, and diacetyl derivative 0.79). For these experiments 5′-O-acetylthymidine and 3′-O-5′-O-diacetylthymidine were prepared by acetylation of thymidine[4] and 3′-O-acetylthymidine was prepared from 5′-O-tritylthymidine by the method of Michelson and Todd.[16]

**Reaction of Bis-2,4-dinitrobenzenesulfenyl-5′-O-tritylthymidine with Sodium Thiosulfate.**—To a solution of 0.110 g. (0.127 mmole) of bis-2,4-dinitrobenzenesulfenyl-5′-O-tritylthymidine in 750 ml. of methanol was added 2.0 g. of sodium thiosulfate in 250 ml. of water. After 4 hr. the volume was reduced to 50 ml. under vacuum; then 50 ml. of water was added and the solution was extracted three times with 25-ml. portions of chloroform. Evaporation of the chloroform extracts and recrystallization of the resulting solid from benzene afforded 0.0447 g. (63%) of the monobenzene adduct of 5′-O-tritylthymidine, m.p. 118–123°; m.p. 160–162° after heating to remove the benzene; $R_f$ 0.91 on Whatman 40 in isopropyl alcohol–ammonia–water (7:1:2 v./v.). The half-life for reaction of III with sodium thiosulfate in aqueous alcohol under the conditions used for the reactions in Table II was 9.6 min. measured at 340 m$\mu$ and 9.8 min. measured at 415 m$\mu$.

**Conversion of Tris-2,4-dinitrobenzenesulfenylthymidine to Thymidine.**—A solution of 0.040 g. of the tris derivative in 50 ml. of methanol was mixed with 0.15 g. of sodium thiosulfate in 20 ml. of water. After 2 hr. of stirring, the solution, originally yellow, had turned deep orange. Chromatography of a sample on Whatman 40 in isopropyl alcohol–water–ammonia (7:2:1) and on diethylaminoethylcellulose thin layer plates with 0.02 *M* hydrochloric acid as the liquid phase afforded in each case an ultraviolet fluorescent spot with the same $R_f$ as thymidine ($R_f$ 0.72 and 0.93, respectively).

When a drop of thiophenol was added to a solution of the tris derivative (17.5 mg.) in 1 ml. of pyridine, the solution turned a deep red immediately. Paper chromatography and thin layer chromatography again showed that thymidine was the product of the reaction.

In confirmation of the fact that thymidine was formed in the thiophenol reaction, the pyridine solution was concentrated under vacuum and the products were added to a Dowex-1 ion-exchange column in the formate form at pH 8.8. The column was washed with 0.01 *M* ammonium formate at pH 8.8 and eluted with 0.01 *M* ammonium formate at pH 7.5. The elution pattern corresponded exactly to that of thymidine and the spectrum of the product eluted (fractions 8–12, 17-ml. fractions) was that of thymidine. The optical density corresponded to an 80% yield of thymidine based on the tris ester (V).

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