Two New Protected Acyl Protecting Groups for Alcoholic Hydroxy Functions

Jonathan M. Brown, Chris Christodoulou, Colin B. Reese,* and Giovanni Sindona
Department of Chemistry, King's College, Strand, London WC2R 2LS

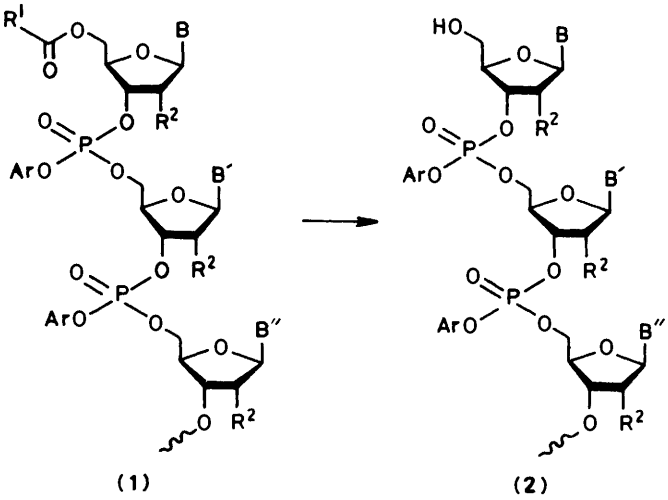
4-(Methylthiomethoxy)butyric acid (**13a**) and 2-(methylthiomethoxymethyl)benzoic acid (**14a**) have been prepared in 53 and 64% overall yields from γ-butyrolactone and phthalide, respectively. Thymidine reacts regioselectively with (**13a**) and (**14a**), in the presence of an appropriate condensing agent, to give the corresponding 5'-*O*-acyl derivatives [(**20**) and (**21**), respectively], both in 70% yield. The latter compounds undergo deacylation relatively slowly when treated with concentrated aqueous ammonia but, following treatment with mercury(II) perchlorate in the presence of 2,4,6-collidine in slightly wet tetrahydrofuran, are both converted back into thymidine under very mild conditions of basic hydrolysis.

In the phosphotriester approach [1] to oligonucleotide synthesis it is of crucial importance to use 5'-*O*-acyl protecting groups which are removable under neutral or, at most, very mildly basic conditions. This is particularly so in the case of oligoribonucleotide synthesis.[2,3] Thus, it must be possible to convert compound (**1**) (Scheme 1) into (**2**) without concomitant removal of any of the aryl protecting groups from the internucleotide linkages.

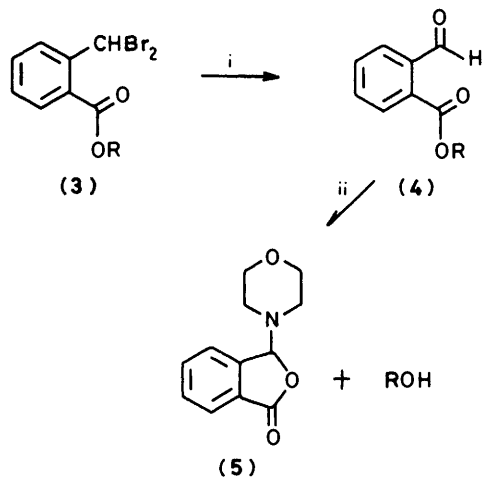
A number of years ago, we found that even relatively sensitive acyl groups, such as *p*-chlorophenoxyacetyl[4] [as in (**1**; R^1 = 4-$ClC_6H_4COCH_2$)], were too stable to alkaline hydrolysis or ammonolysis to be used in the synthesis of oligonucleotides containing more than three or four nucleotide residues. While acyl protecting groups containing additional functional groups, such as β-benzoylpropionyl[5] and more especially laevulinyl,[6] have been used in oligonucleotide synthesis, we have favoured the use of protected acyl protecting groups. The first such protected protecting group which we developed was the 2-dibromomethylbenzoyl group[7] [as in structure (**3**), Scheme 2].

2-Dibromomethylbenzoyl (DBMB) derivatives such as (**3**) may easily be prepared[7,8] by treating alcohols (ROH) with DBMB chloride,[8] a readily obtainable crystalline solid, in the presence of base; these derivatives (**3**) may be regarded as protected 2-formylbenzoate esters (**4**). Treatment of the DBMB derivatives (**3**) with silver perchlorate in slightly wet tetrahydrofuran or acetone, in the presence of 2,4,6-collidine at room temperature, leads[7] to the 'unblocking' of the masked formyl group, that is to the formation of the corresponding 2-formylbenzoate esters (**4**). The latter intermediates (**4**) are extremely sensitive to hydroxide ions[9] and certain other nucleophiles. Thus, treatment with morpholine at room temperature leads[7] (Scheme 2) to the rapid release of unblocked alcohols (ROH). If the DBMB derivatives (**3**) are treated directly with alkali, they undergo hydrolysis at approximately the same rate[7] as the corresponding acetates.

We next turned our attention to protected acyl protecting groups containing masked neighbouring hydroxy functions. Hydroxy functions may be masked in a number of ways, but it seemed to us that the methylthiomethyl (MTM) group,[10] which is stable under a wide range of reaction conditions but removable by mercury(II) ion catalysed hydrolysis, would be particularly suitable for the present purpose. In a preliminary study, we showed[11] that when 2-(methylthiomethoxy)ethoxycarbonyl (MTMEC) derivatives (**6**) of alcohols are treated (Scheme 3) with mercury(II) perchlorate in the presence of 2,4,6-collidine in acetone–water, removal of the MTM group occurs and the putative 2-hydroxyethoxycarbonyl derivatives (**7**) are obtained. The latter intermediates (**7**) are very sensitive to base; thus, when they are treated with ammonia or potassium



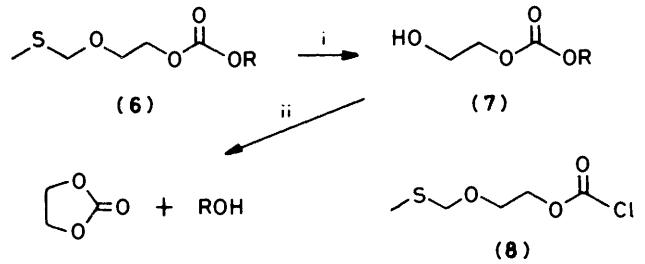
Scheme 1. For oligodeoxyribonucleotides, R^2 = H; for oligoribonucleotides, R^2 = OR^3; Ar = aryl (*e.g.* 2-ClC_6H_4)



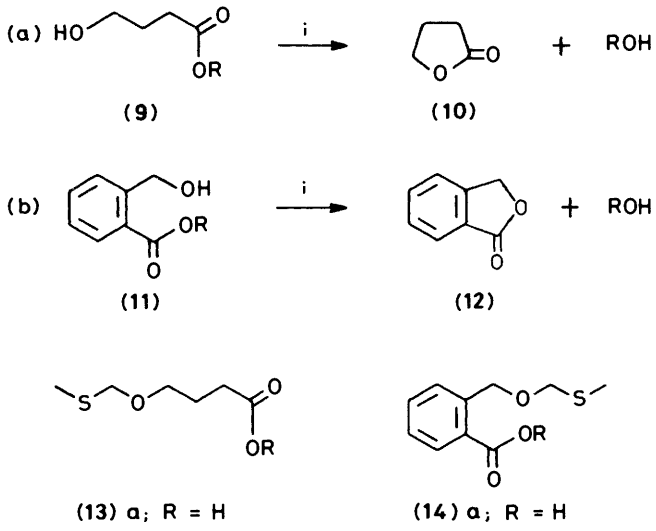
Scheme 2. *Reagents:* i, $AgClO_4$, 2,4,6-collidine, H_2O; ii, morpholine

carbonate in aqueous dioxane under relatively mild conditions, the corresponding unblocked alcohols (ROH) are rapidly formed.[11]

Although the preliminary study relating to the MTMEC group [as in compound (**6**)] was encouraging, we came to the conclusion that this particular protected protecting group was



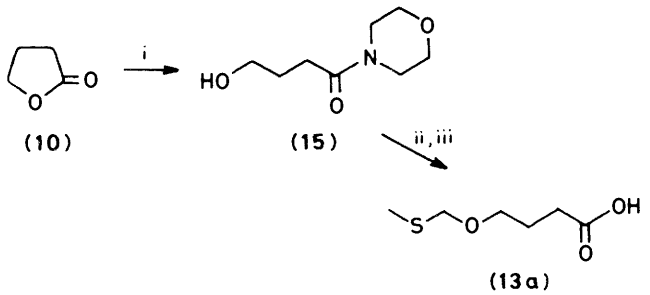
Scheme 3. *Reagents:* i, $Hg(ClO_4)_2$, 2,4,6-collidine, H_2O; ii, base



Scheme 4. *Reagents:* i, base

not entirely satisfctory for use in oligonucleotide synthesis. Perhaps the main drawback to the use of the latter group is that the required acylating agent, 2-(methylthiomethoxy)ethyl chloroformate (**8**) is unstable and needs to be prepared freshly from 2-(methylthiomethoxy)ethanol[11] and phosgene virtually each time that it is used. Another rather unsatisfactory feature of the MTMEC protecting group is that the initial mercury(II) perchlorate promoted removal of the MTM group, to give the putative intermediate 2-hydroxyethoxycarbonyl derivative (**7**), is rather slow when 2,4,6-collidine is added to the reaction medium to prevent it from becoming acidic. Finally, although the base-catalysed cyclization of the intermediate 2-hydroxyethoxy carbonate (**7**), leading to the release of the desired alcohol (ROH), proceeds rapidly under relatively mild conditions,[11] it would clearly have been more satisfactory if this second unblocking step had occurred spontaneously during the removal of the MTM group. For these reasons, we have investigated the chemistry of two related protected acyl protecting groups which also have neighbouring hydroxy functions masked with MTM groups. We now report the results of these studies.

An examination of the literature[12] revealed that esters of 4-hydroxybutyric acid (**9**; R = H) very readily undergo[13] base-catalysed cyclization (Scheme 4a) to give γ-butyrolactone (**10**) and that esters of 2-(hydroxymethyl)benzoic acid (**11**) are even more susceptible[14] to base-catalysed cyclization (Scheme 4b). We then thought that 4-(methylthiomethoxy)butyryl and 2-(methylthiomethoxymethyl)benzoyl [as in compounds (**13**) and (**14**), respectively] might well prove to be useful protected acyl protecting groups in oligonucleotide synthesis. In the first place, it seemed likely that the corresponding carboxylic acids [(**13a**) and (**14a**)] would prove to be stable compounds.



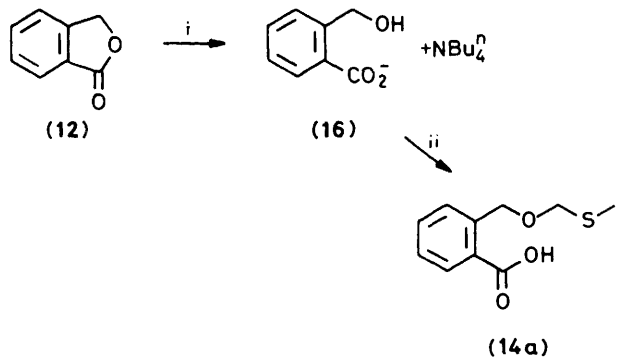
Scheme 5. *Reagents:* i, morpholine–ethanol, reflux; ii, (a) NaH–tetrahydrofuran, (b) $MeSCH_2Cl$, NaI; iii, (a) KOH–ethanol–water, reflux, (b) dilute H_2SO_4

Secondly, from electronic considerations, it seemed probable that the MTM group in compound (**13**) would undergo mercury(II) perchlorate promoted hydrolysis more rapidly than the MTM group in (**6**). Thirdly, it was expected that the cyclization reactions in the unblocking processes (Scheme 4) would both prove to be faster than the cyclization of 2-hydroxyethyl carbonates [structure (**7**), Scheme 3]. Finally, it was hoped that activated 2-(methylthiomethoxymethyl)-benzoic acid (**14a**) in particular might react regioselectively with the primary hydroxy functions of 2′-deoxyribonucleosides and their *N*-acyl derivatives, and that the resulting products might crystallize without difficulty.

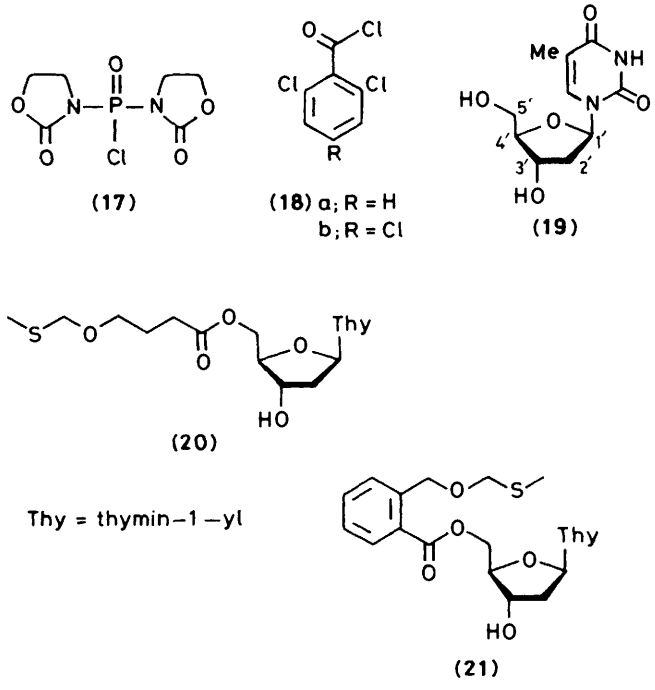
4-(Methylthiomethoxy)butyric acid (**13a**) (MTMB acid) was prepared in essentially three steps (Scheme 5) from γ-butyrolactone (**10**) in 53% overall yield. γ-Butyrolactone (**10**) was first heated, under reflux, for 6 h with a small excess of morpholine in ethanol solution to give *N*-(4-hydroxybutyryl)-morpholine (**15**).[15] The crude amide (**15**) was converted into its MTM derivative by a slight modification of the procedure of Corey and Bock[10] in which only *ca.* 0.14 mol equiv. of sodium iodide with respect to chloromethyl methyl sulphide was used. The resulting product was heated with an excess of potassium hydroxide in aqueous ethanol, under reflux, to give the potassium salt of MTMB acid (**13a**). Free MTMB acid (**13a**) was isolated as a pale yellow liquid in the usual way (see Scheme 5 and Experimental section). As expected, MTMB acid (**13a**) is a stable compound at room temperature, but care must be taken in its distillation. Nevertheless, when 1.0 g of acid was distilled in a Kugelrohr apparatus, 0.92 g of pure, colourless MTMB acid was recovered. Fortunately, however, the undistilled material is quite pure enough to be used in acylation reactions (see below).

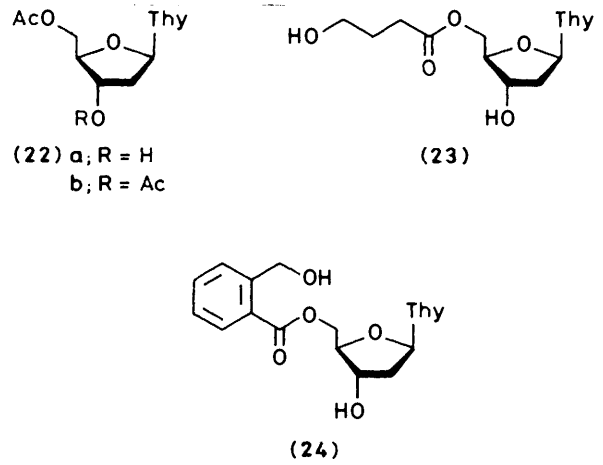
It did not prove possible to prepare 2-(methylthiomethoxy-methyl)benzoic acid (**14a**) (MTMT acid *) from phthalide (**12**) by the above morpholine procedure. Indeed, phthalide was regenerated when the intermediate *N*-(2-hydroxymethyl-benzoyl)morpholine was treated with sodium hydride. However, the tetra-n-butylammonium salt of 2-hydroxymethylbenzoic acid (**16**), which was readily obtained by treating phthalide (**12**) with a slight excess of aqueous tetra-n-butylammonium hydroxide (Scheme 6), was found to be a a useful intermediate in the preparation of MTMT acid (**14a**). When the ammonium salt (**16**) was treated first with sodium hydride in glyme, and then with chloromethyl methyl sulphide and sodium iodide,[10] the conjugate base of MTMT acid was obtained. Following acidification of the products, the free acid (**14a**) was isolated as a crystalline solid, m.p. 66 °C. The overall yield of MTMT acid (**14a**), based on phthalide, was 64%. It should be mentioned

* Compound (**14a**) may be regarded as a methylthiomethoxy derivative of *o*-toluic acid, and hence MTMT acid would seem to be a reasonable abbreviation for it.



Scheme 6. *Reagents:* i, $Bu^n_4N^+$ OH^-–H_2O; ii, (a) NaH–$MeOCH_2CH_2OMe$, (b) $MeSCH_2Cl$, NaI, (c) dilute H_2SO_4





that, many years ago, von Braun and his co-workers[16] reported that 2-methoxymethylbenzoic acid was obtained when phthalide (**12**) was treated with an excess of dimethyl sulphate and an even larger excess of sodium hydroxide.

Following the successful synthesis of both MTMB and MTMT acids [(**13a**) and (**14a**), respectively], we turned our attention towards their activation and the preparation of the nucleoside building blocks required in oligonucleotide synthesis. It seemed unlikely to us that the MTM protecting group would survive the reaction conditions normally used in the preparation of acyl chlorides, or indeed that MTMB and MTMT chlorides would be particularly stable even if it were possible to prepare them. Furthermore, it was decided not to attempt to prepare the anhydrides of (**13a**) and (**14a**) as the use of these reagents in acylation would clearly be wasteful. Instead, we investigated the possibility of using some of the other methods which have recently been suggested for the activation of carboxylic acids. *N,N*-Bis(2-oxo-oxazolidin-3-yl)phosphorodiamidic chloride[17] (**17**) and 2,6-dichlorobenzoyl chloride (**18a**) were found to be particularly useful condensing agents for the present purposes.

When thymidine (**19**) was allowed to react with MTMB acid (**13a**) (1.5 mol equiv.) and *N,N*-bis(2-oxo-oxazolidin-3-yl)phosphorodiamidic chloride[17] (**17**) (3.0 mol equiv.) in pyridine–acetonitrile for 6 h at room temperature and the products worked up and chromatographed, pure 5′-*O*-[4-(methylthiomethoxy)butyryl]thymidine (**20**) was obtained. The latter compound (**20**) was isolated in 70% yield and crystallized from ethyl acetate. Fortunately, acylation had, as desired (see above), taken place regioselectively on the 5′-primary hydroxy function of thymidine. When thymidine (**19**) was treated with MTMT acid (**14a**) (1.2 mol equiv.) and 2,6-dichlorobenzoyl chloride (**18a**) (2.0 mol equiv.) in pyridine at room temperature, regioselective 5′-acylation again occurred and 5′-*O*-[2-(methylthiomethoxymethyl)benzoyl]thymidine (**21**) was isolated from the products as a crystalline solid in 70% yield. We are unaware of any previous reports relating to the use of 2,6-dichlorobenzoyl chloride (**18a**) as an activating agent in acylation reactions. 2,4,6-Trichlorobenzoyl chloride (**18b**) has been used[18] for this purpose, but it is less accessible than (**18a**). Indeed, both 2,6-dichlorobenzoyl chloride (**18a**) and 2,6-dichlorobenzoic acid are commercially available.[19] 2,6-Dichlorobenzoyl chloride (**18a**) appears to have greater reactivity as a condensing agent than the phosphorodiamidic chloride (**17**).

The MTMB and MTMT protecting groups were both found to be more stable to ammonolysis than the acetyl group. When 5′-*O*-acetylthymidine (**22a**), 5′-*O*-(MTMB)thymidine (**20**) and 5′-*O*-(MTMT)thymidine (**21**) were treated with concentrated aqueous ammonia at room temperature, the half-times for their conversion into thymidine were found to be < 5 min, *ca.* 15 min, and *ca.* 6 h, respectively. However, the MTMB and MTMT protecting groups were found to be removable from (**20**) and (**21**), respectively, under very mild conditions of basic hydrolysis after the substrates had first been treated with mercury(II) perchlorate.[11]

When a 0.05M-solution of 5′-*O*-(MTMB)thymidine (**20**) in tetrahydrofuran–water (98:2 v/v) was treated with mercury(II) perchlorate (2 mol equiv.) and 2,4,6-collidine (5 mol equiv.) at room temperature, removal of the MTM group appeared to be complete after 5 min. Thioacetamide was then added to remove the excess of mercury(II) ions and the putative intermediate 4-hydroxybutyryl derivative (**23**) was treated with 1M-potassium carbonate in tetrahydrofuran–water (*ca.* 46:54 v/v) at room temperature. After 10 min, t.l.c. analysis of the products revealed thymidine (**19**) as the sole product. The second unblocking step [*i.e.* the conversion of (**23**) into (**19**)] appeared to be complete after 30 min when 1M-triethylamine/tetrahydrofuran–water (*ca.* 1:1 v/v) was used instead of potassium carbonate. When compound (**20**) [1.0 mmol] was unblocked by the latter mercury (II) perchlorate–triethylamine procedure and the thymidine (**19**) obtained was acetylated, 3′,5′-di-*O*-acetylthymidine (**22b**) was isolated from the products in 66% yield.

The course of the mercury(II) perchlorate–potassium carbonate unblocking of 5′-*O*-(MTMT)thymidine (**21**) differed from that of the corresponding unblocking of 5′-*O*-(MTMB)thymidine (**20**) (see above) in that, while the first step leading to the putative 2-hydroxymethylbenzoyl derivative (**24**) required a longer time (*ca.* 3 h) under the same reaction conditions, the second step was complete within 30 s under milder conditions (0.125M-potassium carbonate). When compound (**21**) (1.0 mmol) was unblocked under the same conditions used (see above) in the recovery experiment based on 5′-*O*-(MTMB)thymidine (**20**) and the products again acetylated, 3′,5′-di-*O*-acetylthymidine (**22b**) was isolated in 73% yield.

Preliminary studies[20] suggest that the MTMT group may prove to be of value as an alternative to the DBMB protecting group[7] [as in (**3**)] in oligoribonucleotide synthesis.[2,3] The MTMT is most probably to be preferred to the MTMB protecting group in this context as the conditions of basic hydrolysis required for the cyclization of 2-hydroxymethylbenzoate esters [Scheme 4(b)] are milder[12] than those required for the cyclization of 4-hydroxybutyrate esters [Scheme 4(a)]. However, in an ideal 'protected' protecting group of this type, both the facile mercury(II) ion-promoted first unblocking step of the MTMB group and the fast base-catalysed second unblocking step of the MTMT group would be combined. We are now searching for a protecting group with such unblocking characteristics. Finally, it should be noted that the neighbouring hydroxy functions of 4-hydroxybutyrate (**9**) and 2-hydroxymethylbenzoate (**11**) esters need not necessarily be 'protected' with an MTM or related group. Thus it should be possible to devise a number of useful but quite differently 'protected' protecting groups, based on 4-hydroxybutyryl and 2-hydroxymethylbenzoyl. Work along these lines is now in progress in this laboratory.

Experimental

^{1}H N.m.r. spectra were measured at 60 MHz with a Jeol PMX 60 SI spectrometer and at 250 MHz with a Bruker WM 250 spectrometer; tetramethylsilane was used as an internal standard. ^{13}C N.m.r. spectra were recorded with Perkin-Elmer 257 and 297 spectrometers. Mass spectra were obtained with a VG Micromass ZAB-1F spectrometer. Merck silica gel 60 F_{254} plates were used for t.l.c.; Merck silica gel H was used for short column chromatography.[21] Acetonitrile, tetrahydrofuran, 1,2-dimethoxyethane (glyme), and pyridine were dried by heating with calcium hydride, under reflux; they were then distilled and stored over no. 4A molecular sieves. Tetrahydrofuran and glyme were further dried, by distillation from lithium aluminium hydride, before use. Ether refers to diethyl ether.

4-(*Methylthiomethoxy*)*butyric Acid* (**13a**).—A solution of γ-butyrolactone (77 ml, 86.2 g, 1.0 mol) and morpholine (105 ml, 104.5 g, 1.2 mol) in ethanol (200 ml) was heated, under reflux, for 6 h. The ethanol was removed in a rotatory evaporator under reduced pressure, and the excess of morpholine was removed by distillation [bath temperature 40 °C, oil-pump] to give crude *N*-(4-hydroxybutyryl)morpholine as a viscous yellow oil (152 g, 88%); δ_H[$CDCl_3$; 60 MHz] 1.95 (2 H, m), 2.52 (2 H, m), 3.68 (10 H, m), and 4.18 (1 H, s); $\nu_{max.}$(film) 1 635 and 3 400 cm^{-1}.

Sodium hydride (10.8 g of 80% dispersion in oil, 0.36 mol) was placed in a 250-ml three-necked round-bottomed flask in an atmosphere of nitrogen and was washed with dry ether (2 × 30 ml). Dry tetrahydrofuran (75 ml) was then added. To the cooled [−5 to −10 °C; dry ice–methanol bath] stirred suspension of sodium hydride was added, dropwise during 20—25 min, a solution of *N*-(4-hydroxybutyryl)morpholine (31.2 g, 0.18 mol) in anhydrous tetrahydrofuran (25 ml). After the addition of sodium iodide (4.5 g, 30 mmol), a solution of chloromethyl methyl sulphide (18.0 ml, 20.75 g, 0.215 mol) in tetrahydrofuran (35 ml) was added dropwise during 15 min. The reactants were then stirred at −5 to −10 °C for 2 h and at room temperature for a further 2 h. The products were cooled (ice–water bath) and water (100 ml) was added cautiously to give a clear two-phase system. The latter was extracted with ether (75 ml), and the aqueous layer was separated and re-extracted with ether (2 × 50 ml). The combined ether layers were dried ($MgSO_4$) and evaporated to give a yellow oil (34 g) which was dissolved in a solution of potassium hydroxide (54 g, 0.96 mol) in ethanol–water (9:1 v/v, 360 ml). The solution obtained was heated, under reflux, with stirring for 3 h. The cooled products were then concentrated under reduced pressure until most of the ethanol had been removed. The resulting solution was diluted with water (200 ml) and extracted with ether (2 × 100 ml). The aqueous layer was retained and acidified (to *ca.* pH 2) by the addition of 2M-sulphuric acid (*ca.* 240 ml); it was then filtered through a plug of glass wool and extracted with ether (3 × 200 ml). The combined ether extracts were dried ($MgSO_4$) and evaporated to give 4-(*methylthiomethoxy*)*butyric acid* as a yellow oil (18.1 g, 53% based on γ-butyrolactone) (Found: M^+ at *m/z* 164.0500. $C_6H_{12}O_3S$ requires *M*, 164.0507); δ_H ($CDCl_3$; 250 MHz) 1.93 (2 H, m), 2.14 (3 H, s), 2.47 (2 H, t, *J* 7.3 Hz), 3.58 (2 H, t, *J* 6.0 Hz), 4.63 (2 H, s), and 11.12 (1 H, br s); δ_C($CDCl_3$) 13.9, 24.5, 30.9, 66.8, 75.2, and 179.2 p.p.m.; $\nu_{max.}$(film) 1 710 cm^{-1}.

The above 4-(methylthiomethoxy)butyric acid was used without further purification in the acylation of thymidine (see below). Normal distillation under reduced pressure led to extensive decomposition and to a low recovery of acid. However, when the above acid (1.0 g) was distilled in a Kugelrohr apparatus (170 °C, 0.2 mmHg), purified 4-(methylthiomethoxy)butyric acid (0.92 g, 92%) was obtained as a colourless liquid.

2-(*Methylthiomethoxymethyl*)*benzoic Acid* (**14a**).—Phthalide (13.4 g, 0.10 mol) and aqueous tetra-n-butylammonium hydroxide (40% w/v, 68.1 ml; 0.105 mol) were heated together, under reflux. After 90 min, the products were cooled, concentrated (to *ca.* one-half volume) under reduced pressure, and then extracted with dichloromethane (6 × 100 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure (water-pump, followed by oil-pump) to constant weight. An oily product (*ca.* 37—39 g) was obtained.

Sodium hydride (80% dispersion in oil, 12.0 g, *ca.* 0.4 mol), contained in a three-necked 500-ml round-bottomed flask (fitted with a mechanical stirrer, dropping funnel, and reflux condenser) was washed with light petroleum (b.p. 30—40 °C, 2 × 150 ml). The flask was flushed with nitrogen and anhydrous glyme (40 ml) was added. The stirred contents of the flask were cooled (ice–sodium chloride bath) and a solution of the above oily tetra-n-butylammonium 2-(hydroxymethyl)-benzoate in glyme (40 ml) was added dropwise during 1 h. After a further 1 h, the reactants were allowed to warm up to room temperature. A slow effervescence, accompanied by the development of a green coloration, was then observed. When the effervescence ceased (after *ca.* 4 h), the reaction mixture was cooled (ice–sodium chloride bath) again. After 30 min, sodium iodide (15 g, 0.10 mol) was added. A solution of chloromethyl methyl sulphide (11.0 ml, 12.7 g, 0.13 mol) in glyme (40 ml) was then added dropwise during 30 min, followed by more glyme (40 ml). The reactants were then stirred for 16 h, during which time they were allowed to warm up to room temperature. The products were cooled (ice–sodium chloride bath) again, and water was added cautiously until the effervescence ceased. Dilute sulphuric acid (*ca.* 2M) was then added until the resulting emulsion was slightly acidic (*ca.* pH 3, indicator paper). The

mixture was extracted with chloroform (6 × 100 ml), and the combined extracts were evaporated under reduced pressure. Ether (200 ml) was added to the residual oil and the mixture obtained was extracted with very dilute sulphuric acid (pH *ca.* 4; 8 × 500 ml) until the undissolved interfacial material had been completely removed. The remaining ether layer was then extracted with 1M-aqueous sodium hydroxide (4 × 100 ml). Dilute sulphuric acid (*ca.* 2M) was added cautiously to the combined alkaline extracts until the pH dropped to *ca.* 2 (indicator paper). The mixture obtained was then extracted with ether (4 × 200 ml) and the combined, dried ($MgSO_4$) ether extracts were evaporated under reduced pressure. Crystallization of the residue from water–ethanol (*ca.* 15:1 v/v) gave 2-(*methylthiomethoxymethyl*)*benzoic acid* (13.6 g, 64%, based on phthalide) (Found: C, 56.7; H, 5.65. $C_{10}H_{12}O_3S$ requires C, 56.6; H, 5.7%), m.p. 66 °C; δ_H[$(CD_3)_2SO$–D_2O; 250 MHz] 2.14 (3 H, s), 4.79 (2 H, s), 4.92 (2 H, s), 7.40 (1 H, m), 7.60 (2 H, m), and 7.88 (1 H, d, *J* 7.3 Hz); δ_C($CDCl_3$) 13.6, 67.5, 74.7, 127.3, 127.7, 129.5, 130.4, 132.1, 139.7, and 168.4; $\nu_{max.}$(KBr) 1 670 cm^{-1}.

5′-O-[4-(*Methylthiomethoxy*)*butyryl*]*thymidine* (**20**).—Thymidine (0.242 g, 1.0 mmol), 4-(methylthiomethoxy)butyric acid (0.246 g, 1.5 mmol), *N,N*-bis(2-oxo-oxazolidin-3-yl)-phosphorodiamidic chloride[17] (0.764 g, 3.0 mmol) were stirred together in acetonitrile–pyridine (1:1 v/v; 6 ml) at room temperature. After 6 h, water (*ca.* 0.1 ml) was added and, after a further 30 min when a clear solution was obtained, chloroform (30 ml) was added. The products were extracted with saturated aqueous sodium hydrogen carbonate (30 ml), and the chloroform layer was then dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was then chromatographed on a short column of silica gel. Evaporation of the appropriate fractions gave 5′-O-[4-(*methylthiomethoxy*)*butyryl*]*thymidine* (0.27 g, 70%) as a colourless oil which subsequently solidified. The colourless crystals obtained (Found: C, 49.3; H, 6.1; N, 7.15. $C_{16}H_{23}N_2O_7S$ requires C, 49.6; H, 6.0; N, 7.2%) following recrystallization from ethyl acetate had m.p. 111—112 °C; R_F[$CHCl_3$–MeOH (9:1 v/v)] 0.47; $\lambda_{max.}$ (95% EtOH) 265 (ε 9 200); $\lambda_{min.}$ 233 (ε 2 200); δ_H [$(CD_3)_2SO$; 250 MHz] 1.80 (5 H, m), 2.06 (3 H, s), 2.17 (2 H, m), 2.41 (2 H, t, *J* 7.3 Hz), 3.46 (2 H, t, *J* 6.2 Hz), 3.91 (1 H, m), 4.23 (3 H, m), 4.61 (2 H, s), 5.42 (1 H, m), 6.19 (1 H, t, *J* 6.9 Hz), 7.44 (1 H, s), and 11.33 (1 H, br s).

5′-O-[2-(*Methylthiomethoxymethyl*)*benzoyl*]*thymidine* (**21**).—A solution of 2-(methylthiomethoxymethyl)benzoic acid (0.255 g, 1.2 mmol) in acetonitrile (2 ml) was added dropwise during 1 h to a solution of thymidine (0.242 g, 1.0 mmol) and 2,6-dichlorobenzoyl chloride (0.42 g, 2.0 mmol) in pyridine (2 ml) at room temperature. The products were then partitioned between chloroform (30 ml) and saturated aqueous sodium hydrogen carbonate (30 ml). The crude products were fractionated by short column chromatography. Evaporation of the appropriate fractions, eluted with $CHCl_3$–EtOH (94:6 v/v), and crystallization of the residue from ethyl acetate gave 5′-O-[2-(*methylthiomethoxymethyl*)*benzoyl*]*thymidine* (0.307 g, 70%) (Found: C, 54.9; H, 5.5; N, 6.3. $C_{20}H_{24}N_2O_7S$ requires C, 55.0; H, 5.5; N, 6.4); m.p. 136 °C; R_F[$CHCl_3$–MeOH (9:1 v/v)] 0.51; $\lambda_{max.}$ (95% EtOH) 267 (ε 9 200); $\lambda_{min.}$ 248 (ε 6 500); δ_H [$(CD_3)_2SO$; 250 MHz] 1.61 (3 H, s), 2.12 (3 H, s), 2.20 (2 H, m), 4.06 (1 H, m), 4.38 (1 H, m), 4.46 (2 H, m), 4.76 (2 H, s), 4.88 (2 H, s), 5.49 (1 H, d, *J* 4.6 Hz), 7.40 (1 H, s), 7.44 (1 H, m), 7.62 (2 H, m), 7.88 (1 H, d, *J* 7.8 Hz), and 11.32 (1 H, s).

Action of Aqueous Ammonia on 5′-O-Acyl Derivatives of Thymidine.—Substrate (0.01 mmol) was dissolved in concentrated aqueous ammonia (*d* 0.88) at room temperature, and deacylation was monitored by t.l.c. [$CHCl_3$–MeOH (85:15 v/v)]. 5′-*O*-Acetylthymidine was completely deacylated in 20 min ($t_{\frac{1}{2}}$ < 5 min); 5′-*O*-[4-(methylthiomethoxy)butyryl]thymidine (**20**) was completely deacylated in 1.5 h ($t_{\frac{1}{2}}$ *ca.* 15 min); and 5′-*O*-[2-(methylthiomethoxymethyl)benzoyl]thymidine (**21**) was completely deacylated in 46 h ($t_{\frac{1}{2}}$ *ca.* 6 h).

Deacylation of 5′-O-[4-(*Methylthiomethoxy*)*butyryl*]*thymidine* (**20**) *by the Procedure Involving Mercury* (II) *Perchlorate Treatment.*—(*a*) To a stirred solution of substrate (**20**) (0.05 mmol, 0.019 g) in tetrahydrofuran–water (98:2 v/v; 0.5 ml) at room temperature was added 2,4,6-collidine (0.033 ml, 0.25 mmol), followed by a solution of mercury(II) perchlorate trihydrate (0.045 g, 0.10 mmol) in tetrahydrofuran–water (98:2 v/v; 0.5 ml). After 5 min, t.l.c. [$CHCl_3$–MeOH (85:15 v/v)] revealed that the starting material (**20**) (R_F 0.65) had disappeared and that a product with R_F 0.37 had been formed. 5% Aqueous thioacetamide (0.15 ml, 0.1 mmol) was then added to the products and, after 15 min, the resulting black precipitate was removed by centrifugation. 2M-Aqueous potassium carbonate (1.15 ml) was added, with stirring, to the supernatant. After *ca.* 10 min, t.l.c. revealed a single component with the same R_F as thymidine (R_F 0.30).

(*b*) The experiment was repeated under the same conditions as above except that 2M-aqueous triethylamine (0.9 ml) was used in the final unblocking step instead of 2M-aqueous potassium carbonate. Conversion into a single component with the same R_F as thymidine was complete within 30 min.

(*c*) To a stirred solution of substrate (**20**) (0.388 g, 1.0 mmol) in tetrahydrofuran–water (98:2 v/v; 10 ml) at room temperature was added 2,4,6-collidine (0.66 ml, 5.0 mmol), followed by a solution of mercury(II) perchlorate trihydrate (0.907 g, 2.2 mmol) in tetrahydrofuran–water (98:2 v/v; 10 ml). After 20 min, 5% aqueous thioacetamide (3.0 ml, 2.0 mmol) was added and, after a further 15 min, the products were centrifuged. The supernatant was retained and the residue was washed with tetrahydrofuran–water (98:2 v/v; 2 × 20 ml). The combined supernatant and washings were concentrated under reduced pressure and redissolved in tetrahydrofuran (19.6 ml). Water (11.4 ml) and triethylamine (5.58 ml, 40 mmol) were then added and the resulting solution was stirred at room temperature. After 1 h, the solution was evaporated under reduced pressure, the residue dissolved in anhydrous pyridine (20 ml), and the solution was re-evaporated. After this process had been repeated once more, the residue was dissolved in pyridine (5 ml) at room temperature and acetic anhydride (0.94 ml, 10 mmol) was added. After 2 h, methanol was added and, after a further 30 min, the products were partitioned between chloroform (20 ml) and saturated aqueous sodium hydrogen carbonate (20 ml). The chloroform layer was separated, and the aqueous layer was re-extracted with chloroform (2 × 20 ml). The combined chloroform layers were dried ($MgSO_4$) and evaporated under reduced pressure. The residue obtained was fractionated by chromatography on silica gel to give 3′,5′-di-*O*-acetylthymidine (0.215 g, 66%). After crystallization from ethanol, the latter material was identical (t.l.c., m.p., ^1H n.m.r.) with authentic 3′,5′-di-*O*-acetylthymidine.

Deacylation of 5′-O-[2-(*Methylthiomethoxymethyl*)*benzoyl*]*thymidine* (**21**) *by the Procedure Involving Mercury*(II) *Perchlorate Treatment.*—(*a*) To a stirred solution of substrate (**21**) (0.05 mmol, 0.022 g) in tetrahydrofuran–water (98:2 v/v; 0.5 ml) at room temperature was added 2,4,6-collidine (0.033 ml, 0.25 mmol), followed by a solution of mercury(II) perchlorate trihydrate (0.045 g, 0.10 mmol) in tetrahydrofuran–water (98:2 v/v; 0.5 ml). After 3 h, t.l.c. [$CHCl_3$–MeOH (85:15 v/v)] revealed that the starting material (**21**) (R_F 0.68) had disappeared and that a product with R_F 0.51 had been formed. 5% Aqueous thioacetamide (0.15 ml, 0.1 mmol) was then added

to the products and, after 15 min, the resulting black precipitate was removed by centrifugation. 0.25M-Aqueous potassium carbonate (1.15 ml) was added, with stirring, to the supernatant. After *ca.* 30 s, t.l.c. revealed a single component with the same R_F as thymidine (R_F 0.30).

(*b*) The relatively large (1.0 mmol) scale unblocking reaction described for 5′-*O*-[4-(methylthiomethoxy)butyryl]thymidine (**20**) under heading (*c*) above was repeated with 5′-*O*-[2-(methylthiomethoxymethyl)benzoyl]thymidine (**21**) (0.437 g, 1.0 mmol). The quantities of reagents and solvents used were exactly the same as described above. The reaction with mercury(II) perchlorate was quenched with thioacetamide after 2 h and the reaction between the putative intermediate 2-hydroxymethylbenzoyl derivative (**24**) {R_F [$CHCl_3$–MeOH (85:15 v/v)] 0.51} with triethylamine in tetrahydrofuran–water was allowed to proceed for 5 min. Following chromatography of the crude acetylated products on silica gel, 3′,5′-di-*O*-acetylthymidine (0.241 g, 73%) was obtained. After crystallization from ethanol, the latter material was identical (t.l.c., m.p., ^{1}H n.m.r.) with authentic 3′,5′-di-*O*-acetylthymidine.

Acknowledgements

One of us (G. S.) thanks the University of Calabria for leave of absence, and N.A.T.O. for the award of a Science Fellowship; we also thank the S.E.R.C. for the award of research studentships (to J. M. B. and C. C.), and G. D. Searle and Co. Ltd. for financial support.

References

1 For a review, see C. B. Reese, *Tetrahedron*, 1978, **34**, 3143.
2 S. S. Jones, B. Rayner, C. B. Reese, A. Ubasawa, and M. Ubasawa, *Tetrahedron*, 1980, **36**, 3075.
3 S. S. Jones, C. B. Reese, and S. Sibanda in 'Current Trends in Organic Synthesis,' Proceedings of the Fourth International Conference on Organic Synthesis, ed. H. Nozaki, Pergamon, Oxford, 1983, p. 71.
4 R. Arentzen and C. B. Reese, *J. Chem. Soc., Perkin Trans. 1*, 1977, 445.
5 R. L. Letsinger and P. S. Miller, *J. Am. Chem. Soc.*, 1969, **91**, 3356.
6 J. H. van Boom and P. M. J. Burgers, *Tetrahedron Lett.*, 1976, 4875.
7 J. B. Chattopadhyaya, C. B. Reese, and A. H. Todd, *J. Chem. Soc., Chem. Commun.*, 1979, 987.
8 J. B. Chattopadhyaya and C. B. Reese, *Nucleic Acids Res.*, 1980, **8**, 2039.
9 M. L. Bender and M. S. Silver, *J. Am. Chem. Soc.*, 1962, **84**, 4589; M. L. Bender, J. A. Reinstein, M. S. Silver, and R. Mikulak, *ibid.*, 1965, **87**, 4545.
10 E. J. Corey and M. G. Bock, *Tetrahedron Lett.*, 1975, 3269.
11 S. S. Jones, C. B. Reese, and S. Sibanda, *Tetrahedron Lett.*, 1981, **22**, 1933.
12 A. J. Kirby in 'Advances in Physical Organic Chemistry,' eds. V. Gold and D. Bethell, Academic Press, London, 1980, vol. 17, p. 245.
13 B. Capon, S. T. McDowell, and W. V. Raftery, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1118.
14 T. H. Fife and B. M. Benjamin, *J. Am. Chem. Soc.*, 1973, **95**, 2059.
15 J. Nordmann, G. Mattioda, and G. Loiseau, *Chim. Ther.*, 1973, **8**, 342 (*Chem. Abstr.*, 1973, **79**, 115411h).
16 J. von Braun, E. Anton, and K. Weiszbach, *Chem. Ber.*, 1930, **63**, 2847.
17 J. Diago-Meseguer, A. L. Palomo-Coll, J. R. Fernandez-Lizarbe, and A. Zugaza-Bilbao, *Synthesis*, 1980, 547.
18 J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, and M. Yamaguchi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1989.
19 Aldrich Chemical Co. Ltd., 1983—1984 Catalogue, p. 394.
20 J. M. Brown, C. Christodoulou, and C. B. Reese, unpublished observations.
21 B. J. Hunt and W. Rigby, *Chem. Ind.*, 1967, 1868.

Received 17*th January* 1984; *Paper* 4/093