Table VI. Physical Properties of I

No.	R[a]	Solvent[b]	Mp, °C	Yield, %	Formula[c]
7	4-$CH_3C_6H_4CH_2CH_2$	A	241-244	11	$C_{19}H_{16}ClNO_3$
8	o-$C_6H_5C_6H_4CH_2CH_2$	B	274-277	28	$C_{24}H_{18}ClNO_3$
9	m-$C_6H_5C_6H_4CH_2CH_2$	B	270-272	75	$C_{24}H_{18}ClNO_3$
10	p-$C_6H_5C_6H_4CH_2CH_2$	B	272 dec	73	$C_{24}H_{18}ClNO_3$
11	α-$C_{10}H_7CH=CH$	B	284 dec	36	$C_{22}H_{14}ClNO_3 \cdot 0.5C_3H_8O_2$
12	α-$C_{10}H_7CH_2CH_2$	C	255-256 dec	78	$C_{22}H_{16}ClNO_3 \cdot C_2H_5OH$
13	β-$C_{10}H_7CH_2CH_2$	B	274-276 dec	41	$C_{22}H_{16}ClNO_3$
14	3,4-$CH_2O_2C_6H_3CH=CH$	B	294 dec	62	$C_{19}H_{12}ClNO_5$
15	3,4-$CH_2O_2C_6H_3CH_2$-CH_2	B	260-261 dec	69	$C_{19}H_{14}ClNO_5$
16	3-$FC_6H_4CH_2CH_2$	B	264-265	74	$C_{18}H_{13}ClFNO_3$
17	3-$ClC_6H_4CH_2CH_2$	B	270-271 dec	30	$C_{18}H_{13}Cl_2NO_3$
18	4-$ClC_6H_4CH_2CH_2$	B	257-258 dec	47	$C_{18}H_{13}Cl_2NO_3$
19	2,6-$Cl_2C_6H_3CH=CH$	B	286-289 dec	54	$C_{18}H_{10}Cl_3NO_3$
20	2,6-$Cl_2C_6H_3CH_2CH_2$	C	259-262	41	$C_{18}H_{12}Cl_3NO_3$
21	2,5-$Cl_2C_6H_3CH_2CH_2$	B	265-266 dec	45	$C_{18}H_{12}Cl_3NO_3$
22	2,4-$Cl_2C_6H_3CH_2CH_2$	B	265-266 dec	51	$C_{18}H_{12}Cl_3NO_3$
23	3,4-$Cl_2C_6H_3CH_2CH_2$	B	274-275 dec	54	$C_{18}H_{12}Cl_3NO_3$

[a]Prepared by previously described method, ref 2. [b]Recrystallization solvents: A, C_6H_5N-hexane; B, 2-methoxyethanol; C, EtOH. [c]Analyzed for C, H, N.

of these nitro compounds to the anilinomethylenemalonates, the ethyl 4-hydroxyquinoline-3-carboxylates, and the 4-hydroxyquinoline-3-carboxylic acids (Tables IV, V, and VI, respectively) has also been previously reported.[2,4]

2-Phenylbenzyl Triphenylphosphonium Chloride (24). A mixt of 5.0 g (25.2 mmoles) of 2-phenylbenzoic acid and 1.5 g of LAH in 200 ml of THF was refluxed for 4 hr. The excess LAH was decompd by adding in order: 1.5 ml of H_2O, 1.5 ml of 15% NaOH, and 4.5 ml of H_2O. The crystalline salts were filtered off, and the THF was concentrated to an almost colorless oil of 4.9 g (105%) of 2-phenylbenzyl alcohol. To this alcohol was added 6 ml (10 g, 84 mmoles) of $SOCl_2$ and 20 ml of C_6H_6. The reaction mixt was placed on the steam bath for 15 min then spin evaporated. More benzene was added, and the mixt was spin evaporated again. Then 100 ml of toluene contg 6.9 g (25 mmoles) of $P(C_6H_5)_3$ was added, and the reaction refluxed for 18 hr to give a first crop of 5.53 g (47%), mp 269-275°. Refluxing the mother liquors for an addl 24 hr gave 1.56 g (13%), mp 275-278°. *Anal.* ($C_{31}H_{26}ClP$) C, H. In like manner a third crop of 0.93 g, mp 275-278°, was collected for a total of 8.02 g (68%) of useable material.

***trans*-3-Nitro-4,2',5'-trichlorostilbene (38) (Method A).** To a mixt of 12.5 g (25 mmoles) of 26 and 4.6 g (25 mmoles) of 4-chloro-3-nitrobenzaldehyde in 50 ml of DMF was added 3.2 g (25 mmoles) of diazobicyclononane, and the mixt was stirred at room temp for 18 hr. The soln was poured into 50 ml of H_2O and stirred until all the oil had solidified. This yellow semisolid was collected and recrystallized from EtOH to give 1.83 g (22%) of yellow crystals, mp 157-158°. *Anal.* ($C_{14}H_8Cl_3NO_2$) C, H, N. The mother liquors (EtOH) were concentrated to an oil, dissolved in benzene, and placed on 100 g of silica. The silica was eluted with benzene until the tlc indicated no more product was present. The benzene was concentrated to 6.1 g of oily cis-trans mixt that was suitable for further transformations, total yield 7.9 g (96%).

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Irreversible Enzyme Inhibitors. 195.†,[1] Inhibitors of Thymidine Kinase from Walker 256 Carcinoma Derived from Thymidine 5'-Acetate

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Seventeen derivatives of thymidine 5'-carbamate and 27 5'-esters of thymidine were synthesized and investigated as inhibitors of thymidine kinase from Walker 256 rat tumor. Derivatives of thymidine 5'-acetate were good inhibitors of the enzyme. The inhibition displayed was attributed in part to an interaction of the inhibitor with an enzymic hydrophobic region adjacent to the active site. The binding exhibited by thymidine 5'-α-thionaphthyloxyacetate and thymidine 5'-*p*-benzyloxyphenoxyacetate was approximately equal to that of thymidine. These compounds could serve as prototypes in the design of more potent inhibitors.

There are two metabolic pathways to the intracellular thymidylate needed for DNA synthesis. The first is the thymidylate synthetase-dihydrofolate reductase pathway using deoxyuridylate and the second is the scavenge pathway from thymidine kinase. A blockade of dihydrofolate reductase, in effect, prevents formation of thymidylate from deoxyuridylate and has been extensively studied in this laboratory.[2-5]

Some studies on inhibition of thymidine kinase from *Escherichia coli* B were reported earlier from this laboratory;[6,7] apparently both the 3'- and 5'-hydroxyls of thymidine were binding points to the enzyme. No bulk tolerance for the large substituents needed for the design of active-site-directed irreversible inhibitors could be found that

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‡Deceased.

gave less than a 50-fold loss in binding;[6] similarly, 1-substituted uracils were not good inhibitors of the enzyme.[7]

Later studies on guanine deaminase showed that a loss of a binding point on the substrate could be tolerated if the substituent causing the loss could enhance binding by some other interaction such as hydrophobic bonding; thus, the 21-fold loss in binding caused by 9-methylation of guanine could be recouped by the hydrophobic bonding of a 9-phenyl substituent.[8,9] We therefore investigated 5′-acyl derivatives of thymidine for inhibition of thymidine kinase. The loss in binding by substitution on the 5′-hydroxyl was regained by use of an acyl group that gave a hydrocarbon interaction with the enzyme. A series of thymidine 5′-carbamates and 5′-esters of thymidine was synthesized and tested as inhibitors of Walker 256 thymidine kinase. The results are the subject of this paper.

Enzyme Results. Thymidine kinase activity was found in an extract from Walker 256 rat tumor when assayed with 25 μM [^{14}C]thymidine. At a concentration of 300 μM, no inhibition was observed with a number of 1-alkyl-,[7,10-12] 1-aralkyl-,[7,12] or 1-hydroxyalkyluracils.[7] Similarly, no inhibition was observed at 300 μM with a number of 5-substituted or 6-substituted uracils.[13-17] Therefore, attention was directed to derivatives of thymidine (Table I).

The inhibition of thymidine kinase from Walker carcinoma shown by two 5-halo-2′-deoxyuridines, thymidine, and 2′-deoxyuridine (**1-4**) was in general agreement with the finding of Bresnick and Thompson.[18] Thus, 5-bromo-2′-deoxyuridine was most effective and 2′-deoxyuridine was least effective. These results indicate that the 5-methyl of thymidine might be a binding point to the kinase since bromo is nearly isosteric with methyl.

The losses in binding observed with the 3-alkylthymidines (**5-7**) are similar to those encountered with thymidine kinase from *E. coli* B.[6]

Compound **8**, 5′-deoxythymidine, suffered a 6-fold loss in binding compared with thymidine. Since **8** possesses no hydrogen-bonding capability at the 5′ position it is conceivable that such an interaction might contribute to the binding of thymidine.

The 32-fold loss in binding observed with 5′-*O*-carbamoylthymidine (**9**) is some indication of the amount of binding that must be regained by a thymidine 5′-carbamate in order to have good inhibition. This loss was not completely recovered by any of the carbamates (**10-26**). However, 5′-*O*-(*N*-phenethylcarbamoyl)thymidine (**12**) did give some inhibition of the kinase.

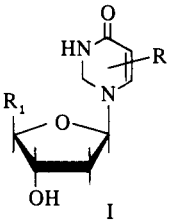
Compound **18**, 5′-*O*-(*N*-*n*-propylcarbamoyl)thymidine, did not bind, even though the enzyme should possess more bulk tolerance for it than for **12**. Therefore, it is possible that the phenyl of **12** binds to the enzyme more strongly than its $(CH_2)_2$ bridge. When substituted phenethylcarbamates (**21-26**) failed to inhibit at 500 μM, the carbamate series was abandoned and a series of 5′-esters of thymidine (**27-54**) was developed.

Significant inhibition of the kinase was observed with all 5′-aryloxyacetates and 5′-thioaryloxyacetates tested. The remaining esters in the series were ineffective.

Preliminary studies involved compounds **27-33**. Of these esters only thymidine 5′-phenoxyacetate (**31**) gave substantial inhibition.

Two other observations are noteworthy. Thymidine 5′-β-phenylpropionate (**29**) was ineffective. However, thymidine 5′-thiophenoxyacetate (**44**) was 3 times as potent as compound **31**. Compounds **29**, **31**, and **44** differ only in the linkage at the phenyl moiety. The marked variance in

Table I. Inhibition[a] of Thymidine Kinase from Walker Carcinoma by I (R_1 = R′CH_2)



I

No.	R	R′	I_{50},[b] μM
1	H	OH	160
2	5-Me	OH	12
3	5-F	OH	87
4	5-Br	OH	9
5[c]	3-(*n*-C_5H_{11})-5-Me	OH	140
6[c]	3-$C_6H_5CH_2$-5-Me	OH	170
7[c]	3-$C_6H_5(CH_2)_3$-5-Me	OH	160
8[c]	5-Me	H	160
9[d]	5-Me	NH_2CO_2	800
10	5-Me	$C_6H_5NHCO_2$	>500
11	5-Me	$C_6H_5CH_2NHCO_2$	>500
12[c]	5-Me	$C_6H_5(CH_2)_2NHCO_2$	370
13	5-Me	$C_6H_5(CH_2)_3NHCO_2$	>300
14	5-Me	$C_6H_5(CH_2)_4NHCO_2$	>500
15	5-Me	$C_6H_5O(CH_2)_2NHCO_2$	>500
16	5-Me	$C_6H_5O(CH_2)_3NHCO_2$	>500
17	5-Me	$C_6H_5O(CH_2)_4NHCO_2$	>500
18	5-Me	*n*-$C_3H_7NHCO_2$	>500
19	5-Me	*s*-$C_4H_9NHCO_2$	>500
20	5-Me	*i*-$C_5H_{11}NHCO_2$	>500
21	5-Me	*o*-$ClC_6H_4(CH_2)_2NHCO_2$	>500
22	5-Me	*m*-$ClC_6H_4(CH_2)_2NHCO_2$	>500
23	5-Me	*p*-$ClC_6H_4(CH_2)_2NHCO_2$	>500
24	5-Me	*o*-$MeOC_6H_4(CH_2)_2NHCO_2$	>500
25	5-Me	*m*-$MeOC_6H_4(CH_2)_2NHCO_2$	>500
26	5-Me	*p*-$MeOC_6H_4(CH_2)_2NHCO_2$	>500
27	5-Me	$C_6H_5CO_2$	>500
28	5-Me	$C_6H_5CH_2CO_2$	>500
29	5-Me	$C_6H_4(CH_2)_2CO_2$	>500
30	5-Me	$C_6H_5(CH_2)_3CO_2$	>500
31[e]	5-Me	$C_6H_4OCH_2CO_2$	147
32	5-Me	$C_6H_5O(CH_2)_2CO_2$	890
33	5-Me	$C_6H_5O(CH_2)_3CO_2$	>500
34	5-Me	*o*-$ClC_6H_4OCH_2CO_2$	220
35	5-Me	*m*-$ClC_6H_4OCH_2CO_2$	73
36	5-Me	*p*-$ClC_6H_4OCH_2CO_2$	90
37	5-Me	2,4-$Cl_2C_6H_3OCH_2CO_2$	85
38	5-Me	3,4-$Cl_2C_6H_3OCH_2CO_2$	80
39	5-Me	3,5-$Cl_2C_6H_3OCH_2CO_2$	68
40	5-Me	*m*-$CF_3C_6H_4OCH_2CO_2$	110
41	5-Me	*p*-$CH_3OC_6H_4OCH_2CO_2$	91
42	5-Me	*p*-*n*-$C_4H_9OC_6H_4OCH_2CO_2$	77
43	5-Me	4-Cl-3,5-$Me_2C_6H_2OCH_2CO_2$	58
44	5-Me	$C_6H_5SCH_2CO_2$	55
45	5-Me	*p*-$BrC_6H_4SCH_2CO_2$	42
46	5-Me	3,4-$Cl_2C_6H_3SCH_2CO_2$	40
47	5-Me	α-$C_{10}H_7OCH_2CO_2$	60
48	5-Me	β-$C_{10}H_7OCH_2CO_2$	62
49	5-Me	α-$C_{10}H_7SCH_2CO_2$	20
50	5-Me	β-$C_{10}H_7SCH_2CO_2$	38
51	5-Me	*m*-$C_6H_5OC_6H_4OCH_2CO_2$	38
52	5-Me	*p*-$C_6H_5OC_6H_4OCH_2CO_2$	49
53	5-Me	*m*-$C_6H_5CH_2OC_6H_4OCH_2CO_2$	30
54	5-Me	*p*-$C_6H_5CH_2OC_6H_4OCH_2CO_2$	19

[a]The technical assistance of Julie Beardslee, Maureen Baker, Nancy Middleton, Janet Wood, and Pauline Minton is acknowledged. [b]I_{50} = concentration of inhibitor giving 50% inhibition when assayed with 25 μM [^{14}C]thymidine as described in the Experimental Section. [c]See ref 6 for synthesis. [d]See ref 20 for synthesis. [e]This compound was subjected to the assay conditions in the absence of enzyme to demonstrate that it did not suffer hydrolysis to thymidine. No thymidine was detected on tlc when an aliquot contg approx 90 μg of the compound was spotted.

effectiveness among these three compounds cannot be accounted for solely on the basis of hydrophobicity since **31** has the more polar OCH_2 bridge compared with the less polar $(CH_2)_2$ bridge of **29**. There are at least two rationalizations that are supported by the data in Table I. First, more torsional strain might be encountered in rotation of the phenyl about the CH_2-CH_2 bond of **29** than in rotation about the O-CH_2 bond of **31** or the S-CH_2 bond of **44**. Secondly, since a methylene is less polarizable than an oxygen, which is in turn less polarizable than a sulfur, the observed trend could result from greater binding energy contributions by Van der Waal's interactions for **31** and **44** than for **29**.

Compounds **34–46** include phenoxy- and thiophenoxyacetates that have small substituents attached to the phenyl moiety. With the exception of the methoxy of compound **41**, these substituents are all hydrophobic. Among these compounds, the best phenoxyacetate was compound **43**, which was about 3-fold more effective than **31**. As the number and hydrophobicity of these substituents was increased, a slight trend toward greater inhibition was noted. The observed differences in binding are too small to warrant discussion. The results obtained with these compounds show that the enzyme possesses ample bulk tolerance to accommodate small ring substituents.

The larger, more hydrophobic compounds **(47–54)**, were good inhibitors. In particular, the α-thionaphthyloxyacetate **(49)** and the *p*-benzyloxyphenoxyacetate **(54)** were about as effective in binding to the kinase as thymidine.

It has not escaped our attention that 5′-esters of nucleosides might be hydrolyzed by nonspecific esterases *in vivo* before they could reach the target enzymes in cancer cells. However, a recent report by Wechter, *et al.*,[19] on 5′-esters of 1-β-D-arabinofuranosylcytosine states that some of them are orally active. Perhaps intracellular deposition of 5′-esters of thymidine can occur before extensive hydrolysis is suffered.

Efforts are underway to obtain more potent reversible inhibitors as well as candidate irreversible inhibitors, by use of these thymidine 5′-acetates as prototypes.

Chemistry. The displacement of phenol from 5′-carbophenoxythymidine[20] with phenethylamine to afford **12** has been reported.[6] Compounds **11** and **13–26** were prepared in a similar manner from the same carbonate. This reaction failed when aniline was used in an effort to prepare compound **10**. It was obtained by displacement of *p*-nitrophenol from *p*-nitrophenyl thymidine 5′-carbonate.[21]

Agarwal and Dhar have discussed the selective esterification of unblocked thymidine with carboxylic acid chlorides in MeCN containing 2.5 equiv of pyridine.[22] 5′-Esters of thymidine have also been prepared from an acid chloride in neat pyridine.[23,24] Pfitzner and Moffatt isolated 5′-*O*-*p*-nitrobenzoylthymidine by nonchromatographic methods, and, by subsequent chromatography, obtained some 3′,5′-di-*O*-*p*-nitrobenzoylthymidine and a small amount of 3′-*O*-*p*-nitrobenzoylthymidine.[24]

The reaction of acetic anhydride and thymidine, from which Gilham and Khorana isolated 5′-acetate and 3′,5′-diacetate,[25] was repeated in this laboratory and monitored on tlc. A trace product, which moved between 5′-acetate and 3′,5′-diacetate, had an R_f identical with that of authentic thymidine 3′-acetate.[26] In this way, 5′-esters of thymidine can be distinguished from their less polar 3′-isomers.

Compounds **27**, **29–31**, **33**, and **34** were prepared from the appropriate acid chlorides in neat pyridine. This method failed to give compound **28** from phenylacetyl chloride. Thus, in repeated attempts we observed several spots on tlc and were unable to isolate the desired product. Fieser and Fieser have mentioned the sensitivity of phenylacetyl chloride.[27] Similarly, we were unable to obtain **32** from β-phenoxypropionyl chloride. In our hands, this acid chloride was very unstable, and Powell has discussed its mode of decomposition.[28]

Compounds **28** and **32** as well as **35–54** were prepared by coupling the appropriate carboxylic acid with thymidine in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC). As monitored on tlc, the DCC coupling procedure gives essentially the same result as the acid chloride reaction.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had an ir spectrum compatible with its structure and moved as one spot on tlc on Brinkmann silica gel GF with chloroform–EtOH (4:1, v/v).

5′-*O*-(*N*-Phenylcarbamoyl)thymidine (10). Method A. To a

Table II. Physical Properties of I (R = 5-Me; R_1 = R′NHCOCH$_2$)

No.	R′	Method	Mp, °C	% yield[a]	Formula[b]
10	C_6H_5	A	208–210	5[c]	$C_{17}H_{19}N_3O_6$
11	$C_6H_5CH_2$	B	215–216	40[d]	$C_{18}H_{21}N_3O_6$
13	$C_6H_5(CH_2)_3$	B	185–186	30[d]	$C_{20}H_{25}N_3O_6$
14	$C_6H_5(CH_2)_4$	B	168–169	37[d]	$C_{21}H_{27}N_3O_6$
15	$C_6H_5O(CH_2)_2$	C[e]	189–191	18[f]	$C_{19}H_{23}N_3O_7$
16	$C_6H_5O(CH_2)_3$	C[g]	172–173	43[f]	$C_{20}H_{25}N_3O_7$
17	$C_6H_5O(CH_2)_4$	B[h]	176–177	42[f]	$C_{21}H_{27}N_3O_7$
18	*n*-C_3H_7	B	214–215	32[c]	$C_{14}H_{21}N_3O_6$
19	*sec*-C_4H_9	B	166–168	28[d]	$C_{15}H_{23}N_3O_6$
20	*i*-C_5H_{11}	B	172–173	39[d]	$C_{16}H_{25}N_3O_6$
21	*o*-$ClC_6H_4(CH_2)_2$	B	129–131	36[i]	$C_{19}H_{22}ClN_3O_6$
22	*m*-$ClC_6H_4(CH_2)_2$	C[j]	149–150	21[f]	$C_{19}H_{22}ClN_3O_6$
23	*p*-$ClC_6H_4(CH_2)_2$	C[k]	188–191	32[f]	$C_{19}H_{22}ClN_3O_6$
24	*o*-$MeOC_6H_4(CH_2)_2$	B[l]	137–139	15[f]	$C_{20}H_{25}N_3O_7$
25	*m*-$MeOC_6H_4(CH_2)_2$	B[l]	120–121	30[d]	$C_{20}H_{25}N_3O_7$
26	*p*-$MeOC_6H_4(CH_2)_2$	C[k]	178–180	23[m]	$C_{20}H_{25}N_3O_7$

[a] Yield of analytically pure material, and yield is minimum. [b] Analyzed for C, H, and N within 0.4% of theoretical. [c] Recrystallized from EtOH contg a little water. [d] Recrystallized from abs EtOH. [e] For starting 2-phenoxypropyl amine hydrochloride see ref 32. [f] Recrystallized from EtOH contg a little petroleum ether (bp 65–110°). [g] From 3-phenoxypropylamine *via* 3-phenoxypropyl bromide and potassium phthalimide in DMF followed by hydrazinolysis. For hydrazinolysis method used see ref 33. For original Gabriel synthesis of this amine see ref 34. [h] For starting 4-phenoxybutylamine see ref 35. [i] Recrystallized from *n*-PrOH contg a little petroleum ether (bp 65–110°). [j] From *m*-chloro-2-phenethylamine hydrochloride *via* catalytic redn of *m*-chlorophenylacetonitrile with PtO_2 in MeOH and HCl by the general method of Buck.[36] See ref 37. [k] For the starting phenethylamine hydrochloride see ref 36. [l] From the distd phenethylamine *via* the phenylacetonitrile by modification of the general method of Buck.[36] AcOH was substituted for alcohol and HCl. See ref 38. [m] Recrystallized from *n*-BuOH.

Table III. Physical Properties of I (R = 5-Me; R_1 = $R'CO_2CH_2$)

No.	R'	Method	Mp, °C	% yield[a,b]	Formula[c]
27	C_6H_5	D[d]	171–172	41[e]	$C_{17}H_{18}N_2O_6$
28	$C_6H_5CH_2$	E[f]	139–140	8	$C_{18}H_{20}N_2O_6$
29	$C_6H_5(CH_2)_2$	D[d]	147–150	30[g]	$C_{19}H_{22}N_2O_6$
30	$C_6H_5(CH_2)_3$	D[h]	122–123	10	$C_{20}H_{24}N_2O_6$
31	$C_6H_5OCH_2$	D[d]	134–135	10	$C_{18}H_{20}N_2O_7$
32	$C_6H_5O(CH_2)_2$	E[f]	152–153	8	$C_{19}H_{22}N_2O_7$
33	$C_6H_5O(CH_2)_3$	D[h]	138–139	12	$C_{20}H_{24}N_2O_7$
34	o-$ClC_6H_4OCH_2$	D[h]	143–144	12	$C_{18}H_{19}ClN_2O_7$
35	m-$ClC_6H_4OCH_2$	E[i]	158–159	9	$C_{18}H_{19}ClN_2O_7$
36	p-$ClC_6H_4OCH_2$	E[f]	198–199	11	$C_{18}H_{19}ClN_2O_7$
37	2,4-$Cl_2C_6H_3OCH_2$	E[f]	198–200	11	$C_{18}H_{18}Cl_2N_2O_7$
38	3,4-$Cl_2C_6H_3OCH_2$	E[f]	190–191	14	$C_{18}H_{18}Cl_2N_2O_7$
39	3,5-$Cl_2C_6H_3OCH_2$	E[i]	179–180	5	$C_{18}H_{18}Cl_2N_2O_7$
40	m-$CF_3C_6H_4OCH_2$	E[i]	166–167	3	$C_{19}H_{19}F_3N_2O_7$
41	p-$CH_3OC_6H_4OCH_2$	E[f]	171–172	8	$C_{19}H_{22}N_2O_8$
42	p-n-$C_4H_9OC_6H_4OCH_2$	E[i]	137–139	4	$C_{22}H_{28}N_2O_8$
43	4-Cl-3,5-$Me_2C_6H_2OCH_2$	E[i]	204–206	6	$C_{20}H_{23}ClN_2O_7$
44	$C_6H_5SCH_2$	E[i]	121–122	8	$C_{18}H_{20}N_2O_6S$
45	p-$BrC_6H_4SCH_2$	E[i]	169–171	11	$C_{18}H_{19}BrN_2O_6S$
46	3,4-$Cl_2C_6H_4SCH_2$	E[i]	168–170	16	$C_{18}H_{18}Cl_2N_2O_6S$
47	α-$C_{10}H_7OCH_2$	E[i]	170–171	12[e]	$C_{22}H_{22}N_2O_7$
48	β-$C_{10}H_7OCH_2$	E[j]	160–161	6	$C_{22}H_{22}N_2O_7$
49	α-$C_{10}H_7SCH_2$	E[i]	123–125	9[k]	$C_{22}H_{22}N_2O_6S$
50	β-$C_{10}H_7SCH_2$	E[i]	166–167	20	$C_{22}H_{22}N_2O_6S$
51	m-$C_6H_5OC_6H_4OCH_2$	E[i]	112–113	4[l]	$C_{24}H_{24}N_2O_8$
52	p-$C_6H_5OC_6H_4OCH_2$	E[i]	173–175	5	$C_{24}H_{24}N_2O_8$
53	m-$C_6H_5OC_2H_4OCH_2$	E[i]	132–134	11	$C_{25}H_{26}N_2O_8$
54	p-$C_6H_5CH_2OC_6H_4OCH_2$	E[i]	162–163	4	$C_{25}H_{26}N_2O_8$

[a]Yield of analytically pure material; yield is minimum. [b]Recrystallized from abs EtOH unless otherwise noted. [c]Analyzed for C, H, and N within 0.4% of theoretical. [d]Starting acid chloride is commercially available. [e]Recrystallized from EtOH contg a little petroleum ether (bp 65–110°). [f]Starting carboxylic acid is commercially available. [g]Recrystallized from *n*-PrOH contg a little petroleum ether (bp 65–110°). [h]Starting acid chloride prepd *via* reaction of the carboxylic acid with neat refluxing $SOCl_2$, 1.25 equiv, for 5 hr, followed by removal of volatile materials *in vacuo*. The product was purified by vacuum distn and had a compatible ir spectrum. [i]Starting carboxylic acid prepd from the phenol and *tert*-butyl chloroacetate. See Table IV. [j]Starting carboxylic acid prepd from the commercial Na salt. [k]Recrystallized from EtOH contg a little water. [l]Recrystallized from *n*-PrOH.

Table IV. Physical Properties of Intermediates, RCH_2CO_2H, Prepared from *tert*-Butyl Chloroacetate

No.[a]	R	Solvent[b]	Mp, °C	Lit. mp, °C	% yield
35	m-ClC_6H_4O	A	108–111	110–111[c]	40
39	3,5-$Cl_2C_6H_3O$	B	113–115	117.5–118.5[d]	22
40	m-$CF_3C_6H_4O$	B	94–96	94.5–95.5[e]	20
42	p-n-$C_4H_9OC_6H_4O$	C	114–115	112[f]	13
43	4-Cl-3,5-$Me_2C_6H_2O$	B	150–151	148–150[g]	21
44	C_6H_5S			63.5[h]	3[i]
45	p-BrC_6H_4S	A	115–117	118–119[j]	10
46	3,4-$Cl_2C_6H_3S$	A	75–78	71–72[k]	6
47	α-$C_{10}H_7O$	C	190–194	193.5[l]	29
49	$\alpha C_{10}H_7S$	B	106–107	107[m]	33
50	β-$C_{10}H_7S$	B	92–93	92–93[n]	15
51	m-$C_6H_5OC_6H_4O$	D	70–71	67–67.4[o]	33
52	p-$C_6H_5OC_6H_4O$	B	124–126	125[p]	21
53	m-$C_6H_5CH_2OC_6H_4O$	C	117–118[q]		50
54	p-$C_6H_5CH_2OC_6H_4O$	B	139–142	140[r]	18

[a]Compound numbers correspond to final thymidine 5'-acetate derivatives. See Tables I and III. [b]Recrystallization solvents: A, benzene; B, toluene; C, abs EtOH; D, petroleum ether (bp 65–110°). [c]See ref 39. [d]See ref 40. [e]See ref 41. [f]See ref 42. [g]See ref 43. [h]See ref 44. [i]Thiophenoxyacetic acid was difficult to isolate and was used without recrystallization. It had a compatible ir spectrum. [j]See ref 45. [k]See ref 46. [l]See ref 47. [m]See ref 48. [n]See ref 49. [o]See ref 50. [p]See ref 51. [q]*m*-Benzyloxyphenoxacetic acid is a new compound. See the Experimental Section. [r]See ref 52.

stirred soln of 484 mg (2 mmoles) of thymidine in 25 ml of pyridine was added 403 mg (2 mmoles) of *p*-nitrophenylchloroformate. After 5 hr, 186 mg (2 mmoles) of freshly distd aniline was added. After an addnl 16 hr, the pyridine was removed *in vacuo*, and the residue was shaken with 30 ml of H_2O. The yellow aqueous layer was decanted off, and the residual red oil was triturated with ether to give a tan solid. Addn of petroleum ether (bp 65–110°) gave more solid. The solid was filtd off and recrystd from EtOH–H_2O after charcoal treatment to yield 33 mg (5%) of off-white crystals, mp 208–210°.

5'-*O*-(*N*-*sec*-Butylcarbamoyl)thymidine (19). Method B. A soln of 121 mg (0.33 mmole) of 5'-*O*-carbophenoxythymidine[20] and 73 mg (1 mmole) of *sec*-butylamine in 3 ml of THF was allowed to stand approx 3 days. The reaction was monitored on tlc. The THF was removed *in vacuo*, and the residue triturated with ether to give a white solid that was filtd off and recrystd from EtOH to yield 30 mg (28%) of white powder, mp 166–168°.

For addnl compounds prepd by this method, see Table II.

Method C was the same as method B except that triethylamine was used to liberate the free amine from the HCl salt.

Thymidine 5'-Benzoate (27). Method D. To a chilled, stirred soln of 484 mg (2.0 mmoles) of thymidine in 7.5 ml of pyridine was added 0.25 ml (2.2 mmoles) of benzoyl chloride. The reaction mixt was protected from moisture. After 18 hr, the pyridine was removed *in vacuo*. The residue was triturated with approx 20 ml of H_2O, and the resultant solid was filtd off, dried, and recrystd from EtOH contg a little petroleum ether (bp 65–110°) to yield 278 mg (41%) of white

crystals, mp 171–172°. The product was homogeneous on tlc when 100 μg was spotted. §,[26]

For addnl compounds prepd by this method see Table III.

Thymidine 5'-(2,4-Dichlorophenoxy)acetate (37). Method E. To a stirred soln of 484 mg (2.0 mmoles) of thymidine and 442 mg (2.0 mmoles) of 2,4-dichlorophenoxyacetic acid in 4 ml of DMF was added 413 mg (2.0 mmoles) of *N,N*-dicyclohexylcarbodiimide. The reaction mixt was protected from moisture. After 24 hr, the ppt was filtd off. The DMF was removed by vacuum distn over warm H_2O. The residue was triturated with H_2O, and the resultant solid was filtd off and dried. The crude product was leached with hot toluene, cooled, filtd off, and recrystd from EtOH to yield 100 mg (11%) of white powder, mp 198–200°. The product was homogeneous on tlc when 100 μg was spotted. §,[26]

***m*-Benzyloxyphenoxyacetic Acid.** To a stirred soln of 4.9 g (25 mmoles) of *m*-benzyloxyphenol[29] in 15 ml of DMF in the presence of 3.8 g (27.5 mmoles) of K_2CO_3 was added 3.8 g (25 mmoles) of *tert*-butyl chloroacetate. The mixt was stirred overnight at 70°, then poured into 300 ml of H_2O, extd 2 × 100 ml of EtOAc, and dried over $MgSO_4$, and the solvent removed *in vacuo*. The residue was dissolved in 25 ml of toluene, and 100 mg of *p*-toluenesulfonic acid was added. The mixt was refluxed 12 hr, then chilled to give crystals, and recrystd from EtOH to give 3.23 g (50%) of white powder, mp 117–118°. An nmr spectrum was compatible with the structure.# *Anal.* ($C_{15}H_{14}O_4$) C, H.

For addnl carboxylic acids prepd by this method see Table IV.

Preparation of Enzyme and Assay Method. The enzyme prepn was a modification of the method of Bresnick and Thompson.[18] A mixt of 30 g of tissue and 50 ml of 0.05 *M* cold Tris (pH 7.4) was homogenized in a precooled head of a Waring blender for 2 min, and the resultant homogenate was centrifuged at 20,000 rpm for 20 min. To the crude supernatant was added 4.3 ml of 5% streptomycin per 73 ml of extract. The mixt was stirred in an ice bath for 10 min and then centrifuged at 20,000 rpm for 10 min. To the supernatant was slowly added 277 g of $(NH_4)_2SO_4$ per liter of extract, and the mixt was stirred in an ice bath to dissolve the $(NH_4)_2SO_4$ and then centrifuged at 20,000 rpm for 10 min. The resultant 0–45% $(NH_2)_2SO_4$ ppt fraction was resuspended in 30 ml of cold Tris (pH 7.4) and could be frozen for storage in 2-ml aliquots. The 0–45% $(NH_4)_2SO_4$ fraction was adjusted to pH 4.5 with 1 *M* AcOH and centrifuged at 20,000 rpm for 10 min. The supernatant was returned to pH 7.4 with 1 *M* KOH.

Thymidine kinase activity was measured by modification of the DEAE-cellulose disk method described by Bollum and Potter[30] and by Breitman.[31] The assay was run in the presence of 10 vol % DMSO, and inhibitors were added in this solvent. The reaction mixt included 40 vol % enzyme extract; 0.05 *M* Tris (pH 8.0); 5 m*M* ATP; 5 m*M* $MgCl_2$; and 25 μ*M* thymidine contg [^{14}C]thymidine. The total vol of the mixt was 0.1 ml.

After 30 min at room temp, the reaction mixt was spotted on a 16-mm DEAE-cellulose disk. Each disk was washed three times with 1 m*M* ammonium formate and once with abs EtOH, dried under an ir lamp, immersed in a toluene–phosphor mixt, and counted in a Packard Tri-Carb liquid scintillation spectrometer.

§ In this way less than 2% thymidine can be detected.

#Starting *m*-benzyloxyphenol is labile to rearrangement under certain conditions (see ref 29). The product was characterized by nmr to ascertain that it had not suffered a similar fate.

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