Nucleoside Analogs. 14. The Synthesis and Antitumor Activity in Mice of Molecular Combinations of 5-Fluorouracil and *N*-(2-Chloroethyl)-*N*-nitrosourea Moieties Separated by a Three-Carbon Chain

R. Stanley McElhinney,*,† Joan E. McCormick,† Mike C. Bibby,‡ John A. Double,‡ Marco Radacic,§ and Patrick Dumont⊥

Screening and Pharmacology Group, EORTC, and University Chemical Laboratory, Trinity College, Dublin 2, Ireland, Clinical Oncology Unit, University of Bradford, Bradford, West Yorkshire, BD7 1DP, U.K., Ruder Boškovic Institute, Bijenicka 54, 41001 Zagreb, Croatia, and Institut Jules Bordet, Rue Héger-Bordet, 1000 Brussels, Belgium

Received September 29, 1995⊗

5-Fluorouracil (5-FU) seco-nucleosides having as the "sugar" moiety a two-carbon (C_2) side chain carrying a *N*-(2-chloroethyl)-*N*-nitrosourea group were designed as molecular combinations of antimetabolite and alkylating agent, but hydrolytic release of free 5-FU was not fast enough for significant contribution to the high activity they showed against colon and breast tumors in mice. In the present study of the synthesis of the more reactive C_3 seco-nucleosides, it emerged that, of various groups attached to the aldehydic center in the precursor phthalimides, only the alkoxy/uracil-1-yl type was conveniently obtained by the standard method. The methylthio/uracil-1-yl analog required relatively large amounts of reagent methanethiol, and exploration of alternatives involving α-chlorination of alkyl methyl sulfide or Pummerer rearrangement of its *S*-oxide, or successive hydrolysis and methylation of isothiouronium bromide, gave disappointing yields. For successful preparation of the alkoxy/uracil-3-yl compounds, the route used for C_2 homologs required considerable experimental modification. In addition to these *O*,*N*- and *S*,*N*-acetals, some *N*,*N*-acetals bearing two 5-FU residues were prepared. The new drugs have been tested against a panel of experimental tumors in mice. Although it is evident from a parallel study that even these C_3 seco-nucleosides release free 5-FU too slowly *in vivo*, several of them have shown impressive anticancer activity. Reviewing their performance in comparison with earlier molecular combinations, a short list of seven [B.4152 (**6**), B.4015 (**5**), B.4030 (**10**), B.3999 (**4**), B.3995 (**2**), B.4083 (**3**), and B.3996 (the N^3-substituted analog of **1**)] should be investigated further. This is particularly appropriate in light of the present understanding of the mode of action of chloroethylating agents. Following a prolonged period of clinical impatience with nitrosoureas because of limited selectivity of action, a new era is confidently anticipated as these powerful drugs are increasingly studied in combination with O^6-benzylguanine and other more efficient inhibitors of repair enzymes like O^6-alkylguanine-DNA-alkyltransferase now being developed.

Alkylation was one of the first principles successfully exploited in cancer chemotherapy, and the value of nitrogen mustards like Melphalan and Cyclophosphamide and sulfonates (Busulfan) has endured[1] to the present day. *N*-(2-Chloroethyl)-*N*-nitrosoureas (CNUs) such as Carmustine (BCNU) and Chlorozotocin are also of this 30−40-year-old vintage.[2] Although very many congeners were made and tested, the clinical usefulness of these agents reached a plateau because of limited selectivity between cancer cells and bone marrow.

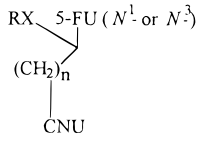
During the past decade improved experimental techniques have led to a greater understanding of the mechanism of action of alkylating drugs on biological macromolecules, particularly DNA. The site of alkylation by CNUs and the function and significance of O^6-alkylguanine-DNA-alkyltransferase (ATase) have been intensively studied.[3] It can be claimed with some confidence that a new era in the history of alkylating agents is opening up, and not only will the performance of early CNUs be greatly enhanced but the availability of several specially-designed nitrosoureas[4] may prove something of an Aladdin's cave, with the prospect of tailor-made treatment for individual patients.

One of these classes has been the molecular combinations incorporating the antimetabolite 5-fluorouracil (5-FU) and the CNU alkylating function.[4−6] Our development of this program was facilitated by a novel synthetic route devised for seco-nucleosides[7] (nucleoside analogs in which the "sugar" is linear instead of the normal cyclic), enabling a pyrimidine to be readily linked to a nitrosourea moiety. It was hoped that the administered molecular combination would gradually release 5-FU to make its own antitumor contribution superimposed on that of the nitrosourea. This would differ subtly from a physical combination (mixture) of two drugs in that the single molecule of the molecular combination is transported *in vivo* initially as such, presenting a challenge for pharmacokinetic study. By chemically adjusting the rate of release of 5-FU, in principle the overall antitumor effect could be varied.

The seco-nucleoside molecular combinations tested up to the present had $n = 1$ in the general structure

* Corresponding author.
† Trinity College, Dublin.
‡ University of Bradford.
§ Ruder Boškovic Institute, Zagreb.
⊥ Institut Jules Bordet, Brussels. Present address: CNRS, Laboratoire de Pharmacologie et de Toxicologie Fundamentales, 205, Route de Narbonne, 31077 Toulouse Cedex, France.
⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1996.

0022-2623/96/1839-1403$12.00/0



where R = small alkyl group, X = O (as in natural sugars and nucleosides) or S, and the pyrimidine ring was linked by N^1 (as in natural nucleosides) or N^3 (designated 5-FU and 5-FU^3 respectively in the formulas in the schemes). When n = 1, 5-FU and CNU are separated by a two-carbon chain, with the CNU substituent attached at the position corresponding to 2′ of a furanose ring. Many of these C_2 drugs [examples are B.3839 (**1**), B.3995 (**2**), and B.4083 (**3**)][8] strongly inhibited the growth of certain colon tumors in mice (the MAC series), well-established as models for clinical disease.

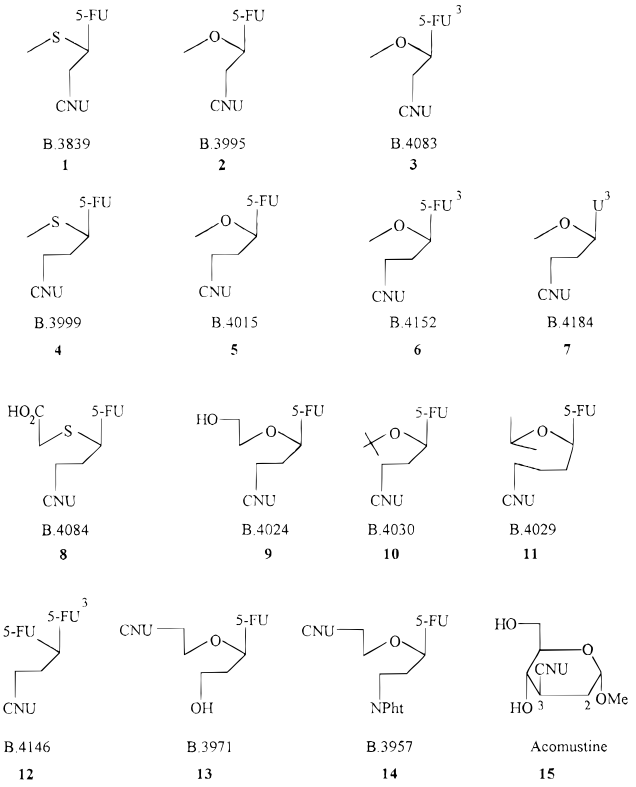
Acid-catalyzed hydrolysis releasing 5-FU from these seco-nucleosides proceeds in the order of reactivity MeO > MeS and 5-FU^3 > 5-FU, thus B.4083 (**3**) is hydrolyzed much faster than B.3839 (**1**)[6,9] (see Scheme 1). However the hydrolysis rates are greatly decreased at physiological pH, and it became likely that even B.4083 (**3**) may not release 5-FU at a rate sufficient to contribute significantly to the antitumor activity. This would indicate that all these C_2 drugs act principally as alkylating agents, in many cases having particularly effective carriers. Such a conclusion was already implied by the activity of the corresponding uracil (U) seco-nucleosides which was often similar to that observed in the 5-FU drugs at a high level.[6,10]

Since 5-FU seco-nucleosides with nitrogen or oxygen functional groups separated from the pyrimidine by a three-carbon chain, *i.e.*, at the position corresponding to 3′ of a furanose ring, are more readily hydrolyzed than their homologs with a two-carbon chain,[11] we undertook a final attempt to prepare a molecular combination whose antitumor action would conclusively involve a contribution from released antimetabolite. Recognizing the other factors governing reactivity, the C_3 analog **6** of B.4083 (**3**) would be an ideal target. To complete the picture in this series it was also desirable to assess the antitumor activity of the analogs **4** and **5** of B.3839 (**1**) and B.3995 (**2**), together with some water-soluble drugs **8** and **9** and more hydrophobic compounds such as the *tert*-butyl ether (**10**) and its C_4 isomer (**11**). We also chose analogs of **6** with a uracil (**7**) or a second 5-FU residue (**12**).

Comparative data for earlier drugs with a three-carbon chain resembling that in **6** are included in this study. B.3971 (**13**)[9] is isomeric with **9**, and the phthalimide B.3957 (**14**)[12] has a similar molecular framework. It is noteworthy that the glycoside nitrosourea Acomustine (**15**),[13] with the 2-deoxy-3-CNU fragment characteristic of all these C_3 seco-nucleosides, has properties superior to earlier hexopyranose analogs bearing a 2-CNU group such as Chlorozotocin.

In the present paper we describe the synthesis of the proposed C_3 drugs and compare their activity against a panel of experimental tumors in mice with that of the C_2 drugs and of standard nitrosoureas like Semustine (MeCCNU) and Tauromustine (TCNU) in the light of recent significant developments in the use of chloroethylating agents.[3,4]

Scheme 1



5-FU = 5-fluorouracil-1-yl, 5-FU^3 = 5-fluorouracil-3-yl, U^3 = uracil-3-yl, CNU = NHCON(NO)CH_2CH_2Cl, Pht = phthaloyl.

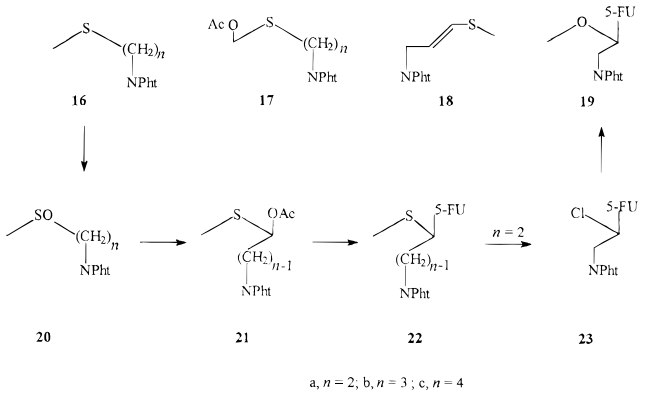
Chemistry

Synthesis of these nitrosoureas was achieved by the general route for C_2 homologs: low-temperature hydrazinolysis of the corresponding phthalimides (thereby avoiding ring closure of the liberated amine to a bicyclic isomer), followed by reaction with 2,4,5-trichlorophenyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate (leading exclusively to the desired *N*-nitroso isomer of the generated urea).[14,15]

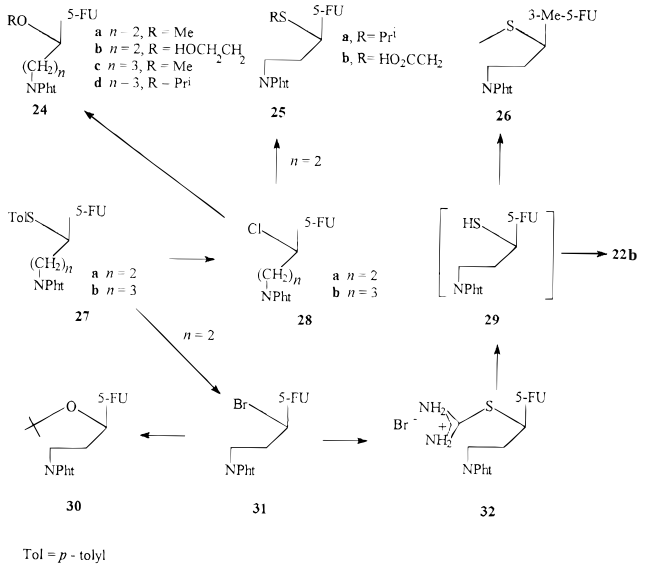
However, preparation of the precursor phthalimides in the C_3 series required a modified approach. Thus, the precursors **22a** and **19** of B.3839 (**1**) and B.3995 (**2**), respectively, were readily obtained from the sulfoxide (**20a**) *via* Pummerer rearrangement which, influenced by the β-phthalimido group, gave the required acetate **21a** and the isomer **17a** in the ratio 65:35[5,16] (see Scheme 2). Rearrangement of the homolog **20b** with acetic anhydride yielded (60:35:5, respectively) mainly the "normal" acetoxymethyl isomer **17b** as anticipated, together with unsaturated product **18** and only traces of the acetate **21b** necessary for the synthesis of **22b** and ultimately B.3999 (**4**). Trifluoroacetic anhydride and **20b** afforded only the **17b** type of isomer and **18** (80:20).

Alternative approaches to the seco-nucleoside **22b** involved α-chlorination studies[17−19,21] (see the Experimental Section), which proved very unsatisfactory when applied to **16b**. Further, we investigated the adaptation of an established route to thioglycosides containing the fragment OCHSMe *via* sugar bromides (OCHBr) and their reaction products with thiourea, isothiouronium bromides. These salts are hydrolyzed to thiols (OCHSH) which can be alkylated without isolation.[20] We found

Scheme 2



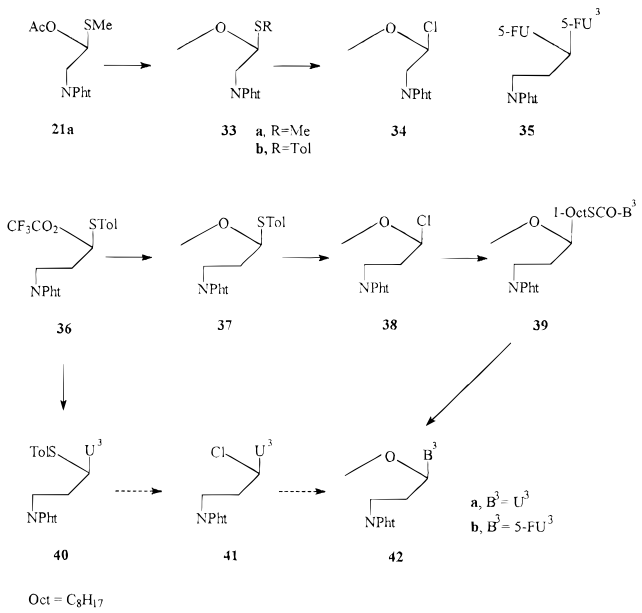
Scheme 3



that this sequence could be applied to seco-nucleoside bromides (NCHBr) such as **31** (see below), and the sulfide (NCHSMe, **22b**) was obtained from the salt **32** (formed in 89% yield) *via* the thiol **29** (see Scheme 3). However the sensitivity of the phthalimide group in alkali is a complication, and some nuclear (N^3) methylation leading to **26** also occurs. After much experimentation the best yield of sulfide **22b** from the salt **32** was 17%.

Accordingly we reverted, reluctantly, to introducing the methylthio group directly at the final stage in the synthesis of **22b**. We had earlier obtained C_3 ethers (**24**, $n = 2$) from the chloride **28a**, derived like the bromide **31** from the aryl sulfide **27a**.[11] This was available without complication by a route corresponding to **16** → **20** → **21** → **22**, since rearrangement of aryl sulfoxides can produce only a single isomer.[21] However, thiols do not react satisfactorily with the chloride **28a** in the form of their sodio derivatives, a great disadvantage for very volatile compounds. We had obtained[15] the sulfide **25b** in 58% yield using neat mercaptoacetic acid, and we now found that simpler thiols (*e.g.*, propane-2-thiol giving **25a**) also reacted well, in nitromethane solvent, in this acid-catalyzed transformation, but temperatures lower than 90−100 °C were unsatisfactory. The sulfide **22b** was finally obtained successfully in 82% yield, although considerable amounts of methanethiol (bp 6 °C) are needed to maintain the concentration necessary for efficient reaction.

Scheme 4



The alkoxyphthalimides **24a** and **24b** described earlier have now been obtained on a preparative scale from the chloride **28a**, and **30** from the bromide **31**; these ethers are the precursors respectively of B.4015 (**5**),[14] B.4024 (**9**), and B.4030 (**10**). The C_4 homolog (**27b**) was made, from *N*-(4-bromobutyl)phthalimide, in the same manner[11] as **27a** and converted *via* the chloride into the isopropoxyphthalimide (**24d**) precursor of B.4029 (**11**).

The N^3-substituted compound **42b** needed for B.4152 (**6**) required a different approach from the isomer **24a**, since reaction of silylated 5-FU with precursors such as **36** affords (at least in practice) only N^1-isomer **27**.[11] The C_2 homolog of **42b** was eventually obtained very satisfactorily[15] by a route based on reaction of the useful reagent N^1-(octylthio)carbonyl-5-FU[22b] with the chloro ether **34** derived from the *O,S*-acetal **33a** (see Scheme 4). Application to the C_3 series would indicate **37** → **38** → **39b** → **42b**. Attempted preparation of **37** revealed differences in the reactivity of C_2 and C_3 aldehyde derivatives in addition to those already observed.[11,25]

Compound **33a** is obtained (86% yield) from the Pummerer acetate **21a** in refluxing methanol with acid catalysis.[15] In the C_3 series the *S*-(*p*-tolyl)acetal **37** is necessary because the *S*-methyl compound **21b** is so inaccessible. The very convenient Pummerer rearrangement of the appropriate sulfoxide in trifluoroacetic anhydride leads to **36**, but reaction of this with refluxing methanol afforded **37** in low and unreproducible yields. However, room temperature gave a very satisfactory result in this evidently more reactive system.[26]

The next steps (**37** → **38** → **39b**) also proved more difficult in the C_3 series. While chlorinolysis of *O,S*-acetals in general proceeds smoothly, for **33a** (C_2) the byproduct methanesulfenyl chloride is relatively volatile and easily removed. The chloro ether **34** is stable and reaction with the protected 5-FU (18 h at room temperature) straightforward and high-yielding.[15] For **37** the (characteristically bright yellow) arenesulfenyl chloride had to be extracted into petroleum ether, and attempted reaction of the chloro ether **38** with N^1-[(octylthio)-carbonyl]-5-FU at room temperature usually afforded no **39b** or on one occasion *ca.* 20% yield, and the reagent could be recovered (60−70%) as such or as free 5-FU.

In case **38** was unstable[27] even at room temperature and/or very sensitive to generated HCl, we carried out the condensation at 0 °C and at −15 °C, using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to remove acid, and obtained **39b** in yields respectively of 28% and 55% (based on **37**).

It was convenient or even desirable to avoid the extraction of sulfenyl chloride because of the considerable solubility of **38**, and a technique used in the synthesis of unsaturated chloromethyl ethers from the corresponding formaldehyde *O,S*-acetals suggested itself. Cyclohexene as scavenger rapidly formed a *trans*-chlorocyclohexyl sulfide adduct which did not interfere with subsequent nucleophilic reaction with the chloromethyl ether.[28] We found that by inclusion of an equivalent of cyclohexene during the chlorinolysis, the product **38** subsequently afforded **39b** in 76% (overall) yield in 1 h at −15 °C. This demonstrates the reactivity of the C_3 derivative **38** relative to **34**,[15] although in the presence of cyclohexene reaction temperature was less significant (63% yield of **39b** at room temperature). The deprotected phthalimide **42b** was then obtained very smoothly using isopropylamine in ether.

Reaction of the chloro ether **38** with N^1-[(octylthio)-carbonyl]uracil readily provided **42a** and in turn B.4184 (**7**), underlining the great simplicity of seco-nucleoside synthesis with these reagents.[22b,29] An alternative route to **42a** seemed promising since, unlike 5-FU, uracil tends to give mainly N^3-seco-nucleosides, *e.g.*, the transformation corresponding to **36** → **40**, using the silylated reagent and tin(IV) chloride, is accomplished in the C_2 series in 60−70% yield.[30] However the C_3 ester **36** gave an inseparable mixture of approximately equal amounts of **40** and its N^1-isomer.

The versatility of the N^1-(octylthio)carbonyl reagents also suggested the synthesis of a "double-headed" seco-nucleoside bearing two pyrimidine residues. Reaction with the chloride **28a** was slower than with the ether **38** but satisfactorily yielded the phthalimide **35**, the precursor of B.4146 (**12**). We have been unable to find any references in the literature to double-headed nucleoside analogs with geminal pyrimidinyl substituents (*N,N*-acetals), although a dialdehydic xylopyranose derivative[31] has purine and pyrimidine residues attached to the respective "anomeric" carbons (by analogy, an *N,O,N*-hemialdal). *N,N*-Acetals have a long pedigree,[32] and compounds bearing geminal hydantoin rings have been described.[33]

Results and Discussion

The effects of drugs against the various tumors following ip administration at maximum tolerated doses are presented in Tables 1−4. Earlier results[6] for the C_2 drugs B.3839 (**1**), B.3995 (**2**), and B.4083 (**3**) are included to make comparisons easier. We have attempted to correlate the observed activity of the new C_3 drugs against the panel of tumors with chemical reactivity and physicochemical properties. Details of pharmacokinetics of B.4152 (**6**)[34] and other very active compounds are reported elsewhere, following those[35] of B.3839 (**1**), the original 5-FU/CNU molecular combination.

It transpired that even B.4152 was not cleaved *in vivo* to pyrimidine to a significant extent.[34] Accordingly, reaction of the nitrosourea moiety is the first and principal event with all these seco-nucleoside drugs, and any occurrence of high antitumor activity in compounds which readily release 5-FU at lower pH's must be regarded as coincidental. Most of the trends discernible for each tumor in the panel are probably due to

Table 1. Effects against MAC 13 Tumors

compound	vehicle	dose, mg/kg	no. deaths/ treated	*T/C*, %[a]
B.3839 (**1**)	arachis oil	31.25	0/10	122
B.3999 (**4**)	arachis oil	135	0/5	0
B.3995 (**2**)	arachis oil	50	0/5	7
B.4015 (**5**)	arachis oil	150	0/5	0.8
B.4083 (**3**)	arachis oil	50	0/5	11
B.4152 (**6**)	10% DMSO/arachis oil	100	0/5	1
B.4184 (**7**)	10% DMSO/arachis oil	100	0/5	43
B.4146 (**12**)	10% DMSO/arachis oil	100	0/5	87
B.4084 (**8**)	arachis oil	200	0/5	31
B.4024 (**9**)	10% ethanol/arachis oil	100	0/5	23
B.3971 (**13**)	arachis oil	100	0/5	27
B.3957 (**14**)	arachis oil	200	0/10	103
B.4030 (**10**)	arachis oil	200	0/5	1
B.4029 (**11**)	arachis oil	200	0/5	33
MeCCNU	10% ethanol/arachis oil	20	0/5	8
TCNU	saline	30	0/5	1

[a] *T* represents mean weight of treated tumors and *C* represents mean weight of control tumors.

Table 2. Effects against MAC 15A Tumors

compound	vehicle	dose, mg/kg	*T/C*, %[a]
B.3839 (**1**)	arachis oil	62.5	156
B.3999 (**4**)	arachis oil	90	200
B.3995 (**2**)	arachis oil	50	200
B.4015 (**5**)	arachis oil	100	233
B.4083 (**3**)	arachis oil	100	>850
B.4152 (**6**)	10% DMSO/arachis oil	100	360
B.4184 (**7**)	10% DMSO/arachis oil	100	200
B.4146 (**12**)	10% DMSO/arachis oil	100	182
B.4084 (**8**)	10% DMSO/arachis oil	200	275
B.4024 (**9**)	10% ethanol/arachis oil	100	233
B.3971 (**13**)	arachis oil	100	136
B.3957 (**14**)	arachis oil	200	148
B.4030 (**10**)	arachis oil	300	280
B.4029 (**11**)	arachis oil	300	250
MeCCNU	saline	20	154
TCNU	saline	30	210

[a] *T* represents median survival times of treated animals and *C* represents median survival times of control animals.

Table 3. Effects against MCa Mammary Carcinoma

compound	vehicle	dose, mg/kg	LT S[a]	*T/C*, %[b]
B.3839 (**1**)	10% DMSO/water	60	1/6	189
B.3999 (**4**)	10% DMSO/water	80	0/8	195
B.3995 (**2**)	10% DMSO/water	100	3/6	311
B.4015 (**5**)	10% DMSO/water	100	6/7	
B.4083 (**3**)	10% DMSO/water	50	5/6	
B.4024[c] (**9**)	10% DMSO/water	150	0/8	168
B.4030[c] (**10**)	10% DMSO/water	100	5/6	
B.3957 (**14**)	10% DMSO/water	100	0/6	121

[a] Long term survival. [b] *T* represents median survival times of treated animals and *C* represents median survival times of control animals. [c] Treated on day 1.

Table 4. Effects against Colon 38 Adenocarcinoma

compound	vehicle	dose, mg/kg	*T/C* %[a]
B.3839 (**1**)	saline	100	0
B.3999 (**4**)	saline	100	63
B.3995 (**2**)	saline	100	38
B.4015 (**5**)	saline	100	47
B.4083 (**3**)	saline	50	17
B.4084 (**8**)	saline/Tween 80 (9/1)	100	0
B.4030 (**10**)	saline/Tween 80 (9/1)	100	42
B.3971 (**13**)	saline/Tween 80 (9/1)	50	68

[a] *T* represents median tumor volume of treated animals and *C* represents median tumor volume of controls.

Table 5. *N*-(2-Chloroethyl)-*N*-nitrosoureas (CNUs)

starting phthalimide	CNU	yield, %	mp, °C[a]	UV λ_{max}[b] (nm)	IR υ_{max} (cm^{-1})	formula[c]
24a	**5**, B.4015[d]	49	129.5−130	264 (262)	1536 1488	$C_{11}H_{15}ClFN_5O_5$
24b	**9**,[e] B.4024	31	63−64.5[f]	264 (263)	3520 1530 1488	$C_{12}H_{17}ClFN_5O_6 \cdot 0.7EtOAc$
30	**10**, B.4030	35	118−119	266 (265)	1530 1489	$C_{14}H_{21}ClFN_5O_5$
24d	**11**, B.4029	31	121−121.5	266 (266)	1530 1495	$C_{14}H_{21}ClFN_5O_5$
22b	**4**, B.3999	34	120−121	269 (270)	1534 1492	$C_{11}H_{15}ClFN_5O_4S$[g]
42a	**7**, B.4184[h]	52	121.5−122	259 (294)		$C_{11}H_{16}ClN_5O_5$
42b	**6**,[j] B.4152[h]	18	122.5−123	266 (305)	1528 1490	$C_{11}H_{15}ClFN_5O_5$
35	**12**,[k] B.4146	45	190−210	267 (270, 305)	1535 1495	$C_{14}H_{14}ClF_2N_7O_6 \cdot 0.75EtOH$

[a] With effervescence. [b] In parentheses: those in the presence of NaOH. [c] Anal. C, H, N in all cases except for **9** (N: calcd, 15.8; found, 15.3) and **12** (C: calcd, 38.45; found, 37.7). [d] Reference 14. [e] ^{1}H NMR δ 11.7 (d, J = 5, ring 3-H), 8.75 (t, J = 6.5, CH_2NH), 7.94 (d, J = 7, ring 6-H), 5.71 (t, J = 6.5, OCHN), and 1.99 (s, CH_3CO_2Et; solvent content, 0.7 mol/mol CNU). [f] Effervescence at 70 °C. [g] S: calcd, 8.7; found, 9.0. [h] Reference 34. [j] ^{1}H NMR δ 10.94 (bs, ring 1-H) 8.78 (t, J = 5.6, CH_2NH), 7.82 (d, J = 5.5, ring 6-H), 5.87 (t, J = 6.3, OCHN), and 3.22 (s, OMe); [k] ^{1}H NMR δ 11.83 (bs, 3-H in 5-FU), 11.12 (bs, 1-H in 5-FU[3]), 8.80 (t, J = 5.3, CH_2NH), 8.27 (d, J = 8.0, 6-H in 5-FU), 7.83 (d, J = 5.5, 6-H in 5-FU[3]), 6.87 (t, J = 6.5, NCHN), and 1.06 (t, CH_3CH_2OH; solvent content, 0.5−0.75 mol/mol CNU).

physicochemical factors. There are consistent patterns, and some anomalies, in each table, but from a survey of all the results a choice of several outstanding drugs can be made for more detailed study.

MAC 13 is a subcutaneous tumor to which drugs administered intraperitoneally have to be transported in order to exert any effect, enabling bioavailability to be assessed. The methoxy C_2 drugs B.3995 (**2**) and B.4083 (**3**) are much more active against this tumor than the methylthio analog B.3839 (**1**) of B.3995 (although B.3839 is effective if administered orally). However, all three C_3 homologs B.3999 (**4**), B.4015 (**5**), and B.4152 (**6**) are equally effective. Dose levels are higher than for the C_2 drugs, and the effect on the tumor is marginally greater, almost eliminating it after 21 days. More water-soluble drugs like B.4084 (**8**), B.4024 (**9**), and B.3971 (**13**) are less active, as is the uracil-containing B.4184 (**7**) and the C_4 compound B.4029 (**11**). On the other hand, the *tert*-butoxy C_3 drug B.4030 (**10**) isomeric with B.4029 is highly active. The relatively insoluble (water and lipid) compounds B.3957 (**14**) and B.4146 (**12**), of higher than average molecular weight, are inactive against this tumor.

In the ascitic tumor MAC 15A, tumor cells are located within the peritoneal cavity into which the drugs are injected, so the transport factor characteristic of MAC 13 is eliminated and drugs have merely to penetrate the cell membrane. Of the C_2 drugs, B.4083 (**3**) is outstandingly more active than B.3839 (**1**) and B.3995 (**2**). Incidentally, its water-solubility (4 mg/mL) is higher than the less active drugs (both 1 mg/mL). The C_3 homologs follow a similar though less marked trend, but again B.4152 (**6**) is very active [and its uracil analog B.4184 (**7**) less so]. Water-soluble drugs B.4084 (**8**) and B.4024 (**9**) have good activity, but B.3971 (**13**) has not. The lipid-soluble isomers B.4029 (**11**) and B.4030 (**10**) also show useful activity, and as against MAC 13 the insoluble drugs B.3957 (**14**) and B.4146 (**12**) are relatively ineffective.

The MCa mammary carcinoma like MAC 13 requires transport of the drugs as administered, and the DMSO-containing aqueous vehicle used may exert an influence on bioavailability. Again the methoxy C_2 drugs B.3995 (**2**) and B.4083 (**3**) are considerably more active than B.3839 (**1**), as was observed for MAC 13. B.4152 was not available for comparison against this tumor, and while the methoxy C_3 drug B.4015 (**5**) maintained good activity with the characteristic high proportion of long-term survivors, the methylthio-C_3 B.3999 (**4**) was much less active, resembling B.3839. Water-solubility [B.4024 (**9**)] was a disadvantage, but again the *tert*-butoxy compound B.4030 (**10**) showed excellent activity. The drug B.3957 (**14**) was once more inactive.

Against Colon 38 adenocarcinoma the methylthio C_2 compound B.3839 (**1**) had high activity, more so than the methoxy drugs B.3995 (**2**) and B.4083 (**3**). The C_3 homologs B.3999 (**4**) and B.4015 (**5**) were less active, especially the former. All these drugs were administered in saline, which may influence bioavailability. Preliminary studies with B.3839 suspended in Klucel (hydroxypropylcellulose) resulted in increased toxicity. Addition of some Tween 80 to the vehicle gave mixed results: B.3971 (**13**) was inactive, B.4030 (**10**) barely so, and B.4084 (**8**) very active, with no tumor measurable on day 20 as in the case of B.3839.

Thus it appears that the principal target drug B.4152 (**6**) has impressive activity against the MAC tumors, significantly higher than its uracil analog B.4184 (**7**) and than standard nitrosoureas like CCNU[12] and PCNU, MeCCNU, TCNU,[6,16] which show activity in these tumors only close to the maximum tolerated dose and accompanied by normal tissue toxicities. Additional evidence[34] makes it a prime candidate for further evaluation, together with three other C_3 drugs which have emerged from the present study: B.4015 (**5**), B.4030 (**10**), and B.3999 (**4**), all outstandingly active against the subcutaneous MAC 13 (colon) tumor when administered ip in an oily vehicle. Treatment of the MCa (mammary) carcinoma with B.4015 and B.4030 in aqueous dimethyl sulfoxide leads to long-term survival in 85% of the test animals, and the relative inactivity of B.3999 in this system poses an interesting question. All three drugs are relatively ineffective against the Colon 38 tumor when administered in saline [but the behavior of B.4084 (**8**) under these conditions would indicate that this drug too should be short-listed].

Finally, three of the earlier C_2 drugs also have a range of high activity[6] which requires further investigation: B.3995 (**2**), B.4083 (**3**), and B.3996, the 5-FU[3] analog of B.3839 (**1**).

It is important to study the effects of administration of O^6-benzylguanine, and other more powerful O^6-alkylguanine-alkyltransferase inhibitors now being developed, in conjunction with a spectrum of chloroethylating agents with differing biological properties. Bioavailability, selectivity for tumor over normal tissue, administration vehicle and site, and scheduling of dosage will all be relevant in potential clinical application. While the usefulness of familiar if inefficient nitrosoureas like BCNU will no doubt be improved in what appears to be an exciting new phase in the study of these alkylating agents, the availability of more effective or selective analogs should prove a considerable advantage.

Experimental Section

UV spectra were measured in MeOH on a Unicam SP-800 spectrophotometer, IR spectra using KBr disks on a Unicam SP1000 instrument, and NMR spectra (80 MHz) in $(CD_3)_2SO$ (J values are given in hertz) on a Bruker WP-80. Melting points, uncorrected, were determined in capillaries. Petroleum ether had bp 40−60 °C unless otherwise stated. Column chromatography was performed on Merck silica gel 60 (35−70 mesh ASTM, Art. 7733). Microanalyses of all compounds were within ±0.4% of theory unless otherwise indicated.

***N*-[3-Alkoxy-3-(5-fluorouracil-1-yl)propyl]phthalimides. a.** The sulfide **27a**[11] (7.46 g, 17 mmol) and SO_2Cl_2 (1.51 mL, 18.7 mmol) were stirred for 2 h in CH_2Cl_2 (187 mL). The chloride **28a** (5.32 g, 89%) was filtered from the yellow solution of *p*-toluenesulfenyl chloride and taken up in boiling MeOH (250 mL). Concentration (to 175 mL) and cooling yielded the methyl ether **24a** (4.63 g, 88%), mp 204−209 °C (lit.[11] mp 202.5−203.5 °C).

b. The chloride **28a** (6.83 g, 19.4 mmol) was stirred in ethylene glycol (38.8 mL) for 15 min in a bath at 80 °C. Cooling, addition of water (230 mL), and filtration gave the 2-hydroxyethyl ether **24b** (7.23 g, 98%), showing a single spot in TLC [$CHCl_3$−MeOH (19:1)] and with the same IR spectrum as that of an aliquot (mp 168−170 °C) recrystallized from EtOAc (lit.[11] mp 169−170 °C).

c. The sulfide **27a** (4.39 g, 10 mmol) suspended in CH_2Cl_2 (200 mL) was treated with bromine (0.55 mL, 10.75 mmol) in CH_2Cl_2 (40 mL). After being stirred for 2 h the bromide **31** (3.23 g, 81%) was filtered off and added to boiling *tert*-butyl alcohol (800 mL). The solution was refluxed for 10 min, cooled, and evaporated *in vacuo*. Trituration of the residue with saturated aqueous $NaHCO_3$ (240 mL) and filtration yielded the product (2.96 g). Recrystallization from MeOH (200 → 100 mL) gave first some unsaturated material (104 mg, discarded) and then the *tert*-butyl ether **30** (2.17 g, 70%), mp 205−209 °C (lit.[11] mp 208−209 °C).

***N*-[4-(5-Fluorouracil-1-yl)-4-isopropoxybutyl]phthalimide (24d).** The intermediate ***N*-[4-(5-fluorouracil-1-yl)-4-(*p*-tolylthio)butyl]phthalimide (27b)** was prepared in the same way as the lower homologs.[11] Thus, *N*-(4-bromobutyl)-phthalimide afforded in turn ***N*-[4-(*p*-tolylthio)butyl]phthalimide** (using *p*-toluenethiol and NaOMe), mp 54.5−56 °C (MeOH) [Anal. ($C_{19}H_{19}NO_2S$) C, H, N, S]; ***N*-[4-(*p*-tolylsulfinyl)butyl]phthalimide** [using 30% (w/v) aqueous H_2O_2 in EtOH−AcOH], mp 85−87 °C (benzene−petroleum ether) [Anal. ($C_{19}H_{19}NO_3S$) C, H, N, S]; ***N*-[4-acetoxy-4-(*p*-tolylthio)butyl]phthalimide** (by Pummerer rearrangement; 64% yield based on starting bromide using crude sulfide and sulfoxide both obtained in quantitative yield), mp 88−89 °C (petroleum ether, bp 80−100 °C) [Anal. ($C_{21}H_{21}NO_4S$) C, H, N, S]; and **27b** (using silylated 5-FU and $SnCl_4$; 62% yield), mp 207−209.5 °C (MeCN); UV λ_{max} (nm) 271 [Anal. ($C_{23}H_{20}FN_3O_4S$) C, H, N, S], also obtained in the alternative way[11] from the sulfoxide using the crude oily Pummerer trifluoroacetate directly (38% yield based on crude sulfoxide). Hydrazinolysis in $MeOCH_2CH_2OH$ (3 h, 100 °C) of phthalimide **27b** yielded (16%) a sample of the parent amine hydrochloride, mp 257−258 °C dec (EtOH); UV λ_{max} (nm) 270, 258 (sh). Anal. ($C_{15}H_{18}FN_3O_2S$·HCl) C, H, N, S.

Finally **27b** (6.80 g, 15 mmol) was chlorinolyzed using SO_2Cl_2 (1.34 mL, 16.5 mmol) in CH_2Cl_2 (360 mL). After stirring for 15 min, the clear solution was evaporated, leaving the chloride (**28b**) and *p*-toluenesulfenyl chloride. The mixture was treated with hot 2-propanol (150 mL) and refluxed for 20 min, the solution evaporated, and the residue triturated with Et_2O (60 mL). The isopropyl ether **24d** (4.50 g, 77%, sufficiently pure for further reaction) had mp 175.5−177.5 °C (*i*-PrOH); UV λ_{max} (nm) 269 (271 in presence of NaOH). Anal. ($C_{19}H_{20}FN_3O_5$) C, H, N. The methyl ether **24c** prepared similarly had mp 205−207 °C (MeOH); UV λ_{max} (nm) 268 (267 in presence of NaOH). Anal. ($C_{17}H_{16}FN_3O_5$) H,N; C: calcd 56.5, found 55.7.

***N*-[3-(5-Fluorouracil-1-yl)-3-(methylthio)propyl]phthalimide (22b). a.** Methanethiol (about 35 g was used) was passed into a stirred slurry of chloride **28a** (10.55 g, 30 mmol) in $MeNO_2$ (150 mL) for 18 min at room temperature and then 40 min at 100 °C. The resulting solution was evaporated, finally with addition of benzene (100 mL). The crumbly solid was stirred with boiling PhMe (170 mL) and the hot mixture filtered from impurity (0.99 g). The filtrate deposited **22b** (9.55 g, 82%), recrystallization from PhMe giving a solvate, mp 152−155.5 °C [Anal. ($C_{16}H_{14}FN_3O_4S$·0.25C_7H_8) C, H, N]. Compound **22b** had mp 157−158 °C (MeOH); UV λ_{max} (nm) 274 (274, decrease in intensity, in presence of NaOH); ^{1}H NMR δ 11.83 (s, ring 3-H), 8.16 (d, J = 7.0, ring 6-H), 5.60 (t, J = 6.4, SCHN) and 2.04 (s, MeS). Anal. ($C_{16}H_{14}FN_3O_4S$) H, N; C: calcd 52.9, found 53.4.

b. Equimolar amounts of the bromide **31** and thiourea in acetone (10 mL mmol^{-1}) were refluxed for 1 h. The isothiouronium bromide **32** (89%) had mp 197−198 °C (MeOH) [Anal. ($C_{16}H_{15}BrFN_5O_4S$) C, H, N, S]. A mull of **32** (2.36 g, 5 mmol) in dimethyl sulfoxide[36] (2.5 mL) was chilled in ice−water while 2 M NaOH (12.5 mL) was added with stirring. After 15 min at room temperature the solution of the sodium salt of the thiol **29** (phthalamate form) was treated with MeI (2.49 mL, 40 mmol) and acetone (12.5 mL) and stirred 30 min longer. Acetone and excess MeI were evaporated, and the residual cloudy solution (containing very little free 5-FU, from UV spectrum) was brought to pH 4−5, with cooling, using 2 M HCl. The *S*-methylated phthalamic acid was extracted into CH_2Cl_2 (3 × 50 mL), washed with saturated brine (17 mL), and dried ($MgSO_4$). Evaporation gave a gum (2.14 g) which was refluxed in AcOH (20 mL) for 2 h to reform the phthalimide ring. The brown gum remaining after evaporation was triturated with water (5 mL), leaving an insoluble portion (869 mg) showing two main spots in TLC [$CHCl_3$−MeOH (19:1)] of which the slower running was due to the phthalimide **22b**. Its methanolic solution gradually deposited crystals (in all, 494 mg) at −18 °C. Recrystallization from PhMe yielded **22b** (314 mg, 17%), mp 154.5−157 °C.

Application of this reaction requence to the isothiouronium chloride made from chloride **28a** gave **22b** in 11% yield.

The water-insoluble gum (549 mg) from another experiment on the bromide (5 mmol, as above) was carefully chromatographed on a column of silica gel (27.4 g) using CH_2Cl_2 containing increasing amounts of MeOH. Only fractions showing the two spots in TLC were obtained. However, recrystallization of these from PhMe yielded **22b** (165 mg, 9%), mp 151−154 °C, and from the mother liquor the material (99 mg, 5%) faster moving in TLC. This was the N^3-methyl-seconucleoside **26**, mp 145−146 °C (MeOH); UV λ_{max} (nm) 274 (spectrum identical in presence of NaOH, characteristic of N^1,N^3-dialkyl-5-FU). Anal. ($C_{17}H_{16}FN_3O_4S$) C, H, N, S.

***N*-[3-(5-Fluorouracil-1-yl)-3-(isopropylthio)propyl]-phthalimide (25a).** The chloride **28a** (70 mg, 0.2 mmol) and propane-2-thiol (0.37 mL, 4 mmol) were stirred for 2 h in $MeNO_2$ (1 mL) at 100 °C. After cooling, evaporation, and addition of water (2.4 mL) the product (74 mg) was filtered off. Recrystallization from MeOH gave **25a** (39 mg, 50%), mp

192.5−194.5 °C; UV λ_{max} (nm) 275 (274 in presence of NaOH). Anal. ($C_{18}H_{18}FN_3O_4S$) C, H, N.

Reaction at 70 °C or the use of AcOH or DMF as solvents gave unsatisfactory results.

Methyl 3-Phthalimidopropyl Sulfoxide (20b). Methanethiol (5.85 mL, 0.11 mol) was added to 1 M NaOMe (100 mL) and the solution treated with a warm (40 °C) solution of *N*-(3-bromopropyl)phthalimide (26.8 g, 0.1 mol) in MeOH (100 mL). The next day MeOH was replaced by water (100 mL), and methyl 3-phthalimidopropyl sulfide (**16b**), mp 54−57 °C (lit.[37] 56 °C) was filtered off in quantitative yield. Oxidation of a portion (11.75 g, 0.05 mol) in EtOH (120 mL) and AcOH (60 mL) with aqueous H_2O_2 (30% w/v; 5.65 mL) for 3 days in the usual way, finally drying ($MgSO_4$) a $CHCl_3$ solution of the product, gave the sulfoxide **20b**, again in quantitative yield: mp 144−146 °C (EtOH). Anal. ($C_{12}H_{13}NO_3S$) C, H, N, S.

Pummerer Rearrangement of Sulfoxide 20b. a. The sulfoxide (2.51 g, 10 mmol) and NaOAc (3.43 g) were stirred for 3 h in refluxing Ac_2O (46 mL). After cooling and evaporation of solvent, the oily product (2.88 g) was isolated using CH_2Cl_2. No residual sulfoxide would be expected under these reaction conditions, and the product consisted essentially of the isomeric acetates **21b** and **17b**, together with the *trans*-olefin **18**: ^{1}H NMR δ 7.65−7.92 (m, C_6H_4; **21b**, **17b**, and **18**), 5.15 (s, OCH_2S; **17b**), 2.22 (s, MeS; **18**), 2.19 (s, MeS; **21b**), 2.09 (s, $OCOCH_3$; **21b**), and 2.08 (s, $OCOCH_3$; **17b**). Integration at the OCH_2S signal shows the content of **17b** to be about 60%; at MeS, **18** 30−35% and **21b** 5−10%; and at $OCOCH_3$, the percent values for **21b** and **17b** are confirmed.

Treatment of the total product with silylated 5-iodouracil in the usual way resulted merely in recovery of 5-iodouracil (46%).

b. When the sulfoxide **20b** (2.51 g) in CH_2Cl_2 (40 mL) was treated with trifluoroacetic anhydride (1.82 mL) for 5 min, followed by dry pyridine (1.85 mL) for 1.5 h, and the solution washed successively with 1 M HCl and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated, the product (3.04 g) consisted mainly (75−80%) of the linear trifluoroacetate (**17b**, CF_3CO for Ac): ^{1}H NMR δ 7.65−7.92 (m, C_6H_4), 6.48 (s, OCH_2S), 2.15 and 2.14 (both s, MeS). The total content of MeS-containing products (15−20%) is considerably less than in the reaction product from Ac_2O.

Reaction of the total product with silylated 5-FU gave a crude solid product (502 mg), mp 115−125 °C, consisting mainly of N^3-substituted 5-FU derivative. Pure ***N*-{3-[[(5-fluorouracil-3-yl)methyl]thio]propyl}phthalimide** (**17b**, 5-FU[3] for AcO) (143 mg, 4%) has mp 145−147 °C (MeOH); UV λ_{max} (nm) 270 (302 in presence of NaOH). Anal. ($C_{16}H_{14}FN_3O_4S$) C, H, N, S.

Seco-Nucleoside Formation from 3-Phthalimidopropyl *p*-Tolyl Sulfide *via* α-Chlorination. A mixture of this sulfide[11] (311 mg, 1 mmol) and *N*-chlorosuccinimide (NCS) (139 mg, 1.04 mmol) in Et_2O (2.5 mL) and benzene (5 mL) was stirred for 24 h. After filtration, solvents were replaced by CH_2Cl_2, and a little more (total 56 mg) insoluble material filtered off. Evaporation gave a semisolid residue which, in CH_2Cl_2 (7 mL), was added to silylated 5-FU (from 130 mg, 1 mmol). The mixture was treated at −15 °C with $SnCl_4$ (0.1 mL) and left overnight at room temperature. It was filtered through Celite into 1 M HCl (4 mL). A solid (188 mg) separated, mainly N^1-substituted seco-nucleoside. Impurities were extracted into boiling MeOH (50 mL), leaving compound **27a** (143 mg, 33%), mp 243−248 °C (lit.[11] mp 245−247 °C), identified by UV spectral data and TLC.

When the sulfide (**16b**, TolS for MeS) (1 mmol) in CCl_4 (3 mL) was treated with SO_2Cl_2 (0.081 mL, 1 mmol) in CCl_4(1 mL) at 4 °C and stirring continued for 4 h at room temperature, the crystals initially separating mainly redissolved. The solvent was replaced by CH_2Cl_2 (7 mL) and the reaction with silylated 5-FU (1 mmol) carried out as above. The gummy product (96 mg) gradually solidified and gave seco-nucleoside **27a** (60 mg, 14%), mp 245−252 °C (MeOH).

***N*-[3-Methoxy-3-(*p*-tolylthio)propyl]phthalimide (37).** A solution of 3-phthalimidopropyl *p*-tolyl sulfoxide[11] (9.81 g, 30 mmol) in CH_2Cl_2 (120 mL) cooled in ice−water was treated with trifluoroacetic anhydride (5.4 mL, 39 mmol). The bath was removed for 5 min and replaced again. Dry pyridine (5.6 mL) was added and the bath removed once more. After 20 min the solution was shaken successively with 1 M HCl (120 mL) and saturated aqueous $NaHCO_3$ (120 mL), dried ($MgSO_4$), and evaporated. The resulting gummy trifluoroacetate **36** (13.0 g) in MeOH (120 mL) was treated with $TsOH\cdot H_2O$ (2.1 g). Next day the solution had deposited product (5.61 g), mp 62−65 °C. Evaporation of the filtrate, trituration of the solid residue with aqueous $NaHCO_3$, and filtration yielded a second fraction (4.12 g) recrystallized from petroleum ether (bp 60−80 °C) to give further methoxyphthalimide **37** (in all, 8.28 g, 81%). Another recrystallization did not raise the melting point. Anal. ($C_{19}H_{19}NO_3S$) C, H, N.

A solution of the *O*-acetate[11] (554 mg, 1.5 mmol) analogous to **36** on similar treatment deposited the ether **37** (182 mg), and workup gave a second fraction (total 295 mg, 58%). Reaction of the trifluoroacetate **36** in refluxing MeOH (2 h), or at room temperature omitting TsOH, gave products in lower yield which were more difficult to purify.

***N*-[2-Methoxy-2-(*p*-tolylthio)ethyl]phthalimide (33b).** 2-Phthalimidoethyl *p*-tolyl sulfoxide[11] (313 mg, 1 mmol) was converted into the trifluoroacetate (homolog of **36**) as above. This was treated with MeOH (4 mL) and $TsOH\cdot H_2O$ (70 mg). Refluxing for 2 h, evaporation, and trituration of the solid residue with aqueous $NaHCO_3$ yielded a product (261 mg), mp 75−80 °C, showing three spots in TLC [C_6H_6−MeOH (4:1)]. The strongest corresponded to the *O*,*S*-acetal, with a slower to the *O*,*O*-dimethyl acetal and the fastest to a byproduct such as di(*p*-tolyl) disulfide. Recrystallization from MeOH gave the main component **33b** (103 mg, 32%): mp 123.5−125.5 °C (benzene−petroleum ether); ^{1}H NMR δ 7.63−7.90 [m, $C_6H_4(CO)_2$], 7.06−7.46 (m, C_6H_4S), 5.01 (t, J = 6.8, SCHO), 3.96(d, J = 7.1, CH_2), 3.47 (s, MeO), and 2.33 (s, MeC). Anal. ($C_{18}H_{17}NO_3S$) C, H, N.

A similar reaction solution after 24 h at room temperature yielded only a gummy mixture (305 mg) containing much starting material. Repetition of the latter experiment with the analogous *O*-acetate[11] (355 mg, 1 mmol) showed a similar pattern (TLC), and this solution was kept a further 24 h before evaporation and washing in CH_2Cl_2 with aqueous $NaHCO_3$. The resulting gum (311 mg) consisted mainly of *O*,*S*-acetal and *O*,*O*-acetal. Recrystallization from MeOH afforded only the latter, *N*-(2,2-dimethoxyethyl)phthalimide (67 mg, 28%), mp 105−108 °C (MeOH) (lit.[38] mp 103 °C); ^{1}H NMR δ 7.6−7.9 [m, $C_6H_4(CO)_2$], 4.78 (t, J = 5.7, OCHO), 3.83 (d, J = 5.7, CH_2) and 3.38 (s, 2MeO). For C_6H_6−MeOH (4:1) using Merck aluminum plates (Art. 5554) precoated with silica gel 60 F_{254}, R_f values for the *O*,*O*-dimethyl acetal, starting *S*-*p*-tolyl *O*-acetate, and *O*-methyl *S*-*p*-tolyl acetal are respectively 0.73, 0.80, and 0.84.

The *S*-*p*-tolyl *O*-acetate (355 mg) in refluxing MeOH was comparable to the trifluoroacetate, yielding (from MeOH) *O*,*S*-acetal (101 mg, 31%) followed by *O*,*O*-acetal (31 mg, 12%) from the mother liquor.

Reaction of the Trifluoroacetate 36 with Silylated Uracil. The trifluoroacetate **36**, made as above from the sulfoxide (654 mg, 2 mmol), in CH_2Cl_2 (10 mL) was added to silylated uracil (from 224 mg, 2 mmol) and the mixture treated at −15 °C with $SnCl_4$ (0.2 mL). The cooling bath was removed, and next day shaking with 1 M HCl (8 mL) yielded a foam (837 mg) from the organic layer. This crystallized very slowly from MeOH, giving material (643 mg, *ca*. 75%) showing two principal spots of equal intensity and very similar mobility in TLC [C_6H_6−MeOH (4:1)]. The UV spectrum [λ_{max} (nm) 261 (287 in presence of NaOH)] confirmed the presence of N^3-substituted seco-nucleoside **40** and N^1-isomer (ca. 1:1). Recrystallization (MeOH, C_6H_6, or MeCN) did not change the TLC pattern.

***N*-[3-(5-Fluorouracil-3-yl)-3-methoxypropyl]phthalimide (42b).** To a solution of the sulfide **37** (938 mg, 2.75 mmol) and cyclohexene (0.30 mL, 3 mmol) in CH_2Cl_2 (12.5 mL) cooled in ice−water was added dropwise, with hand-swirling, SO_2Cl_2 (0.24 mL, 3 mmol) in CH_2Cl_2 (2.5 mL) (internal temperature, 2 °C → 7 °C → 2 °C). After 30 min in the bath, the solution (always virtually colourless) was evaporated, and the oily chloro ether **38** in DMF (3.75 mL) at −15 °C treated all

at once with a solution of N^1-[(octylthio)carbonyl]-5FU[22b] (755 mg, 2.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.41 mL, 2.75 mmol) in DMF (2.5 mL) from a funnel, continuing to exclude moisture. Crystals soon separated, and after 1 h at −15 °C as much solvent as conveniently possible was removed below 40 °C *in vacuo*, and the residue triturated with aqueous AcOH (5%; 25 mL). The resulting solid was filtered off, washed with water, taken up in CH_2Cl_2, dried ($MgSO_4$), and isolated by evaporation. Trituration with Et_2O (25 mL) yielded the N^1-(octylthio)carbonyl derivative **39b** (982 mg, 76%), mp 151.5−152.5 °C (MeCN) [Anal. ($C_{25}H_{30}FN_3O_6S$) C, H, N]; UV λ_{max} (nm) 271, shifted immediately to 304 on addition of NaOH, corrresponding to deprotection. In contrast, the spectrum of the reagent N^1-[(octylthio)carbonyl]-5-FU, with UV λ_{max} (nm) 267 (263, 280 sh in present of NaOH), shifts completely to λ_{max} (nm) 298 in alkali only after 20−30 min.

The derivative in MeCN (15 mL) was filtered from a trace of insoluble material and treated at room temperature with isopropylamine (0.18 mL, 2.1 mmol). After 30 min, evaporation, trituration with Et_2O (10 mL), and filtration yielded **42b** (550 mg, 84%), mp 182−184 °C (EtOAc); UV λ_{max} 273 (303 in presence of NaOH). Anal. ($C_{16}H_{14}FN_3O_5$) C, H, N.

Reaction of the chloro ether **38** for 1 h at room temperature afforded **39b** in 63% yield. In two other experiments on sulfide **37** (3.3 mmol) cyclohexene was omitted and the bright yellow oily products extracted with petroleum ether (5 + 3 mL) to remove much of the sulfenyl chloride; reaction (overnight) of the relatively insoluble **38** at 0 °C and at −15 °C respectively gave **39b** in yields of 28% and 55%.

***N*¹-[(Octylthio)carbonyl]uracil.** No details about the preparation or properties of this compound are available.[29] Uracil (784 mg, 7 mmol) was dissolved in DMF (28 mL) at 110 °C and the solution cooled (just) to room temperature. *S*-Octyl chlorothioformate (1.61 g, 7.7 mmol) in DMF (7 mL) was added in one portion, followed by pyridine (5.6 mL). The next day the solution was filtered from a trace of solid and evaporated. Trituration of the crystalline residue with aqueous AcOH (5%; 42 mL) yielded crude product (1.40 g), reduced (to 1.07 g) by washing with petroleum ether (12 mL). Treatment with water (14 mL) at 100 °C removed a little uracil, leaving N^1-[(octylthio)carbonyl]uracil (947 mg, 48%), mp 163.5−167 °C. The analytical sample (from MeCN) had mp 169−171 °C [Anal. ($C_{13}H_{20}N_2O_3S$) C, H, N]; UV λ_{max} (nm) 262 (261 in presence of NaOH; after 20−30 min, shifts completely to 287 indicating cleavage to uracil). A reaction time of 3 days did not increase the yield, nor addition of various proportions of MeCN as solvent.

***N*-[3-(Uracil-3-yl)-3-methoxypropyl]phthalimide (42a).** The sulfide **37** (2.73 g, 8 mmol) was converted into the chloride **38** as described before. This was treated in DMF (11 mL) with a solution of N^1-[(octylthio)carbonyl]uracil (2.27 g, 8 mmol) and DBU (1.32 mL, 8.8 mmol) in DMF (27 mL) at room temperature. After 1 h the solvent was evaporated, and workup gave crude **39a** (3.10 g), contaminated with some reagent. This was treated in MeCN (51 mL) with isopropylamine (0.8 mL) for 30 min. Uracil (200 mg, 22%) was filtered off, and evaporation, trituration with Et_2O (30 mL), and filtration yielded **42a** (1.65 g, 62% based on **37**). Recrystallization from MeOH gave a product (1.36 g), mp 159−172 °C, containing some solvent, and the analysis sample (from MeCN; dried at 100 °C *in vacuo*) had mp 171.5−174.5 °C; UV λ_{max} (nm) 264, 298 sh (294 in presence of NaOH). Anal. ($C_{16}H_{15}N_3O_5$) C, H, N.

***N*-[3-(5-Fluorouracil-1-yl)-3-(5-fluorouracil-3-yl)propyl]phthalimide (35).** A mixture of the chloride **28a** (4.39 g, 12.5 mmol) and DMF (25 mL) was treated dropwise over 10 min with a solution of N^1-[(octylthio)carbonyl]-5-FU (3.40 g, 11.25 mmol) and NEt_3 (1.74 mL, 12.5 mmol) in DMF (18 mL). Stirring was continued overnight and the solvent then evaporated. Addition of aqueous AcOH (5%; 112 mL) gave a semisolid which was taken up in CH_2Cl_2 (125 mL). Extraction of the aqueous layer with further CH_2Cl_2 (2 × 30 mL), drying ($MgSO_4$), and evaporation yielded the crude (octylthio)carbonyl intermediate as a syrup which was dissolved in MeCN (150 mL) and clarified by filtration. It was stirred with isopropylamine (1.07 mL, 12.5 mmol) for 30 min (the solid which initially separated redissolved) and the solvent then replaced by Et_2O (240 mL). The resulting gummy solid (4.91 g) hardened and was recrystallized from MeOH (10 mL). The *N*,*N*-acetal **35** (2.43 g, 48%) had mp 175−215 °C (MeOH); UV λ_{max} (nm) 268 (274, 304 in presence of NaOH); ^{1}H NMR δ 11.66 (bs, 3-H in 5-FU and 1-H in 5-FU[3]), 8.29 (d, J = 7.9, 6-H in 5-FU), 7.83 (m, 6-H in 5-FU[3] and C_6H_4) and 6.80 (t, J = 8.1, NCHN). Anal. ($C_{19}H_{13}F_2N_5O_6.0.5H_2O$) C, H, N.

***N*-(2-Chloroethyl)-*N*-nitrosoureas (Table 5).** The starting phthalimides were hydrazinolyzed either in DMF[10,15] (1.5 mL mmol^{-1}) at −15 °C overnight (**24a**,**b**, **22b**, **35**; **30** at 3 °C), or during 1 h in refluxing MeOH (8 mL mmol^{-1}) for N^3-substituted seco-nucleosides (**42a**,**b**) where formation of a bicyclic isomer[30] is not a potential problem. Hydrazine hydrate (1.1 equiv) was used initially (**24a**,**b**, **30**, **22b**), filtering off phthalohydrazide (from water) after acidification and evaporation of DMF. When $N_2H_4{\cdot}H_2O$ (2 equiv) was used (**42a**,**b**, **35**), excess reagent was subsequently removed as benzalazine.[15] The aqueous methanolic solution of amine hydrochloride was treated[10,15] with NaOMe and 2,4,5-trichlorophenyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate for 2 h at 0−5 °C. After quenching and extraction into CH_2Cl_2 (**5**, **10**, **4**) or EtOAc, 2,4,5-trichlorophenol was removed by trituration of total product with Et_2O. The CNUs crystallized except in the case of **9** (solvate from EtOAc) and **10** which required chromatography on a column of silica gel [elution with CH_2Cl_2−MeOH (9:1)] and crystallized from H_2O−EtOH (6:4). The other compounds were recrystallized from EtOH.

For the C_4 seco-nucleoside **24d,** bicyclic isomer[30] formation is also unlikely, and hydrazinolysis (using 1.1 equiv in $MeOCH_2CH_2OH$ during 1 h at 100 °C), followed by evaporation, acidification with 0.25 N HCl and filtration, and reevaporation, left crude amine hydrochloride which with trifluoroacetic anhydride in EtOAc yielded (20−30%) the trifluoroacetamide (**24d**, CF_3CONH for PhtN), mp 150.5−152 °C (EtOAc−petroleum ether); UV λ_{max} (nm) 268 (267 in presence of NaOH) [Anal. ($C_{13}H_{17}F_4N_3O_4$) C, H, N]. The product of hydrazinolysis of **24d** at −15 °C in DMF was very impure, and best results were obtained by warming **24d** (3.50 g, 9 mmol) in 0.1 N NaOMe (90 mL) and $N_2H_4{\cdot}H_2O$ (1.34 mL, 27 mmol) for 30 min at 45 °C. Acidification, evaporation, and benzalazine workup and reaction with the nitrosocarbamate as before gave a product which was extracted into CH_2Cl_2, chromatographed on a silica gel column and crystallized from EtOH, yielding 1.09 g (31%).

Biological Evaluation. Tumor Systems: MAC Tumors (Bradford). The MAC system has been shown to be a good model of human colorectal cancer in terms of its chemosensitivity[39] and has been shown to have the appropriate characteristics to be used in the development of new combination regimens.[40] MAC 15A (1 × 10^6 cells) was injected intraperitoneally (ip) in male NMRI mice, and MAC 13 tumor fragments were implanted subcutaneously (sc) in the flank of female NMRI mice by means of a trocar.

Mammary Carcinoma (MCa) (Zagreb). A total of 1 × 10^6 viable MCa tumor cells were injected intramuscularly (im) into the right flank of inbred 10−14-week-old CBA/H mice at the Ruder Boškovic Institute, Zagreb.[41]

Colon 38 Adenocarcinoma (Brussels). The Colon 38 adenocarcinoma originated from the NCI Frederick Cancer Research Facility (Frederick, MD) and was serially maintained in C57B1/6 mice. Tumor fragments of 3 mm × 3 mm were transplanted sc in B6D2F1 mice (weighing 19−22 g) on day 0. The B6D2F1 mice used for testing were purchased also from Frederick.

Experimental Chemotherapy: MAC Tumors. (i) MAC 13. Two days after transplantation, when tumors were ∼2 mm^3 in volume, mice were allocated into groups by restricted randomization. Compounds for injection were made up freshly, and treatment (0.1 mL/10 g of body weight) was ip. Chemosensitivity was assessed by tumor weight inhibition 21 days later and presented as *T*/*C* % where *T* represents mean weight of treated tumors and *C* represents mean weight of control tumor.

(ii) MAC 15A. Animals bearing MAC 15A tumor cells were allocated into groups and treated (0.1 mL/10 g of body weight) on day 2. Antitumor activity was determined by comparison

of the lifespan of treated and control groups. Deaths were recorded and the median survival time (MST) determined.

Mammary Carcinoma (MCa). Drugs were administered ip as a single dose at a constant volume of 0.02 mL/g of body weight on day 1 after tumor cell implantation. Effects of therapy were assessed by comparison of MST of treated and control groups.

Colon 38 Adenocarcinoma. All compounds were prepared freshly on the day of injection. B.3958, B.3970, and B.3971 were dissolved in saline (0.9% NaCl) while the other compounds were suspended in saline by adjunction of Tween 80 (0.1%). All treatments were intraperitoneally (ip) administered on days 2 and 9 as volumes of 0.1 mL/10 g of mouse body weight and all individual animal body weights recorded on days 2 and 20.

The tumor size measured on day 20 was converted into tumor volume by using the formula $P = L(W^2/2)$ where P is the tumor weight in mg when the length (L) and width (W) are given in millimeters. The median tumor volume was calculated for both treated and untreated control groups. The T/C, *i.e.*, the median tumor volume of the treated group divided by that of the control group, indicated antitumor activity. A T/C of <42% was considered necessary to demonstrate activity.

Acknowledgment. We are grateful to Rhône-Poulenc Ltd. (Dagenham) for microanalyses and IR spectra, to Professor T. B. H. McMurry of the University Chemical Laboratory, Trinity College, Dublin, for NMR spectra, and to Neil Lucey for preparation of intermediates. We acknowledge with thanks financial support from the Irish Cancer Society, Bradford's War on Cancer, and the Screening and Pharmacology Group of the EORTC. We thank Mr. George Curran of BASF Ireland Ltd. for a generous gift of *S*-octyl chlorothioformate.

References

(1) Haddow, A.; Timmis, G. M. Myleran (GT41) in Chronic Myeloid Leukaemia. Chemical Constitution and Biological Action. *Lancet* **1953**, *264* (i), 207−208. Bergel, F.; Stock, J. A. Cyto-active Amino-acid and Peptide Derivatives. Part I. Substituted Phenylalanines. *J. Chem. Soc.* **1954**, 2409−2417. Arnold, H.; Bourseaux, F. Cytostatic Active Cyclic *N′,O*-Alkylene-*N,N*-bis-(β-chloroethyl) Diamidophosphates. *Angew. Chem.* **1958**, *70*, 539−544.

(2) Schabel, F. M., Jr.; Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A.; Laster, W. R., Jr; Skipper, H. E. Experimental Evaluation of Potential Anticancer Agents. VIII. Effects of Certain Nitrosoureas on Intracerebral L1210 Leukaemia. *Cancer Res.* **1963**, *23*, 725−733. Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. Synthesis of Chlorozotocin, the 2-Chloroethyl Analog of the Anticancer Antibiotic Streptozotocin. *J. Med. Chem.* **1975**, *18*, 104−106.

(3) Baer, J. C.; Freeman, A. A.; Newlands, E. S.; Watson, A. J.; Rafferty, J. A.; Margison, G. P. Depletion of O^6-Alkylguanine-DNA-Alkyltransferase Correlates with Potentiation of Temozolomide and CCNU Toxicity in Human Tumour Cells. *Br. J. Cancer* **1993**, *67*, 1299−1302. Arris, C. E.; Bleasdale, C.; Calvert, A. H.; Curtin, N. J.; Dalby, C.; Golding, B. T.; Griffin, R. J.; Lunn, J. M.; Major, G. N.; Newell, D. R. Probing the Active Site and Mechanism of Action of O^6-Methylguanine-DNA-Methyltransferase with Substrate Analogues (O^6-Substituted Guanines). *Anti-Cancer Drug Des.* **1994**, *9*, 401−408. Clark, A. S.; Deans, B.; Stevens, M. F. G.; Tisdale, M. J.; Wheelhouse, R. T.; Denny, B. J.; Hartley, J. A. Antitumor Imidazotetrazines. 32. Synthesis of Novel Imidazotetrazinones and Related Bicyclic Heterocycles to Probe the Mode of Action of the Antitumor Drug Temozolomide. *J. Med. Chem.* **1995**, *38*, 1493−1504. Chae, M.-Y.; Swenn, K.; Kanugula, S.; Dolan, M. E.; Pegg, A. E.; Moschel, R. C. 8-Substituted O^6-Benzylguanine, Substituted 6(4)-(Benzyloxy)-pyrimidine, and Related Derivatives as Inactivators of Human O^6-Alkylguanine-DNA-Alkyltransferase. *J. Med. Chem.* **1995**, *38*, 359−365.

(4) McCormick, J. E.; McElhinney, R. S. Nitrosoureas from Chemist to Physician: Classification and Recent Approaches to Drug Design. *Eur. J. Cancer* **1990**, *26*, 207−221.

(5) McCormick, J. E.; McElhinney, R. S. Thio-sugars. Part 7. Secouridines with Amino-groups in the "Carbohydrate" Component: Intramolecular Addition across the 5,6-Double Bond and Molecular Combination of 5-Fluorouracil and *N*-(2-Chloroethyl)-*N*-nitrosourea Residues. *J. Chem. Res.* (*S*) **1981**, 310−311; (*M*) 3601−3641.

(6) Bibby, M. C; Double, J. A.; McCormick, J. E.; McElhinney, R. S.; Radacic, M.; Pratesi, G.; Dumont, P. Nucleoside Analogues. 13. The Effect on Anti-tumour Activity of Varying the Uracil 5-Substituent and the Point of Attachment (N^1 or N^3) of the Uracil Moiety in Seco-nucleoside Nitrosoureas. *Anti-Cancer Drug Des.* **1993**, *8*, 115−128.

(7) (a) McCormick, J. E.; McElhinney, R. S. Thio-sugars. Part 3. 4-Thiotetrofuranose Nucleosides. *J. Chem. Soc.* **1978**, 500−505. (b) McCormick, J. E.; McElhinney, R. S. Thio-sugars. Part 6. Seco-nucleosides related to 4-Thio-DL-ribofuranose. *J. Chem. Res.* (*S*) **1981**, 12−13; (*M*) 256−281. (c) McElhinney, R. S.; McCormick, J. E.; Lucey, C. M. Evolution of Molecular Combinations Involving 5-Fluorouracil. *Cancer Treat. Rev.* **1988**, *15*, 73−81.

(8) McCormick, J. E.; McElhinney, R. S.; McMurry, T. B. H.; Maxwell, R. J. Nucleoside Analogues. Part 12. The Anomalous ^{19}F NMR Spectrum of B.3996, a Molecular Combination of 5-Fluorouracil and *N*-(2-Chloroethyl)-*N*-nitrosourea and Synthesis of its *N′*-Nitroso Isomer and Related Compounds. *J. Chem. Soc., Perkin Trans. 1* **1991**, 877−880.

(9) McElhinney, R. S.; McCormick, J. E.; Bibby, M. C.; Double, J. A.; Atassi, G.; Dumont, P.; Pratesi, G.; Radacic, M. Nucleoside Analogues: 7. Effect on Colon, Breast, and Lung Tumours in Mice of 5-Fluorouracil/Nitrosourea Molecular Combinations Incorporating Alkoxy and Oxidized Sulphur Functions. *Anti-Cancer Drug Des.* **1989**, *3*, 255−269.

(10) McElhinney, R. S.; McCormick, J. E.; Bibby, M. C.; Double, J. A.; Atassi, G.; Dumont, P.; Pratesi, G.; Radacic, M. Nucleoside Analogues. 9. Seco-nucleoside Analogues of some 5-Fluorouracil/Nitrosourea Molecular Combinations having Uracil as Base: Synthesis and Anti-Tumour Activity. *Anti-Cancer Drug Des.* **1989**, *4*, 191−207.

(11) McCormick, J. E.; McElhinney, R. S. Nucleoside Analogues. Part 1. Some 5-Fluorouracil Seco-nucleosides and their Hydrolysis by Dilute Acid. *J. Chem. Res.* (*S*) **1983**, 176−177; (*M*) 1736−1769.

(12) Double, J. A.; Bibby, M. C.; McCormick, J. E.; McElhinney, R. S. Nucleoside Analogues. 5. Molecular Combination of Anticancer Drugs: Activity of 5-Fluorouracil/Nitrosourea Combinations against Mouse Colon Tumours. *Anti-Cancer Drug Des.* **1986**, *1*, 133−139.

(13) Roger, P.; Monneret, C.; Fournier, J.-P.; Choay, P.; Gagnet, R.; Gosse, C.; Letourneux, Y.; Atassi, G.; Gouyette, A. Rationale for the Synthesis and Preliminary Biological Evaluation of Highly Active New Antitumour Nitrosoureido Sugars. *J. Med. Chem.* **1989**, *32*, 16−23.

(14) McCormick, J. E.; McElhinney, R. S. Nucleoside Analogues. Part 6. The Preparation by Dephthaloylation and Properties of *N*-(ω-Aminoalkyl)uracils, Key Intermediates in the Synthesis of Molecular Combinations of Certain Anti-tumour Agents. *Proc. R. Irish. Acad.* **1989**, *89B*, 213−228.

(15) Lucey, N. M.; McCormick, J. E.; McElhinney, R. S. Nucleoside Analogues. Part 10. Synthesis of Three Unusual 5-Fluorouracil Seco-nucleosides Incorporating *N*-(2-Chloroethyl)-*N*-nitrosourea Residues. *J. Chem. Soc., Perkin Trans. 1* **1990**, 795−802.

(16) McElhinney, R. S.; McCormick, J. E.; Bibby, M. C.; Double, J. A.; Atassi, G.; Dumont, P.; Pratesi, G.; Radacic, M. Nucleoside Analogues: 8. Some Isomers of B.3839, the Original 5-Fluorouracil/Nitrosourea Molecular Combination, and Their Effect on Colon, Breast and Lung Tumours in Mice. *Anti-Cancer Drug Des.* **1989**, *4*, 1−20.

(17) Dilworth, B. M.; McKervey, M. A. Organic Synthesis with α-Chlorosulfides. *Tetrahedron* **1986**, *42*, 3731−3752.

(18) Tuleen, D. L.; Stephens, T. B. Chlorination of Unsymmetrical Sulfides. *J. Org. Chem.* **1969**, *34*, 31−35.

(19) Meffre, P.; Lhermitte, H.; Vo-Quang, L.; Vo-Quang, Y.; Le Goffic, F. A New Class of Unusual α-Aminoacids: α-Aminoacid β,γ-Enol Thioethers. *Tetrahedron Lett.* **1991**, *32*, 4717−4720.

(20) Cerny, M.; Pacák, J. Eine Neue Methode zur Darstellung Acetylierter β-D-Thioglucopyranoside. (A New Method for the Preparation of Acetylated β-D-Thioglucopyranosides.) *Collect. Czech. Chem. Commun.* **1959**, *24*, 2566−2569. Durette, P. L.; Shen, T. Y. Insulin-like, and Insulin-Antagonistic, Carbohydrate Derivatives. The Synthesis of Aryl and Aralkyl D-Mannopyranosides and 1-Thio-D-mannopyranosides. *Carbohydr. Res.* **1980**, *81*, 261−274.

(21) **27a** is obtained (50−60% yield overall) from TolS$(CH_2)_3$NPht *via* sulfoxide and Pummerer trifluoroacetate **36**;[11] *via* α-chlorination using NCS the yield of **27a** is 33%, using sulfuryl chloride 14%. The NCS sequence on MeS$(CH_2)_3$NPht (**16b**) gave only a trace (3% yield) of **22b**, isolated with some difficulty. Reaction of α-chloro sulfides with nitrogen nucleophiles has been infrequent,[17,22] and their use in nucleoside synthesis[23] has been superseded.[7a,24]

(22) (a) Worley, J. W. *N*-(α-Chloroalkyl)phthalimides. *J. Org. Chem.* **1979**, *44*, 1178−1180. (b) Ozaki, S.; Watanabe, Y.; Fujisawa, H.; Hoshiko, T. 5-Fluorouracil Derivatives. VI. Alkylation of 5-Fluorouracil Using the (Octylthio)carbonyl Group as a Protecting Function. *Heterocycles* **1984**, *22*, 527−530.

(23) Bobek, M.; Block, A.; Parthasarathy, R.; Whistler, R. L. Synthesis and Biological Activity of 5-Fluoro-4′-thiouridine and Some Related Nucleosides. *J. Med. Chem.* **1975**, *18*, 784−787 and references cited.
(24) Uenishi, J.; Motoyama, M.; Nishiyama, Y.; Wakabayashi, S. Stereocontrolled Preparation of Cyclic Xanthate; a Novel Synthetic Route to 4-Thiofuranose and 4′-Thionucleoside. *J. Chem. Soc., Chem. Commun.* **1991**, 1421−1422. Secrist, J. A., III; Riggs, R. M.; Tiwari, K. N.; Montgomery, J. A. Synthesis and Anti-HIV Activity of 4′-Thio-2′,3′-dideoxynucleosides. *J. Med. Chem.* **1992**, *35*, 533−538.
(25) Chloride in **28a** (C_3) is displaced in good yield by alkoxy by refluxing briefly in lower alcohols, while compound **23** (C_2) can be recrystallized from methanol and only reacts after longer heating in the presence of Ag^+. In turn the ether **24a** (C_3) is hydrolyzed by dilute acid very much faster than **19** (C_2). The deactivating effect of a 2-substituent has long been known in sugar chemistry, confirmed by the great difference in rates of acid hydrolysis of 5-fluorouridine and 5-fluoro-2′-deoxyuridine.[11] The *S*-oxides of **27a** (C_3) and of **22a** (C_2) also differ greatly in the rate of acid hydrolysis to 5-FU; further, the C_3 sulfoxide yields olefin by elimination of sulfenic acid smoothly in boiling toluene, whereas the C_2 sulfoxide requires fusion at 215 °C *in vacuo*.
(26) In a direct comparison, the C_2 homolog of the *p*-tolyl trifluoroacetate **36** was found to react very incompletely with methanol at room temperature, forming several products; at reflux temperature *O,S*-acetal **33b** (32%) was obtained and some *O,O*-dimethyl acetal identified. The corresponding C_2 *p*-tolyl acetate[11] (*i.e.*, **21a** with Tol for Me) had similar reactivity: at room temperature displacement of arylthio was extensive and *O,O*-dimethyl acetal (28% after 48 h) was the only product isolated by recrystallization. The C_3 *p*-tolyl acetate[11] analogous to **36** was only slightly less reactive than **36**, giving **37** in 58% yield.
(27) Summers, L. The α-Haloalkyl Ethers. *Chem. Rev.* **1955**, *55*, 301−353 (p 337).
(28) Antonsen, O.; Benneche, T.; Hagelin, G.; Undheim, K. Synthesis of Unsaturated Chloromethyl Ethers. *Acta Chem. Scand.* **1989**, *43*, 56−61.
(29) Ozaki, S.; Watanabe, Y.; Hoshiko, T.; Fujisawa, H.; Uemura, A.; Ohrai, K. 5-Fluorouracil Derivatives. VII. Tert-amine Promoted *N*-Glycosidation of Pyrimidines. *Tetrahedron Lett.* **1984**, *25*, 5061−5062.
(30) McCormick, J. E.; McElhinney, R. S. Nucleoside Analogues. Part 2. Further Molecular Combinations of (5-Substituted) Uracil and *N*-(2-Chloroethyl)-*N*-nitrosourea Residues as Anticancer Agents. *J. Chem. Soc., Perkin Trans. 1* **1985**, 93−100.
(31) Ferrier, R. J.; Tyler, P. C. Photobromination of Carbohydrate Derivatives. Part 5. Preparation and Reactions of (5*S*)-1,2,3,4-Tetra-*O*-acetyl-5-bromo-β-D-xylopyranose; a New Type of "Double-Headed" Nucleoside. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2767−2773.
(32) Stenhouse, J. *Liebigs Ann. Chem.* **1840**, *33*, 92. Zaugg, H. E.; Martin, W. B. α-Amidoalkylations at Carbon. *Org. React.* **1965**, *14*, 52−269 (p 107).
(33) Johnson, H. E.; Crosby, D. G. Reaction of Hydantoin with Acetals. *J. Org. Chem.* **1962**, *27*, 2077−2080.
(34) Loadman, P. M.; Matthew, A. M.; McCormick, J. E.; McElhinney, R. S.; Bibby, M. C. Pharmacokinetics and Anti-Tumour Activity of B.4152, a Reactive Molecular Combination of 5-Fluorouracil and *N*-(2-Chloroethyl)-*N*-nitrosourea, and its Uracil Analogue. *Anti-Cancer Drug Des.*, in the press.
(35) Loadman, P. M.; Bibby, M. C.; Double, J. A.; McElhinney, R. S. Pharmacokinetics and Cytotoxicity of B.3839, a Molecular Combination of 5-Fluorouracil and *N*-(2-Chloroethyl)-*N*-nitrosourea, in a Mouse Model. *Invest. New Drugs* **1992**, *10*, 149−158.
(36) Pan, H.-L.; Fletcher, T. L. A Facile Preparation of Sulphydryl Compounds in Dimethyl Sulphoxide. *Chem. Ind. (London)* **1968**, 546.
(37) Karrer, P.; Scheitlin, E.; Siegrist, H. Über Homologe des Sulforaphans und über ω-Aminoalkyl-sulfoxyde. (A Study of Homologs of Sulforaphan and of ω-Aminoalkyl Sulfoxides.) *Helv. Chim. Acta* **1950**, *33*, 1237−1245.
(38) Zábransky, J.; Houska, M.; Kálal, J. Aldehydes and Acetals Based on Acryl- and Methacrylamides as Potential Monomers. *Makromol. Chem.* **1985**, *186*, 223−229.
(39) Double, J. A.; Ball, C. R. Chemotherapy of Transplantable Adenocarcinomas of the Colon in Mice. *Cancer Chemother. Rep.* **1975**, *59*, 1083−1089.
(40) Cowen, D. M.; Siegerstetter, J.; Janik, A. C.; Double, J. A. The Assessment of Response of Murine Transplantable Colon Tumours to Combination Chemotherapy. *J. Cancer Res. Clin. Oncol.* **1982**, *103*, 119−126.
(41) Radacic, M.; Boranic, M.; Basic, I. A Mouse Mammary Carcinoma as a Model for Evaluation of Drug Effects on Primary Tumour Growth and Metastases. In *Tumour Progression and Markers*; Lapis, K., Jeney, A., Eds.; Kluger Publications: Amsterdam, 1982; pp 217−223.

JM9507237