by filtration and thoroughly washed with water and Et_2O. The solid was then stirred with 50 mL of saturated $NaHCO_3$, followed by filtration. The filter cake was washed with water and dried to leave 1.59 g. The solid was twice extracted with hot (100 °C) DMF and the solvent was evaporated in vacuo to leave 0.96 g (41%) of pale yellow solid diester **11a**; MS *m/e* 509 ($C_{26}H_{31}N_5O_6$).

A suspension of **11a** (609 mg, 1.20 mmol) in 22 mL of EtOH was treated with 1.74 mL (22.6 mmol) of CF_3COOH to give a solution. PtO_2 (890 mg) was added and the mixture was stirred under 1 atm of H_2 for 44 h. The catalyst was removed by filtration through Celite and the filtrate was evaporated to dryness. The residue was dissolved in 30 mL of $CHCl_3$, washed with 15 mL of saturated $NaHCO_3$, and dried over $MgSO_4$, and the $CHCl_3$ was evaporated to leave 0.43 g of yellow gum. The material was chromatographed on 14 g of silica gel with elution by $CHCl_3$–MeOH (95:5) to afford 0.24 g of **12a** as a pale yellow semisolid; MS *m/e* 513 ($C_{26}H_{35}N_5O_6$).

The tetrahydro diester (225 mg) was treated with 10 mL of EtOH and 20 mL of 0.1 N NaOH and the solution was heated at 100 °C for 15 min. The solution was cooled, adjusted to pH 5 with HOAc, and evaporated in vacuo at 25 °C until precipitation of the product occurred. The precipitate was collected, washed with H_2O, and dried to leave 131 mg (66%) of **13a**; UV_{max} (pH 13) 240 (ε 19746), 269 nm (ε 11504); MS *m/e* 457; NMR (DMSO-d_6) δ 1.2 (3 H, d, CH_3), 1.45 (3 H, C6 + 9-CH_2), 2.0 (2 H, m, CH_2-glu), 2.3 (2 H, d, CH_2COOH) 2.8–3.3 (5 H, m, C-5-CH_2, 10-H, 7-CH_2), 4.36 (1 H, d, NHC*H*), 5.9 (2 H, s, NH_2), 7.3 (2 H, d, 3′,5′-ArH), 7.76 (2 H, d, 2′,6′-ArH), 8.45 (1 H, d, CONH). Anal. Calcd for $C_{22}H_{27}N_5O_6 \cdot 2H_2O$: C, H, N.

10-Ethyl-5,6,7,8-tetrahydro-5,10-dideazafolic acid (13b) was similarly obtained from **10b**. Crude diester **11b** was obtained in 39% yield: MS *m/e* 253 ($C_{27}H_{33}N_5O_6$). Hydrogenation over PtO_2 gave tetrahydro diester **12b** in 25% yield as the trifluoroacetate salt: mp 191–193 °C; MS *m/e* 527 ($C_{27}H_{37}N_5O_6$); NMR ($CDCl_3$) δ 0.73 (3 H, t, CH_3), 1.25 (6 H, m, ester CH_3), 1.65 (5 H, br s, 6-H, 9-CH_2, CH_2CH_3), 2.1–2.5 (4 H, m, $-CH_2CH_2-$), 2.5–3.3 (5 H, m, 5-CH_2, 10-H, 7-CH_2), 4.12 (4 H, q, $-OCH_2$), 4.75 (1 H, m, C*H*NH), 7.22 (2 H, d, 3′,5′-ArH), 7.78 (2 H, d, 2′,6′-ArH), 8.25 (1 H, br s, CONH). Anal. Calcd for $C_{27}H_{37}N_5O_6 \cdot CF_3COOH \cdot {}^1/_2H_2O$: C, H, N, F.

Saponification as above afforded **13b** in 59% yield: UV_{max} (pH 13) 240 (ε 19445), 271 nm (ε 11227). Anal. Calcd for $C_{23}H_{29}N_5O_6 \cdot 1.25H_2O$: C, H, N (calcd 14.2, found 13.7).

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Registry No. **1a**, 123751-61-1; **1b**, 123751-62-2; **2a**, 123751-63-3; **2b**, 123751-64-4; **3a**, 123751-65-5; **3b**, 123751-66-6; **4a**, 123751-67-7; **4b**, 123751-68-8; **5a**, 123751-69-9; **5b**, 123751-70-2; **6a**, 123751-71-3; **6b**, 123775-05-3; **7a**, 123751-72-4; **7b**, 123751-73-5; **8a**, 123751-78-0; **8b**, 123751-79-1; **9a**, 123751-82-6; **9b**, 123751-83-7; **10a**, 123751-74-6; **10b**, 123751-75-7; **11a**, 123751-76-8; **11b**, 123751-77-9; **12a**, 123751-80-4; **12b**, 123751-81-5; **13a**, 105580-37-8; **13b**, 105580-33-4; DHFR, 9002-03-3; GAR formyltransferase, 9032-02-4; 4-EtC_6H_4COOH, 619-64-7; 4-PrC_6H_4COOH, 2438-05-3; 3-MeOCH=$CHCH_2Cl$, 80986-54-5; $K^+ \cdot {}^-C(CN)_2CHO$, 118834-52-9; AcOCH=$C(CN)_2$, 86843-46-1; H-Glu(OEt)-OEt·HCl, 1118-89-4.

Novel Prodrugs Which Are Activated to Cytotoxic Alkylating Agents by Carboxypeptidase G2

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The synthesis of three novel prodrugs, 4-[bis[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid (**7**), 4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid (**8**), and 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid (**9**), for use as anticancer agents, is described here. Each is a bifunctional alkylating agent in which the activating effect of the ionized carboxyl function is masked through an amide bond to the glutamic acid residue. These relatively inactive prodrugs are designed to be activated to their corresponding nitrogen alkylating agents (**10**, **11**, and **12**, respectively) at a tumor site by prior administration of a monoclonal antibody conjugated to the bacterial enzyme carboxypeptidase G2 (CPG2). The viability of two different tumor cell lines was monitored with each prodrug in the presence of CPG2. All three compounds showed substantial prodrug activity—with conversion to the corresponding active drug leading to greatly increased cytotoxicity.

Over the years, many cytotoxic compounds have been discovered which are of potential use in cancer chemotherapy.[1] Nitrogen mustards form one important family of such cytotoxic compounds.[2] The clinical effectiveness of cytotoxic compounds in general and nitrogen mustards in particular has been limited by the poor selectivity in the cytotoxic effect between tumor cells and normal cells. One approach to overcome this problem has involved the development of prodrugs which are potential precursors of the cytotoxic drug and whose cytotoxic properties are considerably reduced compared to those of the parent drug.[3]

Numerous proposals have been made for the administration of such prodrugs to patients under regimes whereby the prodrug is only converted into the cytotoxic drug in the region of the intended site of action.[4] However, such approaches typically entail activation by an organ (e.g. the liver) rich in drug-metabolizing enzymes in the hope that organ-derived tumor cells will be selectively killed.[5] It has rarely proved possible to achieve prodrug activation specifically at cancer sites because human cancers do not in general exhibit intrinsic metabolic properties sufficiently

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distinctive from normal tissues. An apparent example of such selectivity is the activation of the glucuronide of *p*-hydroxyaniline mustard in tumors rich in β-glucuronidase.[6]

There have been many attempts to achieve selectivity by conjugating cytotoxic substances to antibodies which are directed at tumor-associated antigens and which localize selectively, though not exclusively, at cancer sites.[7,8] The general strategy has been to conjugate the toxic component to the antibody in a single bifunctional molecule. Analysis of this strategy suggested that it would be advantageous to separate these two functions.[9] In such a two-phase approach the selective component would be delivered first, with time allowed to optimize localization in the tumor and clearance from the blood before injecting the toxic component in the form of a prodrug.[10]

This approach prompted the synthesis of three novel prodrugs, 4-[bis[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid (**7**), 4-[(2-chloroethyl)[2-(mesyloxy)ethyl]-amino]benzoyl-L-glutamic acid (**8**) and, 4-[bis(2-chloroethyl)amino]benzoyl-L-glutamic acid (**9**). Each is a bifunctional alkylating agent in which the activating effect of the ionized carboxyl function is masked by linking the benzoic acid moiety through an amide bond to the glutamic acid residue. These prodrugs are designed to be activated to their corresponding bifunctionally alkylating benzoic acid derivatives (**10**, **11**, and **12**, respectively) at the tumor site by prior administration of a monoclonal antibody–enzyme conjugate. The studies described here employ activation of the prodrugs with the bacterial enzyme carboxypeptidase G2 (CPG2). This enzyme catalyzes the hydrolytic cleavage of reduced and nonreduced folates to pteroates and L-glutamic acid and has no known equivalent activity acting on its specific substrate in mammals.[11]

In order to evaluate the potential for conversion of the prodrug to a cytotoxic drug, the viability of two different human tumor cell lines was monitored with each prodrug in turn in the presence of CPG2. The ID_{50} results were compared to the viability of the two cell lines when incubated with each prodrug alone or with one of the parent drugs, 4-[bis(2-chloroethyl)amino]benzoic acid (**12**).

Results and Discussion

Chemistry. Three prodrugs were synthesized as shown in Scheme I. Structures **3–8** are new compounds. The synthesis of compound **9** was performed previously[12] by a different route (vide infra). Further analytical data for this compound is presented here.

The starting material, di-*tert*-butyl 4-nitrobenzoyl-L-glutamate (**1**) was hydrogenated over 10% palladium on charcoal to give di-*tert*-butyl 4-aminobenzoyl-L-glutamate (**2**). This was N-alkylated with ethylene oxide to give di-*tert*-butyl-4-[bis(2-hydroxyethyl)amino]benzoyl-L-glutamate (**3**), which was purified by column chromatography on silica gel. Compound **3** was employed as a common intermediate for the synthesis of protected derivatives **4–6**. Methanesulfonyl (mesyl) chloride was used in each case, but different reaction conditions with respect to temperature and time gave rise to a different ratio of products. Reaction of **3** with mesyl chloride at 2 °C gave bis(mesyloxyethyl) compound **4** as the major product. Raising the temperature to 80 °C led to formation of bis(chloroethyl) compound **6** almost exclusively. With use of intermediate temperatures, compound **5** of mixed functionality was isolated.

Diacids **7–9** were prepared by hydrolysis of esters **4–6**, with trifluoroacetic acid, although trifluoroacetic acid remained in the isolated products. This problem has been noted by others[13] who therefore used concentrated HCl, elevated temperatures, and extended time as an alternative method. However, diesters **4–6** were not stable in HCl under these conditions.

Previous attempts to obtain diacids **7** and **8** via diethyl esters with alkaline hydrolysis led to impure products due to the lability of the more reactive mesyloxy derivatives

Table I. Biological Assay of the Compounds in Cell Culture with and without CPG2 (6 Units/mL)

	ID_{50} for JAR/μM		ID_{50} for LS174T/μM	
compd	compd	compd + CPG2	compd	compd + CPG2
7	>800	60	800	65
8	630	10	285	10
9	>800	8	>800	25
12	20		26	

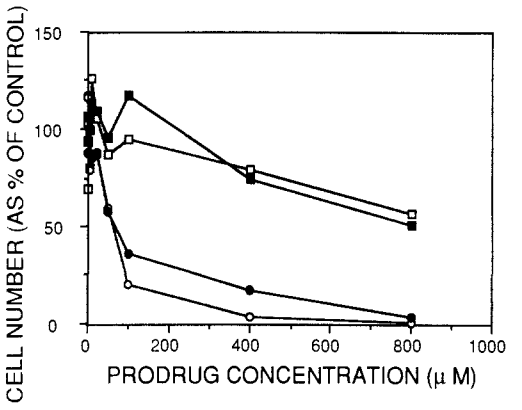

Figure 1. Cell viability in the presence of compound **7**. JAR line, without enzyme (□) and with enzyme (○) (6 units mL^{-1}); LS174T line, without enzyme (■) and with enzyme (●) (6 units mL^{-1}).

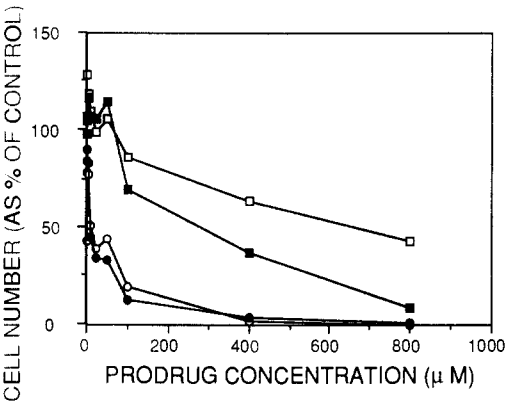

Figure 2. Cell viability in the presence of compound **8**. JAR line, without enzyme (□) and with enzyme (○) (6 units mL^{-1}); LS174T line, without enzyme (■) and with enzyme (●) (6 units mL^{-1}).

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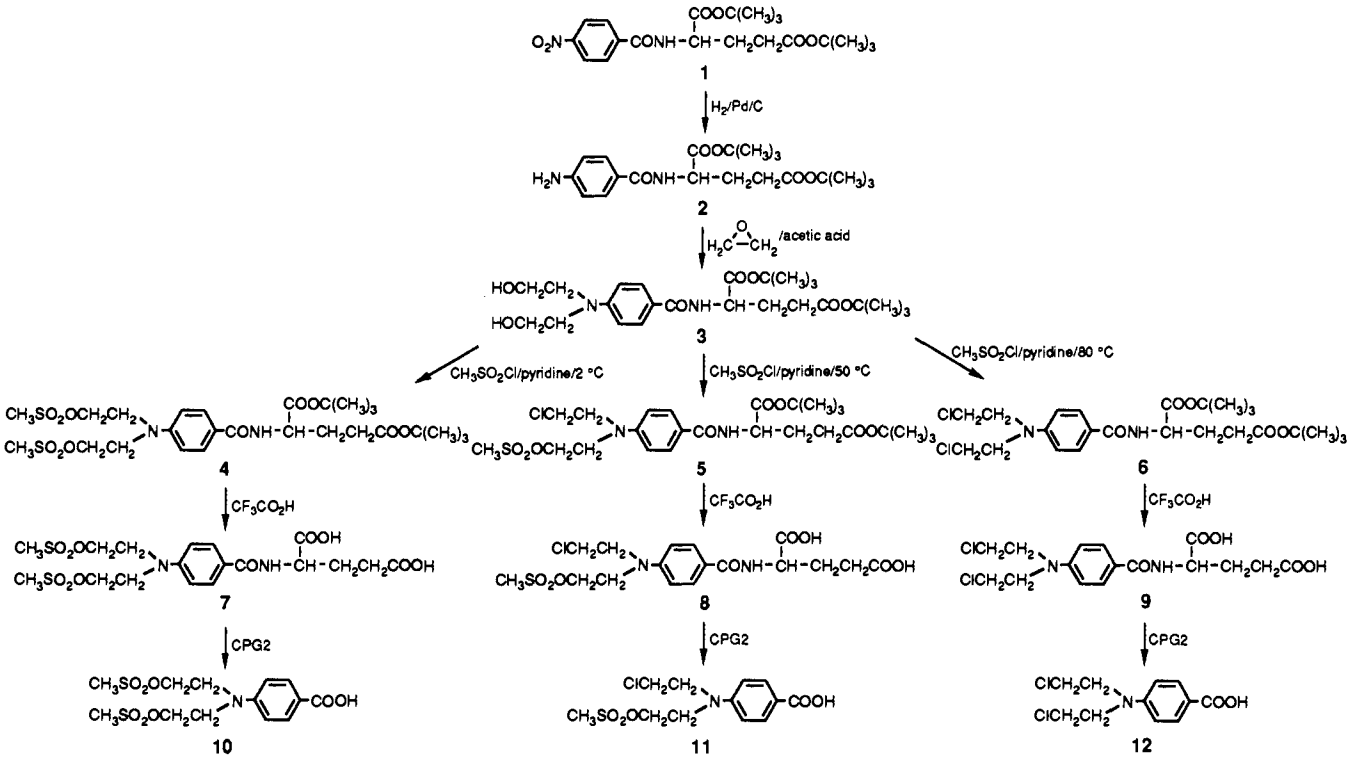
Scheme I



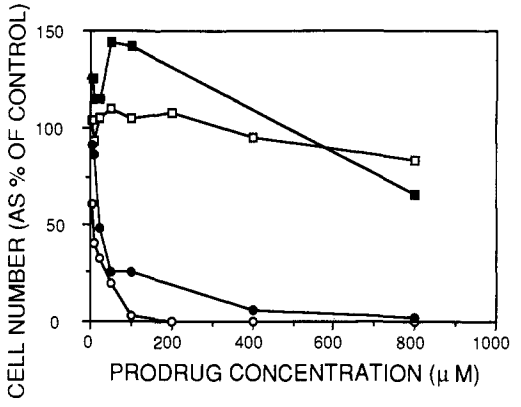


Figure 3. Cell viability in the presence of compound **9**. JAR line, without enzyme (□) and with enzyme (○) (6 units mL^{-1}); LS174T line, without enzyme (■) and with enzyme (●) (6 units mL^{-1}).

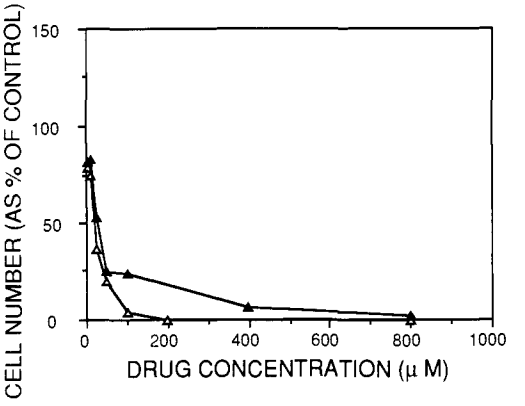


Figure 4. Cell viability in the presence of compound 12: JAR line (△), LS174T line (▲).

in alkaline conditions. Compound **9**, being the least reactive diacid in the series, could be made with either the diethyl or the di-*tert*-butyl esters. The superiority of *tert*-butyl protecting groups in protecting glutamic acid residues has been exemplified previously. In the synthesis of N^{10}-(cyanomethyl)-5,8-dideazafolate, the alkaline hydrolysis of the diethyl ester led to a mixture of products (owing to basic hydrolysis of the cyano group) whereas acid hydrolysis of the di-*tert*-butyl ester gives the desired product.[14]

Biological Evaluation. Diacids **7–9** were tested for prodrug action by measuring their cytotoxicity with and without CPG2 in two human cell lines, JAR, a choriocarcinoma line,[15] and LS174T, a colorectal line.[16] The results are shown in Table I, and in Figures 1–3. No loss of viability was detected in the presence of enzyme alone. All three diacids showed substantial prodrug activity with the conversion to the corresponding drug leading to increased cytotoxicity. In the JAR cell line, the ID_{50} was decreased by >16-fold for compound **7**, 63-fold for compound **8**, and >100-fold for compound **9** on addition of CPG2. In the LS174T cell line, the ID_{50} was decreased by 12-fold for **7**, 29-fold for **8**, and >32-fold for **9**. As an additional control, compound **12** was incubated with the two cell lines. The results are shown in Table I and in Figure 4. In the JAR line the ID_{50} gave 2.5 times higher than and in the LS174T line the ID_{50} gave the same value as the ID_{50} of prodrug **9** + enzyme.

Both models indicate that enzyme conversion of the three diacids exemplified (**7–9**) yield compounds of greatly increased cytotoxicity. There is no significant difference in ID_{50} between compound **9** + enzyme and compound **12**. This indicates that addition of the glutamic acid moiety

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is successful in deactivating benzoic acid mustards 10–12 and that there is complete subsequent conversion to the parent drug on addition of the carboxypeptidase G2 enzyme.

Conclusion. The results presented indicate that compounds can be synthesized as prodrugs which may be activated by nonmammalian enzymes. These compounds may be utilized as prodrugs to be cleaved to cytotoxic agents at tumor sites by prior localization of a monoclonal antibody conjugated to enzyme. At present this two-phase approach is being evaluated for all three diacids. Promising results have been obtained in athymic mice with transplanted choriocarcinoma or colorectal xenografts. The advantage of using a nonmammalian enzyme which has no known equivalent activity acting on its specific substrate ensures that prodrug is not activated by host enzymes at nontumor sites.

The general strategy of employing an antibody conjugated to a nonmammalian enzyme to activate a prodrug to give antitumor activity has been validated by the work in our laboratories.[9,10] Palpable resistant human choriocarcinoma xenografts in nude mice showed marked suppression of tumor growth when treated with antibody–enzyme conjugate followed by prodrug 9. Tumors in mice receiving conventional cytotoxic agents showed similar increasing growth to saline controls. We are currently evaluating the efficacy of prodrugs 7 and 8 in the same system. Recently another group of workers[17] have used a mammalian enzyme, alkaline phosphatase, conjugated to an antibody to activate the relatively nontoxic etoposide phosphate to the free drug, etoposide, which shows an antitumor effect.

Experimental Section

Merck silica 60 (Art 7734, 9385, and 15111) were used in gravity columns. TLC was performed on precoated sheets of silica $60F_{254}$ (Merck Art 5735). TLC spots were developed with Epstein spray.[18] Melting points were determined on a Kofler block (Reichert Thermovar) melting point apparatus and are uncorrected. Electron impact spectra were determined with a VG 7070H spectrometer and a VG 2235 data system using the direct-insertion method, an ionizing voltage of 70 eV, trap current of 100 μA, and an ion-source temperature at 180–200 °C. FAB mass spectra were determined using xenon gas. Reported spectra are by electron impact unless otherwise stated. NMR spectra were determined in Me_2SO-d_6 on a Bruker AC250 (250 MHz) with Me_4Si as internal standard. Elemental analyses were determined by CHN Analysis Ltd., S. Wigston, Leicester, England.

Di-*tert*-butyl 4-Aminobenzoyl-L-glutamate (2). The di-*tert*-butyl ester of 4-nitrobenzoyl-L-glutamic acid was prepared by a modification of the literature method.[19] To a solution of di-*tert*-butyl L-glutamate hydrochloride (20 g, 68 mmol) in Et_3N (19 mL, 137 mmol) was added dropwise a solution of 4-nitrobenzoyl chloride (13 g, 70 mmol) in CH_2Cl_2 (160 mL). Extractive workup gave a solid (1), hydrogenation of which gave the desired amine 2. This was crystallized from EtOH–cyclohexane (45:55); yield 85%.

Di-*tert*-butyl 4-[Bis(2-hydroxyethyl)amino]benzoyl-L-glutamate (3). Amine 2 (21.97 g, 58 mmol) in HOAc (120 mL) was stirred with ethylene oxide (12 mL, 240 mmol) at room temperature for 48 h. The solvent was removed at 45 °C in vacuo and the residue was partitioned between CH_2Cl_2 and H_2O. The organic phase was separated, washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The crude oil was chromatographed on silica gel (Merck, Art 9385) eluting with EtOAc–CH_2Cl_2 (1:1). The product (21.17 g, 78%) melted at 64–65 °C. 3: NMR (Me_2SO-d_6) δ 1.38 (s, 9 H, *t*-Bu), 1.39 (s, 9 H, *t*-Bu), 1.97 (m, 2 H, $CH_2CH_2CO_2$-*t*-Bu), 2.31 (t, 2 H, J = 7.4 Hz, $CH_2CH_2CO_2$-*t*-Bu), 3.51 (m, 8 H, 2 $HOCH_2CH_2$), 4.29 (m, 1 H, CH), 4.80 (t, 2 H, J = 4.8 Hz, 2 HO), 6.69 (AB q, 2 H, J = 8.9 Hz, arom H-3,5), 7.70 (AB q, 2 H, arom H-2,6), 8.15 (d, 1 H, J = 7.6 Hz, NH); mass spectrum m/z 466 (M^+, 16), 435 (M – $HOCH_2$, 65), 208 (M – NHCH(CO_2-*t*-Bu)$CH_2CH_2CO_2$-*t*-Bu, 100). Anal. ($C_{24}H_{38}N_2O_7 \cdot 0.25H_2O$) C, H, N.

Di-*tert*-butyl 4-[Bis[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamate (4), Di-*tert*-butyl 4-[(2-Chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamate (5), and Di-*tert*-butyl 4-[Bis(2-chloroethyl)amino]benzoyl-L-glutamate (6). A solution of 3 (2.00 g, 4.29 mmol) in pyridine (9.5 mL) was stirred with methanesulfonyl chloride (1.3 mL, 17.2 mmol) at 2 °C for 20 min, followed by 50 °C for 10 min. The reaction mixture was partitioned between CH_2Cl_2 and H_2O. The organic phase was separated, washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The concentrate contained three reaction products, each of which gave a positive result (blue color) with the Epstein reagent. The mixture was chromatographed on silica gel (Merck, Art 15111) eluting with EtOAc–CH_2Cl_2 (1:9). The slowest eluting was bis[2-(mesyloxy)ethyl] derivative 4 (0.6 g, 22%): mp 114–116 °C; NMR (Me_2SO-d_6) δ 1.39 (s, 9 H, *t*-Bu), 1.40 (s, 9 H, *t*-Bu), 1.97 (m, 2 H, $CH_2CH_2CO_2$-*t*-Bu) 2.32 (t, 2 H, J = 7.4 Hz, $CH_2CH_2CO_2$-*t*-Bu), 3.16 (s, 6 H, 2 CH_3SO_3), 3.81 (t, 4 H, J = 5.5 Hz, 2 $CH_3SO_3CH_2CH_2$), 4.34 (m, 5 H, 2 $CH_3SO_3CH_2CH_2$ and CH), 6.84 (AB q, 2 H, J = 8.9 Hz, arom H-3,5), 7.72 (AB q, 2 H, arom H-2,6), 8.27 (d, 1 H, J = 7.7 Hz, NH); mass spectrum (FAB) m/z 623 ([M + H^+], 19) 513 (M – $CH_3SO_3CH_2$, 10), 364 (M – NHCH(CO_2-*t*-Bu)$CH_2CH_2CO_2$-*t*-Bu, 100). Anal. ($C_{28}H_{42}N_2O_{11}S_2$) C, H, N, S.

Eluting second was (2-chloroethyl)[2-(mesyloxy)ethyl] derivative 5 (0.2 g, 8%): mp 71–73 °C; NMR (Me_2SO-d_6) δ 1.39 (s, 9 H, *t*-Bu), 1.40 (s, 9 H, *t*-Bu), 2.00 (m, 2 H, $CH_2CH_2CO_2$-*t*-Bu), 2.31 (t, 2 H, J = 7.3 Hz, $CH_2CH_2CO_2$-*t*-Bu), 3.15 (s, 3 H, CH_3SO_3), 3.77 (s, 4 H, $ClCH_2CH_2$), 3.82 (t, 2 H, J = 5.5 Hz, $CH_3SO_3CH_2CH_2$), 4.33 (m, 3 H, $CH_3SO_3CH_2CH_2$ and CH), 6.82 (AB q, 2 H, J = 8.9 Hz, arom H-3,5), 7.77 (AB q, 2 H, arom H-2,6), 8.24 (d, 1 H, J = 7.6 Hz, NH); mass spectrum m/z 562 (M^+, 100) 453 (M – $CH_3SO_3CH_2$, 9) 304 (M – NHCH(CO_2-*t*-Bu)$CH_2CH_2CO_2$-*t*-Bu, 17). Anal. ($C_{25}H_{39}N_2O_8ClS$) C, H, N, Cl, S.

The fastest eluting, bis(2-chloroethyl) derivative 6 was present in such low yield (0.01 g, 0.4%) that a different procedure for production was followed. A solution of 3 (2.53 g, 5.43 mmol) was treated with methanesulfonyl chloride (1.7 mL, 22.5 mmol) in pyridine (13 mL) at 2 °C for 20 min, followed by 80 °C for 80 min. The mixture was partitioned as before, dried, and concentrated to give two products which gave a positive result (blue color) with the Epstein spray. The mixture was chromatographed on silica gel (Merck, 7734) and eluted in solvents as before. The slower eluting (2-chloroethyl)[2-(mesyloxy)ethyl] derivative 5 was present in very low yield, (0.01 g, 0.3%). Eluting faster was bis(2-chloroethyl) derivative 6 (2.11 g, 77%): mp 142–143 °C; NMR (Me_2SO-d_6) δ 1.39 (s, 9 H, *t*-Bu), 1.40 (s, 9 H, *t*-Bu), 1.99 (m, 2 H, $CH_2CH_2CO_2$-*t*-Bu), 2.31 (t, 2 H, J = 7.4 Hz, $CH_2CH_2CO_2$-*t*-Bu), 3.78 (t, 8 H, J = 5.0 Hz, 2 $ClCH_2CH_2$), 4.34 (m, 1 H, CH), 6.70 (AB q, 2 H, J = 9.0 Hz, arom H-3,5), 7.77 (AB q, 2 H, arom H-2,6), 8.26 (d, 1 H, J = 7.6 Hz, NH) mass spectrum m/z 502 (M^+, 4), 453 (M – $ClCH_2$, 4), 244 (M – NHCH(CO_2-*t*-Bu)$CH_2CH_2CO_2$-*t*-Bu, 100). Anal. ($C_{24}H_{36}N_2O_5Cl_2$) C, H, N, Cl.

Preparation of Diacids—General Method. Compound 4 (0.213 g), 5 (0.127 g), or 6 (0.402 g) was suspended in TFA (1–2% w/v) and stirred for 30 min at room temperature. The acid was removed under reduced pressure at 30–35 °C. The remaining oil was diluted with ethyl acetate (1 mL) which was evaporated at the same temperature. This dilution/evaporation step was repeated a further 19 times. Compound 7 (0.19 g, 83%), 4-[bis[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid, was obtained as a pure product from 4: NMR (Me_2SO-d_6) δ 1.99 (m, 2 H, $CH_2CH_2CO_2H$), 2.34 (t, 2 H, J = 7.4 Hz, $CH_2CH_2CO_2H$), 3.16 (s, 6 H, 2 CH_3SO_3), 3.81 (t, 4 H, J = 5.4 Hz, 2 $CH_3SO_3CH_2CH_2$), 4.33 (m, 5 H, 2 $CH_3SO_3CH_2CH_2$ and CH), 6.84 (AB q, 2 H, J = 8.9 Hz, arom H-3,5), 7.75 (AB q, 2 H, arom H-2,6), 8.29 (d, 1 H, J

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= 7.7 Hz, NH) (The presence of EtOAc, noted in the elemental analysis, was confirmed by NMR); mass spectrum (FAB) m/z 511 ([M + H$^+$], 20), 364 (M - NHCH(CO$_2$H)CH$_2$CH$_2$CO$_2$H, 100). Anal. ($C_{18}H_{26}N_2O_{11}S_2$·1.1TFA·0.45EtOAc) C, H, N, S, F. This compound reacted positively (blue color) with the Epstein spray reagent.

Compound **8** (0.09 g, 82%), 4-[(2-chloroethyl)[2-(mesyloxy)-ethyl]amino]benzoyl-L-glutamic acid, was similarly obtained from **5**: NMR (Me$_2$SO-d_6) δ 1.99 (m, 2 H, CH_2CH$_2$CO$_2$H), 2.33 (t, 2 H, J = 7.3 Hz, CH$_2$CH_2CO$_2$H), 3.16 (s, 3 H, CH$_3$SO$_3$), 3.77 (s, 4 H, ClCH$_2$CH$_2$), 3.83 (t, 2 H, J = 5.7 Hz, CH$_3$SO$_3$CH$_2$CH_2), 4.33 (m, 3 H, CH$_3$SO$_3$CH_2CH$_2$ and CH), 6.82 (AB q, 2 H, J = 9.0 Hz, arom H-3,5), 7.77 (AB q, 2 H, arom H-2,6), 8.29 (d, 1 H, J = 7.7 Hz, NH); mass spectrum (FAB) m/z 451 ([M + H$^+$], 5), 401 (M - ClCH$_2$, 4), 341 (M - CH$_3$SO$_3$CH$_2$, 7). Anal. ($C_{17}H_{23}N_2O_8ClS$·0.25 TFA) C, H, N, Cl, F, S. This compound reacted positively (blue color) with the Epstein spray reagent.

Compound **9** (0.33 g, 94%), 4-[bis(2-chloroethyl)amino]-benzoyl-L-glutamic acid, was likewise obtained from **6**: NMR (Me$_2$SO-d_6) δ 2.00 (m, 2 H, CH_2CH$_2$CO$_2$H), 2.34 (t, 2 H, J = 7.4 Hz, CH$_2$CH_2CO$_2$H), 3.78 (t, 8 H, J = 5.2 Hz, 2 ClCH$_2$CH$_2$), 4.34 (m, 1 H, CH), 6.80 (AB q, 2 H, J = 9.0 Hz, arom H-3,5), 7.77 (AB q, 2 H, arom H-2,6), 8.29 (d, 1 H, J = 7.8 Hz, NH); mass spectrum m/z 372 (M - H$_2$O, 12), 244 (M - NHCH(CO$_2$H)CH$_2$CH$_2$CO$_2$H, 45). Anal. ($C_{16}H_{20}N_2O_5Cl_2$·0.4 TFA) C, H, N, Cl, F. This compound reacted positively (blue color) with the Epstein spray reagent.

Biological Methods. Each prodrug compound (**7-9**) and the parent drug, compound **12**, was incubated at a range of concentrations (0.5-800 μM) with the two cell lines (5×10^4 mL^{-1}, cells grown in DMEM). Prodrug or parent drug was made up just prior to use, adjusted to pH 7.4, and added in the same concentration three times at 24-h intervals. Each concentration was performed in duplicate. CPG2 (6 units mL^{-1} final concentration) was added to test wells in equivalent cultures with each dose of prodrug to achieve active drug in situ. Cell viability was determined by hemocytometry 24 h after the last treatment and the results were compared to those of untreated controls.

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Registry No. 1, 88050-23-1; **2**, 76282-66-1; **3**, 123724-54-9; **4**, 123724-55-0; **5**, 123724-56-1; **6**, 123724-57-2; **7**, 123724-58-3; **8**, 122665-73-0; **9**, 3086-06-4; **10**, 93985-39-8; 11, 122665-70-7; **12**, 1141-37-3; CPG2, 9074-87-7; di-*tert*-butyl L-glutamate hydrochloride, 32677-01-3; 4-nitrobenzoyl chloride, 122-04-3.

The Preparation of 2′-Deoxy-2′-fluoro-1′,2′-seconucleosides as Potential Antiviral Agents[1]

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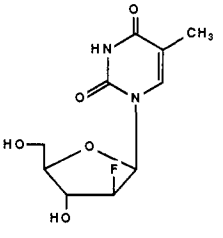
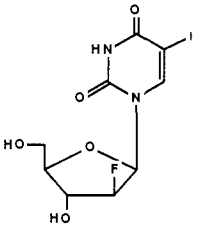
The preparation of (*R*,*R*)-1,3-dibenzyl-4-fluorobutane-1,2,3-triol (**6**) from D-isoascorbic acid and subsequent chloromethylation of this chiron made possible the synthesis of a series of 2′-deoxy-2′-fluoro-1′,2′-seconucleosides. Among them were the uridine (**10**), thymidine, (11), 5-iodouridine (14), ribavirin (17), and guanosine (19) analogues. They were evaluated for antiviral activity primarily against RNA viruses and found to be inactive. In addition to the aforementioned acyclonucleosides, the 3′,5′-cyclic phosphates of the uridine (**22**) and thymidine (**23**) analogues were prepared from their respective 4-nitrophenyl 3′,5′-cyclic phosphate triesters. The triesters were also examined for antiviral activity, but like their nucleoside counterparts exhibited only marginal activity.

The synthesis of sugar-fluorinated nucleoside analogues continues to attract attention as potential antiviral agents.[2-5] To date, those which have demonstrated significant activity possess a 2′-*ara*-fluoro substituent,[6-8] e.g., 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU). Prompted by these reports, we initiated a program aimed at the synthesis of chiral 2′-deoxy-2′-fluoro-1′,2′-seconucleosides and -nucleotides, acyclonucleic acid components which incorporate similar structural features of FMAU, FIAU, and their analogues.[9]



FMAU



FIAU

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(9) The structural formulas of the 2′-deoxy-2′-fluoro-1′,2′-seconucleosides/nucleotides depicted in this paper are drawn in the "arabinose-like" conformation to point out their similarity with certain 2′-fluoroarabinosylpyrimidine nucleosides, e.g., FIAU and FMAU, that have demonstrated significant antiviral activity. In the text the naming and numbering of these analogues follow nucleoside nomenclature, however; in the Experimental Section the acyclic side chains of the 1′,2′-seconucleosides/nucleotides are named and numbered in accordance with butanetriol nomenclature.

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