

## Novel Prodrugs of Alkylating Agents Derived from 2-Fluoro- and 3-Fluorobenzoic Acids for Antibody-Directed Enzyme Prodrug Therapy

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The synthesis of six novel fluorinated potential prodrugs for antibody-directed enzyme prodrug therapy is described. The [2- and 3-fluoro-4-[bis(2-chloroethyl)amino]benzoyl]-L-glutamic acid (**9** and **21**), their bis(mesyloxy)ethyl derivatives (**7** and **19**), and their chloroethyl (mesyloxy)-ethyl derivatives (**8** and **20**) are bifunctional alkylating agents in which the activating effect of the ionized carboxyl function is masked through an amide bond to the glutamic acid residue. These compounds were designed to be activated to their corresponding benzoic acid alkylating agents at a tumor site by prior administration of a monoclonal antibody conjugated to the bacterial enzyme carboxypeptidase G2 (CPG2). The synthesis of the analogous novel parent drugs 2- and 3-fluoro-4-[bis(2-chloroethyl)amino]benzoic acid (**12** and **24**), their bis(mesyloxy)-ethyl derivatives (**10** and **22**), and their chloroethyl (mesyloxy)ethyl derivatives (**11** and **23**) is also described. The viability of a colorectal cell line was monitored with the six potential prodrugs in the presence of CPG2 and with the parent drugs alone. Compounds **19–21** demonstrated substantial prodrug activity, with activation by CPG2 leading to cytotoxicities comparable to those of **22–24**, respectively. The  $K_m$  and  $k_{cat}$  values for **7–9** and **19–21** were determined for CPG2. All potential prodrugs except **7** proved to be excellent substrates. A comparison of the relative chemical reactivity of the compounds as determined by their half-life measurements showed that the 2-fluoro substituent deactivated while the 3-fluoro substituent activated the alkylating moieties.

### Introduction

Many cytotoxic drugs have been developed for use in cancer chemotherapy.<sup>1</sup> Nitrogen mustards form a substantial category of such drugs in common clinical use.<sup>2</sup> However, the clinical efficacy has been limited by the low therapeutic index and lack of selectivity of cytotoxic compounds in general and nitrogen mustards in particular. As a consequence, selective generation of a cytotoxic nitrogen mustard from an inactive prodrug has become a significant objective.<sup>3</sup>

There have been numerous approaches to achieve selectivity by conjugating nitrogen mustards to antibodies which are directed at tumor-associated antigens and which localize selectively at tumor sites. The general strategy is to conjugate the toxic component to the antibody in a single cytotoxic bifunctional agent, which has targeting potential. It has been demonstrated that an *N*-acetylmelphalan–monoclonal antibody conjugate had greater antitumor activity than *N*-acetylmelphalan, melphalan, or the monoclonal antibody alone.<sup>4</sup> However, one of the main drawbacks of this strategy is that the alkylating agent–antibody conjugates are unable to gain sufficient tumor access. Analysis of this approach led to the suggestion that it would be an advantage to separate these two functions.<sup>5</sup> Antibody-directed enzyme prodrug therapy (ADEPT)<sup>6</sup> separates the cytotoxic function from the targeting function in a two-phase system that has benefits over a one-phase chemo- or radioimmunoconjugate or an immunotoxin.

In this two-phase approach, the selective component is delivered first, with time allowed to optimize localization and clearance from blood before administration of the second component in the form of the prodrug.<sup>7,8</sup> The amplification feature (one conjugate molecule can catalyze the conversion of many molecules of the prodrug into the cytotoxic parent drug) inherent in the enzyme component of the antibody–enzyme conjugate provides additional advantages. The tumor interstitial transport of low molecular weight cytotoxic agents thus generated has greater access, compared to those of the antibody conjugates. Nitrogen mustard agents are good candidates for ADEPT in that their cytotoxicity is dose-related and they can be given often with less induced resistance than other classes of anticancer agents.<sup>9</sup> Other groups have also utilized nitrogen mustard prodrugs in different ADEPT systems.<sup>10–15</sup> To date, there has been only one pilot clinical trial in ADEPT, on the prodrug [4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid (**40**), with promising results.<sup>16,17</sup> The three-stage trial (a galactosylated antienzyme antibody was used as an additional second step to clear antibody–enzyme conjugate from plasma) has confirmed the feasibility of this approach.

The synthesis of six novel potential prodrugs, [2- and 3-fluoro-4-[bis[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid (**7** and **19**), [2- and 3-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid (**8** and **20**), and [2- and 3-fluoro-4-[bis(2-chloroethyl)amino]benzoyl]-L-glutamic acid (**9** and **21**), for use in ADEPT is described. Each of these compounds is a bifunctional alkylating agent in which the activating effect of the ionized carboxyl function is

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masked through an amide bond to the glutamic acid residue. These compounds are designed to be activated to their corresponding benzoic acid alkylating agents at a tumor site by prior administration of a monoclonal antibody conjugated to the bacterial enzyme carboxypeptidase G2 (CPG2). The synthesis of the analogous novel parent drugs corresponding to the six new potential prodrugs is also described. These are 2- and 3-fluoro-4-[bis(2-mesyloxy)ethyl]amino]benzoic acid (**10** and **22**), 2- and 3-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoic acid (**11** and **23**), and 2- and 3-fluoro-4-[bis(2-chloroethyl)amino]benzoic acid (**12** and **24**), respectively.

The rationale for the synthesis of the six novel potential prodrugs was to broaden the range of different chemical half-lives in order to favor potential improvements on the previously synthesized prodrugs [4-[bis(2-(mesyloxy)ethyl)amino]benzoyl]-L-glutamic acid (**39**), [4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid (**40**), and [4-[bis(2-chloroethyl)amino]benzoyl]-L-glutamic acid (**41**). There is convincing evidence that these diacids are converted by CPG2 in vivo as well as in vitro to their corresponding active drugs 4-[bis(2-(mesyloxy)ethyl)amino]benzoic acid (**42**), 4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoic acid (**43**), and 4-[bis(2-chloroethyl)amino]benzoic acid (**44**).<sup>8</sup>

The effect of 2- and 3-fluoro ring substituents on the chemical reactivities, the substrate specificities, and the cytotoxicities was unknown. In position 2, the fluorine should have a  $-I$  effect associated with the rather high  $\sigma_m = 0.34$ . Accordingly, the hydrolysis rate of the substituted nitrogen mustard would be expected to decrease. In position 3, there are three conflicting effects of the fluorine: the  $-I$  effect which tends to decrease the hydrolysis rate, the  $+R$  effect which tends to increase the hydrolysis rate, and a steric effect due to the fluorine which causes steric hindrance of resonance of the substituted amino group, decreasing its basicity. It was therefore difficult to predict the overall effect of the fluorine substitutions, and the situation was made even more unpredictable by the effect of the  $\text{CO}_2^-$  group.

Accordingly, chemical half-life measurements were performed to determine the relative chemical reactivities of the fluorine compounds. All the 2-fluoro parent drugs, **10–12**, were deactivated compared to the non-fluorinated derivatives, **42–44**, respectively. Conversely, the three 3-fluoro potential prodrugs, **19–21**, and their three corresponding parent drugs, **22–24**, were greatly activated with respect to the corresponding 2-fluoro and the non-fluorinated analogues. Thus, a wide range of differing reactivities was obtained by 2- and 3-fluoro substitution.

Certain effects of the 2-fluoro and 3-fluoro ring substituents on the substrate reactivity of benzoic acid mustard glutamates for CPG2 were predictable. Thus, the unfluorinated prodrug would be expected to have the lowest  $K_m$ , suggesting tight binding with the enzyme. Introduction of the F at either position 2 or 3 would be expected to diminish the binding due to steric reasons, and this was found to be the case. Introduction of F at position 2 would be expected to reduce the  $k_{cat}$  due to the  $+R$  effect of fluorine (with respect to the amide group) which was found to be the case as demonstrated by  $k_{cat}$  values of **20** > **8**, **19** > **7**, and **21**

> **9**. Introduction of F at position 3 results in a  $-I$  effect. The combined  $+R$ ,  $-I$  effect justifies the observed trend for  $k_{cat}$  values of **20** > **40** > **8**. In conclusion, the 3-fluoro potential prodrugs were good substrates and two of the 2-fluoro potential prodrugs were moderate substrates for CPG2. Compound **7** was a poor substrate. The non-fluorinated prodrugs were the best substrates for the enzyme.

The cytotoxicity of the benzoic acid mustard glutamates with and without CPG2 was measured to assess the 2-fluoro and 3-fluoro potential prodrugs. The parent drugs were also assayed in the same system. The 3-fluoro diacids proved to have good prodrug potential, with activation by CPG2 leading to enhanced cytotoxicity comparable with that of the parent drugs. The 2-fluoro analogues were not cytotoxic even when activated by CPG2.

Therefore, the six novel diacids provide a selection of activated and deactivated potential prodrugs which, in the case of five of the analogues, are cleaved by CPG2 to the corresponding parent drugs. It is anticipated that this variety of potential prodrugs may provide assistance in the selection of the best compound for ADEPT.

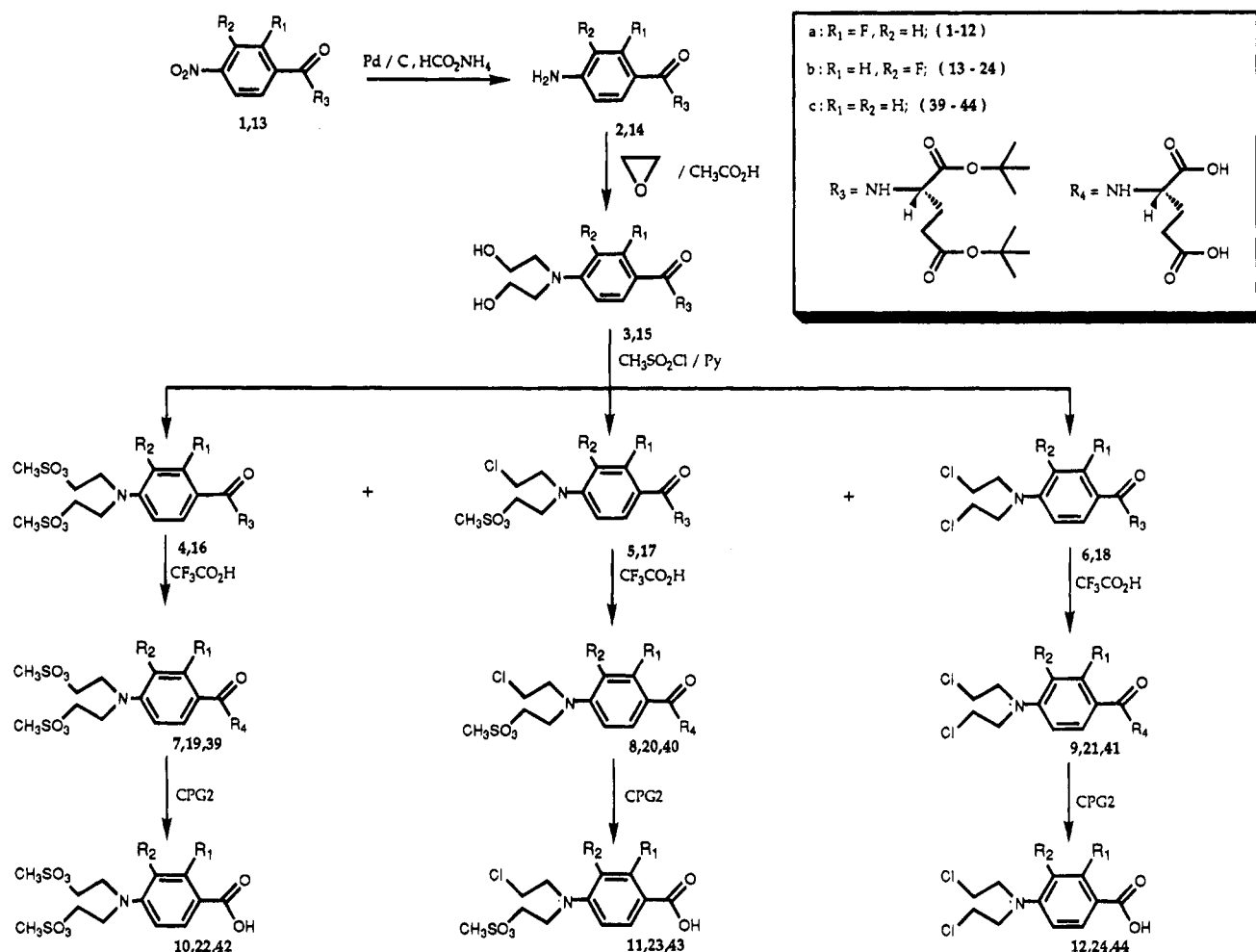
## Results and Discussion

**Chemistry.** Six potential prodrugs were synthesized as shown in Scheme 1. Structures **1–24** and **28–38** are new compounds.

The starting material for the 2-fluoro potential prodrugs was di-*tert*-butyl (2-fluoro-4-nitrobenzoyl)-L-glutamate (**1**), which was obtained by the condensation of di-*tert*-butyl L-glutamate hydrochloride and 2-fluoro-4-nitrobenzoyl chloride. Di-*tert*-butyl (3-fluoro-4-nitrobenzoyl)-L-glutamate (**13**) was made similarly from 3-fluoro-4-nitrobenzoyl chloride. Both nitro compounds were reduced using catalytic hydrogen transfer from ammonium formate to give di-*tert*-butyl (2- and 3-fluoro-4-aminobenzoyl)-L-glutamate (**2** and **14**). These amines were N-alkylated using excess ethylene oxide in glacial acetic acid over long time periods to di-*tert*-butyl [2- and 3-fluoro-4-[bis(2-hydroxyethyl)amino]benzoyl]-L-glutamate (**3** and **15**). The products thus obtained required purification on silica gel chromatography columns. Compound **3** was used as a common intermediate with methanesulfonyl (mesyl) chloride for the synthesis of **4–6**, which were separated and purified by column chromatography on silica gel. Similarly, **15** was employed for the synthesis of **16–18**. The final deprotection of *tert*-butyl esters **4–6** leading to diacids **7–9** was carried out with trifluoroacetic acid. Likewise, **19–21** were formed from **16–18**. Trifluoroacetic acid remained in the isolated products of compounds containing glutamic acid deprotected as above as noted by ourselves<sup>18</sup> and others.<sup>19</sup> The latter used concentrated HCl, elevated temperatures, and extended time as an alternative method. However, neither the alkylating moieties nor the amide bonds of **7–9** and **19–21** are stable to these conditions.

The corresponding six parent drugs were also synthesized as shown in Schemes 2 and 3. The starting material for the 2-fluoro parent drugs was 2-fluoro-4-nitrotoluene. This was oxidized to the 2-fluoro-4-nitrobenzoic acid followed by its chlorination ( $\text{SOCl}_2$ ) to the intermediate acid chloride **25** according to literature

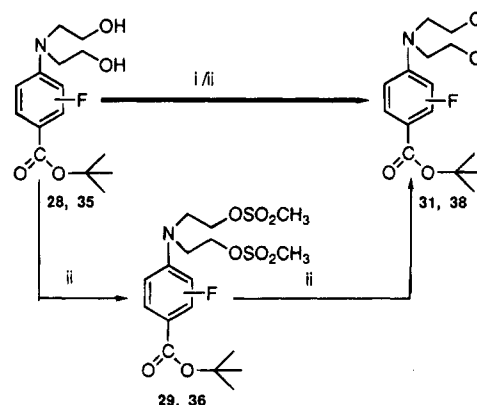
## Scheme 1



methods.<sup>20</sup> The starting material for the 3-fluoro parent drugs was 3-fluoro-4-nitrotoluene which was transformed to the intermediate acid chloride **32** by the same two-step procedure. Both products (**25** and **32**) were esterified using lithium *tert*-butoxide.

The resulting *tert*-butyl 2- and 3-fluoro-4-nitrobenzoates (**26** and **33**, respectively) were reduced using transfer hydrogenation, with ammonium formate and 10% Pd/C in a modification of the literature method.<sup>18</sup> Ethanol was used instead of methanol to avoid the risk of ignition, especially important in the preparation of bulk batches. A good yield of the 2- and 3-fluoro amino derivatives **27** and **34** was obtained. These amines were *N*-alkylated with ethylene oxide in glacial acetic acid to afford the desired *tert*-butyl 2- and 3-fluoro-4-[bis(2-hydroxyethyl)amino]benzoates (**28** and **35**).

The *tert*-butyl 2-fluoro-4-[bis(2-chloroethyl)amino]benzoate (**31**) and *tert*-butyl 3-fluoro-4-[bis(2-chloroethyl)amino]benzoate (**38**) intermediates were each prepared by two different routes as shown in Scheme 2. The direct chlorination of the corresponding hydroxyethyl precursors with  $\text{SOCl}_2$  (method a) or by mesyl chloride in a one-step procedure (method b) was used to obtain the bis(2-chloroethyl) intermediates. The chlorination (mesyl chloride) of the 3-fluorobis(2-mesyloxy)ethyl derivative **36** afforded the corresponding bis(2-chloroethyl) derivative **38** in good yield. Mesyl chloride was also used to yield the other desired products. In the 2-fluoro syntheses, a mixture of *tert*-butyl 2-fluoro-4-[(2-chloroethyl)(2-mesyloxy)ethyl]amino]benzoate (**30**),

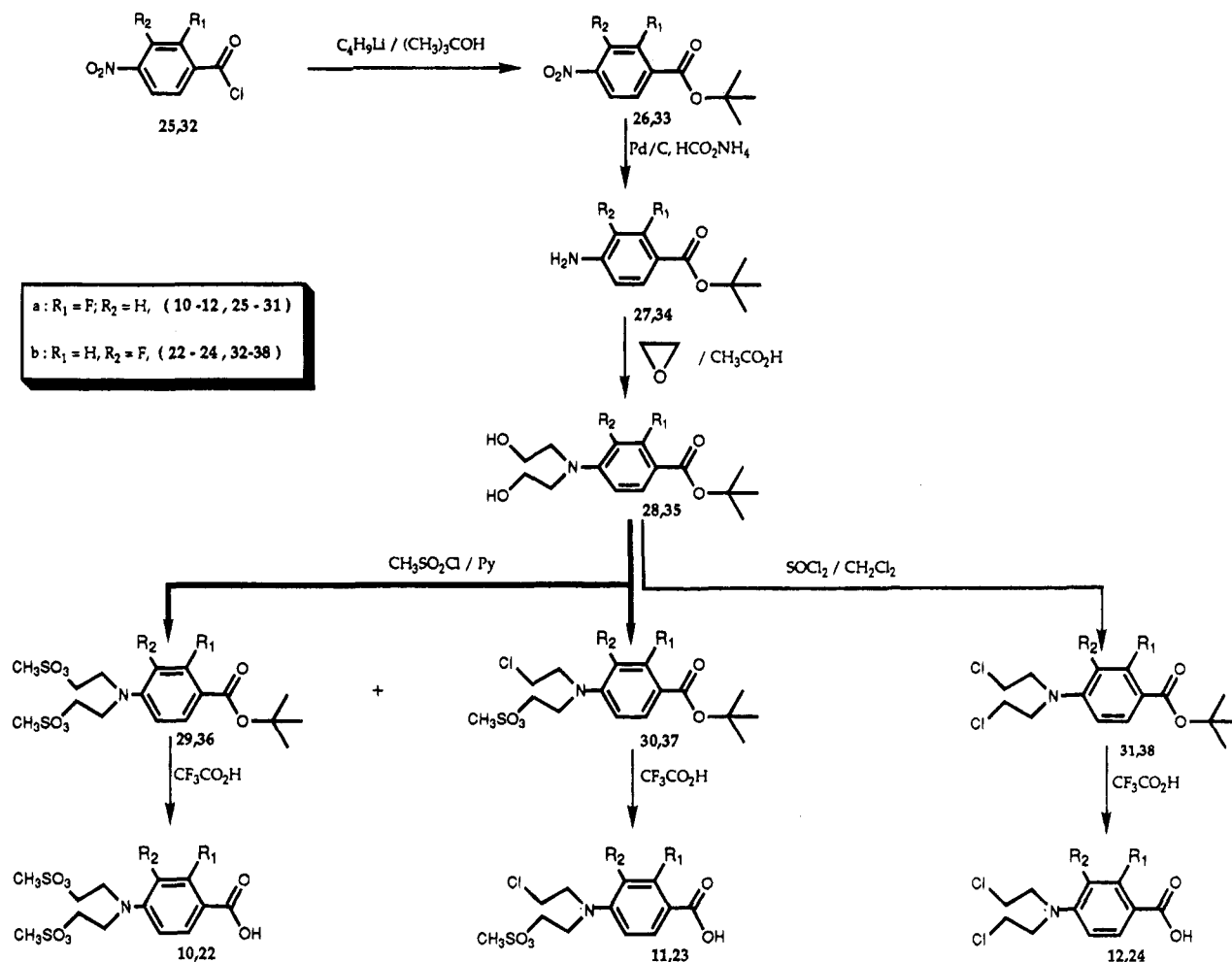
Scheme 2<sup>a</sup>

<sup>a</sup> (i) Thionyl chloride (method a); (ii) mesyl chloride/pyridine (method b).

*tert*-butyl 2-fluoro-4-[bis[2-(mesyloxy)ethyl]amino]benzoate (**29**), and *tert*-butyl 2-fluoro-4-[bis(2-chloroethyl)amino]benzoate (**31**) was obtained as shown in Scheme 3.

Correspondingly, in the 3-fluoro syntheses, mixtures of *tert*-butyl 3-fluoro-4-[(2-chloroethyl)(2-mesyloxy)ethyl]amino]benzoate (**37**), *tert*-butyl 3-fluoro-4-[bis[2-(mesyloxy)ethyl]amino]benzoate (**36**), and *tert*-butyl 3-fluoro-4-[bis(2-chloroethyl)amino]benzoate (**38**) were obtained. The desired products were separated by column chromatography on silica gel.

## Scheme 3

**Table 1.** Chemical Half-Lives in Perchlorate at 37 °C

prodrug	$t_{1/2}$ (min)	active drug	$t_{1/2}$ (min)
7	nd <sup>a</sup>	10	93
8	nd	11	192
9	nd	12	1242
19	9	22	1.9
20	122	23	2.4
21	147	24	72
39 <sup>b</sup>	42	42 <sup>b</sup>	21
40 <sup>b</sup>	984	43 <sup>b</sup>	58
41 <sup>b</sup>	1158	44 <sup>b</sup>	324

<sup>a</sup> nd—not determined. <sup>b</sup> From ref 21.

Acids 10–12 and 22–24 were prepared by treatment of the esters 29–31 and 36–38, respectively, with trifluoroacetic acid. When solid compounds were obtained, the TFA was removed readily by crystallization.

The chemical half-lives of the potential prodrugs 19–21 and the parent drugs 10–12 and 22–24 were determined as described previously for the  $t_{1/2}$  of the non-fluorinated prodrugs 39–41 and their active drugs 42–44.<sup>21</sup> The results are shown in Table 1. All the 2-fluoro acids exhibited longer half-lives (3–4-fold) than their analogous non-fluorinated counterparts (cf. 10 vs 42, 11 vs 43, 12 vs 44), presumably due to the inductive (–I) effect of the fluorine.

However, by contrast, all the 3-fluoro compounds had substantially shorter half-lives (5–24-fold) with respect to those of their analogous non-fluorinated counterparts (cf. 19 vs 39, 20 vs 40, 21 vs 41, 22 vs 42, 23 vs 43, 24 vs 44). As expected, all the parent drugs showed shorter

**Table 2.** Kinetics of Potential Prodrugs as Substrates for CPG2

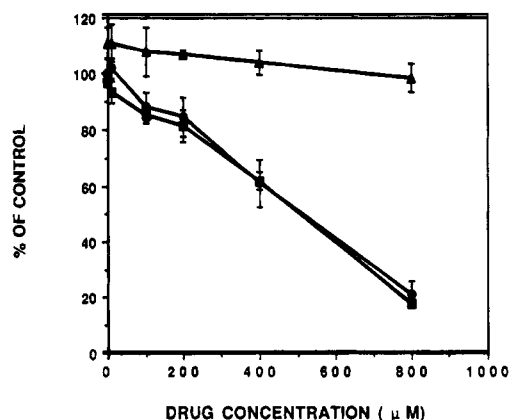
prodrug	$K_m$ (±SD) (μM)	$k_{cat}$ (±SD) (s <sup>−1</sup> )	$k_{cat}/K_m$ (s <sup>−1</sup> μM <sup>−1</sup> )
7	very poor substrate		
8	11 (2.2)	213 (9.0)	19
9	15 (2.7)	462 (28)	31
19	17 (7.3)	565 (126)	33
20	6.0 (1.7)	614 (63)	102
21	10 (1.5)	1028 (76)	103
40	3.4 (0.5)	583 (27)	171

half-lives than their corresponding prodrugs. The greatest divergence in half-lives between potential prodrug and corresponding parent drug was obtained for the 3-fluoro 2-chloroethyl 2-(mesyloxy)ethyl compounds (cf. 20 vs 23), with a differential of ca. 50-fold.

**Biological Evaluation.** The  $K_m$  and  $k_{cat}$  with CPG2 were determined using each of the novel potential prodrugs 7–9 and 19–21 and compared to those of [4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid (40), currently in clinical trial. The kinetics were determined by measuring the decrease in the absorption spectrum after addition of CPG2 to each prodrug. This is a result of the hydrolysis of the amide bond of the benzoylglutamic acid moiety. All the potential prodrugs were substrates for CPG2. However, compound 7 had such a slow rate of conversion that its kinetics could not be measured. For all the other compounds, plots of initial reaction velocity versus each

**Table 3.** Biological Assay of the Compounds in Cell Culture with and without CPG2

prodrug	IC <sub>50</sub> (μM)	active drug	IC <sub>50</sub> (μM)
7	>800	10	>800
7 + CPG2	>800		
8	>800	11	>800
8 + CPG2	>800		
9	>800	12	>800
9 + CPG2	>800		
19	>800	22	480
19 + CPG2	480		
20	>800	23	280
20 + CPG2	350		
21	>800	24	270
21 + CPG2	390		
40	>800	43	185
40 + CPG2	200		

**Figure 1.** Cell viability in the presence of parent drug 22 (●) or with prodrug 19, without enzyme (▲) and with enzyme (■).

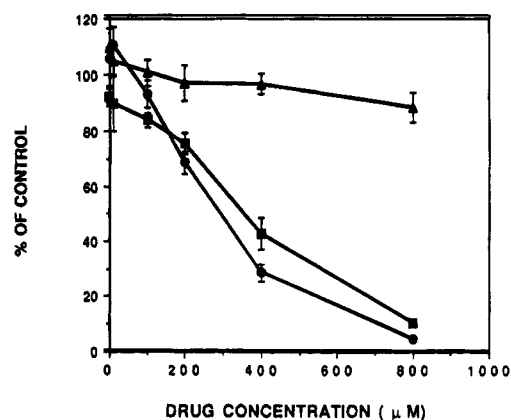
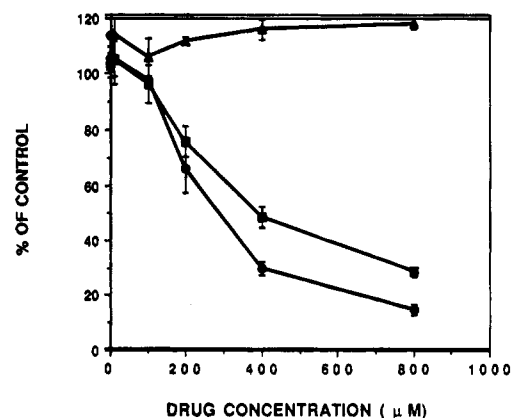
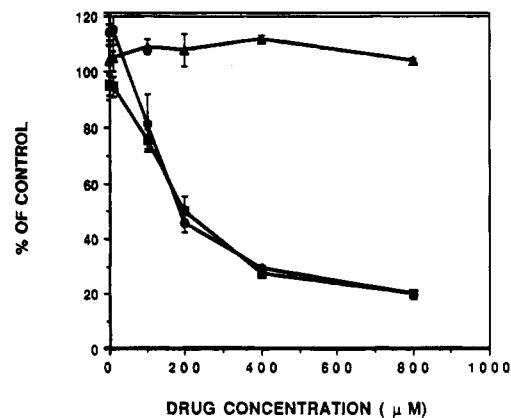
prodrug substrate concentration followed Michaelis-Menten kinetics.

The results are shown in Table 2. The 2-fluoro substituent had a detrimental effect on the  $k_{cat}/K_m$  ratios compared to the non-fluorinated diacids. The bis[2-(methoxy)ethyl] diacids were the poorest substrates in both the 2-fluoro and 3-fluoro series. However, the effect of the 3-fluoro substitution was much less marked. The diacids 20 and 21 were excellent substrates.

The novel diacids, 7–9 and 19–21, and the non-fluorinated diacid, 40, were tested for prodrug activity by measuring their cytotoxicity with and without CPG2 in the colorectal cell line LS174T for 1 h.<sup>22</sup> The novel corresponding parent drugs, 10–12, 22–24, and 43, respectively, were screened under the same conditions.

The results are shown in Table 3 and Figures 1–4. All the 3-fluoro diacids, 19–21, showed substantial prodrug activity as did the non-fluorinated diacid, 40. In each case, the potential prodrug was completely noncytotoxic even at 800 μM and conversion to its corresponding parent drug by CPG2 led to increased cytotoxicity. The cytotoxicity of each of the parent drugs alone, 22–24, was not significantly different from that of its potential prodrug + CPG2, 19–21, respectively. Although all the 2-fluoro diacids alone were nontoxic, none exhibited prodrug activity since they were not converted to a cytotoxic species in the diacid + CPG2 tests. These data were in good agreement with the cytotoxicity experiments using the 2-fluoro parent drugs.

These results indicate that fluorinated compounds can be synthesized as prodrugs to be activated by the bacterial enzyme CPG2. The introduction of the 2- and

**Figure 2.** Cell viability in the presence of parent drug 23 (●) or with prodrug 20, without enzyme (▲) and with enzyme (■).**Figure 3.** Cell viability in the presence of parent drug 24 (●) or with prodrug 21, without enzyme (▲) and with enzyme (■).**Figure 4.** Cell viability in the presence of parent drug 43 (●) or with prodrug 40, without enzyme (▲) and with enzyme (■).

3-fluoro substituent into the benzene ring of prodrugs which have proven activity in ADEPT<sup>6-8,16-18</sup> has led to wide diversity in the chemical and biological characteristics of the novel compounds. Since these novel diacids are all substrates for the same CPG2 enzyme, they will be tested *in vivo* in the ADEPT system alone and in combination.

The prodrug 40, currently undergoing clinical evaluation in ADEPT, has demonstrated efficacy. However, the 3-fluoro prodrug 20 which is also an excellent substrate for the CPG2 enzyme and shows a similar cytotoxicity profile releases an active drug with a shorter chemical half-life.

## Experimental Section

All starting materials and reagents are commercially available (Aldrich or Sigma) unless otherwise stated. Silica was used in gravity columns (Art 9385 and 15111, Merck). TLC was performed on precoated sheets of silica 60 F<sub>254</sub> (Art 5735, Merck). TLC spots were developed with Epstein spray.<sup>23</sup> Melting points were determined on a Kofler hot-stage (Reichert Thermovar) melting point apparatus and are uncorrected. Electron impact spectra were determined with a VG 7070H mass spectrometer and a VG 2235 data system using the direct-insertion method, an ionizing energy of 70 eV, a trap current of 100  $\mu$ A, and an ion-source temperature of 180–200 °C. FAB mass spectra were determined using xenon gas. Reported spectra are by FAB unless otherwise stated. NMR spectra (<sup>1</sup>H and <sup>19</sup>F) were determined in Me<sub>2</sub>SO-*d*<sub>6</sub> on a Bruker AC250 spectrometer (250 MHz) at 30 °C (303 K) unless otherwise stated. Elemental analyses were determined by Butterworth Laboratories Ltd. (Teddington, Middlesex, England). Half-life determinations were performed on an automatic titrator (Radiometer, Copenhagen). Kinetic analyses were performed on a spectrophotometer (Perkin-Elmer Lambda 2) fitted with a heat controller. Results were calculated using a nonlinear regression program (Enzfitter).

**Di-*tert*-butyl [2-Fluoro-4-nitrobenzoyl]-L-glutamate (1).** To a solution of di-*tert*-butyl L-glutamate hydrochloride (5.00 g, 16.9 mmol) in Et<sub>3</sub>N (5.0 mL, 34.0 mmol) was added dropwise a solution of 2-fluoro-4-nitrobenzoyl chloride (3.50 g, 17.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The reaction mixture was evaporated to dryness, and the resulting oil was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and H<sub>2</sub>O (3 × 300 mL). The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give 1 as an oil (6.80 g, 94%): <sup>1</sup>H NMR  $\delta$  1.40 (s, 9 H, *t*-Bu), 1.43 (s, 9 H, *t*-Bu), 1.95 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 2.34 (t, 2 H, *J* = 7.4 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 4.35 (m, 1 H, CH), 7.85 (ddd, 1 H, *J*<sub>H-6,H-5</sub> = 1.1 Hz, *J*<sub>H-6,F</sub> = 7.1 Hz, *J*<sub>H-6,H-5</sub> = 8.2 Hz, H-6), 8.18 (m, 2 H, H-3, H-5), 8.92 (d, 1 H, *J* = 7.5 Hz, NH) (The presence of CH<sub>2</sub>-Cl<sub>2</sub> noted in the elemental analysis was confirmed by NMR.); <sup>19</sup>F NMR  $\delta$  -110.41 (dd); MS *m/z* 427 ([M + H<sup>+</sup>], 18), 315 (M - *t*-Bu<sub>2</sub>, 100). Anal. (C<sub>20</sub>H<sub>27</sub>N<sub>2</sub>O<sub>7</sub>F·0.04CH<sub>2</sub>Cl<sub>2</sub>) C, H, N, F.

**Di-*tert*-butyl [2-Fluoro-4-aminobenzoyl]-L-glutamate (2).** A slurry of the nitro compound 1 (5.80 g, 13.6 mmol) and Pd/C (10%, 1.00 g) in MeOH (60 mL) was stirred with ammonium formate (4.50 g, 71.5 mmol). The catalyst was removed by filtration and the filtrate washed with MeOH and concentrated. The residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O, and the organic phase was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to dryness to give the amine 2 as an oil (5.2 g, 96%): <sup>1</sup>H NMR  $\delta$  1.38 (s, 9 H, *t*-Bu), 1.41 (s, 9 H, *t*-Bu), 1.95 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 2.29 (t, 2 H, *J* = 8.1 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 4.31 (m, 1 H, CH), 5.98 (s, 2 H, NH<sub>2</sub>), 6.30 (dd, 1 H, *J*<sub>H-3,F</sub> = 14.3 Hz, H-3), 6.40 (dd, 1 H, *J*<sub>H-5,H-6</sub> = 8.6 Hz, H-5), 7.42 (dd, 1 H, *J*<sub>H-6,F</sub> = 8.7 Hz, H-6), 7.69 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 6.8 Hz, *J*<sub>H-N,F</sub> = 13.9 Hz, NH); <sup>19</sup>F NMR  $\delta$  -112.23 (ddd); MS *m/z* 397 ([M + H<sup>+</sup>], 100). Anal. (C<sub>20</sub>H<sub>29</sub>N<sub>2</sub>O<sub>5</sub>F·0.5MeOH) C, H, N, F.

**Di-*tert*-butyl [2-Fluoro-4-[bis(2-hydroxyethyl)amino]benzoyl]-L-glutamate (3).** Amine 2 (1.60 g, 4.0 mmol) in HOAc (10 mL) was stirred with ethylene oxide (13.0 mL, 260 mmol) at room temperature for 112 h. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. The organic phase was separated, washed with H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to dryness. The crude oil was chromatographed on silica gel, eluting with EtOAc-CH<sub>2</sub>Cl<sub>2</sub> to give an oil, 3 (1.00 g, 49%): <sup>1</sup>H NMR  $\delta$  1.39 (s, 9 H, *t*-Bu), 1.42 (s, 9 H, *t*-Bu), 1.93 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 2.29 (t, 2 H, *J* = 7.7 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 3.46 (d, 4 H, *J* = 5.2 Hz, 2 HOCH<sub>2</sub>CH<sub>2</sub>), 3.55 (t, 4 H, *J* = 4.8 Hz, 2 HOCH<sub>2</sub>CH<sub>2</sub>), 4.34 (m, 1 H, CH), 4.75 (t, 2 H, *J* = 4.7 Hz, 2 OH), 6.50 (dd, 1 H, *J*<sub>H-3,F</sub> = 17.1 Hz, H-3), 6.57 (dd, 1 H, *J*<sub>H-5,H-6</sub> = 9.0 Hz, H-5), 7.33 (dd, 1 H, *J*<sub>H-6,F</sub> = 9.1 Hz, H-6), 7.69 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 7.1 Hz, *J*<sub>H-N,F</sub> = 14.1 Hz, NH) (The presence of EtOAc noted in the elemental analysis was confirmed by NMR.); <sup>19</sup>F NMR  $\delta$  -111.03 (ddd); MS *m/z* 485 ([M + H<sup>+</sup>], 4), 226 (M - glu-*t*-Bu<sub>2</sub>, 100). Anal. (C<sub>24</sub>H<sub>37</sub>N<sub>2</sub>O<sub>7</sub>F·0.5EtOAc) C, H, N, F.

**Di-*tert*-butyl [2-Fluoro-4-[bis(2-mesyloxy)ethyl]amino]benzoyl]-L-glutamate (4), Di-*tert*-butyl [2-Fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamate (5), and Di-*tert*-butyl [2-Fluoro-4-[bis(2-chloroethyl)amino]benzoyl]-L-glutamate (6).** A solution of 3 (1.40 g, 2.9 mmol) in pyridine (4.5 mL) was stirred with methanesulfonyl chloride (0.9 mL, 1.8 mmol) at 0 °C for 20 min followed by 80 °C for 20 min. The reaction mixture was partitioned between EtOAc (300 mL) and citric acid (10%, 800 mL). The organic phase was separated, washed with H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to dryness. The concentrate contained three reaction products, each of which gave a positive color with the Epstein reagent. The mixture was chromatographed on silica gel and eluted with EtOAc-CH<sub>2</sub>Cl<sub>2</sub> (1:5). The slowest eluting was the 2-fluoro bis[2-(mesyloxy)ethyl] derivative, the solid 4 (0.10 g, 5%): mp 90–92 °C; <sup>1</sup>H NMR  $\delta$  1.38 (s, 9 H, *t*-Bu), 1.42 (s, 9 H, *t*-Bu), 1.94 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 2.30 (t, 2 H, *J* = 7.8 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 3.16 (s, 6 H, 2 CH<sub>3</sub>SO<sub>3</sub>), 3.80 (t, 4 H, *J* = 5.2 Hz, 2 CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 4.33 (t, 5 H, *J* = 5.3 Hz, 2 CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 6.68 (dd, 2 H, *J*<sub>H-5,H-6</sub> = 8.1 Hz, *J*<sub>H-3,F</sub> = 15.3 Hz, H-3, H-5), 7.54 (dd, 1 H, *J*<sub>H-6,F</sub> = 9.1 Hz, H-6), 7.90 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 5.3 Hz, *J*<sub>H-N,F</sub> = 12.5 Hz, NH); <sup>19</sup>F NMR  $\delta$  -110.52 (dd); MS *m/z* 641 ([M + H<sup>+</sup>], 12), 382 (M - glu-*t*-Bu<sub>2</sub>, 100). Anal. (C<sub>26</sub>H<sub>41</sub>N<sub>2</sub>O<sub>11</sub>FS<sub>2</sub>) C, H, N, F, S.

Eluting second was an oil, the 2-fluoro 2-chloroethyl 2-(mesyloxy)ethyl derivative 5 (0.58 g, 34%): <sup>1</sup>H NMR  $\delta$  1.38 (s, 9 H, *t*-Bu), 1.42 (s, 9 H, *t*-Bu), 1.93 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 2.30 (t, 2 H, *J* = 7.8 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 3.15 (s, 3 H, CH<sub>3</sub>SO<sub>3</sub>), 3.77 (s, 4 H, ClCH<sub>2</sub>CH<sub>2</sub>), 3.82 (t, 2 H, *J* = 5.2 Hz, CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 4.32 (t, 3 H, *J* = 5.2 Hz, CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 6.66 (m, 2 H, H-3, H-5), 7.55 (dd, 1 H, *J*<sub>H-6,H-5</sub> = 8.8 Hz, *J*<sub>H-6,F</sub> = 9.2 Hz, H-6), 7.89 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 5.6 Hz, *J*<sub>H-N,F</sub> = 12.8 Hz, NH); <sup>19</sup>F NMR  $\delta$  -110.45 (m); MS *m/z* 581 ([M + H<sup>+</sup>], 14), 322 (M - glu-*t*-Bu<sub>2</sub>, 100). Anal. (C<sub>25</sub>H<sub>38</sub>N<sub>2</sub>O<sub>8</sub>FCIS) C, H, N, F, Cl, S.

The fastest eluting, the 2-fluoro bis(2-chloroethyl) derivative, was a solid, 6 (0.53 g, 34%): mp 104–106 °C; <sup>1</sup>H NMR  $\delta$  1.38 (s, 9 H, *t*-Bu), 1.42 (s, 9 H, *t*-Bu), 1.96 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 2.29 (t, 2 H, *J* = 7.8 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>-*t*-Bu), 3.78 (dt, 8 H, *J* = 5.3 Hz, 2 ClCH<sub>2</sub>CH<sub>2</sub>), 4.35 (m, 1 H, CH), 6.65 (m, 2 H, H-3, H-5), 7.55 (dd, 1 H, *J*<sub>H-6,F</sub> = 9.1 Hz, *J*<sub>H-6,H-5</sub> = 9.4 Hz, H-6), 7.88 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 5.5 Hz, *J*<sub>H-N,F</sub> = 12.8 Hz, NH); <sup>19</sup>F NMR  $\delta$  -110.55 (ddd, *J*<sub>FH-3</sub> = 11.3 Hz, *J*<sub>FH-5</sub> = 14.1 Hz); MS *m/z* 521 ([M + H<sup>+</sup>], 16), 262 (M - glu-*t*-Bu<sub>2</sub>, 100). Anal. (C<sub>24</sub>H<sub>35</sub>N<sub>2</sub>O<sub>5</sub>Cl<sub>2</sub>) C, H, N, F, Cl.

**Preparation of Diacids—General Method.** Compound 4 (0.13 g, 0.20 mmol), 5 (0.21 g, 0.36 mmol), or 6 (0.20 g, 0.38 mmol) was suspended in TFA (4–8% w/v) and stirred for 40 min at room temperature. The solvent was removed under reduced pressure, and the remaining oil was diluted with ethyl acetate (1.0 mL) which was evaporated. This latter step was repeated 5–20 times. Compound 7, an oil (0.12 g, 100%), [2-fluoro-4-[bis(2-mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid, was obtained as a pure product from 4: <sup>1</sup>H NMR  $\delta$  2.05 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H), 2.32 (t, 2 H, *J* = 7.6 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H), 3.16 (s, 6 H, 2 CH<sub>3</sub>SO<sub>3</sub>), 3.18 (t, 4 H, *J* = 5.0 Hz, 2 CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 4.33 (t, 4 H, *J* = 5.3 Hz, 2 CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 4.41 (t, 1 H, *J* = 4.2 Hz, CH), 6.69 (m, 2 H, H-3, H-5), 7.58 (dd, 1 H, *J*<sub>H-6,F</sub> = 9.1 Hz, *J*<sub>H-6,H-5</sub> = 9.3 Hz, H-6), 7.89 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 6.0 Hz, *J*<sub>H-N,F</sub> = 12.1 Hz, NH) (The presence of EtOAc and TFA noted in the elemental analysis was confirmed by NMR.); <sup>19</sup>F NMR  $\delta$  -110.35 (ddd, *J*<sub>FH-3</sub> = 14.5 Hz); MS *m/z* 529 ([M + H<sup>+</sup>], 12), 382 (M - glu, 100); accurate mass calcd, 529.0961; found, 2.0 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>18</sub>H<sub>25</sub>N<sub>2</sub>O<sub>11</sub>FS<sub>2</sub>·0.40TFA·0.30EtOAc) C, H, N, F, S.

Compound 8 (0.17 g, 92%), [2-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid, was similarly obtained as an oil from 5: <sup>1</sup>H NMR  $\delta$  2.02 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H), 2.32 (t, 2 H, *J* = 7.5 Hz, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H), 3.15 (s, 3 H, CH<sub>3</sub>SO<sub>3</sub>), 3.77 (s, 4 H, ClCH<sub>2</sub>CH<sub>2</sub>), 3.82 (t, 2 H, *J* = 5.1 Hz, CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 4.32 (t, 2 H, *J* = 5.3 Hz, CH<sub>3</sub>SO<sub>3</sub>-CH<sub>2</sub>CH<sub>2</sub>), 4.40 (q, 1 H, *J* = 4.5 Hz, CH), 6.67 (m, 2 H, H-3, H-5), 7.57 (dd, 1 H, *J*<sub>H-6,F</sub> = 9.1 Hz, *J*<sub>H-6,H-5</sub> = 9.4 Hz, H-6), 7.88 (dd, 1 H, *J*<sub>H-N,H-C</sub> = 6.5 Hz, *J*<sub>H-N,F</sub> = 13.1 Hz, NH) (The presence of

EtOAc and TFA noted in the elemental analysis was confirmed by NMR.;  $^{19}\text{F}$  NMR  $\delta$  -110.35 (ddd,  $J_{\text{FH}-3} = 16.2$  Hz); MS  $m/z$  469 ( $[\text{M} + \text{H}^+]$ , 8), 322 ( $\text{M} - \text{glu}$ , 100); accurate mass calcd, 469.0847; found, 3.2 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_8\text{FClS} \cdot 0.26\text{TFA} \cdot 0.15\text{EtOAc}$ ) C, H, N, F, Cl, S.

Compound **9** (0.17 g, 97%), [2-fluoro-4-[bis(2-chloroethyl)-amino]benzoyl]-L-glutamic acid, was likewise obtained as an oil from **6**:  $^1\text{H}$  NMR  $\delta$  1.98 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 2.33 (t, 2 H,  $J = 7.7$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 3.78 (dt, 8 H, 2  $\text{ClCH}_2\text{CH}_2$ ), 4.41 (m, 1 H, CH), 6.65 (m, 2 H, H-3, H-5), 7.58 (dd, 1 H,  $J_{\text{H}-6, \text{H}-5} = 8.8$  Hz,  $J_{\text{H}-6, \text{F}} = 9.1$  Hz, H-6), 7.85 (dd, 1 H,  $J_{\text{H}-\text{N}, \text{H}-\text{C}} = 5.5$  Hz,  $J_{\text{H}-\text{N}, \text{F}} = 12.8$  Hz, NH) (The presence of TFA noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -110.43 (ddd,  $J_{\text{FH}-3} = 15.3$  Hz); MS  $m/z$  409 ( $[\text{M} + \text{H}^+]$ , 3), 262 ( $\text{M} - \text{glu}$ , 100); accurate mass calcd, 409.0733; found, 3.7 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}_5\text{FCl}_2 \cdot 0.40\text{TFA}$ ) C, H, N, F, Cl.

**Di-tert-butyl [3-Fluoro-4-nitrobenzoyl]-L-glutamate (13).** To a solution of di-tert-butyl L-glutamate hydrochloride (20.00 g, 67.6 mmol) in  $\text{Et}_3\text{N}$  (19 mL, 136.0 mmol) was added dropwise a solution of 3-fluoro-4-nitrobenzoyl chloride (13.80 g, 68.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (300 mL). Extractive workup gave an oil, **13** (28.00 g, 97%):  $^1\text{H}$  NMR  $\delta$  1.40 (s, 9 H,  $t\text{-Bu}$ ), 1.42 (s, 9 H,  $t\text{-Bu}$ ), 1.99 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 2.36 (t, 2 H,  $J = 7.5$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 4.35 (m, 1 H, CH), 7.92 (dd, 1 H,  $J_{\text{H}-6, \text{H}-5} = 7.6$  Hz, H-6), 8.01 (m, 1 H, H-2), 8.29 (dd, 1 H,  $J_{\text{H}-5, \text{F}} = 8.1$  Hz, H-5), 8.97 (d, 1 H,  $J = 7.4$  Hz, NH) (The presence of  $\text{CH}_2\text{Cl}_2$  noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -117.98 (dd); MS (CI)  $m/z$  427 ( $[\text{M} + \text{H}^+]$ , 100). Anal. ( $\text{C}_{20}\text{H}_{27}\text{N}_2\text{O}_7\text{F} \cdot 0.25\text{CH}_2\text{Cl}_2$ ) C, H, N, F, Cl.

**Di-tert-butyl [3-Fluoro-4-aminobenzoyl]-L-glutamate (14).** Catalytic transfer reduction of the nitro compound **13** (7.50 g, 17.5 mmol) in MeOH (60 mL) with ammonium formate (5.80 g, 91.6 mmol) on 10% Pd/C gave the amine **14** as an oil which crystallized on standing (6.90 g, 99%): mp 84–85 °C;  $^1\text{H}$  NMR  $\delta$  1.39 (s, 9 H,  $t\text{-Bu}$ ), 1.40 (s, 9 H,  $t\text{-Bu}$ ), 1.97 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 2.31 (t, 2 H,  $J = 7.4$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 4.28 (m, 1 H, CH), 5.71 (s, 2 H,  $\text{NH}_2$ ), 6.77 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.8$  Hz,  $J_{\text{H}-5, \text{F}} = 8.7$  Hz, H-5), 7.49 (dd, 1 H, H-6), 7.56 (dd, 1 H,  $J_{\text{H}-2, \text{F}} = 12.8$  Hz, H-2), 8.19 (d, 1 H,  $J = 7.6$  Hz, NH);  $^{19}\text{F}$  NMR  $\delta$  -135.56 (dd); MS  $m/z$  397 ( $[\text{M} + \text{H}^+]$ , 5), 138 ( $\text{M} - \text{glu}$ , 100). Anal. ( $\text{C}_{20}\text{H}_{25}\text{N}_2\text{O}_5\text{F}$ ) C, H, N, F.

**Di-tert-butyl [3-Fluoro-4-[bis(2-hydroxyethyl)amino]benzoyl]-L-glutamate (15).** Amine **14** (5.30 g, 13.4 mmol) in HOAc (30.0 mL) was stirred with ethylene oxide (60 mL, 1.2 mol) at room temperature for 72 h. The solvent was evaporated under vacuum, and the product was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The organic phase was worked up and purified as for compound **3** above to give the pure oil **15** (3.3 g, 51%):  $^1\text{H}$  NMR  $\delta$  1.39 (s, 9 H,  $t\text{-Bu}$ ), 1.41 (s, 9 H,  $t\text{-Bu}$ ), 1.97 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 2.32 (t, 2 H,  $J = 7.4$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 3.43 (t, 4 H,  $J = 5.9$  Hz, 2  $\text{HOCH}_2\text{CH}_2$ ), 3.54 (d, 4 H,  $J = 5.4$  Hz, 2  $\text{HOCH}_2\text{CH}_2$ ), 4.31 (m, 1 H, CH), 4.67 (s, 2 H, 2 OH), 6.99 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.9$  Hz,  $J_{\text{H}-5, \text{F}} = 8.9$  Hz, H-5), 7.60 (m, 2 H, H-6, H-2), 8.3 (d, 1 H,  $J = 7.5$  Hz, NH) (The presence of EtOAc noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -124.31 (dd,  $J_{\text{FH}-2} = 15.4$  Hz); MS  $m/z$  485 ( $[\text{M} + \text{H}^+]$ , 22), 226 ( $\text{M} - \text{glu}t\text{-Bu}$ , 100). Anal. ( $\text{C}_{24}\text{H}_{37}\text{N}_2\text{O}_7\text{F} \cdot 1.1\text{EtOAc}$ ) C, H, N, F.

**Di-tert-butyl [3-Fluoro-4-[bis(2-mesyloxy)ethyl]amino]benzoyl]-L-glutamate (16), Di-tert-butyl [3-Fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamate (17), and Di-tert-butyl [3-Fluoro-4-[bis(2-chloroethyl)amino]benzoyl]-L-glutamate (18).** A solution of **15** (0.67 g, 1.4 mmol) in pyridine (3.0 mL) was stirred with methanesulfonyl chloride (0.6 mL, 7.7 mmol) at 0 °C for 20 min followed by 80 °C for 15 min. The reaction mixture was worked up as for **4–6** above. The concentrate contained three reaction products, each of which gave a positive color with the Epstein reagent, which was purified as for compounds **4–6**. The slowest eluting oil was the 3-fluoro bis[2-(mesyloxy)ethyl] derivative as the oil **16** (0.31 g, 35%):  $^1\text{H}$  NMR  $\delta$  1.39 (s, 9 H,  $t\text{-Bu}$ ), 1.41 (s, 9 H,  $t\text{-Bu}$ ), 1.98 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 2.32 (t, 2 H,  $J = 7.4$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 3.12 (s, 6 H, 2  $\text{CH}_3\text{SO}_3$ ),

3.72 (t, 4 H,  $J = 5.4$  Hz, 2  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.30 (t, 5 H,  $J = 5.3$  Hz, 2  $\text{CH}_3\text{SO}_3\text{CH}_2$ , CH), 7.16 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.8$  Hz,  $J_{\text{H}-5, \text{F}} = 8.8$  Hz, H-5), 7.67 (dd, 2 H,  $J_{\text{H}-2, \text{F}} = 13.8$  Hz, H-2, H-6), 8.43 (d, 1 H,  $J = 7.6$  Hz, NH) (The presence of EtOAc noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -122.69 (dd); MS  $m/z$  641 ( $[\text{M} + \text{H}^+]$ , 15), 382 ( $\text{M} - \text{glu}t\text{-Bu}$ , 100). Anal. ( $\text{C}_{26}\text{H}_{41}\text{N}_2\text{O}_{11}\text{FS}_2 \cdot 0.6\text{EtOAc}$ ) C, H, N, F, S.

Eluting second was the 3-fluoro 2-chloroethyl 2-(mesyloxy)-ethyl derivative as the oil **17** (0.29 g, 37%):  $^1\text{H}$  NMR  $\delta$  1.39 (s, 9 H,  $t\text{-Bu}$ ), 1.41 (s, 9 H,  $t\text{-Bu}$ ), 1.99 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 2.32 (t, 2 H,  $J = 7.4$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 3.12 (s, 3 H,  $\text{CH}_3\text{SO}_3$ ), 3.71 (s, 6 H,  $\text{ClCH}_2\text{CH}_2$ ,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.30 (t, 3 H,  $J = 5.3$  Hz,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ , CH), 7.13 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.8$  Hz,  $J_{\text{H}-5, \text{F}} = 9.0$  Hz, H-5), 7.66 (dd, 2 H,  $J_{\text{H}-2, \text{F}} = 14.6$  Hz, H-2, H-6), 8.41 (d, 1 H,  $J = 7.5$  Hz, NH);  $^{19}\text{F}$  NMR  $\delta$  -123.40 (m); MS  $m/z$  581 ( $[\text{M} + \text{H}^+]$ , 30), 322 ( $\text{M} - \text{glu}t\text{-Bu}$ , 100). Anal. ( $\text{C}_{25}\text{H}_{38}\text{N}_2\text{O}_8\text{FClS}$ ) C, H, N, F, Cl, S.

The fastest eluting, the 3-fluoro bis(2-chloroethyl) derivative, was the solid **18** (0.11 g, 15%): mp 100–103 °C;  $^1\text{H}$  NMR  $\delta$  1.39 (s, 9 H,  $t\text{-Bu}$ ), 1.41 (s, 9 H,  $t\text{-Bu}$ ), 2.01 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 2.33 (t, 2 H,  $J = 7.3$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2t\text{-Bu}$ ), 3.72 (s, 8 H, 2  $\text{ClCH}_2\text{CH}_2$ ), 4.32 (m, 1 H, CH), 7.11 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.9$  Hz,  $J_{\text{H}-5, \text{F}} = 9.1$  Hz, H-5), 7.65 (m, 2 H, H-2, H-6), 8.40 (d, 1 H,  $J = 7.4$  Hz, NH);  $^{19}\text{F}$  NMR  $\delta$  -123.83 (dd,  $J_{\text{FH}-2} = 14.8$  Hz); MS  $m/z$  521 ( $[\text{M} + \text{H}^+]$ , 19), 262 ( $\text{M} - \text{glu}t\text{-Bu}$ , 100). Anal. ( $\text{C}_{24}\text{H}_{35}\text{N}_2\text{O}_5\text{Cl}_2 \cdot 0.5\text{H}_2\text{O}$ ) C, H, N, F, Cl.

**Preparation of Diacids—General Method.** Compound **16** (0.10 g, 0.16 mmol), **17** (0.08 g, 0.13 mmol), or **18** (0.06 g, 0.11 mmol) was suspended in TFA (4% w/v) and stirred for 40 min at room temperature. The acid was removed under reduced pressure, and the remaining oil was diluted with ethyl acetate (1 mL) which was evaporated. This latter step was repeated five to six times. Compound **19** (0.09 g, 91%), [3-fluoro-4-[bis(2-mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid, was obtained as a pure product from **16**:  $^1\text{H}$  NMR  $\delta$  1.99 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 2.35 (t, 2 H,  $J = 7.5$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 3.13 (s, 6 H, 2  $\text{CH}_3\text{SO}_3$ ), 3.72 (t, 4 H,  $J = 5.34$  Hz, 2  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.31 (t, 4 H,  $J = 5.2$  Hz,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.39 (m, 1 H, CH), 7.16 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.6$  Hz,  $J_{\text{H}-5, \text{F}} = 18.0$  Hz, H-5), 7.68 (dd, 2 H,  $J_{\text{H}-2, \text{F}} = 15.3$  Hz, H-2, H-6), 8.45 (d, 1 H,  $J = 7.7$  Hz, NH) (The presence of EtOAc and TFA noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -122.54 (m); MS  $m/z$  529 ( $[\text{M} + \text{H}^+]$ , 45), 382 ( $\text{M} - \text{glu}$ , 100); accurate mass calcd, 529.0961; found, 5.4 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{18}\text{H}_{25}\text{N}_2\text{O}_{11}\text{FS}_2 \cdot 0.22\text{TFA} \cdot 0.21\text{EtOAc}$ ) C, H, N, F, S.

Compound **20** (0.06 g, 91%), [3-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoyl]-L-glutamic acid, was similarly obtained as an oil from **17**:  $^1\text{H}$  NMR  $\delta$  2.00 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 2.35 (t, 2 H,  $J = 7.43$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 3.13 (s, 3 H,  $\text{CH}_3\text{SO}_3$ ), 3.73 (s, 6 H,  $\text{ClCH}_2\text{CH}_2$ ,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.31 (t, 2 H,  $J = 5.4$  Hz,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.39 (m, 1 H, CH), 7.15 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.8$  Hz,  $J_{\text{H}-5, \text{F}} = 18.2$  Hz, H-5), 7.68 (dd, 2 H,  $J_{\text{H}-2, \text{F}} = 14.8$  Hz, H-2, H-6), 8.45 (d, 1 H,  $J = 7.6$  Hz, NH) (The presence of EtOAc and TFA noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -123.19 (dd); MS  $m/z$  469 ( $[\text{M} + \text{H}^+]$ , 10), 322 ( $\text{M} - \text{glu}$ , 100); accurate mass calcd, 469.0847; found, 4.9 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_8\text{FClS} \cdot 0.20\text{TFA} \cdot 0.21\text{EtOAc}$ ) C, H, N, F, Cl, S.

Compound **21** (0.05 g, 97%), [3-fluoro-4-[bis(2-chloroethyl)-amino]benzoyl]-L-glutamic acid, was likewise obtained as an oil from **18**:  $^1\text{H}$  NMR  $\delta$  2.00 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 2.35 (t, 2 H,  $J = 7.4$  Hz,  $\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$ ), 3.73 (s, 8 H, 2  $\text{ClCH}_2\text{CH}_2$ ), 4.41 (m, 1 H, CH), 7.12 (dd, 1 H,  $J_{\text{H}-5, \text{H}-6} = 8.8$  Hz,  $J_{\text{H}-5, \text{F}} = 18.2$  Hz, H-5), 7.67 (dd, 2 H,  $J_{\text{H}-2, \text{F}} = 15.4$  Hz, H-2, H-6), 8.42 (d, 1 H,  $J = 7.2$  Hz, NH) (The presence of EtOAc and TFA noted in the elemental analysis was confirmed by NMR.);  $^{19}\text{F}$  NMR  $\delta$  -123.65 (dd); MS  $m/z$  409 ( $[\text{M} + \text{H}^+]$ , 48), 262 ( $\text{M} - \text{glu}$ , 100); accurate mass calcd, 409.0733; found, -0.7 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{16}\text{H}_{19}\text{N}_2\text{O}_5\text{FCl}_2 \cdot 0.18\text{TFA} \cdot 0.2\text{EtOAc}$ ) C, H, N, F, Cl.

**2-Fluoro-4-nitrobenzoyl Chloride (25).** A slurry of 2-fluoro-4-nitrobenzoic acid (2.13 g, 11.5 mmol) in dry toluene

(50 mL) was stirred with  $\text{SOCl}_2$  (2.03 g, 17.1 mmol) under reflux for 3 h. The cooled solution was filtered through Celite 531 and concentrated to give the acid chloride as the solid **25**, which was recrystallized from petroleum ether–THF (2.0 g, 86%): mp 37–39 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.04–8.20 (m, 2 H, H-3, H-5), 8.29 (m, 1 H, H-6);  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ )  $\delta$  –103.96 (t,  $J$  = 8.4 Hz); MS (EI)  $m/z$  168 ( $\text{M} - \text{Cl}$ , 83).

**tert-Butyl 2-Fluoro-4-nitrobenzoate (26).** Butyllithium (63 mL (1.6 M in hexane), 100 mmol) was added to *tert*-butyl alcohol (200 mL) over 30–40 min at 20 °C under  $\text{N}_2$  and stirred for 20 min. The acid chloride **25** (20.00 g, 98.2 mmol) in THF (50 mL) was added and the mixture stirred for 22 h. The LiCl precipitate was filtered and the filtrate evaporated to an oil that was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ . The organic phase was separated, dried ( $\text{MgSO}_4$ ), and evaporated to dryness to give an oil which was crystallized from MeOH– $\text{H}_2\text{O}$  (12.39 g, 71%): mp 81–82 °C;  $^1\text{H}$  NMR  $\delta$  1.56 (s, 9H, *t*-Bu), 7.96–8.02 (m, 3H, H-3, H-5, H-6);  $^{19}\text{F}$  NMR  $\delta$  –107.1 (t,  $J$  = 8.4 Hz); MS (EI)  $m/z$  241 ( $\text{M}^+$ , 1), 226 ( $\text{M} - \text{CH}_3$ , 2), 168 ( $\text{M} - \text{O}-t\text{-Bu}$ , 95).

**tert-Butyl 2-Fluoro-4-aminobenzoate (27).** A slurry of the nitro ester **26** (1.00 g, 4.1 mmol) and Pd/C (10%, 0.35 g) in ethanol (12 mL) was stirred with ammonium formate (1.54 g, 24.4 mmol). An exothermic reaction was observed, and the mixture was reacted for 50 min. The catalyst was removed by filtration and the filtrate washed with EtOH and concentrated. The residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ , and the organic phase was separated, dried ( $\text{MgSO}_4$ ), and evaporated to dryness to give a solid, which was recrystallized from MeOH– $\text{H}_2\text{O}$  (0.74 g 93%): mp 99–101 °C;  $^1\text{H}$  NMR  $\delta$  1.49 (s, 9 H, *t*-Bu), 6.20 (s, 2 H,  $\text{NH}_2$ ), 6.26 (q, 1 H,  $J_{\text{H-3,H-5}}$  = 2.2 Hz,  $J_{\text{H-3,F}}$  = 14.2 Hz, H-3), 6.37 (q, 1 H,  $J_{\text{H-5,H-6}}$  = 8.7 Hz, H-5), 7.50 (t, 1 H,  $J_{\text{H-6,F}}$  = 8.8 Hz, H-6);  $^{19}\text{F}$  NMR  $\delta$  –108.6 (q); MS (EI)  $m/z$  211 ( $\text{M}^+$ , 4), 155 ( $\text{M} - t\text{-Bu}$ , 100), 138 ( $\text{M} - \text{O}-t\text{-Bu}$ , 63).

**tert-Butyl 2-Fluoro-4-[bis(2-hydroxyethyl)amino]benzoate (28).** Amine **27** (0.46 g, 3.5 mmol) dissolved in HOAc (10 mL) was stirred with ethylene oxide (1.0 mL, 20.0 mmol) at room temperature for 48 h. The solvent was evaporated to dryness and partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ , and the organic phase was separated, dried ( $\text{MgSO}_4$ ), and evaporated to an oil which was purified by column chromatography eluting with EtOAc– $\text{C}_6\text{H}_{12}$  (1:1). The product **28** (0.80 g, 76%) was recrystallized from petroleum ether–ethyl acetate: mp 102–103 °C;  $^1\text{H}$  NMR  $\delta$  1.57 (s, 9 H, *t*-Bu), 3.47 (m, 4 H, 2  $\text{HOCH}_2\text{CH}_2$ ), 3.53 (m, 4 H,  $J$  = 4.8 Hz, 2  $\text{HOCH}_2\text{CH}_2$ ), 4.82 (t, 2H,  $J$  = 5.2 Hz, 2  $\text{HO}$ ), 6.42–6.56 (m, 2 H, H-3, H-5), 7.58 (t, 1 H, H-6);  $^{19}\text{F}$  NMR  $\delta$  –107.6 (m); MS (EI)  $m/z$  299 ( $\text{M}^+$ , 17), 268 ( $\text{M} - \text{CH}_2\text{OH}$ , 38), 243 ( $\text{M} - t\text{-Bu}$ , 2), 226 ( $\text{M} - \text{O}-t\text{-Bu}$ , 19). Anal. ( $\text{C}_{15}\text{H}_{22}\text{NO}_4\text{F}$ ) C, H, N, F.

**tert-Butyl 2-Fluoro-4-[bis(2-chloroethyl)amino]benzoate (31).** **Procedure a.** To the hydroxyethyl derivative **28** (0.70 g, 2.3 mmol) in dry toluene (25 mL) was added  $\text{SOCl}_2$  (1.78 g, 15.0 mmol). The mixture was refluxed for 1.5 h and the solvent removed. This compound was deprotected without prior purification (see 12).

**Procedure b.** A solution of the hydroxyethyl derivative **28** (2.00 g, 6.7 mmol) dissolved in pyridine (10 mL) was stirred with mesyl chloride (2.1 mL, 26.7 mmol) at 2–4 °C for 20 min followed by 80 °C for 15 min. The solvent was evaporated, and the resultant residue was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The organic phase was separated, dried ( $\text{MgSO}_4$ ), and evaporated to give an oil which was purified by column chromatography eluting with EtOAc– $\text{C}_6\text{H}_{12}$  (1:4) to give **31** as a solid which was recrystallized from petroleum ether (1.20 g, 57%): mp 64–65 °C;  $^1\text{H}$  NMR  $\delta$  1.50 (s, 9 H, *t*-Bu), 3.74–3.83 (m, 8 H, 2  $\text{ClCH}_2\text{CH}_2$ ), 6.56–6.66 (m, 2 H, H-3, H-5), 7.64 (t, 1 H, H-6);  $^{19}\text{F}$  NMR  $\delta$  –107.2 (m); MS (EI)  $m/z$  335 ( $\text{M}^+$ , 10), 279 ( $\text{M} - t\text{-Bu}$ , 8), 262 ( $\text{M} - \text{O}-t\text{-Bu}$ , 15), 230 ( $\text{M} - t\text{-Bu} - \text{CH}_2\text{Cl}$ , 100). This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{15}\text{H}_{20}\text{NO}_2\text{Cl}_2\text{F}$ ) C, H, N, Cl, F.

**tert-Butyl 2-Fluoro-4-[bis[2-(mesyloxy)ethyl]amino]benzoate (29) and tert-Butyl 2-Fluoro-4-[2-(2-chloroethyl)-2-(mesyloxy)ethyl]amino]benzoate (30).** A solution of the hydroxyethyl benzoate **28** (1.00 g, 3.3 mmol) in pyridine (10

mL) was stirred with mesyl chloride (1.0 mL, 13.0 mmol) at 2–4 °C for 40 min and then the temperature raised to 50 °C for 30 min. The solvent was evaporated to dryness and the residue dissolved in dry EtOAc and cooled to 4 °C. The solid pyridine hydrochloride was removed by filtration and the filtrate evaporated to give an oil which was purified by column chromatography, eluting with EtOAc– $\text{CH}_2\text{Cl}_2$  (1:1).

The slowest eluting was the 2-fluoro bis[2-(mesyloxy)ethyl] derivative **29** as a solid (0.49 g, 32%): mp 127–128 °C;  $^1\text{H}$  NMR  $\delta$  1.51 (s, 9 H, *t*-Bu), 3.15 (s, 6 H, 2  $\text{CH}_3\text{SO}_3$ ), 3.81 (t, 4 H,  $J$  = 5.47 Hz, 2  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.34 (t, 4H, 2  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 6.59–6.69 (m, 2 H, H-3, H-5), 7.63 (t, 1 H,  $J$  = 9.1 Hz, H-6);  $^{19}\text{F}$  NMR  $\delta$  –107.3 (m); MS (EI)  $m/z$  455 ( $\text{M}^+$ , 5), 382 ( $\text{M} - \text{O}-t\text{-Bu}$ , 18), 346 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2$ , 23), 290 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2 - t\text{-Bu}$ , 100). This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{17}\text{H}_{26}\text{NO}_6\text{FS}_2$ ) C, H, N, F, S.

The faster eluting was the 2-fluoro 2-chloroethyl 2-(mesyloxy)ethyl derivative **30** (0.45 g, 34%), which was recrystallized from  $\text{C}_6\text{H}_{12}$ –EtOAc: mp 61–62 °C;  $^1\text{H}$  NMR  $\delta$  1.50 (s, 9 H, *t*-Bu), 3.15 (s, 3 H,  $\text{CH}_3\text{SO}_3$ ), 3.77 (m, 4 H,  $\text{ClCH}_2\text{CH}_2$ ), 3.83 (t, 2 H,  $J$  = 5.4 Hz,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.33 (t, 2H,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 6.57–6.67 (m, 2 H, H-3, H-5), 7.64 (t, 1 H,  $J$  = 9.0 Hz, H-6);  $^{19}\text{F}$  NMR  $\delta$  –107.2 (m); MS (EI)  $m/z$  395 ( $\text{M}^+$ , 23), 346 ( $\text{M} - \text{CH}_2\text{Cl}$ , 8), 290 ( $\text{M} - \text{CH}_2\text{Cl} - t\text{-Bu}$ , 53), 286 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2$ , 18), 230 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2 - t\text{-Bu}$ , 100). This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{16}\text{H}_{23}\text{NO}_5\text{ClFS}$ ) C, H, N, Cl, F, S.

**Preparation of Acids—General Method.** Compound **29** (0.74 g, 1.6 mmol), **30** (0.65 g, 1.6 mmol), or **31** (0.25 g, 0.74 mmol) was dissolved in TFA (2–3%, w/v) and stirred for 40 min at room temperature. The TFA was evaporated to dryness.

Compound **10**, 2-fluoro-4-[bis(2-(mesyloxy)ethyl)amino]benzoic acid, was obtained from **29** and purified by column chromatography, eluting with EtOAc– $\text{C}_6\text{H}_{12}$  (1:1) (0.46 g, 79%): mp 154–155 °C;  $^1\text{H}$  NMR  $\delta$  3.15 (s, 6 H, 2  $\text{CH}_3\text{SO}_3$ ), 3.80 (t, 4 H,  $J$  = 5.5,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.33 (t, 4 H,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 6.60–6.69 (m, 2 H, H-3, H-5), 7.69 (t, 1 H,  $J$  = 9.1 Hz, H-6);  $^{19}\text{F}$  NMR  $\delta$  –106.9 (m); MS (EI)  $m/z$  399 ( $\text{M}^+$ , 17), 290 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2$ , 90), 246 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2 - \text{CO}_2$ , 50); accurate mass calcd, 399.0457; found, 1.9 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{13}\text{H}_{18}\text{NO}_6\text{FS}_2$ ) C, H, N, F, S.

Compound **11**, 2-fluoro-4-[(2-chloroethyl)[2-(mesyloxy)ethyl]amino]benzoic acid, was obtained from **30** as an oil which crystallized on standing and was recrystallized from EtOAc– $\text{C}_6\text{H}_{12}$  (1:1) (0.43 g, 77%): mp 142–144 °C;  $^1\text{H}$  NMR  $\delta$  3.15 (s, 3 H,  $\text{CH}_3\text{SO}_3$ ), 3.77 (m, 4,  $\text{ClCH}_2\text{CH}_2$ ), 3.83 (t, 2 H,  $J$  = 5.4 Hz,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 4.33 (t, 2 H,  $\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_2$ ), 6.58–6.68 (m, 2 H, H-3, H-5), 7.69 (t, 1 H,  $J$  = 9.1 Hz, H-6);  $^{19}\text{F}$  NMR  $\delta$  –106.9 (m); MS spectrum (EI)  $m/z$  339 ( $\text{M}^+$ , 23), 290 ( $\text{M} - \text{CH}_2\text{Cl}$ , 38), 230 ( $\text{M} - \text{CH}_3\text{SO}_3\text{CH}_2$ , 84); accurate mass calcd 339.0343; found, 2.0 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{12}\text{H}_{15}\text{NO}_5\text{ClFS}$ ) C, H, N, Cl, F, S.

Compound **12**, 2-fluoro-4-[bis(2-chloroethyl)amino]benzoic acid, was similarly obtained from **31** and crystallized on standing. Recrystallization was from petroleum ether–toluene (0.19 g, 91%): mp 132–133 °C;  $^1\text{H}$  NMR  $\delta$  3.76–3.80 (m, 8 H, 2  $\text{ClCH}_2\text{CH}_2$ ), 6.57–6.67 (m, 2 H, H-3, H-5), 7.92 (t,  $J$  = 9.2 Hz, 1 H, H-6), 12.43 (s, 1 H,  $\text{CO}_2\text{H}$ );  $^{19}\text{F}$  NMR  $\delta$  –106.8 (m,  $J_{\text{F,H-3}}$  = 12.9 Hz); MS (EI)  $m/z$  297 ( $\text{M}^+$ , 230 ( $\text{M} - \text{CH}_2\text{Cl}$ ); accurate mass calcd, 279.0229; found, 3.3 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. ( $\text{C}_{11}\text{H}_{12}\text{NO}_2\text{Cl}_2\text{F}$ ) C, H, N, Cl, F.

**3-Fluoro-4-nitrobenzoyl Chloride (32).** A slurry of 3-fluoro-4-nitrobenzoic acid (5.00 g, 27.0 mmol) in dry toluene (100 mL) was stirred with  $\text{SOCl}_2$  (5.00 g, 42.3 mmol) under reflux for 3 h and worked up, as for **25** above, to give **32** which was crystallized from petroleum ether (3.39 g, 62%): mp 22–23 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.04–8.08 (m, 2 H, H-2, H-6), 8.10–8.22 (m, 1 H, H-5);  $^{19}\text{F}$  NMR ( $\text{CDCl}_3$ )  $\delta$  –115.24 (t,  $J$  = 8.8 Hz); MS (EI)  $m/z$  168 ( $\text{M} - \text{Cl}$ , 100).

**tert-Butyl 3-Fluoro-4-nitrobenzoate (33).** Butyllithium (65 mL (1.6 M in hexane), 103.2 mmol) was added to *tert*-butyl

alcohol (200 mL) over 30–40 min at 20 °C under N<sub>2</sub> and stirred for 20 min. The acid chloride **32** (21.00 g, 103.2 mmol) in THF (30 mL) was added and the mixture stirred for 22 h. The product was worked up, as for **26** above, to give **33** which was crystallized from MeOH–H<sub>2</sub>O (12.88 g, 58%): mp 68–70 °C; <sup>1</sup>H NMR δ as for **26** above except 1.57 (s, 9 H, *t*-Bu), 7.87–7.99 (m, 2 H, H-2, H-6), 8.27 (m, 1 H, H-5); <sup>19</sup>F NMR δ –117.5 (m); MS (EI) *m/z* 241 (M<sup>+</sup>, 2), 226 (M – CH<sub>3</sub>, 5), 1.68 (M – *O*-*t*-Bu, 100).

**tert-Butyl 3-Fluoro-4-aminobenzoate (34).** A slurry of the nitro ester **33** (1.5 g, 6.2 mmol) and Pd/C (10%, 0.65 g) in ethanol (20 mL) was stirred with ammonium formate (3.00 g, 47.5 mmol). The product was worked up as for **27** above and crystallized from MeOH–H<sub>2</sub>O to give **34** (1.28 g, 98%): mp 48–50 °C; <sup>1</sup>H NMR δ 1.50 (s, 9 H, *t*-Bu), 6.00 (s, 2 H, NH<sub>2</sub>), 6.71–6.79 (m, 1 H, H-5), 7.40–7.49 (m, 2 H, H-2, H-6); <sup>19</sup>F NMR δ –135.3 (m); MS (EI) *m/z* 211 (M<sup>+</sup>, 21), 155 (M – *t*-Bu, 100), 138 (M – *O*-*t*-Bu, 81).

**tert-Butyl 3-Fluoro-4-[bis(2-hydroxyethyl)amino]benzoate (35).** Amine **34** (1.30 g, 6.2 mmol) dissolved in HOAc (15 mL) was stirred with ethylene oxide (2.0 mL, 40.0 mmol) at room temperature for 48 h. The product **35** was worked up as for **28** above (1.30 g, 71%): mp 48–49 °C; <sup>1</sup>H NMR δ 1.51 (s, 9 H, *t*-Bu), 3.46 (m, 4 H, 2 HOCH<sub>2</sub>CH<sub>2</sub>), 3.55 (t, 4 H, *J* = 5.3 Hz, 2 HOCH<sub>2</sub>CH<sub>2</sub>), 4.77 (t, 2 H, 2 HO), 6.96 (t, 1 H, H-5), 7.40–7.55 (m, 2 H, H-2, H-6); <sup>19</sup>F NMR δ –124.4 (m); MS (EI) *m/z* 299 (M<sup>+</sup>, 5), 268 (M – CH<sub>2</sub>OH, 50), 226 (M – *O*-*t*-Bu, 9). Anal. (C<sub>15</sub>H<sub>22</sub>NO<sub>4</sub>F) C, H, N, F.

**tert-Butyl 3-Fluoro-4-[bis(2-mesyloxyethyl)amino]benzoate (36) and tert-Butyl 3-Fluoro-4-[(2-chloroethyl)-[2-mesyloxyethyl]amino]benzoate (37).** To a solution of the hydroxyethyl benzoate **35** (2.00 g, 6.7 mmol) in pyridine (10 mL) was added mesyl chloride (2.1 mL, 26.7 mmol) at 2–4 °C. The reaction mixture was stirred at this temperature for 40 min and then the temperature raised to 50 °C for 30 min. The product was worked up and purified as for **29** and **30** above.

The slowest eluting was the 3-fluoro bis[2-(mesyloxy)ethyl] derivative **36** as a solid, which was recrystallized from petroleum ether–toluene (0.97 g, 32%): mp 77–79 °C; <sup>1</sup>H δ 1.52 (s, 9 H, *t*-Bu), 3.12 (s, 6 H, 2 CH<sub>3</sub>SO<sub>3</sub>), 3.74 (t, 4 H, *J* = 5.5 Hz, 2, CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.31 (t, 4 H, 2 CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 7.14 (t, 1 H, *J* = 8.9 Hz, H-5), 7.49–7.62 (m, 2 H, H-2, H-6); <sup>19</sup>F NMR δ –122.5 (m); MS (EI) *m/z* 455 (M<sup>+</sup>, 10), 3.82 (M – *O*-*t*-Bu, 17), 346 (M – CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>, 50), 290 (M – CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>–*t*-Bu, 100). This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>17</sub>H<sub>26</sub>NO<sub>8</sub>FS<sub>2</sub>) C, H, N, F, S.

The faster eluting was the 3-fluoro 2-chloroethyl 2-(mesyloxy)ethyl derivative **37** as an oil (0.60 g, 23%): <sup>1</sup>H NMR δ 1.52 (s, 9 H, *t*-Bu), 3.13 (s, 3 H, CH<sub>3</sub>SO<sub>3</sub>), 3.73 (m, 4 H, ClCH<sub>2</sub>CH<sub>2</sub>), 3.76 (t, 2 H, *J* = 5.4 Hz, CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.31 (t, 2 H, CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 7.11 (t, 1 H, *J* = 8.9 Hz, H-5), 7.49–7.62 (m, 2 H, H-2, H-6); <sup>19</sup>F NMR δ –123.1 (m); MS (EI) *m/z* 395 (M<sup>+</sup>, 5), 346 (M – CH<sub>2</sub>Cl, 5), 321 (M – *t*-Bu, 5), 290 (M – CH<sub>2</sub>Cl – *t*-Bu, 19), 230 (M – CH<sub>3</sub>SO<sub>3</sub>OCH<sub>2</sub>–*t*-Bu, 100). This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>16</sub>H<sub>23</sub>NO<sub>5</sub>ClFS) C, H, N, Cl, F, S.

**tert-Butyl 3-Fluoro-4-[bis(2-chloroethyl)amino]benzoate (38).** Procedure a. To the hydroxyl derivative **35** (0.88 g, 2.9 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added SOCl<sub>2</sub> (1.63 g, 13.7 mmol). The mixture was refluxed for 45 min and the solvent removed. The product **38** was purified by column chromatography eluting with EtOAc–cC<sub>6</sub>H<sub>12</sub> (1:1) to yield an oil (0.49 g, 50%).

**Procedure c.** Mesyl chloride (0.21 mL, 2.6 mmol) was added to the bis[2-(mesyloxy)ethyl] derivative **36** (0.30 g, 0.66 mmol) dissolved in pyridine (3.0 mL). The temperature of the reaction mixture was raised to 90 °C for 1.5 h. The solution was evaporated to dryness and the residue thus obtained partitioned between EtOAc and H<sub>2</sub>O. The organic layer was dried (MgSO<sub>4</sub>) and evaporated, and the resulting oil was purified by column chromatography eluting with EtOAc–cC<sub>6</sub>H<sub>12</sub> (1:1) to give the oil **38** (0.11 g, 48%): <sup>1</sup>H NMR δ 1.52 (s, 9 H, *t*-Bu), 3.74 (s, 8 H, 2 ClCH<sub>2</sub>CH<sub>2</sub>), 7.08 (t, 1 H, *J*<sub>H-5,H-6</sub> = 8.7 Hz, *J*<sub>H-5,F</sub> = 8.92 Hz, H-5), 7.51 (q, 1 H, *J*<sub>H-2,H-6</sub> = 2.0 Hz,

*J*<sub>H-2,F</sub> = 14.9 Hz, H-2), 7.57 (q, 1 H, H-6); <sup>19</sup>H NMR δ –123.70 (m); MS (EI) *m/z* 335 (M<sup>+</sup>, 42), 286 (M – CH<sub>2</sub>Cl, 87), 262 (M – *O*-*t*-Bu, 42), 230 (M – *t*-Bu – CH<sub>2</sub>Cl<sub>2</sub>, 100). This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>15</sub>H<sub>20</sub>NO<sub>2</sub>Cl<sub>2</sub>F) C, H, N, Cl, F.

**Preparation of Acids—General Method.** The deprotection was carried out as for compounds **29–31** above. Briefly, compounds **36** (0.40 g), **37** (0.17 g), and **38** (from **35** (0.89 g) in a two-step nonisolated preparation) were dissolved in TFA (2–3% w/v), and the reaction mixture was worked up as for the 2-fluoro derivatives above.

3-Fluoro-4-[bis(2-(mesyloxy)ethyl)amino]benzoic acid (**22**) was obtained from **36** as a solid and recrystallized from petroleum ether–EtOAc (0.37 g, 100%): mp 105–106 °C; <sup>1</sup>H NMR δ 3.12 (d, 6 H, *J* = 3.16 Hz, 2 CH<sub>3</sub>SO<sub>3</sub>), 3.74 (t, 4 H, *J* = 5.4 Hz, 2 CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.32 (t, 4 H, 2 CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 7.15 (t, 1 H, *J* = 8.8 Hz, H-5), 7.52–7.65 (m, 2 H, H-2, H-6); <sup>19</sup>F NMR δ –122.5 (m); MS (EI) *m/z* 417 ([M + H<sub>2</sub>O], 8), 399 (M<sup>+</sup>, 5), 308 ([M + H<sub>2</sub>O] – CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>, 100), 290 (M – CH<sub>3</sub>SO<sub>3</sub>OCH<sub>2</sub>, 22); accurate mass calcd (for [M + H<sub>2</sub>O]), 417.0563; found, 2.9 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>13</sub>H<sub>18</sub>FO<sub>8</sub>S<sub>2</sub>·0.5TFA) C, H, N.

Compound **23**, 3-fluoro-4-[(2-chloroethyl)[2-mesyloxy]ethyl]amino]benzoic acid, was likewise obtained from **37** and recrystallized from ethyl acetate–petroleum ether (0.14 g, 95%): mp 103–104 °C; <sup>1</sup>H NMR δ 3.13 (s, 3 H, CH<sub>3</sub>SO<sub>3</sub>), 3.73 (s, 4 H, ClCH<sub>2</sub>CH<sub>2</sub>), 3.76 (t, 2 H, *J* = 5.3 Hz, CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 4.32 (t, 2 H, CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>), 7.11 (t, 1 H, *J* = 8.8 Hz, H-5), 7.58–7.65 (m, 2 H, H-2, H-6); <sup>19</sup>F NMR δ –123.3 (m); MS (EI) *m/z* 339 (M<sup>+</sup>, 4), 290 (M – CH<sub>2</sub>Cl, 40), 230 (M – CH<sub>3</sub>SO<sub>3</sub>CH<sub>2</sub>, 100); accurate mass calcd, 339.0343; found, 2.6 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>12</sub>H<sub>15</sub>NO<sub>5</sub>ClFS) C, H, N, F, S.

Compound **24**, 3-fluoro-4-[bis(2-chloroethyl)amino]benzoic acid, was similarly obtained from **38** and recrystallized from petroleum ether–toluene (0.33 g, 36% from **35**): mp 106–107 °C; <sup>1</sup>H NMR δ 3.74 (s, 8 H, 2 ClCH<sub>2</sub>CH<sub>2</sub>), 7.09 (t, 1 H, *J* = 8.9 Hz, H-5), 7.53–7.65 (m, 2 H, H-2, H-6), 12.74 (s, 1 H, CO<sub>2</sub>H); <sup>19</sup>F NMR δ –124.0 (m); MS (EI) *m/z* 279 (M<sup>+</sup>, 8), 230 (M – CH<sub>2</sub>Cl, 100); accurate mass calcd, 279.0229; found, 2.2 ppm. This compound reacted positively (blue color) with the Epstein spray reagent. Anal. (C<sub>11</sub>H<sub>12</sub>NO<sub>2</sub>Cl<sub>2</sub>F) C, H, N, Cl, F.

**Chemical Half-Life Determinations.** The *t*<sub>1/2</sub> was determined according to our previously described method.<sup>21</sup> Briefly, each compound, **10–12**, **19–21**, and **22–24**, was added to sodium perchlorate (0.1 M, 10 mL), and the hydrolysis reaction was followed to effective completion by titration of the released acid with NaOH (0.01 M) under N<sub>2</sub> using an automatic titrator at a constant pH of 7.4 at 37 °C. Each measurement was performed at least twice. Results are expressed as the mean of all determinations.

**Biological Methods. Cytotoxicity Assays.** LS174T cells (2.5 × 10<sup>3</sup>) were plated in Dulbecco's modified Earl's medium (DMEM) + fetal calf serum (10%) in 96-well plates in 5% CO<sub>2</sub> at 37 °C. Each novel potential prodrug, **7–9** and **19–21**, and its corresponding novel parent drug, **10–12** and **22–24**, respectively, were incubated at a range of six concentrations (1–800 μM) with the LS174T cells for 1 h.

CPG2 (6 units mL<sup>−1</sup> final concentration) was added to test wells in equivalent cultures with each potential prodrug to achieve parent drug in situ. Compounds and CPG2 solutions were made up just prior to use and added once. Each concentration of potential prodrug and parent drug was performed in octuplicate. Cells were incubated for 1 h with each parent drug or potential prodrug ± CPG2. The cells were then washed (three times) to remove any remaining compound and reincubated for a further 6 days. At the end of this period, the cells were fixed and stained. The concentration of cellular protein from the remaining viable cells was quantitated by a sulforhodamine B assay.<sup>24</sup> The results of protein concentration in treated samples were compared to those of untreated controls. The prodrug **40** and its parent drug **43** were also assayed under the same conditions. Each experiment was performed at least twice. The standard deviation for each data point was less than or equal to 10%. A representative result

for each potential prodrug and parent drug is shown in Table 3, and the non-fluorinated and 3-F compounds are shown in the figures.

**Enzyme Kinetics.** The  $K_m$  and  $k_{cat}$  of the cleavage of the amidic bond with CPG2 were calculated for each of the potential prodrugs and compared to the non-fluorinated prodrug **40** by modifications of literature CPG2 assay methods.<sup>8,25</sup> The reaction mixture (1.0 mL) containing potential prodrug (100  $\mu$ M), Tris/HCl (100 mM, pH 7.3), and  $ZnCl_2$  (260  $\mu$ M) was incubated at 37 °C in a thermostated spectrophotometer. An absorbance spectrum was recorded for each compound between 240 and 400 nm. The wavelength corresponding to the maximal absorbance difference between each potential prodrug and its parent drug was recorded. This wavelength was used for subsequent prodrug kinetic measurements. The reaction for each potential prodrug was initiated by the addition of CPG2 (0.01–1.0 unit), and the change in the absorbance, at the wavelength determined as above, was measured. Analyses were performed in duplicate. The  $K_m$  and  $k_{cat}$  values were obtained for concentrations of the potential prodrugs in the range 0.1–100  $\mu$ M using a concentration of CPG2 that was kept constant for each determination.

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