Optimization of Alkylating Agent Prodrugs Derived from Phenol and Aniline Mustards: A New Clinical Candidate Prodrug (ZD2767) for Antibody-Directed Enzyme Prodrug Therapy (ADEPT)

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Sixteen novel potential prodrugs derived from phenol or aniline mustards and their 16 corresponding drugs with ring substitution and/or different alkylating functionalities were designed. The [[[4-[bis(2-bromoethyl)- (**1a**), [[[4-[bis(2-iodoethyl)- (**1b**), and [[[4-[(2-chloroethyl)-[2-(mesyloxy)ethyl]amino]phenyl]oxy]carbonyl]-L-glutamic acids (**1c**), their [[[2- and 3-substituted-4-[bis(2-chloroethyl)amino]phenyl]oxy]carbonyl]-L-glutamic acids (**1e–l**), and the [[3-substituted-4-[bis(2-chloroethyl)amino]phenyl]carbamoyl]-L-glutamic acids (**1o–r**) were synthesized. They are bifunctional alkylating agents in which the activating effect of the phenolic hydroxyl or amino function is masked through an oxycarbonyl or a carbamoyl bond to a glutamic acid. These prodrugs were designed to be activated to their corresponding phenol and aniline nitrogen mustard drugs at a tumor site by prior administration of a monoclonal antibody conjugated to the bacterial enzyme carboxypeptidase G2 (CPG2) in antibody-directed enzyme prodrug therapy (ADEPT). The synthesis of the analogous novel parent drugs (**2a–r**) is also described. The viability of a colorectal cell line (LoVo) was monitored with the potential prodrugs and the parent drugs. The differential in the cytotoxicity between the potential prodrugs and their corresponding active drugs ranged between 12 and >195 fold. Compounds **1b–d,f,o** exhibited substantial prodrug activity, since a cytotoxicity differential of >100 was achieved compared to **2b–d,f,o** respectively. The ability of the potential prodrugs to act as substrates for CPG2 was determined (kinetic parameters K_M and k_{cat}), and the chemical stability was measured for all the compounds. The unsubstituted phenols with different alkylating functionalities (**1a–c**) proved to have the highest ratio of the substrates k_{cat}:K_M. From these studies [[[4-[bis(2-iodoethyl)amino]phenyl]oxy]carbonyl]-L-glutamic acid (**1b**) emerges as a new ADEPT clinical trial candidate due to its physicochemical and biological characteristics.

Introduction

Nitrogen mustard drugs are in common clinical use in cancer chemotherapy.[1] Unfortunately, their clinical efficacy has been limited by their toxicity to normal tissues. Therefore, selective tumor generation of a potent cytotoxic nitrogen mustard from a relatively inactive prodrug is desirable. Antibody-directed enzyme prodrug therapy (ADEPT)[2,3] is a two-component system that is designed to generate drugs selectively at the tumor. In the first phase a tumor selective antibody–enzyme conjugate is administered. Time is allowed to optimize conjugate localization at the tumor, with clearance from blood and other normal tissues, before administration of the second component in the form of a prodrug.[3] There is an amplification feature whereby one enzyme conjugate molecule is able to catalyze the conversion of many prodrug molecules to the cytotoxic parent drug, which provides advantages in addition to the selectivity conferred by the antibody. Nitrogen mustard prodrugs are excellent candidates for ADEPT since their cytotoxicity is dose-related and they can be administered repeatedly with less induced resistance than other classes of anticancer agents.[4] Also, an additional advantage is that they are active against quiescent cells in G_0. We have already utilized different nitrogen mustard prodrugs in ADEPT systems.[3,5–10] To date there have been two pilot clinical trials in ADEPT, on the prodrug [4-[(2-chloroethyl)[2-(mesyloxy)ethyl]-amino]benzoyl]-L-glutamic acid (**1s**), with promising results[11–13] which have confirmed the feasibility of this approach.

The rationale for the synthesis of the 16 novel potential prodrugs described herein was to optimize the cytotoxicity differential, enzyme kinetics, and chemical stability in order to provide improvements to the previously synthesized clinical prodrug **1s** and the subsequently synthesized fluoro aromatic substituted prodrugs. A problem associated with ADEPT appeared to be the potential for drugs to leak back into the general circulation after formation at the tumor.[14] In order to circumvent this potential drawback, active drugs with very short half-lives were considered desirable. The synthesis of appropriately substituted nitrogen mustards from benzoic acids is one way to fulfill this requirement and has been explored previously.[8] An alternative possibility[15] is to use the more reactive

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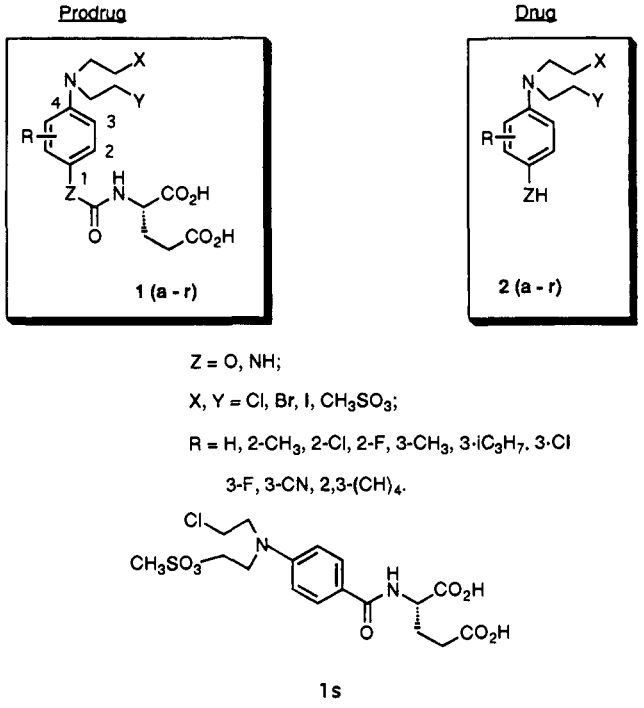
Chart 1



phenol and aniline alkylating agents as the active drugs and to design the corresponding prodrugs for an ADEPT approach using carboxypeptidase G2 (CPG2) as activating enzyme (see Chart 1). A bond cleavable by CPG2 is essential and was constructed in the prodrug between the active drug and the glutamic acid moiety. Prodrugs with linkages −OCO− (oxycarbonyl) for the phenol nitrogen mustards and −NHCO− (carbamoyl) for the aniline nitrogen mustards have been shown to be substrates for CPG2.[15,16] We found previously that different aromatic substitutions influence the reactivity and substrate specificity of the prodrugs.[8,17] Herein we have examined different aromatic substitutions in the phenol and aniline mustard potential prodrugs and drugs.

Finally, the more reactive bisbromo and bisiodo nitrogen mustards were also considered as active drugs. An S_N2 reaction mechanism operates for the latter two types of nitrogen mustards which contrasts with the S_N1 mechanism for the bischloro aromatic mustards.[17]

The purpose of this paper is to explore these potential prodrugs for their efficacy in ADEPT. The synthesis of the 16 novel potential prodrugs **1a−r** and their corresponding active drugs **2a−r** for use in ADEPT is described (see Chart 1). Compounds **1a−r** are bifunctional alkylating agents in which the activating effect of the phenol or aniline function is masked through an oxycarbonyl or carbamoyl bond to the glutamic acid residue. These compounds are designed to be activated to their corresponding phenol or aniline alkylating agents at the tumor site by prior administration of a tumor specific monoclonal antibody conjugated to the bacterial enzyme CPG2.

The 16 novel diacids provide a selection of potential prodrugs of differing reactivity and substrate specificity. Accordingly, they were analyzed for they ability to act as substrates for CPG2. It was anticipated that this variety of compounds could lead to the selection of a new clinical prodrug. The optimal combination of physicochemical and biological parameters suggested prodrug **1b** (ZD2767) to be the candidate of choice.

Results and Discussion

Chemistry. Sixteen potential prodrugs were synthesized as shown in Schemes 1−3. Structures **1a-r** and their corresponding active drugs **2a−r** are new compounds. Their physicochemical and analytical characteristics are given in Tables 1 and 2.

A. Phenol Prodrugs and Active Drugs. The starting materials for the 12 potential phenol prodrugs were the unsubstituted and substituted aminophenols **3a−l**, respectively, which were commercially available except for compounds **3e,g,h,k**. The 3-chloro-4-hydroxyaniline, **3e**, and the 3-fluoro-4-hydroxyaniline, **3h**, were obtained by catalytic hydrogenation of the corresponding nitro derivatives. The 2-chloro-4-hydroxyaniline, **3k**, was prepared by nitration of the 3-chlorophenol and subsequent reduction of the resultant 3-chloro-4-nitrophenol. The routes to the potential phenol prodrugs and active drugs are shown in Schemes 1 and 2.

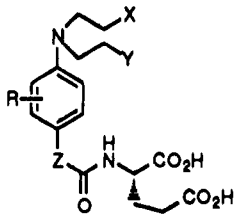
The aminophenols **3a−l** were N-alkylated using excess ethylene oxide in glacial acetic acid to form the corresponding [*N*,*N*-bis(2-hydroxyethyl)amino]phenols **5a−l.** At this stage, protection of the phenolic hydroxyl was necessary in order to avoid secondary reaction in the later steps (halogenation or mesylation of the alcoholic hydroxyls). Benzylation of the phenolic hydroxyl group provided a good protection strategy for the bis(2-chloroethyl) prodrug and active drug precursors, which proved to be stable during deprotection by hydrogenation. The benzylic ethers **6a−l** were obtained by treatment of the corresponding [*N*,*N*-bis(2-hydroxyethyl)amino]phenols **5a−l** with benzyl bromide in the presence of potassium carbonate.

However, since the mesylethyl-, iodoethyl-, and bromoethylamino groups are sensitive to hydrogenolysis, a different protection strategy was required for the synthesis of these prodrugs and their corresponding active drugs. We proposed the use of the (1-adamantyloxy)carbonyl moiety, removable by TFA at room temperature, as an alternative protecting group for phenols carrying strongly electron-withdrawing substituents.[18] Accordingly, the reaction of the 4-nitrophenol and 3-fluoro-4-nitrophenol with 1-adamantyl fluoroformate in THF in the presence of pyridine led to the corresponding carbonates in high yield. The resultant carbonates were reduced using catalytic hydrogen transfer (Pd/C, 10%) from ammonium formate to give the protected aminophenols **4a,h** which were converted to the bis(hydroxyethyl) derivatives **6a,h** as previously described.[18]

The substituted bis(2-chloroethyl) derivatives **7d−h,k,l** were obtained by direct chlorination of the hydroxyethyl precursors with PCl_5 in $CHCl_3$ or mesyl chloride in pyridine. The hydroxyethyl derivatives **6a,h** were used as common intermediates for the synthesis of compounds **7c,m** and **7h,n**, respectively (see Scheme 1). The mesylation of these compounds (**6a,h**) in mild conditions resulted in a mixture of monomesyl and bismesyl derivatives which were purified by column chromatography on silica gel.

Both 1-adamantyl 4-[bis[2-(mesyloxy)ethyl]amino]-phenyl carbonate, **7m**, and 1-adamantyl 3-fluoro-4-[bis-[2-(mesyloxy)ethyl]amino]phenyl carbonate, **7n**, were efficiently transfomed into the corresponding bisbromo (**7a,i**) and bisiodo (**7b,j**) nitrogen mustards by reaction

Table 1. Phenol and Aniline Prodrugs



no.	R	Z	X, Y	mp (°C)	^{1}H NMR (δ, ppm)[a]	MS (*m/z*)	yield (%)	anal
1a	H	O	Br	oil	7.92 (d, 1H, NH, J = 8.0 Hz), 6.94 (d, 2H, H_2, H_6, J = 9.0 Hz), 6.71 (d, 2H, H_3, H_5) 3.76 (t, 4H, CH_2Cl, J = 7.1 Hz), 3.58 (t, 4H, CH_2N)	495 ($M^+ + 1$)[c]	80	C,H,N,Br
1b	H	O	I	oil	7.92 (d, 1H, NH, J = 8.0 Hz), 6.94 (d, 2H, H_2, H_5, J = 8.8 Hz), 6.66 (d, 2H, H_3, H_5), 3.72 (t, 4H, 2 $ClCH_2$, J = 7.6 Hz), 3.31 (t, 4H, 2 CH_2N)	591 ($M^+ + 1$)[c]	82	C,H,N,I
1c	H	O	CH_3SO_3, Cl	oil	7.90 (d, 1H, NH, J = 7.6 Hz), 6.92 (dd, 2H, H_2, H_6, J = 9.0 Hz), 6.75 (d, 2H, H_3, H_5), 4.49 + 4.30 (t, 2H, $CH_3SO_3CH_2$, J = 5.5 Hz), 3.70 (m, 6H, 2 $ClCH_2$ + 4 CH_2N), 3.15 (s, 3H, CH_3SO_3)	467 ($M^+ + 1$)[c]	95	C,H,N,Cl,S[e]
1d	2-CH_3	O	Cl	124–6	7.90 (d, 1H, NH, J = 8.3 Hz), 6.83 (d, 1H, H_6, J = 8.8 Hz), 6.60–6.49 (m, 2H, H_3, H_5), 3.66 (m, 8H, 2 $ClCH_2CH_2N$), 2.07 (s, 3H, 2-CH_3)	420 (M^+)	62	C,H,N
1e	2-Cl	O	Cl	106–8	8.10 (d, 1H, NH, J = 8.3 Hz), 7.06 (d, 1H, H_6, J = 9.0 Hz), 6.85–6.67 (m, 2H, H_3, H_5), 3.73 (m, 8H, 2 $ClCH_2CH_2N$)	441 (M^+)	58	C,H,N
1f	3-CH_3	O	Cl	160–2	8.01 (d, 1H, NH, J = 8.0 Hz), 7.22 (d, 1H, H_5, J = 8.8 Hz), 6.96–6.83 (m, 2H, H_2, H_6), 3.54 (t, 4H, 2 $ClCH_2$, J = 6.0 Hz), 3.34 (t, 4H, 2 CH_2N), 2.27 (s, 3H, 3-CH_3)	420 (M^+)	70	C,H,N
1g	3-*i*-Pr	O	Cl	156–8	8.02 (d, 1H, NH, J = 8.0 Hz), 7.28 (d, 1H, H_5, J = 8.6 Hz), 7.00–6.85 (m, 2H, H_2, H_6), 3.67 (m, 1H, C*H*-*i*-Pr), 3.54 (t, 4H, 2 $ClCH_2$, J = 6.0 Hz), 3.30 (t, 4H, 2 NCH_2), 1.13 (d, 6H, 2 CH_3-*i*-Pr)	448 (M^+)	82	C,H,N
1h	3-F	O	Cl	oil	8.05 (d, 1H, NH, J = 8.3 Hz), 7.13 (t, 1H, H_5), 7.04–6.82 (m, 2H, H_2, H_6), 3.64 (t, 4H, 2 $ClCH_2$, J = 6.3 Hz), 3.56 (t, 4H, 2 NCH_2)	425 (M^+)	36	C,H,N
1i	3-F	O	Br	oil	8.08 (d, 1H, NH, J = 8.0 Hz), 7.12 (t, 1H, H_5, J_H = 8.3 Hz, J_F = 7.5 Hz), 7.05–6.80 (m, 2H, H_2, H_6), 3.65–3.46 (m, 8H, 2 $BrCH_2CH_2N$)	514 (M^+)	93	C,H,N
1j	3-F	O	I	oil	7.01 (t, 1H, H_5, J_H = 10.4 Hz, J_F = 8.3 Hz), 6.95–6.68 (m, 2H, H_2, H_6), 5.87 (d, 1H, NH, J = 8.3 Hz), 3.50 (t, 4H, 2 ICH_2, J = 7.5 Hz), 3.16 (t, 4H, 2 CH_2N)[b]	608 (M^+)	33	C,H,N
1k	3-Cl	O	Cl	oil	7.24 (d, 1H, NH, J = 7.0 Hz), 7.08 (d, 1H, H_5, J = 8.8 Hz), 6.87 (d, 1H, H_2, J = 2.8 Hz), 6.70 (dd, 1H, H_6), 3.50–3.37 (m, 8H, 2 $ClCH_2CH_2N$)	441 (M^+)	92	C,H,N
1l	2,3-$(CH)_4$	O	Cl	180–2	8.50–8.40 (m, 1H, $H_{5(8)}$), 8.04–7.95 (m, 1H, $H_{8(5)}$), 7.53–7.46 (m, 2H, H_{arom}), 7.35–7.23 (m, 2H, H_{arom}), 6.67 (d, 1H, NH, J = 8.3 Hz), 3.65–3.43 (m, 8H, 2 $ClCH_2CH_2N$)	456 (M^+)	65	C,H,N
1o[f]	3-F	NH	Cl	111–4	8.67 (s, 1H, NH-Ph), 7.38 (q, 1H, H_2, J_H = 2.4 Hz, J_F = 14.0 Hz), 7.06 (t, 1H, H_5, J_H = J_F = 9.0 Hz), 6.94 (q, 1H, H_6), 6.45 (d, 1H, NH-G,[d] J = 8.0 Hz), 3.59 (t, 4H, 2 CH_2Cl, J = 6.0 Hz), 3.46 (t, 4H, 2 NCH_2)	nd	75	C,H,N[f]
1p	3-F	NH	Br	oil	8.66 (s, 1H, N*H*-Ph), 7.55 (q, 1H, H_2, J_H = 2.4 Hz, J_F = 15.0 Hz), 7.04 (t, 1H, H_5, J_H = J_F = 9.3 Hz), 6.92 (q, 1H, H_6), 6.44 (d, 1H, NH-G,[d] J = 8.7 Hz), 3.47 (m, 8H, 2 $ClCH_2CH_2N$)	512 ($M^+ - 1$)[c]	62	C,H,N
1q	3-Cl	NH	Cl	136–8	8.73 (s, 1H, NH-Ph), 7.64 (d, 1H, H_2, J_H = 2.2 Hz), 7.24 (d, 1H, H_5, J_H = 8.5 Hz), 7.15 (q, 1H, H_6), 6.49 (d, 1H, NH-G,[d] J = 8.0 Hz), 3.55 (t, 4H, 2 $ClCH_2$, J = 5.6 Hz), 3.42 (t, 4H, 2 CH_2N)	441 (M^+)	77	C,H,N
1r	3-CN	NH	Cl	105–7	8.78 (s, 1H, NH-Ph), 7.77 (d, 1H, H_2, J_H = 1.4 Hz), 7.47 (q, 1H, H_6), 7.25 (d, 1H, H_5, J = 9.2 Hz), 6.55 (d, 1H, NH-G,[d] J = 10.0 Hz), 3.67–3.56 (m, 8H, 2 $ClCH_2CH_2N$)	430 (M^+)	14	C,H,N

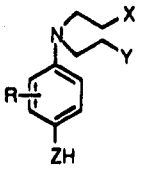
[a] The signals for the glutamic acid protons for **1** are as follows: 4.28–3.98 (m, 1H, CH), 2.45–2.24 (m, 2H, CH_2CO_2H), 2.15–1.70 (2m, 2H, CH_2CH). [b] $CDCl_3$, the signals for the glutamic acid protons are 4.59–4.44 (m, 1H, CH), 2.65–2.50 (m, 2H, CH_2CO_2H), 2.42–2.12 (2m, 2H, CH_2CH). [c] By FAB. [d] G = glutamic acid. [e] $C_{17}H_{23}ClN_2O_9S \cdot 1.2TFA \cdot 0.16EtOAc$ calcd: C = 39.6, H = 4.3, N = 4.7, Cl = 5.9, S = 5.4. Found: C = 39.2, H = 4.5, N = 4.3, Cl = 6.1, S = 5.4. [f] $C_{16}H_{20}Cl_2FN_3O_5$ calcd: C = 45.3, H = 4.75, N = 9.9. Found: C = 45.5, H = 4.9, N = 9.4.

with lithium bromide or sodium iodide, respectively, in CH_3CN at 70 °C. The deprotection of the intermediates **7a–l** to the active drugs **2a–l** was carried out by (a) hydrogenation with Pd/C (30%) catalyst in AcOEt for the benzyl-protected intermediates **7d–h,k,l**, and (b) by dissolution in TFA at room temperature for the (1-adamantyloxy)carbonyl-protected compounds **7a–c,h–j,m,n**.[5] Some of the phenol nitrogen mustards were separated as salts (hydrochlorides or oxalates).

In order to synthesize the potential prodrugs **1d–h,k,l**, the phenolic hydroxyl of the nitrogen mustards **2d–h,k,l** was activated as the 4-nitrophenyl carbonate by reaction with 4-nitrophenyl chloroformate in the presence of triethylamine. The coupling of carbonates **8d–h,k,l** with L-glutamic acid dibenzyl ester, at 60 °C, afforded the protected prodrugs **9d–h,k,l**, which were purified on columns of silica gel. The final deprotection was carried out by catalytic hydrogenation (Pd/C) and led to the potential prodrugs **1d–h,k,l.** The synthesis of the more reactive prodrugs **1a–c,i,j** was performed by an alternative route using L-glutamic acid di-*tert*-butyl ester (see Scheme 2).

Accordingly, 4-nitrophenyl chloroformate was directly coupled to the glutamic acid *tert*-butyl ester leading to

Table 2. Phenol and Aniline Drugs



no.	R	Z	X, Y	mp (°C)	^{1}H NMR (δ, ppm)	MS (*m/z*)	yield (%)	anal.
2a	H	O	Br	oil	6.65 (s, 4H, H_{arom}), 3.64 (t, 4H, 2 $ClCH_2$, J = 5.9 Hz), 3.51 (t, 4H, 2 CH_2N)	324 ($M^+ + 1$)[a]	99	3.7 ppm
2b	H	O	I	oil	6.68 (m, 4H, H_{arom}), 4.34 + 3.23 (m, 4H, 2 ICH_2, J = 5.3 Hz), 3.57 (m, 4H, 2 CH_2N, J = 7.4 Hz)	418 ($M^+ + 1$)[a]	99	4.5 ppm
2c	H	O	CH_3SO_3, Cl	oil	9.96 (bds, 1H, OH), 6.67 (s, 4H, H_{arom}), 4.24 (t, 2H, $CH_3SO_3CH_2$, J = 5.9 Hz), 3.64–3.57 (m, 6H, 4 CH_2N + 2 $ClCH_2$), 3.12 (s, 3H, CH_3SO_3)	311 ($M^+ + H_2O$)	93	4.5 ppm
2d·HCl	2-CH_3	O	Cl	122–4	6.87–6.68 (m, 3H, H_{arom}), 3.64 (s, 8H, 2 $ClCH_2CH_2N$), 2.10 (s, 3H, 2-CH_3)	247 (M^+)	98	C,H,N[c]
2e·HCl	2-Cl	O	Cl	156–8	6.88 (d, 1H, H_6, J = 9.0 Hz), 6.75 (d, 1H, H_2, J = 3.0 Hz), 6.64 (dd, 1H, H_5), 3.64 (m, 8H, 2 $ClCH_2CH_2N$)	267 (M^+)	60	C,H,N
2f·HCl	3-CH_3	O	Cl	164–7	7.05 (d, 1H, H_5, J = 9.2 Hz), 6.66–6.53 (m, 2H, H_2, H_6), 3.50 (t, 4H, 2 $ClCH_2$, J = 5.0 Hz), 3.39 (t, 4H, 2 CH_2N),2.20 (s, 3H, 3-CH_3)	247 (M^+)	56	C,H,N
2g·HCl	3-*i*-Pr	O	Cl	124–7	7.06 (d, 1H, H_5, J = 8.3 Hz), 6.64–6.54 (m, 2H, H_2, H_6), 3.63 (m, 1H, C*H*-*i*-Pr, J = 7.0 Hz), 3.48 (t, 4H, 2 $ClCH_2$, J = 6.0 Hz), 3.24 (t, 4H, 2 CH_2N), 1.08 (d, 6H, 2 CH3-*i*-Pr)	275 (M^+)	58	C,H,N
2h·HCl	3-F	O	Cl	123–5	7.03 (t, 1H, H_5, J_H = 10.4 Hz, J_F = 8.8 Hz), 6.61–6.52 (m, 2H, H_2, H_6), 3.55 (t, 4H, 2 $ClCH_2$, J = 6.0 Hz), 3.38 (t, 4H, 2 CH_2N)	251 (M^+)[a]	73	C,H,N[d]
2i	3-F	O	Br	oil	7.04 (t, 1H, H_5, J_H = 10.4 Hz, J_F = 8.8 Hz), 6.61–6.52 (m, 2H, H_2, H_6), 5.94 (bds, 1H, OH), 3.43 (s, 8H, 2 $ClCH_2CH_2N$)	342 ($M^+ + 1$)[a]	99	4.8 ppm
2j	3-F	O	I	oil	7.06 (t, 1H, H_5, J_H = 10.4 Hz, J_F = 8.8 Hz), 6.61–6.52 (m, 2H, H_2, H_6), 6.00 (bds, 1H, OH), 4.30 + 3.17 (m, 4H, 2 ICH_2, J = 5.0 Hz), 3.41 (m, 4H, 2 CH_2N)	435 ($M^+ + 1$)[a]	99	4.4 ppm
2k·HCl	3-Cl	O	Cl	119–21	7.19 (d, 1H, H_5, J = 9.0 Hz), 6.86 (d, 1H, H_2, J = 3.0 Hz), 6.62 (dd, 1H, H_6), 3.54 (t, 4H, 2 $ClCH_2$), 3.37 (t, 4H, 2 CH_2N)	267 (M^+)	70	C,H,N
2l·HCl	2,3-$(CH)_4$	O	Cl	180–4	8.34–8.27 (m, 1H, $H_{5(8)}$), 8.17–8.09 (m, 1H, $H_{8(5)}$), 7.56–7.41 (m, 2H, H_6, H_7), 7.28 (d, 1H, H_2, J = 8.3 Hz), 6.89 (d, 1H, H_3), 3.62–3.39 (m, 8H, 2 $ClCH_2CH_2N$)	283 (M^+)	44	C,H,N
2o[b]	3-F	NH	Cl	146–8	6.91 (t, 1H, H_5, $J_H = J_F$ = 8.3 Hz), 6.41–6.30 (m, 2H, H_2, H_6), 3.52 (t, 4H, 2 $ClCH_2$, J = 6.2 Hz), 3.32 (t, 4H, 2 CH_2N)	251 ($M^+ + 1$)[a]	97	C,H,N[e]
2p[b]	3-F	NH	Br	134–6	6.92 (t, 1H, H_5), 6.48–6.37 (m, 2H, H_2, H_6), 3.40 (m, 8H, 2 $ClCH_2CH_2N$)	341 ($M^+ + 1$)[a]	72	C,H,N[f]
2q[b]	3-Cl	NH	Cl	118–21	7.04 (d, 1H, H_5, J = 8.4 Hz), 6.63 (d, 1H, H_2, J = 2.4 Hz), 6.50 (dd, 1H, H_6), 3.50 (t, 4H, 2 $ClCH_2$, J = 6.0 Hz), 3.32 (t, 4H, 2 CH_2N)	267 ($M^+ + 1$)[a]	49	C,H,N
2r[b]	3-CN	NH	Cl	116	7.17–7.10 (m, 1H, H_{arom}), 6.90–6.81 (m, 2H, H_{arom}), 6.50 (dd, 1H, H_6), 3.57 (t, 4H, 2 $ClCH_2$, J = 5.6 Hz), 3.44 (t, 4H, 2 CH_2N)	258 ($M^+ + 1$)[a]	77	C,H,N

[a] By FAB. [b] As oxalate, 0.5H_2O. [c] $C_{11}H_{15}Cl_2NO$ calcd: C = 46.4, H = 5.7, N = 4.9. Found: C = 46.9, H = 6.1, N= 4.8. [d] $C_{10}H_{12}Cl_2FNO$·0.4H_2O·1.0HCl calcd: C = 40.6, H = 4.7, N = 4.7. Found: C = 40.2, H = 4.8, N = 5.3. [e] $C_{11}H_{15}Cl_2NO$·1.75H_2O·3.0HCl calcd: C = 39.6, H = 5.1, N = 3.3. Found: C = 39.3, H = 4.6, N = 3.0. [f] $C_{11}H_{15}Cl_2NO$·1.0$H_2C_2O_4$·0.5H_2O calcd: C = 41.3, H = 4.2, N = 7.5. Found: C = 41.2, H = 4.6, N = 8.0.

the di-*tert*-butyl [[(4-nitrophenyl)oxy]carbonyl]glutamate (**11a**). The di-*tert*-butyl [[(3-fluoro-4-nitrophenyl)oxy]-carbonyl]glutamate (**11h**) was obtained by direct reaction of the corresponding 3-fluoro-4-nitrophenol (**10h**) with phosgene to give the chloroformate followed by reaction with the glutamic acid ester.

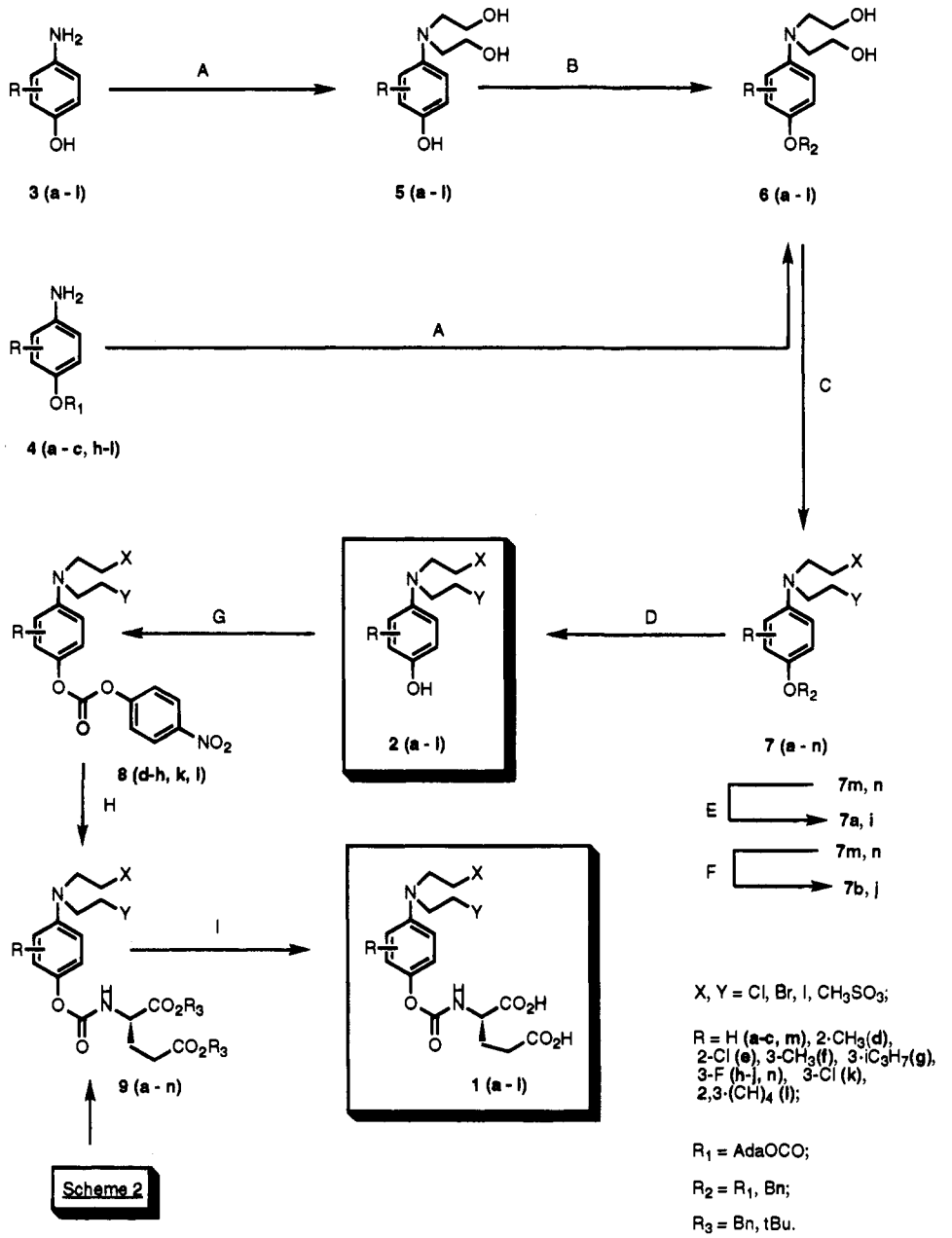
The resulting 4-nitro derivatives **11a,h**, respectively, were reduced using catalytic hydrogen transfer, with ammonium formate and Pd/C (10%), according to our method.[8] Ethanol was used instead of methanol to avoid the risk of ignition, especially important in the preparation of bulk batches. The di-*tert*-butyl [[(4-aminophenyl)oxy]carbonyl]glutamates **12a,h** were obtained in good yield.

The amines **12a,h** were N-alkylated with ethylene oxide in glacial acetic acid to afford the corresponding di-*tert*-butyl [[[4-[bis(2-hydroxethyl)amino]- and [[[3-fluoro-4-[bis(2-hydroxethyl)amino]phenyl]oxy]carbonyl]-glutamates (**13a,h**). Mesylation of these latter compounds led to the mono- and bismesyl derivatives **9c,m,n** which were purified by column chromatography. Reaction of the 4-[bis[2-(mesyloxy)ethyl]amino] and 3-fluoro-4-[bis[2-(mesyloxy)ethyl]amino] derivatives **9m,n** with lithium bromide or sodium iodide, respectively, afforded the corresponding bisbromo (**9a,i**) and bisiodo (**9b,j**) nitrogen mustards. The deprotection to the potential prodrugs **1a–c,i,j** was achieved with TFA.[5,8]

B. Aniline Prodrugs and Active Drugs. The corresponding four prodrugs and active drugs were synthesized as shown in Scheme 3. The substituted 4-fluoronitrobenzenes **14o,q,r** were transformed into the corresponding bis(hydroxyethyl) derivatives **15o,q,r** by heating with diethanolamine. On treatment with $SOCl_2$ in CH_2Cl_2, these hydroxyethyl derivatives afforded the nitrobenzene nitrogen mustards **16o,q,r** except for 3-fluoro-4-[bis(2-bromoethyl)amino]nitrobenzene (**16p**) which was prepared by bromination of its precursor (**15o**) with $SOBr_2$ under similar conditions.

The active drugs **2o–r** were obtained as oxalates after

Scheme 1[a]



[a] (A) ethylene oxide, AcOH, room temperature; (B) BzBr, KOH, EtOH; (C) MesCl, Py or PCl_5, $CHCl_3$; (D) H_2, Pd/C (30%), EtOH or TFA; (E) LiBr, CH_3CN, 70 °C; (F) NaI, CH_3CN, 70 °C; (G) 4-$NO_2C_6H_4OCOCl$, NEt_3; (H) dibenzylglutamyl tosylate, NEt_3; (I) H_2, Pd/C (30%), AcOEt or TFA.

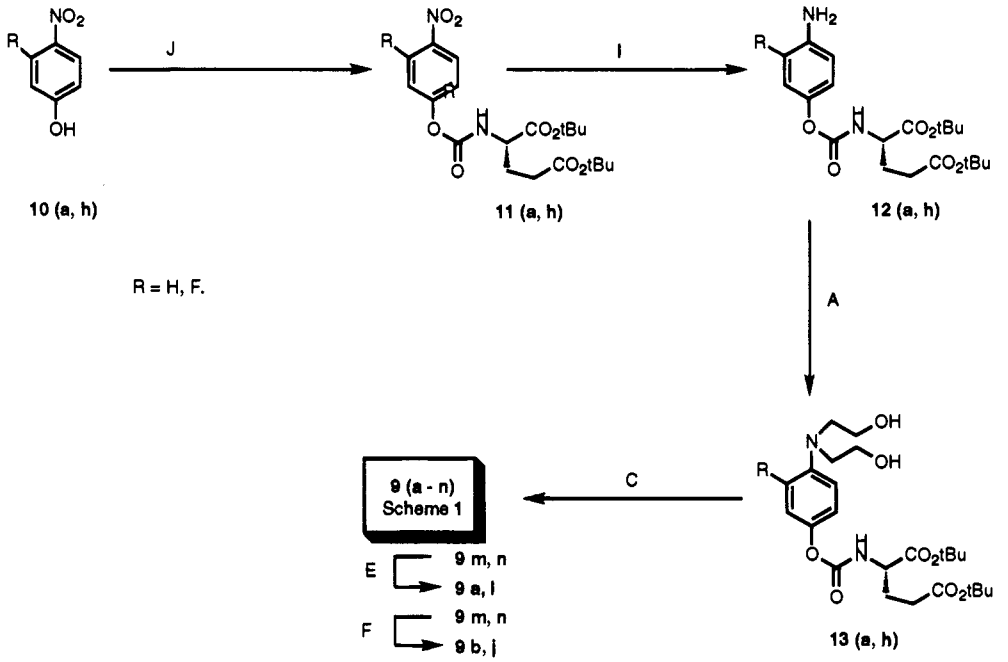
previous catalytic reduction (Pd/C, 30%) of the nitro group. The synthesis of the corresponding di-*tert*-butyl glutamates **17o–r** was carried out in a one-pot procedure, by treating the corresponding aniline drugs **2o–r** with phosgene to give the isocyanates followed by coupling with dibenzyl L-glutamic acid ester. The final deprotection to potential prodrugs **1o–r** was achieved by hydrogenation (Pd/C).

Physicochemical Data. The effect of different alkylating groups and different aromatic ring substitutions on the chemical reactivities, the substrate specificities, and the cytotoxicities was unknown. It was found that the influence of the substituents on these parameters was a function of their position on the aromatic ring, their electronic and steric parameters, and the chemical nature of the alkylating moiety.

The chemical stability of the chloroethyl mustard functionalities of the compounds was determined by an HPLC method different from that described previously for the $t_{1/2}$ measurement of the benzoic acid prodrugs and their active drugs.[19] The results are shown in Tables 3 and 4.

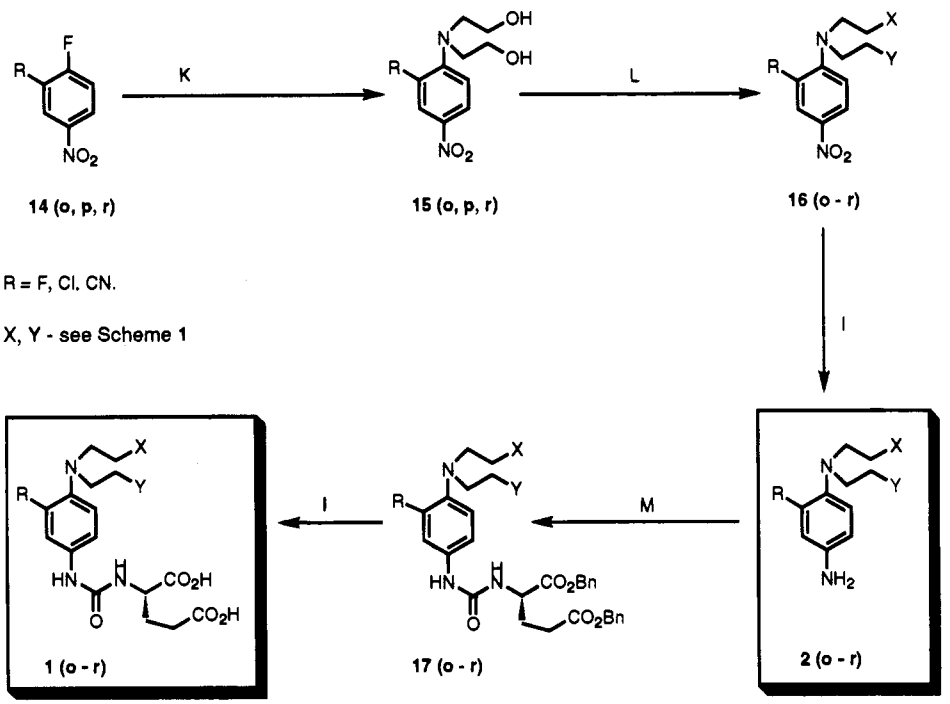
It would be expected that the effect of substitution in the aromatic ring of the phenol and aniline alkylating agents would be similar in the prodrugs to their corresponding active drugs. The mechanism governing the chemical reactivity and chemical stability which operates in nitrogen mustard reactions belongs to the neighboring groups mechanism.[20,21] Therefore, both these parameters depend strongly upon the aromatic amine basicity. The introduction of a methyl group (showing an +I effect associated with a $\sigma_m = -0.07$) in position 2 of the aromatic ring (for numbering see Chart 1) has little effect on the chemical stability of the corresponding nitrogen mustard. When chlorine is located in the same position, the combination of its −I

Scheme 2[a]



[a] (J) Two steps—$COCl_2$, toluene; di-*t*-Bu glutamate, NEt_3. For A, C, and I, see Scheme 1.

Scheme 3[a]



[a] (K) $HN(CH_2CH_2OH)_2$, *t* °C; (L) $SOCl_2$ or $SOBr_2$, CH_2Cl_2, Py; (M) $COCl_2$, toluene, dibenzylglutamyl tosylate, NEt_3. For I, see Scheme 1.

and +R effects significantly decreases the reactivity of the nitrogen mustard.

Substitution in the 3-position with a halogen atom generates three conflicting effects: the −I effect, which tends to increase the chemical stability, the +R effect, which tends to increase the reactivity, and a steric effect, which hinders the resonance of the amino moiety group and therefore increases its basicity. This phenomenon, known as steric hindrance of resonance,[22] is produced by any substituent that is bulkier than hydrogen located in the *ortho*-position (position 3, see Chart 1) with respect to the nitrogen mustard moiety. The plane in which the 2-haloethyl groups are lying is pushed out and twisted (with respect to the aromatic ring) by the *ortho*-substituent, weakening the p−π conjugation at the nitrogen and therefore increasing the availability of the unshared pair of electrons at this atom.

This effect seems to be very important since the increase in the reactivity of the nitrogen mustard is influenced by the bulkiness of the *ortho*-substituent. For instance, despite the small difference of the electronic effects between chlorine and fluorine, measured as σ_m = 0.34 and 0.23, respectively, the chemical stability of the corresponding nitrogen mustards is very different:

Table 3. Prodrugs: Kinetic Characteristics, Half-Lives, and Cytotoxicities[a]

no.	R	X, Y	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($s^{-1}\,\mu M^{-1}$)	$t_{1/2}$ (min)	IC_{50} (μM)
1a	H	Br	6.2	60.0	9.5	nd	9.4 ± 0.2
1b	H	I	2.0	29.5	14.8	nd	47.2 ± 30.9
1c	H	CH_3SO_3, Cl	1.7	151.0	88.8	nd	>500[b]
1d	2-CH_3	Cl	23.5	13.0	0.6	33.0	437 ± 59
1e	2-Cl	Cl	76.5	50.5	0.7	186	86.7 ± 31.8
1f	3-CH_3	Cl	13.0	30.0	2.3	1.6	115.8 ± 30.8
1g	3-iPr	Cl	56.0	12.6	0.2	2.2	36.7 ± 0.4
1h	3-F	Cl	unstable	unstable	unstable	19.0	53.6 ± 17.2
1i	3-F	Br	ns	ns	ns	nd	46.2 ± 21.0
1j	3-F	I	ns	ns	ns	nd	24.5[c]
1k	3-Cl	Cl	ns	ns	ns	1.7	9.4 ± 7.6
1l	2,3-$(CH)_4$	Cl	41.6	25.6	0.6	3.3	7.2 ± 1.7
1o	3-F	Cl	2.7	9.3	3.4	6.8	>500[b]
1p	3-F	Br	5.4	14.5	2.7	nd	298.7 ± 146.8
1q	3-Cl	Cl	1.2	9.4	7.8	2.8	330 ± 170
1r	3-CN	Cl	8.3	8.4	1.0	61	>500[c]

[a] ns = not substrate; nd = not determined. [b] Two determinations. [c] One determination.

Table 4. Drugs: Half-Lives and Cytotoxicities[a]

no.	R	X, Y	$t_{1/2}$ (min)	IC_{50} (μM)	IC_{50}(prodrug)/ IC_{50}(drug)
2a	H	Br	nd	0.40 ± 0.16	23.5
2b	H	I	nd	0.34 ± 0.11	138.8
2c	H	CH_3SO_3, Cl	nd	4.2 ± 2.2	>119
2d	2-CH_3	Cl	4.0	2.25 ± 0.89	194.2
2e	2-Cl	Cl	12.0	7.31 ± 1.04	11.9
2f	3-CH_3	Cl	0.6	0.75 ± 0.32	154.4
2g	3-*i*-Pr	Cl	0.7	1.12 ± 0.22	32.8
2h	3-F	Cl	5.6	2.55 ± 1.58	21.0
2i	3-F	Br	nd	1.34 ± 0.6	34.5
2j	3-F	I	nd	0.74[b]	33.1
2k	3-Cl	Cl	0.6	2.12 ± 1.04	4.4
2l	2,3-$(CH)_4$	Cl	0.9	0.49 ± 0.06	14.7
2o	3-F	Cl	2.2	2.56 ± 1.51	>195
2p	3-F	Br	nd	4.92 ± 1.98	60.7
2q	3-Cl	Cl	1.1	1.78 ± 0.43	89.9
2r	3-CN	Cl	18	9.75 ± 1.65	51.3

[a] nd = not determined. [b] One determination.

$t_{1/2}$ = 0.6 and 5.6 min^{-1}, respectively. However the chemical stability of the chlorine derivative is the same as that of the 3-methyl-substituted nitrogen mustard which exhibited a very different electronic effect (σ_m = −0.07) but a comparable van der Waals volume. For the 3-alkyl sustituents, just the +I and the steric hindrance of resonance effects need be considered. It was therefore impossible to predict the overall effect of the aromatic ring substitution on the chemical reactivity of the new alkylating agents. The situation was made yet more unpredictable by the additional effects of the amino and hydroxyl linkage groups.

The 2-substituted phenol parent drugs **1d−e** were deactivated compared to their unsubstituted counterparts. Conversely, the 3-substituted-phenol potential prodrugs **1f−h,k** and their corresponding parent drugs **2f−h,k** were greatly activated with respect to both the corresponding 2-substituted and nonsubstituted analogues. For the aniline series where only the 3-substituted derivatives were available, a similar behavior was noticed. Thus, a wide range of differing stabilities was obtained by 2- and 3-substitution of the aromatic ring of the phenol and aniline nitrogen mustards.

Kinetic Data. The K_M and k_{cat} with CPG2 were determined using each of the novel potential prodrugs **1a−r**. The kinetic measurements have been described previously[8] and were determined by measuring the decrease in the absorption spectrum which results from the hydrolysis of the bond of the glutamic acid moiety after addition of CPG2 to each prodrug. Not all the potential prodrugs were substrates for CPG2. For those compounds that were substrates, plots of initial reaction velocity versus each prodrug substrate concentration followed Michaelis−Menten kinetics.

The results are shown in Table 3. The substitution of the aromatic ring led to a decrease in the k_{cat}/K_M ratios compared to the nonsubstituted diacids. The [[[4-[2-(mesyloxy)ethyl](2-chloroethyl)amino]phenyl]-oxy]carbonyl]-L-glutamic acid (**1c**) had the highest k_{cat}/K_M in the unsubstituted phenol nitrogen mustard series.

The electronic and steric properties of the substituents play an important role in determining the kinetics of the CPG2 cleavage of the prodrugs. Certain effects of the 2- and 3-ring substitution on the substrate reactivity for CPG2 and of phenol and aniline nitrogen mustard glutamates were predictable. Thus the unsubstituted prodrugs would be expected to have the lowest K_M, suggesting tighter binding with the enzyme. Substitution of either the 2- or 3-position is expected to weaken the binding to the enzyme due to steric reasons, and this was found to be the case. The substitution in the 2-position (which lies close to the CPG2 cleavage position) has the effect of decreasing the tightness of binding. However, even in the 3-position, bulky substituents like *i*-Pr group have the effect of increasing the K_M.

The k_{cat} is more difficult to rationalize in terms of electronic and steric effects of the substituents. Introduction of a chlorine in position 2 would be expected to reduce the k_{cat} due to +R effect (with respect to the amide group) of the halogen. This was found to be the case as demonstrated by the k_{cat} values of **1c** > **1e** (or as compared with the unsubstituted bis(2-chloroethyl) nitrogen mustard, k_{cat} = 168).[16] However this reduction is even more dramatic in the 2-methyl-substituted-phenol nitrogen mustard. A significant reduction in the k_{cat} values was also observed in the case of 3-alkyl-substituted nitrogen mustards. An explanation could be the hindrance of enzyme−substrate interaction by any substituents of the aromatic nucleus. This point of view is supported by the K_M and k_{cat} values obtained for the naphthalene nitrogen mustard **1l**. It is, however, surprising that the modification of the nitrogen mustard moiety seems to affect the k_{cat} values.

The 16 novel diacids synthesized herein thus provide a selection of activated and deactivated potential prodrugs for cleavage by CPG2 to their corresponding active drugs. This variety of potential prodrugs led to the choice of a further candidate for clinical trial, with suitable physicochemical and kinetic parameters.

Biological Evaluation. The novel compounds **1a–r** and **2a–r** were tested for cytotoxicity in 1 h incubation experiments in LoVo cells. All the potential prodrugs **1a–r** were found to be less toxic than their corresponding active drugs **2a–r**, which is an essential prerequisite for ADEPT. The results are shown in Tables 3 and 4. In addition, the drug **2b** of potential prodrug **1b** was incubated under the same conditions but for 1 min instead of 1 h, in order to determine whether the potency of this clinical candidate system would be maintained for the potentially very short time periods available in an in vivo environment. The cytotoxicity, IC_{50}, was found to be 1.7 μM, which is only 7-fold less cytotoxic than that of **1b** + CPG2 when incubated for 1 h (IC_{50} = 0.32 μM). This is in good agreement with the cytotoxicity after incubation of **2b** for 1 h (IC_{50} = 0.34 μM), demonstrating that the IC_{50} obtained when the prodrug is activated by CPG2 is the same as the IC_{50} of the parent drug alone. These results indicate that (a) nonsubstituted and substituted phenol and aniline nitrogen mustards with different alkylating groups can be made into prodrugs to be activated by the bacterial enzyme CPG2 (The introduction of the 2- and 3-fluoro substituent into the benzene ring of benzoic acid prodrugs[8] was also shown previously to lead to wide diversity in the chemical and biological characteristics.) and (b) an amide bond between the substituted aromatic alkylating agent and the L-glutamic acid is not compulsory. The enzyme can also cleave oxycarbonyl or carbamoyl bonds, which confirms previous results.[15]

Summary

The prodrug [4-[(2-chloroethyl)[2-(mesyloxy)ethyl]-amino]benzoyl]-L-glutamic acid (**1s**), currently undergoing clinical evaluation in ADEPT, has demonstrated efficacy.[12,13] Herein we describe the synthesis and the properties of the [[[4-[bis(2-iodoethyl)amino]phenyl]oxy]-carbonyl]-L-glutamic acid (**1b**) which is also a good substrate for the CPG2 enzyme and releases an extremely potent active drug with a much shorter chemical half-life. It emerges as the next candidate for further clinical trials, under the development name ZD2767.

Experimental Section

All reagents were commercially available (Aldrich, Lancaster, or Sigma) unless otherwise stated. Silica gel was used in columns (art. mo. 9385 and 15111, Merck). TLC was performed on precoated sheets of silica gel 60 F_{254} (art. no. 5735, Merck). Melting points were determinated on a Kofler hot-stage (Reichert Thermovar) or Buchi 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 μA, and an ion source temperature at 180–200 °C. EI mass spectra were determined using xenon gas. Reported spectra are by FAB unless otherwise stated. NMR spectra (^1H and ^{19}F) were determined in Me_2SO-d_6 on a Bruker AC250 (250 MHz) or Bruker AM200 spectrometer at 30 °C (303 K) unless otherwise stated. Elemental analyses were determined by Butterworth Laboratories Ltd. (Teddington, Middlesex, England) and are within 0.4% of theory except where stated. Half-life determinations were performed by HPLC. Kinetic analyses were performed on a spectrophotometer (Perkin Elmer, Lambda 2) fitted with a heat controller. Results were calculated using a nonlinear regression program (Enzfit).

All starting materials were commercially available (Aldrich) unless othewise stated. The 3-chloro-4-hydroxyaniline and the 3-fluoro-4-hydroxyanilines were obtained by the reduction of the corresponding nitro derivatives. The 2-chloro-4-hydroxyaniline was prepared by nitration of the 3-chlorophenol and subsequent reduction of the resulted 3-chloro-4-nitrophenol.

One example of each type of reaction is described in detail below. Where the same procedure was used for more than one compound, these are described in the tables.

1-Adamantyl 3-Fluoro-4-aminophenyl Carbonate (4h). To a stirred solution of the 1-adamantyl 3-fluoro-4-nitrophenyl carbonate (0.50 g, 1.5 mmol) in ethanol (5.0 mL) was added Pd/C 10% (0.10 g) followed by ammonium formate (0.45 g, 7.1 mmol). An exothermic effect occurred, and the reaction was complete in 15–20 min [TLC, cyclooxane–toluene (1:1)]. The catalyst was removed by filtration, the filtrate concentrated under vacuum, and the residue partitioned between AcOEt and H_2O. The organic layer was dried ($MgSO_4$) and concentrated to give **4h** (0.45 g, 98%) as a white solid: mp 109–110 °C; IR ν_{max} (cm^{-1}) 3486, 3382 (N–H), 1758 (C=O), 1272 (C–F), 1220 (C–O); ^1H NMR δ 1.63 (s, 6H, 3 CH_2-Ad), 2.08 (s, 6H, 3 CH_2-Ad), 2.17 (s, 3H, 3 CH-Ad), 5.07 (bds, 2H, NH), 6.71–6.74 (m, 2H, H_{arom}), 6.89–6.95 (m, 1H, H_{arom}); ^{19}F NMR δ −132.25 (m, 1F); MS (EI) *m/z* 304 ($M^+ - H$), 261 ($M^+ - CO_2$), 135 (adamantyl). Anal. ($C_{17}H_{20}NO_3F$) C, H, N, F.

Compound **4a** was synthesized by a similar procedure.

3-Methyl-4-[bis(2-hydroxyethyl)amino]phenol (5f). Ethylene oxide (40 g, 0.9 mol) was bubbled into a solution of 4-amino-*m*-cresol (12.3 g, 0.1 mol) in AcOH–H_2O (1:1) (500 mL). The mixture was allowed to stand at room temperature for 48 h and then evaporated to dryness. The residue was purified on silica gel chromatography eluted with AcOEt, to obtain **5f** (6.2 g, 30%) as an oil. For analytical data, see Table 5.

The foregoing procedure was used for the synthesis of compounds **5d–l** (see Table 5).

1-(Benzyloxy)-3-methyl-4-[bis(2-hydroxyethyl)amino]-benzene (6f). Benzyl bromide (4.24 g, 24.8 mol) was added to a mixture of [bis(hydroxyethyl)amino]phenol **5f** (6.0 g, 28.4 mmol), potassium hydroxide (1.6 g, 28.4 mmol), and ethanol (40 mL). The mixture was heated under reflux for 2 h, cooled, and concentrated under vacuum. The residue was poured onto H_2O (100 mL), extracted twice with AcOEt, dried, and evaporated to obtain **6f** (7.20 g, 84%) as a solid. For the analytical data, see Table 6.

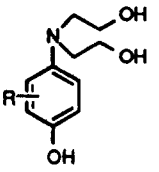
Compounds **6d–l** were obtained according to the same procedure (see Table 6).

1-Adamantyl 3-Fluoro-4-[bis(2-hydroxyethyl)amino]-phenyl Carbonate (6h). Amine **4h** (4.47 g, 14.6 mmol) in AcOH (100 mL) was stirred with ethylene oxide (1.4 mL, 1.26 g, 28.5 mmol) at room temperature for 48 h. The solution was diluted with 150 mL of H_2O, and Na_2CO_3 was added to neutral pH. An oily product separated which was extracted with AcOEt (2 × 150 mL). The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to dryness to give a clear oil (6.31 g) which was chromatographed on silica gel, eluting with AcOEt–cyclohexane (2:1). The white solid **6h** (4.00 g, 70%) resulted. For the analytical data, see Table 6.

The same procedure was used to obtain the intermediate **6a** (see Table 6).

1-(Benzyloxy)-3-methyl-4-[bis(2-chloroethyl)amino]benzene (7f). Procedure a. Phosphorus pentachloride (11.40 g, 54.7 mmol) was added in portions to the compound **6f** (7.00 g, 23.2 mmol) in $CHCl_3$ at 10–20 °C. The mixture was heated at reflux for 90 min and then cooled and poured onto H_2O. The organic phase was separated, washed with aqueous sodium bicarbonate solution and H_2O, dried, and then evaporated. The residue was chromatographed on silica gel. After elution with hexane–AcOEt (2:1), **7f** (2.20 g, 52%) was obtained as an oil. For the analytical data, see Table 7.

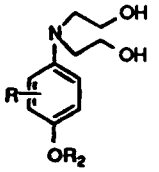
Table 5. Phenols: Hydroxyethyl Derivatives



no.	R	mp (°C)	^{1}H NMR (δ, ppm)	MS (*m/z*)	yield (%)
5d	2-CH_3	oil	6.59 (d, 1H, H_6, J = 8.3 Hz), 6.42–6.30 (m, 2H, H_3, H_5), 4.60 (t, 2H, OH), 3.55–3.40 (m, 4H, 2 HOCH_2, J = 5.2 Hz), 3.36–3.29 (m, 4H, 2 CH_2N), 2.07 (s, 3H, 2-CH_3)	211 (M^+)	30
5e	2-Cl	oil	6.80 (d, 1H, H_6, J = 9.0 Hz), 6.72–6.52 (m, 2H, H_3, H_5), 4.01 (t, 2H, 2 OH, J = 6.6 Hz), 3.45 (q, 4H, 2 HOCH_2), 3.31 (t, 4H, 2 CH_2N, J = 5.8 Hz)	231 (M^+)	94
5f	3-CH_3	oil	7.00 (dd, 1H, H_5, J = 9.2 Hz), 6.54–6.50 (m, 2H, H_2, H_6), 4.30 (t, 2H, 2 OH, J = 5.4 Hz), 3.33 (q, 4H, 2 HOCH_2), 2.93 (t, 4H, 2 CH_2N, J = 6.6 Hz), 2.15 (s, 3H, 3-CH_3)	211 (M^+)	30
5g	3-*i*-Pr	90–2	9.00 (bds, 1H, NH-Ph), 7.05 (d, 1H, H_5, J = 8.5 Hz), 6.61–6.50 (m, 2H, H_2, H_6), 4.32 (t, 2H, 2 OH, J = 5.4 Hz), 3.54 (m, 1H, C*H*-*i*-Pr, J = 7.8 Hz), 3.34 (q, 4H, 2 HOCH_2), 2.93 (t, 4H, 2 CH_2N, J = 6.3 Hz), 1.11 (d, 6H, 2 CH_3-*i*-Pr)	239 (M^+)	49
5h–j	3-F	oil	9.37 (s, 1H, NH-Ph), 6.97 (m, 1H, H_5, J_H = 9.2 Hz, J_F = 8.4 Hz), 6.56–6.45 (m, 2H, H_2, H_6), 4.78 (t, 2H, 2 OH, J = 5.4 Hz), 3.45–3.33 (q, 4H, 2 HOCH_2), 3.08 (t, 4H, 2 CH_2N, J = 7.0 Hz)	215 (M^+)	ni
5k	3-Cl	oil	7.17 (d, 1H, H_5, J = 8.6 Hz), 6.72 (d, 1H, H_2, J = 2.8 Hz), 6.68 (dd, 1H, H_6), 3.37 (t, 4H, 2 HOCH_2, J = 6.6 Hz), 3.05 (t, 4H, 2 CH_2N)	231 (M^+)	60
5l	2,3-$(CH)_4$	oil	8.35–8.25 (m, 1H, $H_{5(8)}$), 8.14–8.05 (m, 1H, $H_{8(5)}$), 7.36–7.15 (m, 2H, H_6, H_7), 7.18 (d, 1H, H_2, J = 8.0 Hz), 6.81 (d, 1H, H_3), 4.39 (bds, 2H, 2 OH), 3.41 (m, 4H, 2 HOCH_2), 3.14 (t, 4H, 2 CH_2N, J = 6.4 Hz)	247 (M^+)	23

[a] ni = not isolated.

Table 6. Protected Phenols: Hydroxyethyl Derivatives



no.	R	R_2	mp (°C)	^{1}H NMR (δ, ppm)[a,b]	MS (*m/z*) (M^+)	yield (%)
6a–c	H	AdOCO	117–8	6.92 (d, 2H, H_3, H_5, J = 9.0 Hz), 6.65 (d, 2H, H_2, H_6), 3.52 (t, 4H, 2 HOCH_2, J = 5.9 Hz), 3.39 (t, 4H, 2 CH_2N)	375 331 (M − 44)	82
6d	2-CH_3	Bn	oil	6.82 (d, 1H, H_6, J = 8.8 Hz), 6.59–6.36 (m, 2H, H_3, H_5), 4.66 (t, 2H, 2 OH, J = 5.0 Hz), 3.52 (q, 4H, 2 HOCH_2), 3.33 (t, 4H, 2 CH_2N), 2.16 (s, 3H, 2-CH_3)	301	87
6e	2-Cl	Bn	oil	7.02 (d, 1H, H_6, J = 9.0 Hz), 6.75 (d, 1H, H_2, J = 3.0 Hz), 6.59 (dd, 1H, H_5), 3.51 (t, 4H, 2 HOCH_2), 3.33 (t, 4H, 2 CH_2N, J = 5.8 Hz)	321	43
6f	3-CH_3	Bn	70–2	7.13 (d, 1H, H_5, J = 8.2 Hz), 6.81–6.75 (m, 2H, H_2, H_6), 4.34 (bds, 2H, 2 OH), 3.34 (t, 4H, 2 HOCH_2), 2.97 (t, 4H, 2 CH_2N, J = 6.4 Hz), 2.21 (s, 3H, 3-CH_3)	301	84
6g	3-*i*-Pr	Bn	oil	7.20 (d, 1H, H_5, J = 8.6 Hz), 6.88–6.76 (m, 2H, H_2, H_6), 3.65 (m, 1H, C*H*-*i*-Pr, J = 7.8 Hz), 3.34 (t, 4H, 2 HOCH_2), 2.97 (t, 4H, 2 CH_2N), 1.90 (d, 6H, 2 CH_3-*i*-Pr)	329	88
6h–j	3-F	Bn	72–4	7.07 (m, 1H, H_5, J_H = 9.0 Hz, J_F = 8.4 Hz), 6.90–6.70 (m, 2H, H_2, H_6), 3.51 (t, 4H, 2 HOCH_2, J = 6.6 Hz), 3.33 (t, 4H, 2 CH_2N)	305	76
6h–j	3-F	AdOCO	82–4	7.08–7.00 (m, 2H, H_2, H_5), 6.99–6.85 (dd, 1H, H_6, J_H = 9.0 Hz, J_H = 2.6 Hz), 4.56 (s, 2H, 2 OH), 3.49 (m, 4H, 2 HOCH_2), 3.26 (t, 4H, 2 CH_2N, J = 6.2 Hz)	393 349 (M − 44)	70
6k	3-Cl	Bn	oil	7.28 (d, 1H, H_5, J = 8.6 Hz), 7.08 (d, 1H, H_2, J = 2.8 Hz), 6.94 (dd, 1H, H_6), 3.48–3.35 (m, 4H, 2 HOCH_2), 3.33 (t, 4H, 2 CH_2N)	321	77
6l	2,3-$(CH)_4$	Bn	oil	8.40–8.32 (m, 1H, $H_{5(8)}$), 8.25–8.18 (m, 1H, $H_{8(5)}$), 7.59–7.33 (m, 7H, H_6, H_5, Bn), 7.30 (d, 1H, H_2, J = 8.3 Hz), 6.91 (d, 1H, H_3), 3.42 (q, 4H, 2 HOCH_2, J = 6.2), 3.14 (t, 4H, 2 CH_2N)	337	40

[a] The proton signals of the benzylic moiety are located for all compounds as follows: 7.50–7.27 (m, 5H, Ph), 5.27–4.98 (s, 2H, CH_2Ph). [b] The signals of adamantanyl protons are located as follows: 2.22–2.19 (s, 3H, 3 CH), 2.12–2.10 (s, 6H, 3 CH_2), 1.65–1.63 (s, 6H, 3 CH_2).

Procedure b. Methanesulfonyl chloride (2.5 mL, 3.63 g, 31.7 mmol) was added at 0–5 °C to a solution of compound **6f** (2.6 g, 8.6 mmol) in pyridine (8 mL). The mixture was then heated at 70 °C for 15 min, cooled, and poured onto dilute citric acid solution (100 mL). The mixture was extracted twice with AcOEt, dried, and evaporated to obtain the compound **7f** (2.60 g, 84%) as an oil. For analytical data, see Table 7.

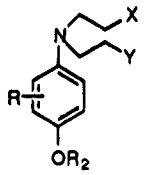
The intermediates **7d–h,k,l** (see Table 7) were obtained using the same procedure.

1-Adamantanyl 4-[Bis[2-(mesyloxy)ethyl]amino]phenyl Carbonate (7m) and 1-Adamantanyl 4-[(2-Chloroethyl)[2-(mesyloxy)ethyl]amino]phenyl Carbonate (7c). A solution of **6a** (1.40 g, 3.7 mmol) in pyridine (8.0 mL) was stirred with methanesulfonyl chloride (1.2 mL, 1.74 g, 15.2 mmol) for 40 min at 0 °C followed by 15–18 min at 50 °C. AcOEt (20 mL) was added and the solution cooled at 0 °C. The precipitate thus formed was filtered and the filtrate evaporated under vacuum to a yellow oil. The operation was repeated using THF as solvent. The residue containing three reaction products, each of which gave a positive color with the Epstein reagent, was chromatographed on silica gel eluting with AcOEt–cyclohexane (1:1). The slowest eluting was the 1-adamantyl bis[2-(mesyloxy)ethyl] derivative **7m** (0.96 g, 48%), as an oil. For the analytical data, see Table 7.

Eluting second was 1-adamantanyl 2-chloroethyl 2-(mesyloxy)ethyl derivative **7c** (0.83 g, 47%), as an oil. For the analytical data, see Table 7.

1-Adamantyl 4-[bis(2-chloroethyl)amino]phenyl carbonate

Table 7. Nitrogen Mustards from Protected Phenols



no.	R	R_2	X, Y	mp (°C)	^{1}H NMR (δ, ppm)	MS (M^+)	yield (%)
7a	H	AdOCO	Br	oil	7.00 (d, 2H, H_3, H_5), 6.72 (d, 2H, H_2, H_6), 3.77 (t, 4H, 2 $BrCH_2$, J = 7.1 Hz), 3.58 (t, 4H, 2 CH_2N)[a]	502 (M^+ + 1) 457 (M^+ − 44)	89
7b	H	AdOCO	I	oil	7.01 (d, 2H, H_3, H_5), 6.67 (d, 2H, H_2, H_5), 3.72 (t, 4H, 2 ICH_2, J = 6.7 Hz), 3.31 (t, 4H, 2 CH_2N)[a]	596 (M^+ + 1) 551 (M^+ − 44)	73
7c	H	AdOCO	CH_3SO_3, Cl	oil	7.00 (d, 2H, H_3, H_5, J = 9.0 Hz), 6.77 (d, 2H, H_2, H_5), 4.31 + 3.77 (m, 8H, 2 $ClCH_2CH_2N$), 3.15 (s, 3H, CH_3SO_3)[a]	427 (M^+ − 44)	43[c]
7d	2-CH_3	Bn	Cl	oil	6.87 (d, 1H, H_6, J = 8.8 Hz), 6.76−6.47 (m, 2H, H_3, H_5), 3.73−3.56 (m, 8H, 2 $ClCH_2CH_2N$), 2.18 (s, 3H, 2-CH_3)[b]	338	34
7e	2-Cl	Bn	Cl	68−70	7.08 (d, 1H, H_6, J = 9.0 Hz), 6.83 (d, 1H, H_2, J = 3.0 Hz), 6.68 (dd, 1H, H_5), 3.67 (m, 8H, 2 $ClCH_2CH_2N$)[b]	357	51
7f	3-CH_3	Bn	Cl	oil	7.15 (d, 1H, H_5, J = 8.3 Hz), 6.89−6.78 (m, 2H, H_2, H_5), 3.51 (t, 4H, 2 $ClCH_2$, J = 6.6 Hz), 3.29 (t, 4H, 2 CH_2N), 2.26 (s, 3H, 3-CH_3)[b]	337	52[d] 84[e]
7g	3-*i*-Pr	Bn	Cl	oil	7.22 (d, 1H, H_5, J = 8.6 Hz), 6.92−6.79 (m, 2H, H_2, H_6), 3.68 (m, 1H, CH-*i*-Pr, J = 7.0 Hz), 3.50 (t, 4H, 2 $ClCH_2$, J = 6.2 Hz), 3.27 (t, 4H, 2 CH_2N), 1.12 (d, 6H, 2 CH_3-*i*-Pr)[b]	367	48
7h	3-F	Bn	Cl	oil	7.12 (t, 1H, H_5, J_H = 8.3 Hz, J_F = 8.6 Hz), 6.94−6.90 (m, 2H, H_2, H_6), 3.57 (t, 4H, 2 $ClCH_2$, J = 5.8 Hz), 3.42 (t, 4H, 2 CH_2N)[b]	341	47
7h′	3-F	AdOCO	Cl	oil	7.18−7.09 (m, 2H, H_{arom}), 6.96−6.92 (dd, 1H, H_{arom}), 3.65 (t, 4H, 2 $ClCH_2$, J = 5.6 Hz), 3.56 (t, 4H, 2 CH_2N)[a]	429 386 (M^+ − 44)	91
7i	3-F	AdOCO	Br	oil	7.18−7.10 (m, 2H, H_2, H_5), 6.95 (dd, 1H, H_6, J_H = 8.8 Hz, J_H = 2.5 Hz), 3.62 (t, 4H, 2 $BrCH_2$), 3.52 (t, 4H, 2 CH_2N)[a]	520 (M^+ + 1)	87
7j	3-F	AdOCO	I	oil	7.16−7.06 (m, 2H, H_2, H_5), 6.95 (dd, 1H, H_6, J_H = 8.9 Hz, J_H = 2.7 Hz), 3.56 (t, 4H, 2 ICH_2, J = 7.3 Hz), 3.26 (t, 4H, 2 CH_2N)[a]	614 (M^+ + 1)	88
7k	3-Cl	Bn	Cl	oil	7.30 (d, 1H, H_5, J = 9.0 Hz), 7.12 (d, 1H, H_2, J = 3.0 Hz), 6.96 (dd, 1H, H_6), 3.55 (t, 4H, 2 $ClCH_2$, J = 5.8 Hz), 3.37 (t, 4H, 2 CH_2N)[b]	357	84
7l	2,3-$(CH)_4$	Bn	Cl	oil	8.39−8.30 (m, 1H, $H_{5(8)}$), 8.28−8.19 (m, 1H, $H_{8(5)}$), 7.64−7.34 (m, 7H, H_6, H_7, Bn), 7.05 (d, 1H, H_2, J = 8.3 Hz), 6.91 (d, 1H, H_3), 3.63−3.44 (m, 8H, 2 $ClCH_2CH_2N$)[b]	374	23
7m	H	AdOCO	$(CH_3SO_3)_2$	oil	7.00 (d, 2H, H3, H_5, J = 9.0 Hz), 6.79 (d, 2H, H_2, H_6), 4.31 (t, 4H, $CH_3SO_3CH_2$, J = 5.7 Hz), 3.73 (t, 4H, 2 CH_2N), 3.16 (s, 6H, 2 CH_3SO_3)[a]	487 (M^+ − 44)	33
7n	3-F	AdOCO	$(CH_3SO_3)_2$	oil	7.22−7.10 (m, 2H, H_2, H_5), 6.98−6.93 (m, 1H, H_6, J_H = 9.3 Hz, J_H = 2.8 Hz), 4.23 (t, 4H, 2 $CH_3SO_3CH_2$, J = 5.5 Hz), 3.55 (t, 4H, 2 CH_2N), 3.10 (s, 6H, 2 CH_3SO_3)[a]	550 (M^+ + 1) 505 (M^+ − 44)	63

[a] The adamantanyl protons are located as follows: 2.17 (s, 3H, 3 CH-Ad), 2.10 (s, 6H, 3 CH_2-Ad), 1.64 (s, 6H, 3 CH_2-Ad). [b] The benzyl protons are located as follows: 7.47−7.29 (m, 5H, Ph), 5.09−5.00 (s, 2H, CH_2-Ph). [c] After column chromatography. [d] According to procedure a. [e] According to procedure b.

(the fastest eluting compound) resulted as an impurity (2−3%). The same procedure was used to obtain the intermediate **7n** (see Table 7).

1-Adamantanyl 3-Fluoro-4-[bis(2-chloroethyl)amino]-phenyl Carbonate (7h′). To the compound **6h** (0.60 g, 1.5 mmol) in dry CH_2Cl_2 (10 mL) and under N_2 was added thionyl chloride (2.5 mL, 4.08 g, 3.2 mmol). The mixture was heated under reflux for 30−40 min and the solvent removed. The product was purified by column chromatography, eluting with AcOEt−cyclohexane (1:1) to yield **7h′** (0.60 g, 91%), as an oil. For the analytical data, see Table 7.

1-Adamantyl 3-Fluoro-4-[bis(2-bromoethyl)amino]phenyl Carbonate (7j). To a solution of **7n** (0.19 g, 0.35 mmol) in CH_3CN (20 mL) was added LiBr (0.12 g, 1.42 mmol). The reaction mixture was kept at 70 °C for 22 h, cooled, and filtered. The filtrate was concentrated under vacuum, and the oily residue thus obtained was chromatographed on silica gel using CH_2Cl_2−AcOEt (19:1) as eluent. The resulted product **7j** (0.16 g, 87%) was an oil. For the analytical data, see Table 7.

The corresponding unsubstituted nitrogen mustard **7a** (see Table 7) was prepared from **7m** in a similar way.

1-Adamantyl 3-Fluoro-4-[bis(2-iodoethyl)amino]phenyl Carbonate (7i). The foregoing procedure was applied to compound **7n** (0.19 g, 0.35 mmol) except that NaI (0.21 g, 1.42 mmol) was used instead of LiBr. The resulted product **7i** (0.19 g, 88%) was an oil. For the analytical data, see Table 7.

The corresponding unsubstituted nitrogen mustard **7b** (see Table 7) was prepared in a similar way.

3-Methyl-4-[bis(2-chloroethyl)amino]phenol (2f). Saturated ethereal HCl was added to the compound **7f** (2.00 g, 5.9 mmol) in EtOH (25.0 mL) until complete dissolution. Hydrogenation was carried out under an atmosphere of H_2 using 30% Pd/C catalyst (0.30 g). The catalyst was removed by filtration and the filtrate evaporated to obtain **2f**·HCl (0.95 g, 57%) as a solid. For the analytical data, see Table 2.

The synthesis of compounds **2d−h,k,l** was performed according to the same procedure (see Table 2).

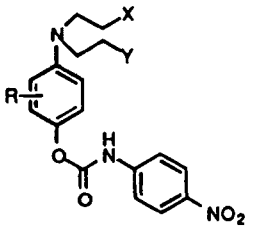
3-Fluoro-4-[bis(2-iodoethyl)amino]phenol (2j). Compound **7j** (0.16 g, 0.31 mmol) was suspended in TFA (4−8%, w/v) and stirred for 50 min at 0 °C. The solvent was removed under reduced pressure, and the oily residue was diluted with AcOEt (1.0 mL) and then evaporated again. This latter step was repeated 5−20 times to give compound **2j** (0.16 g, 100%) as an oil. For the analytical data, see Table 2.

Compounds **2a−c,i** (see Table 2) were prepared according to the same route.

TFA deprotection of compound **7m** (0.300 g, 0.56 mmol) resulted in 4-[bis[2-(mesyloxy)ethyl]amino]phenol as an oil (0.329 g, 100%): IR ν_{max} (cm^{-1}) 3209 (O−H), 1647 (C=C), 1358, 1176 (OSO_2); ^{1}H NMR δ 3.13 (s, 6H, 2 CH_3SO_3), 3.59 (t, 4H, J = 5.8 Hz, 2 CH_2N), 4.25 (t, 4H, 2 $CH_3SO_3CH_2$), 6.75−6.64 (m, 4H, H_{arom}); MS (EI) m/z 389 (M^+ + $2H_2O$); accurate mass calcd 389.0814, found 4.7 ppm. This compound reacted positively (blue color) with the Epstein spray reagent.

TFA deprotection of compound **7n** (0.245 g, 0.5 mmol) resulted in 3-fluoro-4-[bis[2-(mesyloxy)ethyl]amino]phenol as

Table 8. Activated 4-Nitrophenyl Phenol Nitrogen Mustards



no.	R[a]	mp (°C)	^{1}H NMR (δ, ppm)[b]	MS (M^+)	yield (%)
8d	2-CH_3	oil	7.16 (d, 1H, H_6, J = 9.0 Hz), 6.72–6.45 (m, 2H, H_3, H_5), 3.72 (s, 8H, 2 $ClCH_2CH_2N$), 2.21 (s, 3H, 2-CH_3)	412	62
8e	2-Cl	oil	7.30 (d, 1H, H_6, J = 9.0 Hz), 6.98–6.75 (m, 2H, H_3, H_5), 3.75 (ps, 8H, 2 $ClCH_2CH_2N$)	nd	48
8f	3-CH_3	oil	7.33 (d, 1H, H_5, J = 8.6 Hz), 7.27–7.16 (m, 2H, H_2, H_6), 3.57 (t, 4H, 2 $ClCH_2$, J = 6.4 Hz), 3.39 (t, 4H, 2 CH_2N), 2.32 (s, 3H, 3-CH_3)	413 (M^+ + 1)	67
8g	3-*i*-Pr		not isolated		
8h	3-F	oil	7.04 (t, 1H, H_5), 6.61–6.50 (m, 2H, H_2, H_6), 3.57 (t, 4H, 2 $ClCH_2$), 3.37 (t, 4H, 2 CH_2N)	416	77
8k	3-Cl	oil	not purified		44
8l	2,3-$(CH)_4$	oil	8.46–8.37 (m, 3H, $H_{5(8)}$ + $H_{3'}$, $H_{5'}$), 8.16 (m, 1H, $H_{8(5)}$), 7.82–7.46 (m, 6H, H_2, H_3, H_6, H_7, $H_{2'}$, $H_{6'}$), 3.68–3.58 (m, 8H, 2 $ClCH_2CH_2N$)	449	62

[a] X = Y = Cl. [b] The protons of the 4-nitrophenyl group are located as follows: 8.40–8.36 (d, 2H, H_3, H_5, J = 10.0 Hz), 7.85–7.67 (d, 2H, H_2, H_5).

an oil (0.300 g, 95%): IR ν_{max} (cm^{-1}) 3165 (OH), 1647 (C=C), 1352, 1174 (OSO_2); ^{1}H NMR δ 3.11 (s, 6H, 2 CH_3SO_3), 3.38 (t, 4H, 2 CH_2N, J = 5.6 Hz), 4.38 + 4.16 (2t, 4H, 2 $CH_3SO_3CH_2$), 6.51–6.58 (m, 2H, H_{arom}), 7.00–7.07 (m, 1H, H_{arom}); MS (EI) *m/z* 389 (M^+ + H_2O), 371 (M^+), 280 [(M^+ + H_2O) − $CH_3O_2SOCH_2$], 262 (M^+ − $CH_3O_2SOCH_2$); accurate mass calcd 389.0614, found 4.1 ppm. This compound reacted positively (blue color) with the Epstein spray reagent.

4′-Nitrophenyl 4-[Bis(2-chloroethyl)amino]phenyl Carbonate (8f). To a solution of **2f**-HCl (2.15 g, 7.6 mmol) and triethylamine (2.1 mL) in $CHCl_3$ (25 mL) was added 4-nitrophenyl chloroformate (1.56 g, 7.7 mmol) slowly at room temperature, and the mixture was left for 12 h. The reaction mixture was concentrated to a yellow oil. Flash column chromatography eluting with hexane–AcOEt (3:1) yielded **8f** (2.10 g, 67%) as an oil. For the analytical data, see Table 8.

The same procedure was used for the synthesis of compounds **8d–h,k,l** (see Table 8).

Dibenzyl [[[3-Methyl-4-[bis(2-chloroethyl)amino]phenyl]oxy]carbonyl]-L-glutamate (9f). Dibenzyl-L-glutamic acid (*p*-toluenesulfonate salt) (5.08 g, 10.0 mmol) was added at room temperature to a solution of compound **8f** (2.1 g, 5.0 mmol) in $CHCl_3$ (12 mL) and triethylamine (1.4 mL). The reaction mixture was heated at 50 °C for 2.5 h. Dibenzyl-L-glutamic acid (2.45 g, 4.8 mmol) was added again and the heating continued for 2.5 h more. The reaction mixture was cooled and concentrated under vacuum to a yellow oil. Flash chromatography, eluting with chloroform containing AcOEt (3%), yielded **9f** (1.49 g, 50%) as a colorless oil. For analytical data, see Table 9.

The same procedure was used for the synthesis of compounds **9d–h,k,l** (see Table 9).

Di-*tert*-butyl [[(4-Nitrophenyl)oxy]carbonyl]-L-glutamate (11a). A solution of di-*tert*-butyl-L-glutamic acid hydrochloride (4.26 g, 14.4 mmol) and triethylamine (4.0 mL) in dry $CHCl_3$ (30 mL) was stirred with a cooled solution of 4-nitrophenyl chloroformate (2.92 g, 14.4 mmol) for 5 min. After 5 h at ambient temperature, the solvent was evaporated and the residue dissolved in AcOEt (70 mL), filtered, and evaporated to dryness. The residue was chromatographed on silica gel and eluted with $CHCl_3$ to obtain **11a** (5.02 g, 82%) as an oil: IR ν_{max} (cm^{-1}) 3411, 3352 (NH), 2980, 2935 (CH_3), 1731 (C=O), 1526, 1347 (NO_2), 1159 (C–O); ^{1}H NMR δ 1.42, 1.43 (2s, 18H, 2 *t*-Bu), 1.82–2.05 (2m, 2H, CH_2CH), 2.29–2.40 (m, 2H, CH_2CO_2t-Bu), 3.98–4.05 (m, 1H, $CHCH_2$), 7.40 (d, 2H, H-2, H-6), 8.29 (d, 2H, H-3, H-5, J_o = 9.1 Hz), 8.39 (d, 1H, NH, J = 7.7 Hz).

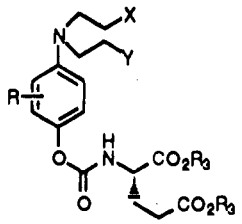
Di-*tert*-butyl [[(3-Fluoro-4-nitrophenyl)oxy]carbonyl]-L-glutamate (11h). Using the same procedure as for **11a**, an oil, **11h** (40%), was obtained: IR ν_{max} (cm^{-1}) 3340 (NH), 2980, 2945 (CH_3), 1732 ($C{=}O_{ester}$), 1673 ($C{=}O_{amide}$), 1536, 1369 (NO_2), 1154 (C–O); ^{1}H NMR δ 1.41, 1.43 (2s, 18H, 2 *t*-Bu), 1.71–2.10 (2m, 2H, CH_2CH), 2.28–2.40 (m, 2H, CH_2CO_2t-Bu), 3.98–4.05 (m, 1H, $CHCH_2$), 7.47 (dd, 1H, H-6, J = 2.4 Hz), 8.23 (t 1H, H-5, J_o = 11.0 Hz), 8.48 (d, 1H, NH, J = 7.8 Hz); MS (EI) *m/z* 422 (M^+).

Di-*tert*-butyl [[(4-Aminophenyl)oxy]carbonyl]-L-glutamate (12a). Compound **11a** (17.3 g, 40.8 mmol) in EtOH (200 mL) was hydrogenated by hydrogen transfer (Pd/C, 10%, 5.7 g) and ammonium formate (11.5 g, 182 mmol) at room temperature. The reaction was complete in 20 min. The catalyst was filtered and the filtrate concentrated under vacuum to an oil which was partitioned between AcOEt and H_2O. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated to an oil, **12a** (13.4 g, 83%): IR ν_{max} (cm^{-1}) 3368 (NH), 2979, 2934 (CH_3), 1729 ($C{=}O_{ester}$), 1668 ($C{=}O_{amide}$), 1155 (C–O); ^{1}H NMR δ 1.38, 1.39 (2s, 18H, 2 *t*-Bu), 1.70–2.07 (2m, 2H, CH_2CH), 2.16–2.30 (m, 2H, CH_2CO_2t-Bu), 3.92–4.02 (m, 1H, $CHCH_2$), 6.49 (d, 2H, H-6, H-2), 6.82 (d, 2H, H-5, H-3, J_o = 9.0 Hz), 7.33 (d, 1H, NH, J = 8.7 Hz).

Di-*tert*-butyl [[[4-[Bis(2-hydroxyethyl)amino]phenyl]oxy]carbonyl]-L-glutamate (13a). Procedure a. A solution of the nitro derivative **11a** (5.01 g, 11.8 mmol) in AcOH (30 mL) was hydrogenated on Pd/C (10%) for 3 days. After filtering, the solution was cooled, and ethylene oxide (5.0 mL) was added and the reaction mixture left at room temperature for 22 h. The solvent was evaporated and the residue partitioned between AcOEt and H_2O. The organic phase was separated, washed with H_2O, dried (Na_2SO_4), and concentrated under vacuum. The residue was chromatographed on silica gel and eluted with AcOEt–$CHCl_3$ (2:1) to obtain **12a** (3.93 g, 69%) as a solid: mp 91–93 °C; ^{1}H NMR δ 1.40, 1.41 (2s, 18H, 2 *t*-Bu), 1.70–2.05 (2m, 2H, CH_2CH), 2.21–2.34 (m, 2H, CH_2CO_2t-Bu), 3.38 (t, 4H, 2 CH_2N, J = 6.1 Hz), 3.54 (q, 4H, 2 $HOCH_2$), 3.93–4.08 (m, 1H, $CHCH_2$), 4.96 (t, 2H, OH, J = 5.3 Hz), 6.64 (d, 2H, H-6, H-2), 6.85 (d, 2H, H-5, H-3, J_o = 8.9 Hz), 8.48 (d, 1H, NH, J = 7.8 Hz); MS (EI) *m/z* 484 (M^+ + 1), 453 [(M^+ + 1) − CH_2OH], 372 [(M^+ + 1) − 2*t*-Bu].

Procedure b. To compound **12a** (6.5 g, 6.5 mmol) dissolved in AcOH (75 mL) was added ethylene oxide (3.1 mL, 65.0 mmol) at room temperature. After 72 h the reaction mixture was diluted with H_2O (150 mL), neutralized with Na_2CO_3 at 4 °C, and extracted with AcOEt. The organic layer was dried and evaporated under vacuum to an oil, which was chromato-

Table 9. Protected Prodrugs Derived from Phenol Mustards



no.	R	R_3	X, Y	mp (°C)	^{1}H NMR (δ, ppm)[a]	MS (M^+)	yield (%)
9a	H	*t*-Bu	Br	oil	7.93 (d, 1H, NH, J = 7.9 Hz), 6.93 (d, 2H, H_2, H_6, J = 9.0 Hz), 6.72 (d, 2H, H_3, H_5), 3.76 (d, 4H, 2 $BrCH_2$, J = 7.1 Hz), 3.58 (d, 4H, 2 CH_2N), 1.43 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu)	609 ($M^+ + 1$)[c]	83
9b	H	*t*-Bu	I	oil	7.91 (d, 1H, NH, J = 7.9 Hz), 6.93 (d, 2H, H_2, H_6, J = 8.8 Hz), 6.67 (d, 2H, H_3, H_5), 3.72 (d, 4H, 2 ICH_2, J = 7.1 Hz), 3.31 (d, 4H, 2 CH_2N), 1.43 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu)	591 ($M^+ + 1$)[c]	64
9c	H	*t*-Bu	CH_3SO_3, Cl	oil	7.92 (d, 1H, NH, J = 7.9 Hz), 6.92 (d, 2H, H_2, H_6, J = 9.1 Hz), 6.75 (d, 2H, H_3, H_5), 4.29 (t, 2H, $CH_3SO_3CH_2$, J = 5.6 Hz), 3.70 (s, 6H, $ClCH_2$ + 2 CH_2N), 3.15 (s, 3H, CH_3SO_3), 1.42 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu)	579 ($M^+ + 1$)[c]	43
9d	2-CH_3	Bn	Cl	oil	8.15 (d, 1H, NH, J = 8.3 Hz), 6.80 (d, 1H, H_6, J = 10.0 Hz), 6.60–6.48 (m, 2H, H_3, H_5), 3.70 (s, 4H, 2 $ClCH_2$), 3.27 (m, 4H, 2 CH_2N), 2.03 (s, 3H, 2-CH_3)[b]	601	53
9e	2-Cl	Bn	Cl	oil	8.34 (d, 1H, NH, J = 8.3 Hz), 7.01 (d, 1H, H_6, J = 9.0 Hz), 6.84–6.76 (m, 2H, H_3, H_5), 3.73 (s, 8H, 2 $ClCH_2CH_2N$)[b]	620 ($M^+ - 1$)	39
9f	3-CH_3	Bn	Cl	oil	7.13 (d, 1H, H_5, J = 8.3 Hz), 6.98–6.86 (m, 2H, H_2, H_6), 5.20 (d, 1H, NH, J = 8.3 Hz), 3.50–3.31 (m, 8H, 2 $ClCH_2CH_2N$), 2.29 (s, 3H, 3-CH_3)[b]	601	50
9g	3-*i*-Pr	Bn	Cl	oil	8.24 (d, 1H, NH, J = 8.3 Hz), 7.27 (d, 1H, H_5, J = 10.0 Hz), 7.00–6.81 (m, 2H, H_2, H_6), 3.67 (m, 1H, C*H*-*i*-Pr, J = 7.00 Hz), 3.60–3.49 (m, 4H, 2 $ClCH_2$), 3.32 (m, 4H, 2 CH_2N), 1.18 (d, 6H, 2 CH_3-*i*-Pr)[b]	628 ($M^+ - 1$)	31
9h	3-F	Bn	Cl	oil	8.50 (d, 1H, NH, J = 8.3 Hz), 7.06 (t, 1H, H_5), 6.68–6.47 (m, 2H, H_2, H_6), 3.54 (t, 4H, 2 $ClCH_2$, J = 6.0 Hz), 3.37 (t, 4H, 2 CH_2N)[b]	605	50
9i	3-F	*t*-Bu	Br	oil	8.12 (d, 1H, NH, J = 8.3 Hz), 7.13 (t, 1H, H_5, $J_H = J_F$ = 10.0 Hz), 7.05–6.81 (m 2H, H_2, H_6), 3.66–3.44 (m, 8H, 2 $BrCH_2CH_2N$), 1.42 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu)	627 ($M^+ + 1$)[c]	52
9j	3-F	*t*-Bu	I	oil	8.10 (d, 1H, NH, J = 8.3 Hz), 7.12 (t, 1H, H_5, J_H = 10.0 Hz, J_F = 10.4 Hz), 7.02–6.80 (m, 2H, H_2, H_6), 3.55 (t, 4H, 2 ICH_2, J = 7.5 Hz), 3.27 (t, 4H, 2 CH_2N), 1.43 (2s, 18H, 2 *t*-Bu)	720	40
9k	3-Cl	Bn	Cl	oil	8.10 (d, 1H, NH, J = 8.3 Hz), 7.13 (d, 1H, H_5, J = 8.6 Hz), 6.89 (d, 1H, H_2, J = 2.8 Hz), 6.70 (dd, 1H, H_6), 3.52 (m, 8H, 2 $ClCH_2CH_2N$)[b]	621	38
9l	2,3-$(CH)_4$	Bn	Cl	oil	not isolated	637	26
9m	H	*t*-Bu	$(CH_3SO_3)_2$	oil	7.92 (d, 1H, NH, J = 7.8 Hz), 6.92 (d, 2H, H_2, H_6, J = 9.0 Hz), 6.78 (d, 2H, H_{3+5}), 4.30 (t, 4H, $CH_3SO_3CH_2$, J = 5.7 Hz), 3.71 (t, 4H, 2 CH_2N), 3.15 (s, 6H, 2 CH_3SO_3), 1.42 (s, 9H, *t*-Bu), 1.41 (s, 9H, *t*-Bu)	639 ($M^+ + 1$)[c]	28
9n	3-F	*t*-Bu	$(CH_3SO_3)_2$		not isolated		

[a] The signals for glutamic acid protons are located for all derivatives **9** as follows: 4.56–3.91 (m, 1H, CH), 2.45–2.24 (m, 2H, CH_2CO_2H), 2.23–1.70 (2m, 2H, CH_2CH). [b] The signals for benzylic protons are located for all derivatives **9** as follows: 7.36–7.35 (m, 5H, Bn), 7.35–7.34 (m, 5H, Bn), 5.20–5.15 (s, 2H, CH_2Bn), 5.11–5.02 (s, 2H, CH_2Bn). [c] By FAB.

graphed on silica gel, eluting with AcOEt–$CHCl_3$ (2:1) to obtain **12a** (3.0 g, 38%).

Di-*tert*-butyl [[[3-fluoro-4-[bis(2-hydroxyethyl)amino]phenyl]-oxy]carbonyl]-L-glutamate (**13h**) (36%) was obtained as an oil, by a similar procedure: ^{1}H NMR δ 1.41 (s, 18H, 2 *t*-Bu), 1.70–2.06 (2m, 2H, CH_2CH), 2.27–2.40 (m, 2H, CH_2CO_2t-Bu), 3.25 (t, 4H, 2 CH_2N), 3.43–3.53 (q, 4H, 2 $HOCH_2$), 3.91–4.04 (m, 1H, $CHCH_2$), 4.55 (t, 2H, OH, J = 6.2 Hz), 6.25–6.94 (m, 2H, H-6, H-2), 7.05 (t, 1H, H-5), 8.05 (d, 1H, NH, J = 8.3 Hz); MS (EI) m/z 500 (M^+).

Di-*tert*-butyl [[[4-[(2-Chloroethyl)[2-(mesyloxy)ethyl]-amino]phenyl]oxy]carbonyl]-L-glutamate (9c). A solution of **13a** (0.86 g, 1.8 mmol) in pyridine (3.0 mL) was stirred with methanesulfonyl chloride (0.6 mL, 0.87 g, 7.6 mmol) at 2 °C for 20 min followed by 50 °C for 10 min. The reaction mixture was concentrated under vacuum and the residue dissolved in AcOEt, washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel, eluting with AcOEt–CH_2Cl_2 (1:9) to obtain **9c** (0.44 g, 43%) as an oil.

Di-*tert*-butyl [[[4-[Bis[2-(mesyloxy)ethyl]amino]phenyl]oxy]carbonyl]-L-glutamate (9m). A solution of **13** (2.53 g, 10.0 mmol) in pyridine (9.0 mL) was stirred with methanesulfonyl chloride (1.8 mL, 2.61 g, 22.8 mmol) at 2 °C for 20 min. The reaction mixture was partitioned between AcOEt and H_2O (10%). The organic phase was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on silica gel and eluted with AcOEt–CH_2Cl_2 (1:9) to obtain **9m** (0.95 g, 28%) as an oil. For analytical data, see Table 9.

Compound **9n** was synthesized according to a similar procedure but was not isolated.

Di-*tert*-butyl [[[4-[Bis(2-bromoethyl)amino]phenyl]-oxy]carbonyl]-L-glutamate (9a). A solution of **9m** (0.48 g, 0.7 mmol) in CH_3CN (30 mL) was stirred with LiBr (0.26 g, 2.9 mmol) at 70 °C for 22 h. The reaction mixture was filtered and the filtrate concentrated under vacuum. The residue was chromatographed on silica gel and eluted with AcOEt–CH_2Cl_2 (1:5) to obtain **9a** (0.37 g, 83%) as an oil. For analytical data, see Table 9.

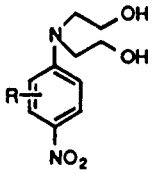
Di-*tert*-butyl [[[4-[Bis(2-iodoethyl)amino]phenyl]oxy]-carbonyl]-L-glutamate (9b). A solution of **9m** (1.00 g, 1.6 mmol) in CH_3CN (50 mL) was stirred with NaI (1.00 g, 6.7 mmol) at 70 °C for 20 h. The reaction mixture was filtered and the filtrate concentrated under vacuum. The same workup as for compound **9a** resulted in compound **9b** (0.75 g, 68%) as an oil. For analytical data, see Table 9.

[[[4-[Bis(2-iodoethyl)amino]phenyl]oxy]carbonyl]-L-glutamic Acid (1b). Compound **9b** (0.19 g, 0.3 mmol) was suspended in TFA (4.0 mL) and stirred for 30 min at room temperature. TFA was removed under reduced pressure; the remaining oil was diluted with AcOEt (3.0 mL) and evaporated to give the **1b** (0.16 g, 82%) as an oil. For analytical data, see Table 1.

The foregoing procedure led to compounds **1a–c,h,j**.

[[[3-Methyl-4-[bis(2-chloroethyl)amino]phenyl]oxy]car-

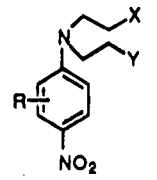
Table 10. 4-Nitro *N,N*-Bis(2-hydroxyethyl) Derivatives



no.	R	mp (°C)	^{1}H NMR (δ, ppm)	MS[a] ($M^+ + 1$)	yield (%)
15o,p	3-F	99–100	7.93–7.83 (m, 2H, H_2, H_6), 7.04 (t, 1H, H_5, $J_H = J_F = 8.8$ Hz), 4.80 (t, 2H, 2 OH, $J = 5.0$ Hz), 3.58 (s, 8H, 2 $ClCH_2CH_2N$)	245	97
15q	3-Cl	oil	8.14 (d, 1H, H_2, $J = 2.40$ Hz), 8.05 (dd, 1H, H_6), 7.29 (d, 1H, H_5, $J_H = 9.2$ Hz), 4.65 (t, 2H, 2 OH, $J = 4.8$ Hz), 3.62–3.45 (m, 8H, 2 $ClCH_2CH_2N$)	nd	100
15r	3-CN	151–4	8.35 (d, 1H, H_2), 8.12 (dd, 1H, H_6), 7.16 (d, 1H, H_5, $J_H = 8.3$ Hz), 4.90 (t, 2H, 2 OH, $J = 4.8$ Hz), 3.83 (t, 4H, 2 $ClCH_2$), 3.69 (t, 4H, 2 NCH_2)	252	99

[a] By FAB.

Table 11. 4-Nitroaniline Mustard Derivatives



no.	R	X, Y	mp (°C)	^{1}H NMR (δ, ppm)	MS[a] ($M^+ + 1$)	yield (%)
16o	3-F	Cl	66–8	8.02–7.89 (m, 2H, H_2, H_6), 6.89 (t, 1H, H_5, $J_H = J_F = 8.8$ Hz), 3.86 (t, 4H, 2 $ClCH_2$, $J = 6.0$ Hz), 3.65 (t, 4H, 2 CH_2N)	281	99
16p	3-F	Br	66–8	8.02–7.88 (m, 2H, H_2, H_6), 6.86 (t, 1H, H_5, $J_H = J_F = 10.0$ Hz), 3.87 (t, 4H, 2 $BrCH_2$, $J = 7.0$ Hz), 3.50 (t, 4H, 2 CH_2N)	371	27
16q	3-Cl	Cl	oil	8.27 (d, 1H, H_2, $J = 2.5$ Hz), 8.09 (dd, 1H, H_6), 7.24 (d, 1H, H_5, $J_H = 9.3$ Hz), 3.75 (t, 4H, 2 $ClCH_2$, $J = 6.5$ Hz), 3.58 (t, 4H, 2 CH_2N)	297	79
16r	3-CN	Cl	106–9	8.48 (d, 1H, H_2), 8.24 (dd, 1H, H_6), 7.25 (d, 1H, H_5, $J_o = 10.0$ Hz), 4.05 (t, 4H, 2 $ClCH_2$, $J = 5.3$ Hz), 3.86 (t, 4H, 2 CH_2N)	288	52

[a] By FAB.

bonyl]-L-glutamic Acid (1f). The dibenzyl ester **9f** (1.49 g, 2.48 mmol) dissolved in EtOH was hydrogenated (Pd/C, 30%, 0.30 g) at room temperature for 3 h. The catalyst was filtered through Celite and the filtrate concentrated under vacuum to a solid. Crystallization from AcOEt–hexane (1:5) afforded **1f** (0.74 g, 71%) as a white solid, mp 160–162 °C. For analytical data, see Table 1.

A similar route was used for the synthesis of compounds **1d–h,k,l** (see Table 1).

3-Fluoro-4-[bis(2-hydroxyethyl)amino]nitrobenzene (15o). A suspension of 3,4-difluoronitrobenzene (15.9 g, 10.0 mmol) in diethanolamine (30.0 mL, 30.0 mmol) was heated for 2 h at 130 °C. The reaction mixture was poured into 1.0 L of water. Filtration of the precipitate from the cooled solution afforded **15o** (22.7 g, 97%) as a solid: mp 99–101 °C. For analytical data, see Table 10.

The same procedure was used in order to obtain compounds **15p–r** (see Table 10).

3-Fluoro-4-[bis(2-chloroethyl)amino]nitrobenzene (16o). To a cooled solution (5–10 °C) of **15o** (2.44 g, 10.0 mmol) and piperidine (0.81 mL, 10.0 mmol) in CH_2Cl_2 was added thionyl chloride (1.8 mL, 25.0 mmol) over 45 min. The reaction mixture was heated to reflux for 1 h, cooled, washed, dried ($MgSO_4$), and concentrated under vacuum to give **16o** (2.60 g, 93%) as an oil. Column chromatography on silica gel, eluting with hexane–ethyl acetate (9:1), afforded **16o** (2.77 g, 99%) as a solid: mp 66–68 °C. For analytical data, see Table 11.

The same procedure was used to obtain compounds **16q,r** (see Table 11).

3-Fluoro-4-[bis(2-bromoethyl)amino]nitrobenzene (16p). The same procedure was carried out for bromination of compound **15o**, except that thionyl bromide was used instead of thionyl chloride. For analytical data, see Table 11.

3-Fluoro-4-[bis(2-chloroethyl)amino]aniline (2o). Compound **16o** (2.50 g, 8.9 mmol) dissolved in AcOEt (200 mL) was hydrogenated (Pd/C, 30%, 2.0 g) for 3 h. The catalyst was filtered and the reaction mixture concentrated under vacuum to give **2o** as an oil. **2o**-HCl (1.80 g, 71%) was precipitated from an etheric solution of HCl as a hygroscopic solid. When the crude **2o** (from 11.0 g, 39.2 mmol of **16o**) dissolved in AcOEt (100mL) was added to an etheric solution of oxalic acid (300 mL, 1% oxalic acid), **2o**-oxalate (11.0 g, 97%) was precipitated, as a white solid: mp 146–148 °C.

The drugs **2p–r** were synthesized according to the same route (see Table 2).

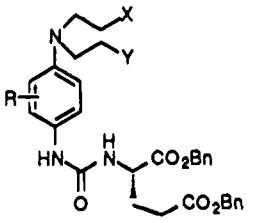
Dibenzyl [[3-Fluoro-4-[bis(2-chloroethyl)amino]phenyl]carbamoyl]-L-glutamate (17o). A suspension of the **2o**-oxalate (3.50 g, 10.3 mmol) in anhydrous AcOEt (200 mL) and K_2CO_3 (5.50 g, 39.8 mmol) was cooled to 5 °C. To this mixture was added, under Ar, a solution of phosgene in toluene (1.9 M) (5.50 mL, 10.5 mmol). The reaction mixture was stirred at room temperature for a further 10 min and filtered and the filtrate dried ($MgSO_4$). The filtrate was added in one portion to a suspension of dibenzylglutamate *p*-toluenesulfonate (5.00 g, 9.7 mmol), K_2CO_3 (2.00 g, 14.4 mmol), and AcOEt (100 mL). After the addition of triethylamine (2.0 mL), the mixture was stirred for 20 min at room temperature, filtered, and evaporated to dryness. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1:2) to obtain compound **17o** (5.5 g, 94%—with respect to the glutamic acid), as a solid. For the analytical data, see Table 12.

The same procedure was used to prepare compounds **17p–r** (see Table 12).

[[3-Fluoro-4-[bis(2-chloroethyl)amino]phenyl]carbamoyl]-L-glutamic Acid (1o). To a solution of **17o** (0.40 g, 0.7 mmol) in AcOEt (10 mL) was added Pd/C (30%) (50% moist) (0.16 g), and the mixture was stirred under a hydrogen atmosphere for 1 h. After filtration of the catalyst, the filtrate was evaporated to dryness. The compound **1o** (0.21 g, 75%) was obtained as a white powder after trituration of the oily residue with AcOEt–hexane. For the analytical data, see Table 1.

Compounds **1p–r** were prepared similarly (see Table 1).

Table 12. Protected Prodrugs Derived from Aniline Mustards



no.	R	X, Y	mp (°C)	^{1}H NMR (δ, ppm)[a]	MS ($M^+ + 1$)	yield (%)
17o	3-F	Cl	81–4	7.24–6.84 (m, 3H, H_{arom}), 5.68 (d, 1H, NH, J = 8.3 Hz), 3.50 (m, 8H, 2 $ClCH_2CH_2N$)[b]	604	91
17p	3-F	Br	oil	7.25–6.83 (m, 3H, H_{arom}), 5.66 (d, 1H, NH, J = 8.3 Hz), 3.54 (t, 4H, 2 $BrCH_2$, J = 6.8 Hz), 3.34 (t, 4H, 2 CH_2N)[b]	639 (M^+)	72
17q	3-Cl	Cl	oil	7.42 (m, 1H, H_{arom}), 7.11 (m, 2H, H_{arom}), 6.87 (d, 1H, NH), 3.46 (m, 8H, 2 $ClCH_2CH_2N$)[b]	621 (M^+)	64
17r	3-CN	Cl	oil	8.22 (d, 1H, NH-Ph), 7.62–7.56 (m, 2H, H_2, H_6), 7.09 (d, 1H, H_5, J = 9.7 Hz), 6.25 (d, 1H, NH-G), 3.62 (t, 4H, 2 $ClCH_2$, J = 6.2 Hz), 3.54 (t, 4H, 2 CH_2N)[b]	nd	70

[a] The protons belonging to glutamic acid moiety are located for all compounds **17** as follows: 4.68–4.57 (m, 1H, CH), 2.51–2.42 (m, 2H, CH_2CO_2R), 2.34–1.96 (2m, 2H, CH_2CH). The benzylic protons are located for all compounds **17** as follows: 7.33–7.31 (2m, 10H, 2 Ph), 5.17–5.16 (s, 2H, CH_2Bn), 5.09–5.07 (s, 2H, CH_2Bn). [b] $CDCl_3$.

Chemical Stability Determination. The rates of hydrolysis of the chloroethyl mustard-containing compounds were measured by HPLC in phosphate buffer (pH 7.4) at 37 °C. This method was not used for the more reactive mustard functionalities (bromo, iodo, and mesyl) since the hydrolysis rates of these latter groups are too fast to measure accurately. Reaction products were separated on a 25 cm S50DS1 HPLC column. The $t_{1/2}$ was calculated using a standard kinetic program.

Biological Methods: Cytotoxicity Assays. The colorectal tumor cell line LoVo was incubated with different concentrations of the potential prodrugs **1a–r** or parent drugs **2a–r** in 96-well (2.5×10^3 cells/well) microtiter plates for 1 h. The cells were then washed and incubated for a further 3 days at 37 °C. At the end of this period, the cells were fixed and stained. The concentration of cellular protein from the remaining viable cells adhering to the plates was quantitated by a sulforhodamine B (SRB) dye.[23] The results of protein concentration in treated samples were compared to those of untreated controls. Potency of the compounds is expressed as the concentration required to inhibit the cell growth by 50% (IC_{50}, μM).

Compounds were made up just prior to use and added once. Each experiment was performed at least twice. The IC_{50} ± the standard deviation was calculated for each data point.

In addition, the drug **2b**, of potential prodrug **1b**, was incubated under the same conditions but for 1 min instead of 1 h, and the prodrug **1b** was incubated with CPG2 for 1 h under the same conditions.

Enzyme Kinetics. The K_m and k_{cat} of the cleavage of amidic bond with CPG2 were calculated for each of the potential prodrugs according to the literature CPG2 assay methods.[24] The absorbances of the prodrug and its corresponding drug were scanned from 200 to 350 nm using a spectrophotometer, and the wavelength was selected where the maximal absorbance difference between prodrug and drug, due to release of glutamate from the prodrug, was obtained. The K_M and V_{max} were then determined by measuring the initial rate of conversion of prodrug at this wavelength using a range of prodrug concentrations and CPG2 enzyme concentrations. The k_{cat} was calculated from the V_{max} by dividing by the concentration of CPG2 in the reaction mixture.

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References

(1) Frei, E., III. Curative cancer chemotherapy. *Cancer Res.* **1985**, *45*, 6532–6538.
(2) Bagshawe, K. D. Antibody directed enzymes revive anti-cancer prodrug concept. *Br. J. Cancer* **1987**, *56*, 531–532.
(3) Bagshawe, K. D.; Springer, C. J.; Searle, F.; Antoniw, P.; Sharma, S. K.; Melton, R. G.; Sherwood, R. F. A cytotoxic agent can be generated selectively at cancer sites. *Br. J. Cancer* **1988**, *58*, 700–703.
(4) Teicher, B. A.; Frei, E., III. Development of alkylating agent resistant human tumor cell lines. *Cancer Chemother. Pharmacol.* **1988**, *21*, 292–298.
(5) Springer, C. J.; Antoniw, P.; Bagshawe, K. D.; Searle, F.; Bisset, G. M. F.; Jarman, M. Novel prodrugs which are activated to cytotoxic alkylating agents by carboxypeptidase G2. *J. Med. Chem.* **1990**, *33*, 677–681.
(6) Mann, J.; Haase-Held, M.; Springer, C. J.; Bagshawe, K. D. Synthesis of an N-mustard prodrug. *Tetrahedron* **1990**, *46*, 5377–5382.
(7) Springer, C. J. CMDA, an antineoplastic prodrug. *Drugs Future* **1993**, *18*, 212–215.
(8) Springer, C. J.; Niculescu-Duvaz, I.; Pedley, R. B. Novel prodrugs of alkylating agents derived from 2-fluoro- and 3-fluoro benzoic acids for antibody-directed enzyme prodrug therapy. *J. Med. Chem.* **1994**, *37*, 2361–2370.
(9) Sharma, S. K.; Boden, J. A.; Springer, C. J.; Burke, P. J.; Bagshawe, K. D. Antibody-directed prodrug therapy (ADEPT). A three-phase study in ovarian tumour xenografts. *Cell Biophys.* **1994**, *24/25*, 219–228.
(10) Eccles, S.; Court, W. J.; Box, G. A.; Dean, C. J.; Melton, R. G.; Springer, C. J. Regression of established breast carcinoma xenografts with antibody-directed enzyme prodrug therapy against C-erbB2 p185. *Cancer Res.* **1994**, *54*, 5171–5177.
(11) Bagshawe, K. D.; Sharma, S. K.; Springer, C. J.; Antoniw, P. Antibody-Directed Enzyme Prodrug Therapy (ADEPT): Pilot Scale Clinical Trial. *Tumour Targeting* **1995**, *1*, 17–29.
(12) Bagshawe, K. D.; Sharma, S. K.; Springer, C. J.; Antoniw, P.; Boden, J. A.; Rogers, G. T.; Burke, P. J.; Melton, R. G.; Sherwood, R. F. Antibody-Directed Enzyme Prodrug Therapy (ADEPT): Clinical report. *Disease Markers* **1991**, *9*, 233–238.
(13) Bagshawe, K. D.; Sharma, S. K.; Springer, C. J.; Rogers, G. T. Antibody-Directed Enzyme Prodrug Therapy (ADEPT): A review of some theoretical, experimental and clinical aspects. *Anal. Oncol.* **1994**, *5*, 879–891.
(14) Springer, C. J.; Poon, G. K.; Sharma, S. K.; Bagshawe, K. D. Identification of prodrug, active drug and metabonates in an ADEPT clinical study. *Cell Biophys.* **1993**, *22*, 1–8.
(15) Springer, C. J.; Niculescu-Duvaz, I.; Mauger, A. B.; Connors, T. A.; Burke, P. J.; Davies, D. H.; Dowell, R. I.; Boyle, F. T.; Blakey, D. C.; Melton, R. G. In *New Antibody Technology and the Emergence of Useful Cancer Therapy*; Begent, R., Hamlin, A., Eds.; Royal Society of Medicine Press: London, 1995; pp 75–77.
(16) Dowell, R.; Springer, C. J.; Davies, D. H.; Hadley, E. M.; Burke, P. J.; Boyle, F. T.; Melton, R. G.; Connors, T. A.; Blakey, D. C.; Mauger, A. B. New mustard prodrugs for antibody directed enzyme prodrug therapy (ADEPT). Alternative to amide link. *J. Med. Chem.*, accepted for publication.

(17) Springer, C. J.; Niculescu-Duvaz, I. Antidody-directed enzyme prodrug therapy (ADEPT) with mustard prodrugs. *Anticancer Drug Des.* **1995**, *10*, 361–372.
(18) Niculescu-Duvaz, I.; Springer, C. J. Adamantylanyloxycarbonyl: a novel protecting group for phenols carrying strongly electron-withdrawing substituents. *J. Chem. Res., Synop.* **1994**, 242–243.
(19) Springer, C. J.; Antoniw, P.; Bagshawe, K. D.; Wilman, D. E. V. Comparison of half-lives and cytotoxicity of N-mesyloxyethyl- and N-chloroethyl-4-amino benzoyl compounds, products of prodrugs in antibody-directed enzyme prodrug therapy (ADEPT). *Anticancer Drug Des.* **1991**, *6*, 467–479.
(20) Panthananical, A.; Hansch, C.; Leo, A.; Quinn, F. R. Structure-activity relationships in antitumor aniline mustards. *J. Med. Chem.* **1978**, *21*, 16–26.
(21) Ross, W. C. J. *Biological alkylating agents*; Butterworths: London, 1962.
(22) Bohm, S.; Decouzon, M.; Exner, O.; Gal, J. F.; Maria, C. P. Sterically hindered resonance in methyl-substituted anilines in the gas phase. *J. Org. Chem.* **1994**, *59*, 8127–8131.
(23) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, D.; Warren, J. T.; Bokedch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
(24) Springer, C. J.; Bagshawe, K. D.; Sharma, S. K.; Searle, F.; Boden, J. A.; Antoniw, P.; Burke, P. J.; Rogers, G. T.; Sherwood, R. F.; Melton, R. G. Ablation of human choriocarcinoma xenografts in nude mice by Antibody-Directed Enzyme Prodrug Therapy (ADEPT) with three novel compounds. *Eur. J. Cancer* **1991**, *27*, 1361–1366.

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