# Self-Immolative Nitrogen Mustards Prodrugs Cleavable by Carboxypeptidase G2 (CPG2) Showing Large Cytotoxicity Differentials in GDEPT

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Nineteen novel potential self-immolative prodrugs and their corresponding drugs have been synthesized for gene-directed enzyme prodrug therapy (GDEPT) with carboxypeptidase G2 (CPG2) as the activating enzyme. The compounds are derived from *o*- and *p*-amino and *p*-methylamino aniline nitrogen mustards. Their aqueous stability, kinetics of drug release by CPG2, and cytotoxicity in the colon carcinoma cell line WiDr, expressing either surface-tethered CPG2 (stCPG2(Q)3) or control $\beta$-galactosidase, are assessed. The effect of various structural features on stability, kinetics of activation, and biological activity is discussed. The *p*-methylamino prodrugs are the most stable compounds from this series, with the largest cytotoxicity differentials between CPG2-expressing and nonexpressing cells. The most potent compounds in all series are prodrugs of bis-iodo nitrogen mustards. 4-{*N*-[4′-Bis(2″-iodoethyl)aminophenyl]-*N*′-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid, compound **39b**, is 124-fold more cytotoxic to WiDr cells expressing CPG2 than to cells expressing $\beta$-galactosidase. An additional six compounds show better cytotoxicity differential than the published *N*-{4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl}-L-glutamic acid (CMDA) prodrug.

## Introduction

A number of targeting strategies has been developed in an attempt to increase the selectivity of anticancer drugs. One approach uses antibodies conjugated with enzymes to target tumor-associated antigens. The enzyme can activate a subsequently administered prodrug in a therapy termed antibody-directed enzyme prodrug therapy (ADEPT).[1,2] Alternatively the gene for the prodrug-activating enzyme is expressed in tumor cells in a strategy called gene-directed enzyme prodrug therapy (GDEPT). This methodology requires prodrugs that are substrates for the expressed enzyme and which, after activation, release cytotoxic drugs.[3] The targeting of the genes is achieved by using viral[4,5] or nonviral delivery vectors.[6−9] Several enzyme/prodrug systems have been investigated and a number of clinical trials using suicide gene therapy are ongoing.[10]

We have developed a GDEPT system based on the enzyme carboxypeptidase G2 (CPG2, glutamate carboxypeptidase, EC 3.4.17.11) from Pseudomonas RS16.[11] CPG2 catalyzes the scission of the amidic,[12] urethanic or ureidic,[13,14] linkage between an aromatic nucleus and L-glutamic acid. CPG2 has been expressed both intracellularly (called CPG2*)[15] and tethered to the outer cell surface (called stCPG2(Q)3)[16] in a range of tumor cell lines. The extracellular tethering overcomes the need for the prodrug to cross the tumor cell membrane for activation. This method of expression is expected to improve the bystander effect, whereby a prodrug is activated to an active drug that kills neighboring tumor cells not expressing the activating enzyme.[17−19]

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Prodrugs where the L-glutamic acid is linked directly onto the drug are already in clinical trials for ADEPT.[20,21] Self-immolative alkylating prodrugs, where the L-glutamic acid is bonded through a linker to the drug, were synthesized for GDEPT, cleavable by CPG2.[22,23] It was demonstrated that the two linkers, L1 and L2 (see Scheme 2), were substrates for CPG2 ($K_M$ = 1.7−3.1 $\mu$M and $k_{cat}$ = 65.4−140 $s^{-1}$).[22] The probable mechanism of drug release from these self-immolative prodrugs[24,25] is shown in Scheme 1.

There are potential advantages of these self-immolative prodrugs. The lipophilicity of the prodrugs and their respective drugs may be altered with minimal effect on the kinetics of activation by CPG2. Improvements can be made in the event of unfavorable kinetics of activation due to unsuitable steric or electronic effects. Also the range of drugs which can be converted to prodrugs can be expanded, unrestricted by the structural substrate requirements of CPG2.

## Rationale of the Design

Previous data suggested that the shape and bulk of the cytotoxic drug moiety attached to the linker would be of importance for the behavior of the self-immolative prodrugs as substrates for CPG2 and, therefore, for their biological activity.[22,23] Modeling of prodrugs shows that the shape and the van der Waals volumes of ortho- and para-self-immolative nitrogen mustards are quite different. To compare these structural features, we report here that both series of self-immolative alkylating agents are synthesized and studied.

An important parameter for a GDEPT system is the differential, or degree of activation, which is defined as the ratio of $IC_{50,prodrug}$ in cells that do not express CPG2

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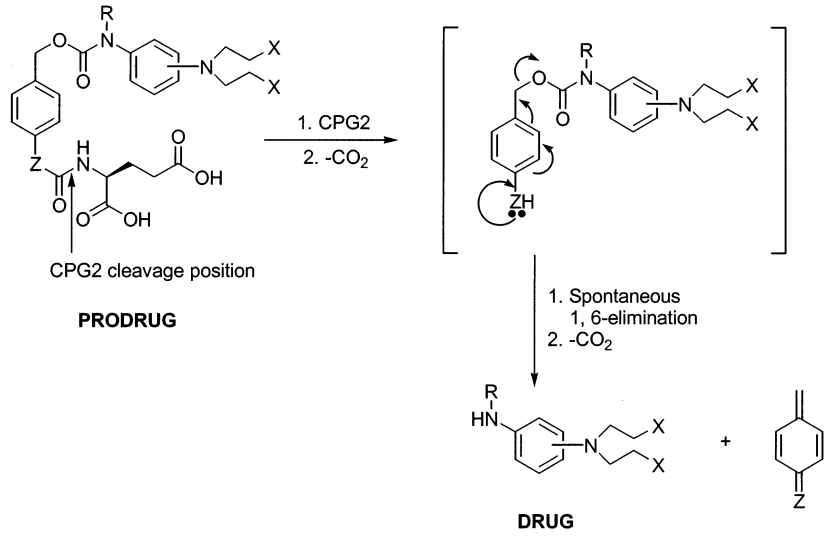
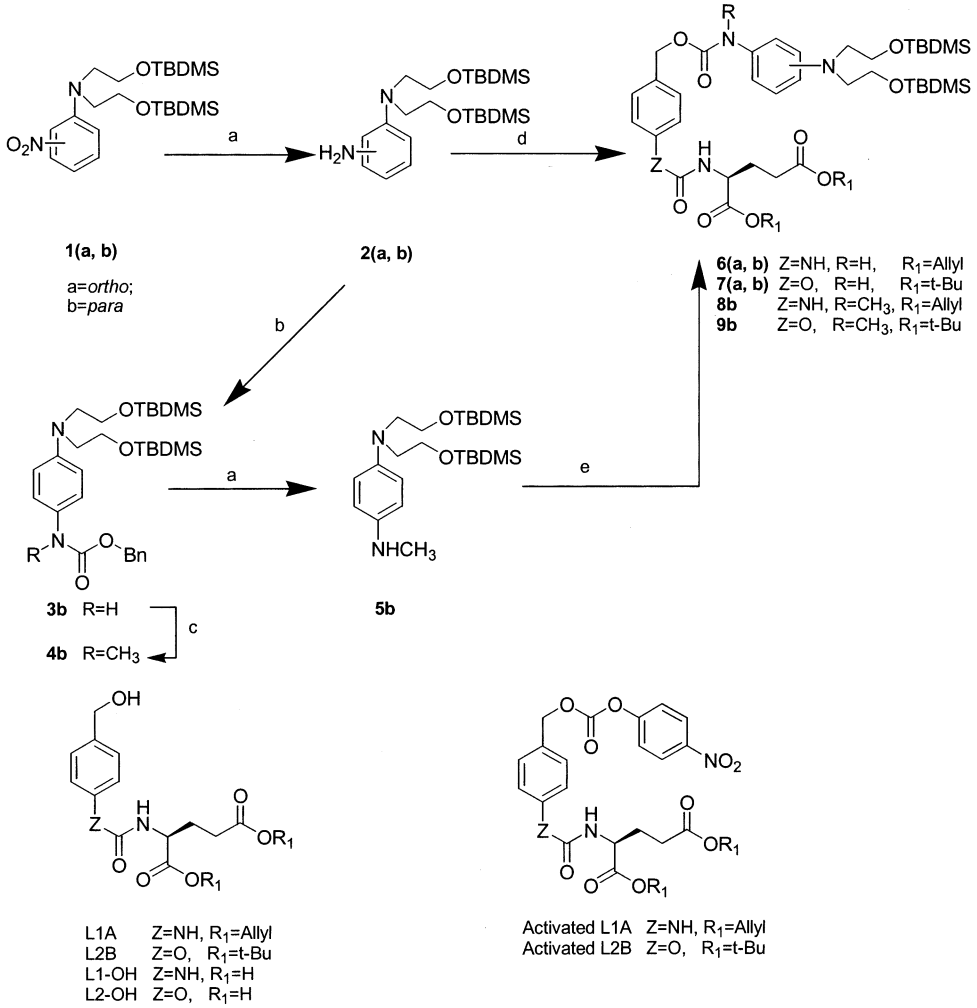
**Scheme 1**



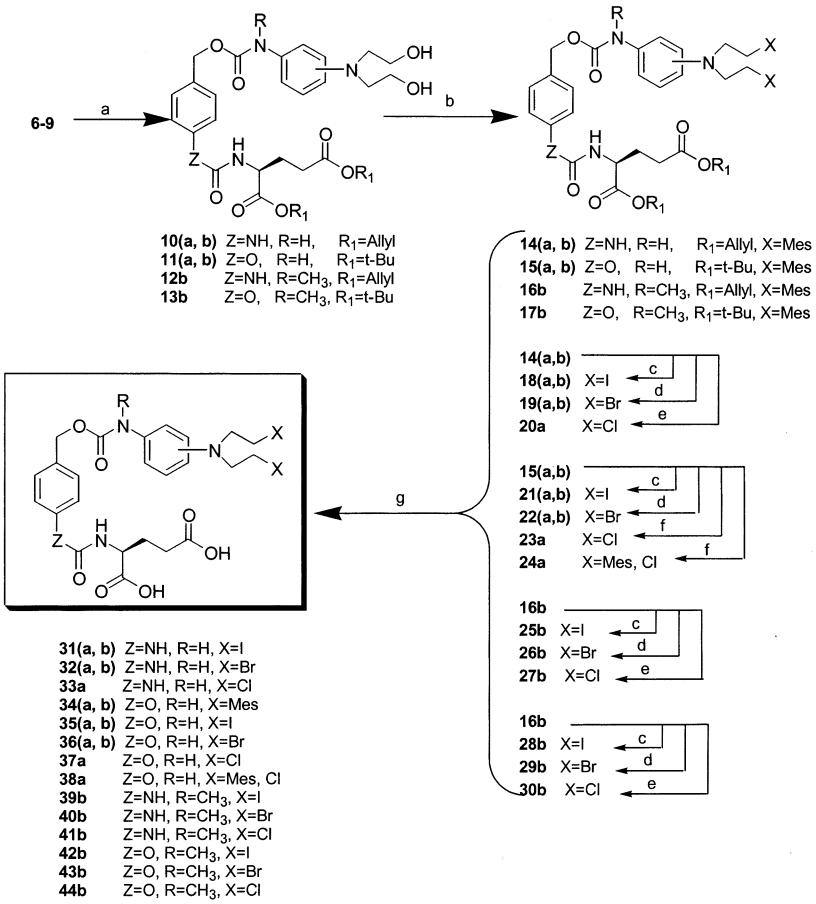

**Scheme 2**[a]




[a] (a) $H_2$, Pd/C 10%, THF; (b) *N*-benzyloxycarbonyloxysuccinimide, THF; (c) MeI, NaH, THF; (d) triphosgene, $NEt_3$, $CH_2Cl_2$, then L1A or L2B, dibutyltin dilaurate, $CH_2Cl_2$; (e) activated L1A or L2B, DMA.

vs $IC_{50,prodrug}$ in cells expressing CPG2.[26] A large differential indicates a good therapeutic window so that a dose for in vivo use can be found that is toxic to the tumor without damage to normal cells. We investigated the relationship between the nature of the leaving groups of the alkylating moiety and the cytotoxic differential of the corresponding prodrugs. We are interested in prodrugs able to release more cytotoxic drugs. It was found that in both series the most effective prodrugs are the bis-iodo nitrogen mustards, which showed significant differentials (49−120-fold) between cells expressing stCPG2(Q)3 and control cells.

**Scheme 3**[a]




[a] (a) $NEt_3 \cdot 3HF$, THF; (b) $Mes_2O$, $NEt_3$, DMAP, $CH_2Cl_2$; (c) NaI, acetone, reflux; (d) LiBr, THF, reflux; (e) LiCl, DMA; (f) NaCl, acetonitrile, 55 °C; (g) $Pd(PPh_3)_4$, morpholine, or pyrrolidine, $CH_2Cl_2$ (for $R_1$ = allyl) or HCOOH (for $R_1$ = t-Bu).

Prodrugs releasing *N*-methylamino aniline mustards were also investigated in comparison with the non-methylated counterparts. It was anticipated that the corresponding drugs would be more reactive as the methylamino group is a stronger electron donor than the amino group and thus a greater differential would be expected.[27] The methylamino prodrugs, as secondary carbamates, are also potentially more stable.[28,29]

The activity of these new prodrugs is assessed in the colon carcinoma cell line WiDr expressing stCPG2(Q)3 or non-CPG2 expressing controls.

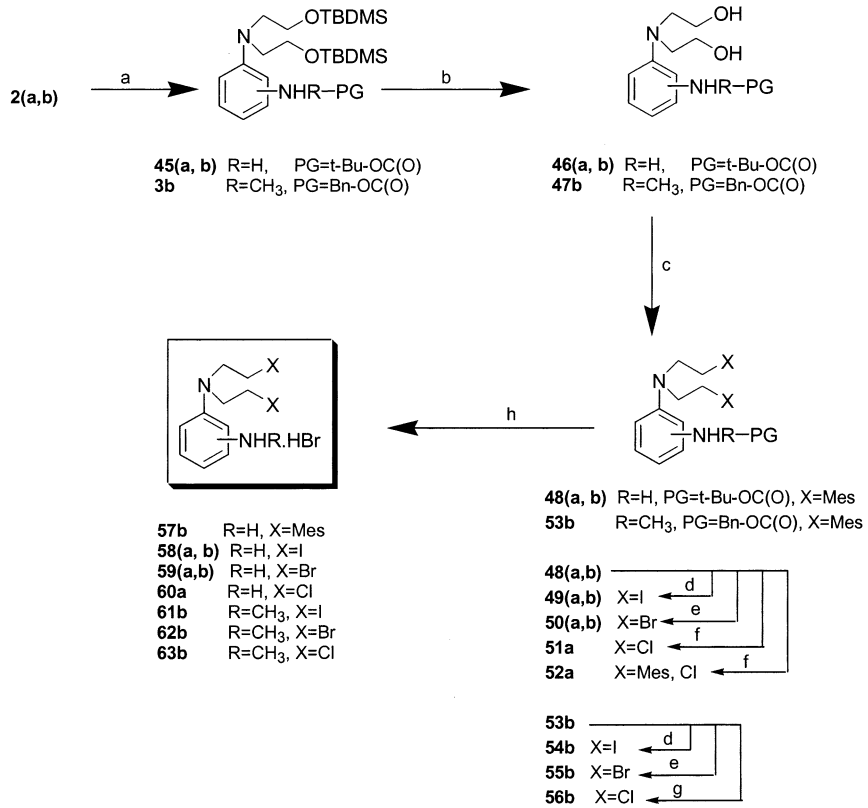
## Results and Discussion

**Chemistry.** Nineteen new self-immolative prodrugs (compounds **31**−**44**, Scheme 3) and their corresponding drugs (compounds **57**−**63**, Scheme 4), derived from *o*- and *p*-amino and *p*-methylamino aniline nitrogen mustards, were synthesized.

2- and 4-[Bis(2′-hydroxyethyl)amino]nitrobenzene were used as starting materials and protected as 2- and 4-bis-(2′-*tert*-butyldimethylsilyloxyethyl)aminonitrobenzene, **1**(a, ortho; b, para) (Scheme 2). After reduction to the corresponding amines, **2a**,**b**, these were converted to isocyanates and coupled with the linkers L1A and L2B (Scheme 2) in the presence of catalytic amounts of dibutyltin dilaurate [30] leading to the protected prodrug intermediates **6** (**a**, **b**; Z = NH; R = allyl) and **7** (**a**, **b**; Z = O; R = *tert*-butyl). To obtain the corresponding methylamino analogues, the 4-amino intermediate **2b** was protected as the benzyloxycarbonyl (Z) derivative **3b**, methylated with methyl iodide and sodium hydride to intermediate **4b**. The Z protection has the purpose of allowing selective monomethylation and increasing the acidity of the N−H bond toward proton removal with NaH. The methylamino compound **5b** was obtained by hydrogenolysis of benzyloxycarbonyl and coupled to the activated L1A and L2B to yield the protected prodrug intermediates **8b** and **9b** respectively.

The *N*,*N*-bis(2-hydroxyethyl)amino function was restored in compounds **10**−**13** by treatment with triethylamine trihydrofluoride in THF [31] in good yield (Scheme 3). These compounds were transformed to the corresponding bis-mesyl derivatives **14**−**17** using mesyl anhydride/triethylamine in dichloromethane. These conditions are preferable to the mesyl chloride/pyridine method,[12] as the yield is improved and there is no risk of formation of unwanted chloro products. The alkylating agents bis-iodo (**18(a,b)**, **21(a,b)**, **25b**, and **28b**), bis-bromo (**19(a,b)**, **22(a,b)**, **26b**, and **29b**) and bis-chloro (**20a**, **27b**, and **30b**) were obtained by exchanging the mesyl leaving group for halogens with NaI/acetone, LiBr/THF, or LiCl/dimethylacetamide, respectively. The bis-chloro-nitrogen mustard, **23a**, and the monomesyl-mono-chloro derivative, **24a**, were obtained as a mixture, by reacting the compound **15a** with NaCl in acetonitrile. The reaction is slow at 55 °C, and conditions could be adapted to obtain either of them as the main product.

**Scheme 4**[a]




[a] (a) $Boc_2O$, THF; (b) $NEt_3$·3HF, THF; (c) $Mes_2O$, $NEt_3$, DMAP, $CH_2Cl_2$; (d) NaI, acetone, reflux; (e) LiBr, THF, reflux; (f) NaCl, acetonitrile, 55 °C; (g) LiCl, DMA; (h) HBr, AcOH.

The final deprotection, with formic acid for the compounds deriving from L2B and Pd(0) with secondary amines for the compounds deriving from L1A, led to the desired self-immolative nitrogen mustard prodrugs **31**−**44**.

The access route to nine new corresponding active drugs is summarized in Scheme 4. Silyl-protected *o*- and *p*-phenylenediamines **2(a,b)** were converted to the corresponding Boc-derivatives **45(a,b)** (Scheme 4). The silyl moieties were removed with triethylamine trihydrofluoride ($NEt_3$·3HF) to obtain the hydroxyethyl-compounds **46(a,b)**. In an alternative procedure compounds **46(a,b)** were obtained directly from the 2- or 4-amino-*N*-bis(2′-hydroxyethyl)anilines by treatment with Boc-pyrocarbonate. The corresponding bis-hydroxy intermediate **47b** for 4-methylamino aniline drugs was obtained by desilylation of **3b**. The Boc- or Z-protected 2-amino, 4-amino, and 4-methylamino alkylating agents **48**−**56** were obtained by a method similar to that used for the corresponding protected prodrugs **14**−**30**, namely mesylation of bis-hydroxy compounds **46(a,b)** and **47b** and subsequent halogen exchange.

The active drugs **57**−**63** were obtained by deprotection with HBr/AcOH. These conditions have a dual role: to remove the protecting groups *tert*-butyloxycarbonyl or benzyloxycarbonyl yielding the final products and to trap the very reactive drugs as they formed as hydrobromides, which otherwise will self-alkylate quickly as free amines. [32] In the salt form they are hygroscopic but stable to storage.

**Physicochemical and Kinetic Data.** The chemical half-lives of the candidate prodrugs and parent drugs were determined by HPLC (See Experimental Section). For the compounds **58b**, **59a**, **59b**, **61b**, and **62b** which proved too labile, a spectrophotometric method was employed. The results are shown in Tables 1 and 2.

Difficulties were encountered in determining the half-lives of the ortho nitrogen mustards. All the ortho nitrogen mustard drugs (**58a**, **59a**, and **60a**) have the correct microanalysis. However $^1$H NMR (in DMSO-$d_6$) and LC-MS (in DMSO buffer) showed rapid cyclization to the corresponding benzopiperidine derivative (see Scheme 5), except for the bis(chloroethyl) derivative **60a**.

The 4-amino aniline mustard prodrugs have shorter half-lives in general than the *N*-methylated and their 2-amino counterparts (see Table 3). The half-lives of the *N*-methylated compounds are consistently 3−4 times longer than the corresponding nonmethylated ones, irrespective of the halogen in the mustard moiety or the linker1/linker2 origin. Similarly the *o*-amino analogues have half-lives 2−3 times longer than *p*-amino prodrugs. However, this was not the case for the correponding drugs. The difference in reactivity between prodrugs and their corresponding drugs varies in the range of 1−20-fold (Table 1).

The kinetics of activation of the self-immolative prodrugs by CPG2 was measured for the bis(chloroethyl) series, and the results are shown in Table 4. This series was chosen in order to minimize the influence of the nitrogen mustard moiety hydrolysis on the measured kinetics.

All prodrugs showed $K_m$'s comparable with those of linker 1 and linker 2.[22] This indicates a good structural fit for the CPG2 active site, even with the ortho derivatives. However, the $k_{cat}$ remains low compared to the directly linked prodrugs and the linkers alone.

**Table 1.** Prodrugs: Half-lives and Cytotoxicity Differentials between StCPG2(Q)3 and $\beta$-gal Expressing WiDr Cells

| compd no. | $T_{1/2}$ prodrug (min) | $T_{1/2}$ prodrug/ $T_{1/2}$ drug | $IC_{50}$ ($\mu$M) LacZ | $IC_{50}$ ($\mu$M) stCPG2(Q)3 | degree of activation (fold) |
|---|---|---|---|---|---|
| CMDA | 59 | 2.3 | 3230 [±120] | 100 [±10] | 32.3 |
| **31a** | 2.7 | - | 149.0 (80.7−275.0) | 3.0 (2.1−4.4) | 49.7 |
| **31b** | 0.97 | 1.9 | 179.8 (71.7−449.4) | 1.8 (1.0−3.2) | 101.6 |
| **32a** | 3.0 | 3.5 | 117.7 (83.8−165.2) | 4.7 (7.8−27.8) | 24.9 |
| **32b** | 0.85 | 0.4 | 204.6 (108.6−384.6) | 3.1 (1.8−5.3) | 66.6 |
| **33a** | 107.5 | 13.9 | 21.1 (12.0−36.9) | 4.5 (2.9−7.1) | 4.7 |
| **34a** | 10.8 | - | 208.2 (86.4−500.7) | 40.0 (22.7−70.3) | 5.2 |
| **35a** | 2.1 | - | 73.3 (45.3−119.8) | 19.0 (11.7−30.9) | 3.9 |
| **35b** | 0.98 | 2.0 | 53.6 (36.9−78.0) | 1.3 (0.8−2.0) | 42.5 |
| **36a** | 3.47 | 3.5 | 183.4 (83.8−400.4) | 95.2 (57.1−158.5) | 1.9 |
| **36b** | 0.9 | 0.4 | 99.7 (19.1−520.6) | 2.6 (1.2−6.0) | 38.3 |
| **37a** | 146.3 | 11.9 | 38.1 (19.1−76) | 28.1 (11.1−43.6) | 1.4 |
| **38a** | 10.0 | - | 148.4 (65.1−338.2) | 28.5 (17.5−47.5) | 5.2 |
| **39b** | 3.9 | 6.5 | 184.7 (105.4−324.4) | 1.5 (0.8−2.6) | 124.0 |
| **40b** | 2.8 | 1.0 | 332.4 (222.6−496.3) | 4.1 (2.5−6.7) | 81.4 |
| **41b** | 173.6 | 20.9 | 39.7 (27.0−58.4) | 2.5 (1.6−3.9) | 15.9 |
| **42b** | 4.6 | 7.7 | 21.5 (16.2−28.4) | 2.6 (1.7−4.1) | 8.3 |
| **43b** | 2.7 | 1.0 | 27.2 (14.1−52.5) | 3.6 (2.2−6.0) | 7.5 |
| **44b** | 173.6 | 20.6 | 19.6 (15.0−25.7) | 1.7 (1.1−2.5) | 11.5 |

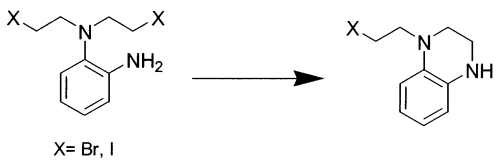
[a] The round bracketed values indicate the 95% confidence intervals. For CMDA, the square bracketed values represent the standard errors of the mean as published in ref 17.

**Table 2.** Half-Lives and Cytotoxicities of Drugs in WiDr Cell Line Expressing Either $\beta$-gal (control) or StCPG2(Q)3[a]

| compd. no | $T_{1/2}$ (min) | $IC_{50}$ ($\mu$M) LacZ | $IC_{50}$ ($\mu$M) stCPG2(Q)3 |
|---|---|---|---|
| **58b** | 0.5 | 10.2 (4.8−18.3) | 8.6 (4.2−14.0) |
| **59a** | 1.0 | 23.4 (12.4−44.1) | 18.6 (10.7−32.2) |
| **59b** | 2.3 | 9.4 (5.7−18.5) | 7.7 (5.0−14.8) |
| **60a** | 12.3 | 3.5 (2.1−5.7) | 2.4 (1.3−4.4) |
| **61b** | 0.6 | 7.7 (4.1−14.5) | 8.4 (4.6−15.5) |
| **62b** | 2.7 | 7.2 (3.6−14.4) | 7.4 (3.5−15.5) |
| **63b** | 8.3 | 9.2 (5.7−15.1) | 8.2 (5.3−12.5) |

[a] The bracketed values indicate the 95% confidence intervals.

**Scheme 5**




**Cytotoxicity Assays.** The prodrugs and some of the corresponding drugs were tested for cytotoxicity in WiDr cells engineered for stable expression of (stCPG2(Q)3) or, as a control, the non-prodrug activating enzyme $\beta$-galactosidase ($\beta$-gal). For comparative purposes with the directly linked prodrugs, the WiDr cell line was treated with the *N*-{4-[(2-chloroethyl)(2-mesyloxyethyl)-amino]benzoyl}-L-glutamic acid prodrug (CMDA), which has undergone clinical trials in ADEPT.[20,21] The results are summarized in Table 1. The degree of activation defined as the ratio of the $IC_{50}$ value of the prodrug in the $\beta$-gal expressing cell line to the $IC_{50}$ value of the prodrug in CPG2 expressing cell line was calculated for the new compounds and for CMDA. The degree of activation ranged between 1.4 and 124-fold (CMDA: 32-fold[19]). Seven of the new prodrugs (**31a**, **31b**, **32b**, **35b**, **36b**, **39b**, and **40b**) give higher differentials than CMDA.

Despite possessing a smaller $k_{cat}$ compared to CMDA, the bis(iodoethyl) and bis(bromoethyl) prodrugs proved to be highly active against the CPG2-expressing WiDr cell line. This could be due to the higher potency of the released drugs ($IC_{50}$ in the range of 0.5−2.3 $\mu$M) compared to the drug derived from CMDA (120 $\mu$M).[19]

**Table 3.** Comparison of Half-Lives between 4-Amino, 4-Methylamino, and 2-Amino Aniline Mustard Prodrugs

| | 4-NH/4-NMe | | 4-NH/2-NH | |
|---|---|---|---|---|
| mustard type | compounds compared | $T_{1/2}$ ratio | compounds compared | $T_{1/2}$ ratio |
| Linker-1 Prodrugs (Z = NH, see Scheme 3) | | | | |
| bis-chloro | *a*/**41b** | 0.28 | *a*/**33a** | 0.45 |
| bis-bromo | **32b**/**40b** | 0.30 | **32b**/**32a** | 0.28 |
| bis-iodo | **31b**/**39b** | 0.25 | **31b**/**31a** | 0.36 |
| Linker-2 Prodrugs (Z = O, see Scheme 3) | | | | |
| bis-chloro | *b*/**44b** | 0.30 | *b*/**37a** | 0.35 |
| bis-bromo | **36b**/**43b** | 0.33 | **36b**/**36a** | 0.26 |
| bis-iodo | **35b**/**42b** | 0.21 | **35b**/**35a** | 0.47 |

[a] 4-{[4′-(*N*,*N*-Bis(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid.[20] [b] 4-{[4′-(*N*,*N*-Bis(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenoxycarbonyl-L-glutamic acid.[20]

**Table 4.** Kinetics of the Linkers and the Bis(2-chloroethyl) Prodrugs

| compd no. | $K_m$ ($\mu$M) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ |
|---|---|---|---|
| L1-OH[(a)] | 3.1 | 65.4 | 21.1 |
| L2-OH[(a)] | 1.7 | 140 | 82.3 |
| L1−4-NHPhN$(CH_2CH_2Cl)_2$[(b)] | <5 | 10−50 | - |
| L2−4-NHPhN$(CH_2CH_2Cl)_2$[(c)] | <5 | <10 | - |
| **33a** | 0.55 | 2.92 | 5.31 |
| **37a** | 1.03 | 4.27 | 4.14 |
| **41b** | 6.05 | 5.50 | 0.91 |
| **44b** | 2.43 | 4.32 | 1.78 |

[a] L1-OH, L2-OH: for structure see Scheme 2.[20] [b] L1−4-NHPhN-$(CH_2CH_2Cl)_2$ = 4-{[4′-(*N*,*N*-bis(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid.[20] [c] L2−4-NHPhN$(CH_2CH_2Cl)_2$ = 4-{[4′-(*N*,*N*-bis(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenoxycarbonyl-L-glutamic acid.[20]

## Discussion

The self-immolative prodrugs[24] (pro-prodrugs) have a number of advantages. The possibility of modifying the activation kinetics by using suitable linkers and the broad range of anticancer agents that can be converted to prodrugs is emphasized.[33−35]

Our strategy to improve the biological efficacy of the self-immolative prodrugs has three approaches:

1. Using more effective leaving groups than chlorine in the nitrogen mustard moiety, such as iodine or bromine.[13]

| Prodrug | Drug | t1/2 drug | Log differential |
|---|---|---|---|
| **39b** | **61b** | 0.6 | 2.09 |
| **31b** | **58b** | 0.5 | 2.01 |
| **40b** | **62b** | 2.7 | 1.91 |
| **32b** | **39b** | 2.3 | 1.82 |
| **41b** | **63b** | 8.3 | 1.2 |

| | | |
|---|---|---|
| Observations | n | 5 |
| Intercept y | | 2.121206 |
| Intercept x | | -0.109446 |
| Correlation coefficient | r | 0.985818 |
| R square | $r^2$ | 0.971837 |
| Standard error | s | 0.068548 |
| F statistic | F | 103.5212 |

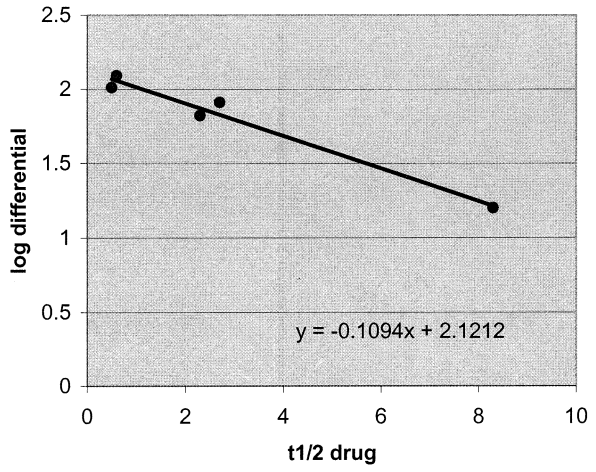



**Figure 1.**

2. Employing ortho nitrogen mustards in order to improve the kinetics by a different positioning of the self-immolative prodrug in the active site of the enzyme.

3. Synthesizing *N*-methylated self-immolative prodrugs in order to improve the stability of the carbamate linkage between the linker and the drug moiety and to increase the basicity and therefore the chemical reactivity of the released mustard.

The use of I and Br instead of Cl as leaving groups in the nitrogen mustards led to drugs with shorter half-lives and increased potency ($IC_{50}$ = 0.5−2.7 $\mu$M). The corresponding prodrugs also exhibited shorter half-lives, but for many of the prodrugs the differentials in the WiDr cell lines are better than those of CMDA (**31a**, **31b**, **32b**, **35b**, **36b**, **39b**, and **40b**). The two most effective prodrugs in terms of differential are the iodo derivatives **31b** and **39b**.

The prodrugs belonging to the ortho series are less effective than their para counterparts. However, even in the ortho series, the I and Br nitrogen mustard prodrugs are the most active and significant differentials were obtained in the transfected WiDr cell line (50 and 25-fold for **31a** and **32a**, respectively). The ortho prodrugs had lower $K_m$'s with respect to the linkers and the para series, which is of potential benefit in in vivo situations.

The *N*-methylation has a beneficial effect on their biological activity despite having no benefit on the kinetics. The prodrugs show lower chemical reactivity than the nonmethylated counterparts, presumably due to increased stability of the secondary carbamate. This effect is minimal at the drug level.

As a general observation, prodrugs incorporating linker 1 are more effective than those containing linker 2. The five most effective prodrugs in terms of differential are all linker 1 based (Table 1).

The differential obtained in tumor cells transfected with CPG2 seems to be dependent on an optimal chemical reactivity of the prodrugs and the corresponding drugs. This is in good agreement with previous observation on the in vitro and in vivo behavior of the direct prodrugs in CPG2-based GDEPT systems. [36] The increased lipophilicity of these prodrugs and drugs could also be important for their improved biological activity.

QSAR was attempted on the synthesized self-immolative prodrugs, using the available in vitro data on WiDr. One important parameter determining the antitumor activity of the prodrugs is the kinetics of activation. However, despite significant differences between ortho and para nitrogen mustard prodrugs in terms of activity, the kinetics of activation is very similar for both classes (see Table 3). Therefore, other parameters should be considered to rationalize the differences in activity between the synthesized prodrugs.

Another parameter important in explaining the range of differentials between CPG2-expressing and nonexpressing cells is assumed to be the chemical reactivity of the released active drugs. For nitrogen mustards, this is known to parallel their half-lives.[37,38] An equation linking the cytotoxicity differentials of the prodrugs **31b**, **32b**, **39b**, **40b**, **41b** with the half-lives of the corresponding released drugs **58b**, **59b**, **61b**, **62b**, **63b** has been computed, which supports our hypothesis (Figure 1),

$$\log \Delta = 2.1212 - 0.1094 T_{1/2}$$

$$n = 5,\ r = 0.986,\ r^2 = 0.970,\ s = 0.068,\ F = 103.5$$

where $\Delta$ = cytotoxicity differential and $T_{1/2}$ = half-life of drug.

No other significant QSAR could be determined for this set of compounds. This equation also point out to the limitations for this type of prodrug, since bis-iodo derivatives are the most reactive nitrogen mustard synthesized.

## Conclusions

Nineteen new self-immolative prodrugs and their corresponding drugs derived from aniline nitrogen mustard have been synthesized. The new prodrugs behave as substrates for CPG2 and yielded significant $IC_{50}$ differential between stCPG2(Q)3 expressing and control $\beta$-gal-expressing WiDr cells. The differential ranged between 1.4 and 124-fold. Seven prodrugs (**31a**, **31b**, **32b**, **35b**, **36b**, **39b**, and **40b**) were more active than CMDA. The released drugs were also 5.4−41.7-fold more potent than the 4-[(2-mesyloxyethyl)(2-chloroethyl)amino]benzoic acid drug released from CMDA. This is the first example of self-immolative prodrugs cleavable by CPG2 that show better differentials than CMDA in a GDEPT system.

## Experimental Section

All starting materials, reagents, and anhydrous solvents (i.e., THF packed under $N_2$) were purchased from Aldrich, unless otherwise stated. Kieselgel 60 (0.043−0.060) was used

in gravity columns (Art 9385 and 15111, Merck). TLC was performed on precoated sheets of Kieselgel 60 $F_{254}$ (Art 5735, Merck). Melting points were determinated on a Kofler hot-stage (Reichert Thermovar) melting point apparatus and are uncorrected. Low resolution EI and FAB spectra were performed on a VG-2AB−SE double focusing magnetic sector mass spectrometer (Fisons Instruments, Warrington, Manchester, UK), operating at a resolution of 1000. High-resolution accurate mass spectra were determined on the same system, but with a resolution set to 8000−10000. Masses are measured by peak matching the unknown with a mass of known composition. Reported spectra are by FAB unless otherwise stated. NMR spectra were determined in $Me_2SO$-$d_6$ on a Brucker AC250 spectrometer (250 MHz) at 30 °C (303 K) unless otherwise stated. IR spectra (film) were recorded on a Perkin-Elmer 1720X FT-IR spectrometer. Elemental analysis were determined by Butterworth Laboratories Ltd. (Teddington, Middlesex, UK) and are within 0.4% of theory except when stated. The chemical stability of the prodrugs and their propensity to behave as substrates for CPG2 were determined by HPLC.

**4-Nitro-bis[2′-(*tert*-butyldimethylsilyloxy)ethyl]aniline (1b).** 4-Nitro-[bis(2′-hydroxyethyl)]aniline (5.0 g, 22.1 mmol) and *tert*-butyldimethylsilyl chloride (7.5 g, 50 mmol) were dissolved in 20 mL of DMF; imidazole (4.76 g, 70 mmol) was added, and the solution was stirred at room temperature for 20 h. The solution was then concentrated and purified by column chromatography (cyclohexane:AcOEt 1:1) to afford **1a** (8.9 g, 89%) as a yellow oil. $^1$H NMR $\delta_H$ (ppm): −0.03 (s, 12H, $SiCH_3$), 0.81 (s, 18H, Si-*t*-Bu), 3.64 (t, 4H, $NCH_2$, $J$ = 5.19 Hz), 3.78 (t, 4H, $CH_2OSi$), 6.83 (d, 2H, $H_{arom2+6}$, $J$ = 9.37 Hz), 8.00 (d, 2H, $H_{arom3+5}$); MS *m*/*z*: 455 ($M^+$ + 1, 88), 477 ($M^+$ + 23, 5), 439 ($M^+$ − Me, 53), 397 ($M^+$ − *t*-Bu, 30); acc. mass: ($C_{22}H_{43}N_2O_4Si_2$) calcd 455.2761, found 455.2767. Anal. ($C_{22}H_{43}N_2O_4Si_2$) C, H, N.

The same procedure affords: **2-Nitro-bis[2′-(*tert*-butyldimethylsilyloxy)ethyl]aniline (1a)**, yellow oil (95.1%). Purified by column chromatography (eluent: cyclohexane:AcOEt 3:1); $^1$H NMR $\delta_H$ (ppm): −0.03 (s, 12H, $SiCH_3$), 0.80 (s, 18H, Si-*t*-Bu), 3.27 (t, 4H, $NCH_2$, $J$ = 5.87 Hz), 3.65 (t, 4H, $CH_2$-OSi), 6.98 (dt, 1H, $H_5$, $J$ = 7.58 Hz), 7.37 (dd,1H, $H_3$, $J$ = 8.43 Hz), 7.48 (dt,1H, $H_4$, $J$ = 7.80 Hz), 7.67 (dd, 1H, $H_6$, $J$ = 8.05 Hz); MS *m*/*z*: 455 ($M^+$ + 1, 32), 397 ($M^+$ − *t*-Bu, 8), 309 ($M^+$ − SiTBDM, 100); acc. mass: ($C_{22}H_{43}N_2O_4Si_2$) calcd 455.2761, found 455.2745. Anal. ($C_{22}H_{43}N_2O_4Si_2$) C, H; N required 6.17, found 6.94%.

**4-Amino-bis[2′-(*tert*-butyldimethylsilyloxy)ethyl]aniline (2b).** 5.50 g (12.1 mmol) of **1b** was dissolved in 90 mL of THF, 1.5 g Pd/C 10% was added and the suspension was stirred under $H_2$ atmosphere for 6 h. The catalyst was then filtered off, the solvent evaporated and the residue submitted to purification by column chromatography (cyclohexane:AcOEt 3:1). **2b** (4.90 g, 95%) resulted as an oil. $^1$H NMR $\delta_H$ (ppm): 0.00 (s, 12H, $SiCH_3$), 0.85 (s, 18H, Si-*t*-Bu), 3.30 (t, 4H, $NCH_2$, $J$ = 6.20 Hz), 3.63 (t, 4H, $CH_2OSi$), 4.34 (s, 2H, $NH_2$), 6.46 (s, 4H, $H_{arom}$); MS *m*/*z*: 424 ($M^+$, 70); acc. mass: ($C_{22}H_{44}N_2O_2Si_2$) calcd 424.2941, found 424.2950. Anal. ($C_{22}H_{44}N_2O_2Si_2$): H, N; C required 62.21, found 62.63%.

**2-Amino-bis[2′-(*tert*-butyldimethylsilyloxy)ethyl]aniline (2a)** was synthesized in a similar way. An oil was obtained (4.60 g, 99%). $^1$H NMR, $\delta_H$ (ppm): 0.04 (s, 12H, $SiCH_3$), 0.85 (s, 18H, Si-*t*-Bu), 3.02 (t, 4H, $NCH_2$, $J$ = 6.19 Hz), 3.56 (t, 4H, $CH_2OSi$), 6.49 (dt, 1H, $H_5$, $J$ = 7.51 Hz), 6.63 (dd, 1H, $H_3$, $J$ = 7.92 Hz), 6.78 (dt, 1H, $H_4$, $J$ = 7.54 Hz), 7.00 (dd, 1H, $H_6$, $J$ = 7.81 Hz); MS *m*/*z*: 425 ($M^+$ + 1, 28), 367 ($M^+$ − *t*-Bu + 1, 15); acc. mass: ($C_{22}H_{45}N_2O_2Si_2$) calcd 425.3020, found 425.3034.

**4-(*N*′-Benzyloxycarbonylamino)-*N*,*N*-bis[(2′-(*tert*-butyldimethylsilyl-oxy)ethyl]aniline (3b).** 4-Amino-*N*,*N*-bis-[(2′-(*tert*-butyldimethylsilyl-oxy)ethyl]-aniline **2b** (3.74 g, 8.8 mmol) was dissolved in THF (100 mL), and *N*-(benzyloxycarbonyloxy)succinimide (2.25 g, 9.0 mmol) was added. The solution was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was purified by column chromatography (cyclohexane:ethyl acetate 1:1) to afford **3b** (4.65 g, 95%) as an oil. $^1$H NMR $\delta_H$: −0.01 (s, 12H, $SiCH_3$), 0.84 (s, 18H, Si-*t*-Bu), 3.41 (t, 4H, $NCH_2$, $J$ = 5.86 Hz), 3.67 (t, 4H, $CH_2OSi$), 5.09 (s, 2H, Ph*CH*$_2$), 6.58 (d, 2H, $H_{arom3+5}$, $J$ = 8.89 Hz), 6.82 (d, 2H, $H_{arom2+6}$), 7.28−7.40 (m, 5H, $H_{arom\ benzyl}$), 9.28 (s, 1H, NH). MS *m*/*z*: 558 ($M^+$, 35), 423 ($M^+$ − $PhCH_2$ − $CO_2$, 25); acc. mass: ($C_{30}H_{50}N_2O_4Si_2$) calcd 558.3309, found 558.3330. Anal. ($C_{30}H_{50}N_2O_4Si_2$) C, H; N required 5.01, found 4.60%

**4-(*N*′-Benzyloxycarbonyl-*N*′-methylamino)-*N*,*N*-bis[(2′-(*tert*-butyldimethylsilyloxy)ethyl]aniline (4b). 3b** (5.2 g, 9.3 mmol) was dissolved in dry THF (60 mL), and NaH (60% in mineral oil, 0.6 g, 15 mmol) was added. After 40 min stirring at room temperature under argon, methyl iodide (586 $\mu$L, 9.3 mmol) was added and the stirring continued for 12 h. The solvent was evaporated and the residue redissolved in ethyl acetate (100 mL) and extracted with distilled water (100 mL). The organic layer was dried and evaporated to afford **4b** (5.34 g, 100%) as an oil. $^1$H NMR $\delta_H$: 0.00 (s, 12H, $SiCH_3$), 0.88 (s, 18H, Si-*t*-Bu), 3.21 (s, 3H, $NCH_3$), 3.45 (t, 4H, $NCH_2$, $J$ = 6.47 Hz), 3.71 (t, 4H, $CH_2OSi$), 5.10 (s, 2H, Ph*CH*$_2$), 6.60 (d, 2H, $H_{arom3+5}$, $J$ = 9.08 Hz), 6.99 (d, 2H, $H_{arom2+6}$, $J$ = 7.25 Hz), 7.20−7.35 (m, 5H, $H_{arom\ benzyl}$). MS *m*/*z*: 572 ($M^+$, 50), 595 ($M^+$ + Na, 7), 437 ($M^+$ − $PhCH_2$ − $CO_2$, 45); acc. mass: ($C_{31}H_{52}N_2O_4Si_2$) calcd 572.3466, found 572.3485.

**4-Methylamino-*N*,*N*-bis[(2′-(*tert*-butyldimethylsilyloxy)-ethyl]aniline (5b). 4b** (2.9 g, 5.06 mmol) was dissolved in ethyl acetate (120 mL), and Pd/C 10% catalyst (1.6 g) was added. The suspension was stirred under $H_2$ atmosphere for 3 h. The catalyst was filtered off, and the filtrate was evaporated to afford **5b** (2.23 g, 100%) as an oil. $^1$H NMR $\delta_H$: −0.01 (s, 12H, $SiCH_3$), 0.84 (s, 18H, Si-*t*-Bu), 2.58 (s, 3H, $NCH_3$), 3.22−3.32 (t, 4H, $NCH_2$), 3.63 (t, 4H, $CH_2OSi$, $J$ = 6.19 Hz), 4.87 (s, 1H, NH), 5.10 (s, 2H, Ph*CH*$_2$), 6.43 (d, 2H, $H_{arom3+5}$, $J$ = 8.92 Hz), 6.65 (d, 2H, $H_{arom2+6}$), 7.20−7.35 (m, 5H, $H_{arom\ benzyl}$). MS *m*/*z*: 438 ($M^+$, 100), 451 ($M^+$ + Na, 25); acc. mass: ($C_{23}H_{46}N_2O_2Si_2$) calcd 438.3098, found 438.311500. Anal. ($C_{23}H_{46}N_2O_2Si_2$) C, H; N required 6.38, found 5.88.

**Diallyl 4-{[4′-Bis(2″-*tert*-butyldimethylsilyloxyethyl) aminophenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamate 6b (Z = NH).** A 2.0 g (4.7 mmol) amount of **2b** was dissolved in 30 mL of DCM, triethylamine (1.24 mL, 9.5 mmol) was added and then triphosgene (0.48 g, 1.57 mmol) in one portion with vigorous stirring. After 15 min, the solvent was evaporated and the residue taken up in THF. The undissolved solid was filtered off, and the filtrate was evaporated to dryness. The corresponding isocyanate was obtained as an oil which was redissolved in 20 mL of $CH_2Cl_2$ and mixed with a solution of diallyl *N*-[4-(hydroxymethyl)phenylcarbamoyl]-L-glutamate (linker1, L1A) (1.76 g, 4.7 mmol) in 30 mL of $CH_2Cl_2$.[22] Dibutyltin dilaurate (120 $\mu$L) was added and the stirring continued for 20 h at room temperature. After the evaporation of the solvent the residue was purified by preparative HPLC (cyclohexane:AcOEt, 3:1) to yield **6b** (3.10 g, 80%) as a foamy solid, mp 50−53 °C. $^1$H NMR, $\delta_H$, (ppm): −0.01 (s, 12H, $SiCH_3$), 0.84 (s, 18H, Si-*t*-Bu), 1.80−2.15 (2m, 2H, *CH*$_2$-CH(NH)), 2.45 (t, 2H, $CH_2CO_2$, $J$ = 7.29 Hz), 3.42 (t, 4H, $NCH_2$, $J$ = 5.31 Hz), 3.68 (t, 4H, $CH_2OSi$), 4.25−4.35 (m, 1H, *CH*(NH)-$CH_2$), 4.55 (d, 2H, $CH_2O$ allyl, $J$ = 5.39 Hz), 4.60 (d, 2H, $CH_2O$ allyl, $J$ = 5.28 Hz), 4.99 (s, 2H, $PhCH_2$), 5.15−5.37 (m, 4H, $CH_2$= allyl), 5.80−6.05 (m, 2H, CH= allyl), 6.58 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.99 Hz), 6.66 (d, 1H, NH-G, $J$ = 7.99 Hz), 7.19 (d, 2H, $H_{arom2'+6'}$), 7.27 (d, 2H, $H_{arom2+6}$, $J$ = 8.46 Hz), 7.39 (d, 2H, $H_{arom3+5}$), 8.69 (s, 1H, PhNH), 9.26 (s, 1H, Ph′NH); MS *m*/*z*: 827 ($M^+$ + 1, 5), 849 ($M^+$ + Na, 55); acc. mass: ($C_{42}H_{67}N_4O_9Si_2$) calcd 827.4447, found 827.4440. Anal. ($C_{42}H_{66}$-$N_4O_9Si_2$): C, H, N.

Using a similar procedure the following compounds were obtained: **diallyl 4-{[2′-bis(2″-*tert*-butyldimethylsilyloxyethyl)aminophenyl]carbamoyloxybenzyl}carbamoyl-L-glutamate 6a (Z = NH)** was obtained as an oil (1.99 g; 65.6%) after purification by preparative HPLC (cyclohexane:AcOEt 7:1). $^1$H NMR, $\delta_H$, (ppm): −0.06 (s, 12H, $SiCH_3$), 0.79 (s, 18H, Si-*t*-Bu), 1.90−2.20 (2m, 2H, *CH*$_2$CH(NH)), 3.00 (t, 4H, $NCH_2$,

$J$ = 5.66 Hz), 3.49 (t, 4H, $CH_2OSi$), 4.29−4.32 (m, 1H, $CH_2CH(NH)$), 4.57 (dd, 4H, $CH_2O$-allyl, $J_1$=10.08 Hz, $J_2$=5.26 Hz), 5.03 (s, 2H, $PhCH_2$), 5.16−5.36 (m, 4H, $CH_2$= allyl), 6.60 (d, 1H, NH-G, $J$ = 8.02 Hz), 7.01 (dt, 1H, $H_{arom4(5)'}$, $J_o$ = 7.66 Hz, $J_m$ = 1.34 Hz), 7.12 (dd, 1H, $H_{arom5(4)'}$), 7.25 (d, 2H, $H_{arom3+5}$, $J$ = 8.53 Hz), 7.37 (d, 2H, $H_{arom2+6}$), 7.95 (dd, 1H, $H_{arom6'}$, $J_o$ = 8.53), 8.43 (s, 1H, PhNH, $J$ = 7.87 Hz), 8.64 (s, 1H, Ph′NH); MS *m*/*z*: 827 ($M^+$ + 1, 5), 849 ($M^+$ + 23, 55); acc. mass: ($C_{42}H_{66}N_4O_9Si_2Na$) calcd: 849.4266, found 849.4240. Anal. ($C_{44}H_{73}N_3O_{10}Si_2$), C, H, N.

**Di-*tert*-butyl 4-{[2′-bis(2″-(*tert*-butyldimethylsilyloxyethyl)-aminophenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate 7a (Z = O)** was obtained from L2B as an oil (0.97 g; 47.5%) after purification by preparative HPLC (cyclohexane: AcOEt 7:1). $^1$H NMR, $\delta_H$, (ppm): −0.06 (s, 12H, $SiCH_3$), 0.79 (s, 18H, Si-*t*-Bu), 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 2.31−2.37 (m, 2H, $CH_2CO_2$), 3.01 (t, 4H, $NCH_2$, $J$ = 5.70 Hz), 3.50 (t, 4H, $CH_2OSi$), 5.12 (s, 2H, $PhCH_2$), 7.01−7.12(m, 4H, $H_{arom3+5,\ 3',\ 5'}$), 7.30 (dd, 1H, $H_{arom4'}$, $J_o$ = 7.15 Hz, $J_m$ = 1.24 Hz), 7.39 (d, 2H, $H_{arom3+5}$, $J$ = 7.39 Hz),), 7.79 (dd, 1H, $H_{arom6'}$, $J_o$ = 8.16 Hz, $J_m$ = 1.18 Hz), 8.07 (d, 1H, NH-G, $J$ = 7.87 Hz), 8.49 (s, 1H, Ph′NH); MS *m*/*z*: 860 ($M^+$ + 1, 37), 882 ($M^+$ + 23, 12); acc. mass: ($C_{44}H_{74}N_3O_{10}Si_2$) calcd: 860.4913, found 860.4900. Anal. ($C_{44}H_{73}N_3O_{10}Si_2$) C, H, N.

**Di-*tert*-butyl 4-{[4′-bis(2″-*tert*-butyldimethylsilyloxyethyl)-aminophenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate 7b (Z = O)** was obtained from L2B as an oil (3.73 g, 66.7%). $^1$H NMR, $\delta_H$, (ppm): 0.00 (s, 12H, $SiCH_3$), 0.85 (s, 18H, Si-*t*-Bu), 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.70−2.00 (2m, 2H, $CH_2CH(NH)$), 2.34 (t, 2H, $CH_2CO_2$, $J$ = 7.81 Hz), 3.42 (t, 4H, $NCH_2$, $J$ = 5.03 Hz), 3.69 (t, 4H, $CH_2$-OSi), 3.95−4.05 (m, 1H, $CH(NH)CH_2$), 5.08 (s, 2H, $PhCH_2$), 6.59 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.86 Hz), 7.10 (d, 2H, $H_{arom2+6}$, $J$ = 8.44 Hz), 7.19 (d, 2H, $H_{arom2'+6'}$), 7.41 (d, 2H, $H_{arom3+5}$), 8.09 (d, 1H, NH-G, $J$ = 7.75 Hz), 9.24 (s, 1H, Ph′NH); MS *m*/*z*: 860 ($M^+$ + 1, 37); acc. mass: ($C_{44}H_{74}N_3O_{10}Si_2$) calcd: 860.4913, found 860.4900. Anal. ($C_{44}H_{73}N_3O_{10}Si_2$): C, H, N.

**Diallyl 4-{*N*-[4′-Bis(2″-*tert*-butyldimethylsilyloxyethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyl-carbamoyl-L-glutamate 8b.** Activated L1A, diallyl *N*-(4-{[4-nitrophenoxycarbonyloxy]methyl}phenylcarbamoyl)-L-glutamate (2.1 g, 3.9 mmol), prepared as described previously,[22] and **5b** (2.2 g, 5 mmol) were dissolved in DMA (50 mL) and stirred for 5 days at room temperature. The solvent was evaporated and the residue purified by column chromatography ($CH_2Cl_2$:AcOEt 9:1) to yield **8b** (0.92 g, 28%) as an oil. $^1$H NMR, $\delta_H$, (ppm): 0.01 (s, 12H, $SiCH_3$), 0.83 (s, 18H, Si-*t*-Bu), 1.80−2.10 (2m, 2H, $CH_2CH(NH)$), 2.44 (t, 2H, $CH_2$-$CO_2$, $J$ = 8.25 Hz), 3.11 (s, 3H, $NCH_3$), 3.46 (t, 4H, $NCH_2$, $J$ = 5.58 Hz), 3.69 (t, 4H, $CH_2OSi$), 4.25−4.35 (m, 1H, $CH(NH)$-$CH_2$), 4.53 (d, 2H, $CH_2O$ allyl, $J$ = 5.45 Hz), 4.59 (d, 2H, $CH_2O$ allyl, $J$ = 5.38 Hz), 4.94 (s, 2H, $PhCH_2$), 5.14−5.38 (m, 4H, $CH_2$= allyl), 5.80−6.00 (m, 2H, CH= allyl), 6.60 (d, 3H, $H_{arom3'+5'}$+ NH-G, $J$ = 8.70 Hz), 6.99 (d, 2H, $H_{arom2'+6'}$), 7.15 (d, 2H, $H_{arom2+6}$), 7.32 (d, 2H, $H_{arom3+5}$, $J$ = 8.20 Hz), 8.60 (s, 1H, PhNH); MS *m*/*z*: 841 ($M^+$ + 1, 5), 864 ($M^+$ + Na, 3), 437 ($M^+$ − L1A$CH_2OCO$, 100); acc. mass: ($C_{43}H_{69}N_4O_9Si_2$) calcd 841.4603, found 841.4630. Anal. ($C_{43}H_{68}N_4O_9Si_2$): C, H; N required 6.66, found 6.22.

Using a similar procedure, **di-*tert*-butyl 4-{*N*-[4′-bis(2″-*tert*-butyldimethylsilyloxyethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenoxycarbonyl-L-glutamate 9b** was obtained from activated L2B, di-*tert*-butyl *N*-(4-{[4-nitrophenoxycarbonyloxy]methyl}phenoxycarbonyl)-L-glutamate as an oil (3.8 g, 92.5%). $^1$H NMR, $\delta_H$, (ppm): 0.00 (s, 12H, $SiCH_3$), 0.85 (s, 18H, Si-*t*-Bu), 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.05 (2m, 2H, $CH_2CH(NH)$), 2.33 (t, 2H, $CH_2CO_2$), 3.14 (s, 3H, $NCH_3$), 3.46 (t, 4H, $NCH_2$, $J$ = 5.03 Hz), 3.69 (t, 4H, $CH_2OSi$), 3.90−4.05 (m, 1H, $CH(NH)$-$CH_2$), 5.03 (s, 2H, $PhCH_2$), 6.62 (d, 2H, $H_{arom3'+5'}$, $J$ = 9.03 Hz), 7.03 (d, 4H, $H_{arom2'+6'}$ + $H_{arom3+5}$, $J$ = 8.90 Hz), 7.27 (d, 2H, $H_{arom2+6}$), 8.09 (d, 1H, NH-G, $J$ = 7.83); MS *m*/*z*: 875 ($M^+$ + 1, 38), 897 ($M^+$ + Na, 12), 819 ($M^+$ − t-Bu, 3); acc. mass: ($C_{45}H_{76}N_3O_{10}Si_2$) calcd: 874.5040, found 874.5069.

**4-Bis(2′-*tert*-butyldimethylsilyloxyethyl)amino-*N*-*tert*-butyloxycarbonylaniline (45b).** A 1.0 g (2.35 mmol) amount of **2b** was dissolved in THF (30 mL), and *tert*-butyl pyrocarbonate (565 mg, 2.6 mmol) was added. The solution was stirred at room temperature for 16 h. The solvent was evaporated, and the residue was purified by preparative HPLC (cyclohexane:AcOEt 6:1) to afford **45b** (1.23 g, 100%) as an oil. $^1$H NMR, $\delta_H$, (ppm): 0.00 (s, 12H, $SiCH_3$), 0.85 (s, 18H, Si-*t*-Bu), 1.47 (s, 9H, O-*t*-Bu), 3.41 (t, 4H, $NCH_2$, $J$ = 5.93 Hz), 3.69 (t, 4H, $CH_2OSi$), 6.57 (d, 2H, $H_{arom3+5}$, $J$ = 8.87 Hz), 7.18 (d, 2H, $H_{arom2+6}$), 8.80 (d, 1H, NH); MS *m*/*z*: 524 ($M^+$, 23), 469 ($M^+$ − t-Bu, 15), 423 ($M^+$ − *t*-Bu − $CO_2$, 18); acc. mass: ($C_{27}H_{52}N_2O_4Si_2$) calcd: 524.3466, found 524.3480. Anal. ($C_{27}H_{52}N_2O_4Si_2$): C, H, N.

**2-Bis(2′-*tert*-butyldimethylsilyloxyethyl)amino-*N*-*tert*-butyloxycarbonylaniline (45a)** was obtained by the same method as an oil (4.27 g, 91.4%) after purification by preparative HPLC (cyclohexane:AcOEt 4:1). $^1$H NMR, $\delta_H$, (ppm): −0.13 (s, 12H, $SiCH_3$), 0.83 (s, 18H, Si-*t*-Bu), 1.46 (s, 9H, $CO_2$-*t*-Bu), 3.01 (t, 4H, $NCH_2$, $J$ = 5.56 Hz), 3.49 (t, 4H, $CH_2OSi$), 6.99 (dt, 1H, $H_{arom4(5)}$, $J_o$ = 7.57, $J_m$ = 1.20 Hz), 7.11 (dd, 1H, $H_{arom5(4)}$, $J_o$ = 7.44 Hz), 7.29 (dd, 1H, $H_{arom3}$, $J_o$ = 8.66, $J_m$ = 1.23 Hz), 7.94 (d, 1H, $H_{arom6}$, $J_o$ = 8.17 Hz), 8.13 (s, 1H, PhNH); MS *m*/*z*: 525 ($M^+$ + 1, 15), 469 ($M^+$ − *t*-Bu + 1, 15); acc. mass: ($C_{27}H_{53}N_2O_4Si_2$) calcd: 525.3544, found 525.3560. Anal. ($C_{27}H_{52}N_2O_4Si_2$) H, N; C required 61.78, found 62.19.

**Diallyl 4-{[4′-(*N*,*N*-Bis(2″-hydroxyethyl)amino)phenyl]-carbamoyloxymethyl}phenylcarbamoyl-L-glutamate 10b (Z = NH).** A 2.0 g (2.42 mmol) amount of **6b** (Z = NH) was dissolved in 60 mL of THF, 6.0 mL of triethylamine trihydrofluoride was added, and the solution was stirred at room temperature for 7 h. The solvent was evaporated, and the residue was diluted with AcOEt, extracted with $H_2O$ (200 mL), saturated aqueous $NaHCO_3$ (200 mL), and again with $H_2O$ (200 mL), dried ($MgSO_4$), and evaporated to yield **10b** (1.4 g, 97%) as a foamy solid, mp 79−81 °C. $^1$H NMR, $\delta_H$, (ppm): 1.85−2.10 (2m, 2H, $CH_2CH(NH)$), 2.46 (t, 2H, $CH_2CO_2$, $J$ = 8.14 Hz), 3.30−3.40 (m, 4H, $NCH_2$, under $H_2O$), 3.45−3.55 (m, 4H, $CH_2OH$), 4.25−4.35 (m, 1H, $CH(NH)CH_2$), 4.55 (d, 2H, $CH_2O$ allyl, $J$ = 5.37 Hz), 4.60 (d, 2H, $CH_2O$ allyl, $J$ = 5.26 Hz), 4.72 (t, 2H, OH, $J$ = 5.21 Hz), 4.99 (s, 2H, $PhCH_2$), 5.15−5.40 (m, 4H, $CH_2$= allyl), 5.80−6.00 (m, 2H, CH= allyl), 6.59 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.76 Hz), 6.64 (d, 1H, NH-G, $J$ = 8.22 Hz), 7.19 (d, 2H, $H_{arom2'+6'}$), 7.27 (d, 4H, $H_{arom2+6}$, $J$ = 8.56 Hz), 7.38 (d, 2H, $H_{arom3+5}$), 8.67 (s, 1H, PhNH), 9.25 (s, 1H, Ph′NH); MS, *m*/*z*: 599 ($M^+$ + 1, 35), 621 ($M^+$ + Na, 7); acc. mass: ($C_{30}H_{39}N_4O_9$) calcd 599.2717, found 599.2707. Anal. ($C_{30}H_{38}$-$N_4O_9$): H, N; C required 60.66, found 60.19.

The following compounds were prepared according to the same procedure:

**Diallyl 4-{[2′-[*N*,*N*-bis(2″-hydroxyethyl)amino)phenyl]-carbamoyloxymethyl}phenylcarbamoyl-L-glutamate 10a (Z = NH)**, obtained as a white foamy solid (1.70 g, 63.5%).$^1$H NMR, $\delta_H$, (ppm): 1.84−2.15 (2m, 2H, $CH_2CH(NH)$), 2.47 (t, 2H, $CH_2CO_2$, $J$ = 8.20 Hz), 2.98 (t, 4H, $NCH_2$, $J$ = 5.68 Hz), 3.33 (t, 4H, $CH_2OH$), 4.30−4.40 (m, 1H, $CH(NH)CH_2$), 4.54−4.66 (m, 4H, $CH_2O$ allyl), 5.07 (s, 2H, Ph-$CH_2$), 5.21−5.38 (m, 4H, $CH_2$= allyl), 5.87−6.05 (m, 2H, CH= allyl), 6.63 (d, 1H, NH-G, $J$ = 7.98 Hz), 6.99−7.09 (m, 2H, $H_{arom4'(5')}$, $H_{arom3'}$, $J$ = 8.76 Hz), 7.26−7.30 (t, 1H, $H_{arom5'(4')}$) 7.30 (d, 2H, $H_{arom3+5}$, $J$ = 8.58 Hz), 7.40 (d, 2H, $H_{arom2+6}$), 7.95 (dd, 1H, $H_{arom6'}$, $J_o$ = 8.67 Hz, $J_m$ = 1.40 Hz), 8.65 (s, 1H, Ph-NH), 9.02 (s, 1H, Ph′-NH); MS, *m*/*z*: 599 ($M^+$ + 1, 75), 621 ($M^+$ + Na, 72); acc. mass: ($C_{30}H_{39}N_4O_9$) calcd 599.2717, found 599.2740. Anal. ($C_{30}H_{38}N_4O_9$• 0.7 $H_2O$): C, H, N.

**Di-*tert*-butyl 4-{[2′-(*N*,*N*-bis(2″-hydroxyethyl)amino)-phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate 11a (Z = O)** was obtained as a white foamy solid (2.35 g, 99%), mp 57−60 °C. $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.70−2.0o (2m, 2H, $CH_2$-$CH(NH)$), 2.30−2.37 (m, 2H, $CH_2CO_2$-*t*-Bu), 2.96 (t, 4H, $NCH_2$, $J$ = 5.74 Hz), 3.20 (t, 4H, $CH_2OH$), 3.97−4.04 (m, 1H, $CH(NH)$-$CH_2$), 4.64 (t, 2H, $N(CH_2CH_2OH)_2$, $J$ = 5.14 Hz), 5.13 (s, 2H, $PhCH_2$), 6.98−7.05 (m, 2H, $H_{arom3'+5'}$, $J$ = 8.87 Hz), 7.10 (d,

2H, $H_{arom3+5}$, $J$ = 8.52 Hz), 7.27 (dd, 1H, $H_{arom4'}$, $J_o$ = 7.75, $J_m$ = 1.53 Hz), 7.43 (d, 2H, $H_{arom2+6}$), 7.94 (dd, 1H, $H_{arom6'}$, $J_o$ = 8.05, $J_m$ = 1.53 Hz), 8.09 (d, 1H, NH-G, $J$ = 7.89 Hz), 9.09 (s, 1H, Ph′-NH); MS *m/z*: 632 ($M^+$ + 1, 100); acc. mass: ($C_{32}H_{46}N_3O_{10}$) calcd 632.3183, found 632.3170. Anal. ($C_{32}H_{45}N_3O_{10}$) C, H, N.

**Di-*t*-butyl4-{[4′-(*N*,*N*-Bis(2″-hydroxyethyl)amino)phenyl]-carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate 11b (Z = O).** The compound was obtained from **7b (Z = O)** by a similar method, as a solid (2.73 g, 99%), mp 47−50 °C. $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.70−2.05 (2m, 2H, $CH_2$CH(NH)), 2.30−2.40 (m, 2H, $CH_2CO_2$-*t*-Bu), 3.35 (t, 4H, $NCH_2$, $J$ = 5.75 Hz), 3.50 (t, 4H, $CH_2$OH), 3.95−4.05 (m, 1H, *CH*(NH)$CH_2$), 4.63 (t, 2H, OH, $J$ = 5.34 Hz), 5.08 (s, 2H, $PhCH_2$), 6.60 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.87 Hz), 7.10 (d, 2H, $H_{arom2+6}$, $J$ = 8.42 Hz), 7.19 (d, 2H, $H_{arom2'+6'}$), 7.41 (d, 2H, $H_{arom3+5}$), 8.10 (d, 1H, NH-G, $J$ = 7.74 Hz), 9.20 (s, 1H, Ph′NH); MS, *m/z*: 632 ($M^+$ + 1, 100); acc. mass: ($C_{32}H_{46}N_3O_{10}$) calcd 632.3183, found 632.3170. Anal. ($C_{32}H_{45}N_3O_{10}$): C, H; N required 6.65, found 6.19.

**Diallyl 4-{*N*-[4′-bis(2″-hydroxyethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamate 12b** was obtained from **8b** by a similar method as a gum (0.58 g, 93.6%). $^1$H NMR, $\delta_H$, (ppm): 1.75−2.15 (2m, 2H, $CH_2$-CH(NH)), 2.44 (t, 2H, $CH_2CO_2$, $J$ = 8.54 Hz), 3.12 (s, 3H, $NCH_3$), 3.38 (t, 4H, $NCH_2$, $J$ = 5.50 Hz), 3.50 (t, 4H, $CH_2$OH), 4.25−4.35 (m, 1H, *CH*(NH)$CH_2$), 4.53 (d, 2H, $CH_2O$ allyl, $J$ = 5.31 Hz), 4.59 (d, 2H, $CH_2O$ allyl, $J$ = 5.28 Hz), 4.71 (t, 2H, OH, $J$ = 5.45 Hz), 4.94 (s, 2H, $PhCH_2$), 5.15−5.38 (m, 4H, $CH_2$= allyl), 5.85−6.00 (m, 2H, CH= allyl), 6.61 (d, 3H, $H_{arom3'+5'}$+NH-G, $J$ = 8.90 Hz), 6.99 (d, 2H, $H_{arom2'+6'}$), 7.17 (d, 4H, $H_{arom2+6}$), 7.33 (d, 2H, $H_{arom3+5}$, $J$ = 8.09 Hz), 8.62 (s, 1H, PhNH); MS, *m/z*: 613 ($M^+$ + 1, 45); acc. mass: ($C_{31}H_{41}N_4O_9$) calcd 613.2874, found 613.2853. Anal. ($C_{31}H_{40}N_4O_9$): C, H, N.

**Di-*tert*-butyl 4-{*N*-[4′-bis(2″-hydroxyethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamate 13b** was obtained from **9b** by a similar method as a glass (2.29 g, 91%). $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.70−2.00 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.14 (s, 3H, $NCH_3$), 3.39 (t, 4H, $NCH_2$), 3.48−3.55 (m, 4H, $CH_2$OH), 3.90−4.05 (m, 1H, *CH*(NH)$CH_2$), 4.72 (t, 2H, OH, $J$ = 5.28 Hz), 5.04 (s, 2H, $PhCH_2$), 6.63 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.85 Hz), 7.03 (d, 2H, $H_{arom2'+6'}$), 7.06 (d, 4H, $H_{arom2+6}$, $J$ = 8.00 Hz), 7.29 (d, 2H, $H_{arom3+5}$), 8.11 (d, 1H, NH-G, $J$ = 7.85 Hz); MS, *m/z*: 645 ($M^+$, 30), 668 ($M^+$ + Na, 10); acc. mass: ($C_{33}H_{47}N_3O_{10}$) calcd 645.3240, found 645.3261. Anal. ($C_{33}H_{47}N_3O_{10}$): C, H, N.

**4-Bis(2′-hydroxyethyl)amino-*N*-*tert*-butyloxycarbonylaniline (46b)** (0.39 g, 69%) was obtained from **45b** by a similar method as a gum. $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, $CO_2$-*t*-Bu), 3.34 (t, 4H, $NCH_2$, $J$ = 6.25 Hz), 3.45−3.55 (m, 4H, $CH_2$OH), 4.65 (t, 2H, OH, $J$ = 5.06 Hz), 6.58 (d, 2H, $H_{arom3+5}$, $J$ = 8.69 Hz), 7.18 (d, 2H, $H_{arom2+6}$), 8.77 (d, 1H, NH-G); MS, *m/z*: 296 ($M^+$, 85), 319 ($M^+$ + Na, 7), 240 ($M^+$ − t-Bu, 52; acc. mass: ($C_{15}H_{24}N_2O_4$) calcd 296.1736, found 296.1750.

In an alternative procedure, 4-amino-*N*-bis(2-hydroxyethyl)-aniline (2.6 g, 13.3 mmol) was dissolved in THF (80 mL), *tert*-butyl pyrocarbonate (2.9 g, 13.5 mmol) was added, and the reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated and the residue was purified by preparative HPLC (AcOEt:ethanol 9:1) to afford **46b** (3.0 g, 76%).

**2-Bis(2′-hydroxyethyl)amino-*N*-*tert*-butyloxycarbonylaniline (46a)** was obtained as an oil (7.2 g, 63.8%). By reacting *tert*-butyl pyrocarbonate with 2-amino-*N*-bis(2-hydroxyethyl)aniline the same intermediate was obtained (7.10 g, 100%). The compound was purified by preparative HPLC (AcOEt). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, $CO_2$-*t*-Bu), 2.96 (t, 4H, $NCH_2$, $J$ = 5.96 Hz), 4.49 (t, 4H, $CH_2$OH), 6.92−7.08 (m, 2H, $H_{arom4+5}$), 7.24 (dd, 1H, $H_{arom3}$, $J_o$ = 7.88, $J_m$ = 1.60 Hz), 7.90 (d, 1H, $H_{arom6}$), 8.65 (s, 1H, PhNH); MS *m/z*: 297 ($M^+$ + 1, 25), 241 ($M^+$ − *t*-Bu + 1, 30); acc. mass: ($C_{15}H_{25}N_2O_4$) calcd 297.1814; found 297.1830. Anal. ($C_{15}H_{24}N_2O_4$) C, H, N.

**4-(*N*′-*Benzyloxycarbonyl-N*′-*methylamino)-N,N-bis(2′-hydroxyethyl)aniline (47b)** was obtained by desilylation of **3b**, as described previously for compound **10b**, as a wax (1.2 g, 88%). $^1$H NMR $\delta_H$: 3.14 (s, 3H, $NCH_3$), 3.38 (t, 4H, $NCH_2$), 3.45−3.55 (t, 4H, $CH_2$OH), 4.71 (t, 2H, OH, $J$ = 5.16 Hz), 5.04 (s, 2H, Ph$CH_2$), 6.62 (d, 2H, $H_{arom3+5}$, $J$ = 8.17 Hz), 7.02 (d, 2H, $H_{arom2+6}$), 7.26−7.36 (m, 5H, $H_{arom\ benzyl}$). MS *m/z*: 344 ($M^+$, 100), 367 ($M^+$ + Na, 20); acc. mass: ($C_{19}H_{24}N_2O_4$) calcd 344.1746, found 344.1736. Anal. ($C_{19}H_{24}N_2O_4$) C, H, N.

**Diallyl 4-{[4′-(*N*,*N*-Bis(2″-mesyloxyethyl)amino)phenyl]-carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (14b).** Over a solution of **10b (Z = NH)** (0.50 g, 0.83 mmol), 4-*N*-(dimethylamino)pyridine (25 mg, 0.2 mmol), and $NEt_3$ (364 μL, 2.6 mmol) in $CH_2Cl_2$ (10 mL), mesyl anhydride (0.435 g, 2.5 mmol) dissolved in $CH_2Cl_2$ (10 mL) was added. After stirring at room temperature for 2.5 h, the solution was diluted with $CH_2Cl_2$ to 50 mL, extracted with 10% aq citric acid (2 × 50 mL), aq $NaHCO_3$ (50 mL), and distilled water (50 mL), dried over $MgSO_4$, and evaporated to afford **14b** (0.62 g, 99%) as a solid, mp 55−58 °C. $^1$H NMR $\delta_H$, (ppm): 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.46 (t, 2H, $CH_2CO_2$, $J$ = 7.92 Hz), 3.15 (s, 6H, $CH_3SO_3$), 3.67 (t, 4H, $NCH_2$, $J$ = 5.45 Hz), 4.27 (t, 5H, *CH*(NH)-$CH_2$ + $CH_2$OMes), 4.55 (d, 2H, $CH_2O$ allyl, $J$ = 5.42 Hz), 4.61 (d, 2H, $CH_2O$ allyl, $J$ = 5.26 Hz), 5.01 (s, 2H, $PhCH_2$), 5.15−5.40 (m, 4H, $CH_2$= allyl), 5.85−6.00 (m, 2H, CH= allyl), 6.64 (d, 1H, NH-G, $J$ = 7.95 Hz), 6.74 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.97 Hz), 7.28 (d, 4H, $H_{arom2+6}$ + $H_{arom2'+6'}$, $J$ = 8.53 Hz), 7.39 (d, 2H, $H_{arom3+5}$, $J$ = 8.49 Hz), 8.68 (s, 1H, PhNH), 9.38 (s, 1H, Ph′NH); MS, *m/z*: 754 ($M^+$, 17), 777 ($M^+$ + Na, 28); acc. mass: ($C_{32}H_{42}N_4O_{13}S_2Na$) calcd 777.2088, found 777.2080. Anal. ($C_{32}H_{42}N_4O_{13}S_2$): C, H, N.

Using the same procedure the following compounds were synthesized:

**Diallyl 4-{[2′-(*N*,*N*-Bis(2″-mesyloxyethyl)amino)phenyl]-carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (14a)** was obtained (1.33 g, 57%) as an oil, purified by preparative HPLC (cyclohexane:AcOEt 1:1). $^1$H NMR $\delta_H$, (ppm): 1.82−2.12 (2m, 2H, $CH_2$CH(NH)), 2.45 (t, 2H, $CH_2CO_2$, $J$ = 8.00 Hz), 3.05 (s, 6H, $CH_3SO_3$), 3.29 (t, 4H, $NCH_2$, $J$ = 5.25 Hz), 4.10 (t, 4H, $CH_2$OMes), 4.25−4.35 (m, 1H, $CH_2$*CH*(NH)), 4.55 and 4.61 (dd, 2H, $CH_2O$ allyl, $J$ = 5.26 Hz), 5.05 (s, 2H, $PhCH_2$), 5.20−5.37 (m, 4H, $CH_2$= allyl), 5.83−6.00 (m, 2H, CH= allyl), 6.62 (d, 1H, NH-G, $J$ = 7.97 Hz), 7.06 (dt, 1H, $H_{arom4'(5')}$, $J_o$ = 7.66 Hz, $J_m$ = 1.38 Hz), 7.19 (dt, 1H, $H_{arom4'(5')}$), 7.29 (d, 2H, $H_{arom3+5}$, $J$ = 8.54 Hz), 7.38 (d, 2H, $H_{arom2+6}$), 7.95 (d. 1H, $H_{arom6'}$ $J$ = 8.14 Hz), 8.68 (s, 1H, PhNH), 8.64 (s, 1H, Ph′NH); MS, *m/z*: 755 ($M^+$ + 1, 1), 777 ($M^+$ + Na, 5); acc. mass: ($C_{32}H_{42}N_4O_{13}S_2Na$) calcd 777.2088, found 777.2055. Anal. ($C_{32}H_{42}N_4O_{13}S_2$): C, H, N.

**Di-*tert*-butyl 4-{[2′-(*N*,*N*-Bis(2″-mesyloxyethyl)amino)-phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (15a)** resulted as a foamy solid (2.20 g, 91.9%), mp 43−44 °C. $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.03 (2m, 2H, $CH_2$CH(NH)), 2.50 (m, 2H, $CH_2CO_2$-*t*-Bu), 3.04 (s, 6H, $CH_3SO_3$), 3.29 (t, 4H, $NCH_2$, $J$ = 5.24 Hz), 3.95−4.05 (m, 1H, *CH*(NH)$CH_2$), 4.10 (t, 4H, $CH_2$-OMes), 5.13 (s, 2H, $PhCH_2$), 7.07−7.11 (m, 3H, $H_{arom3+5}$, $H_{arom\ 4'(5')}$), 7.19 (t, 1H, $H_{arom5'(4')}$, $J_o$ = 8.47 Hz, $J_m$ = 1.36 Hz), 7.45 (d, 3H, $H_{arom2+6,\ 3'}$, $J$ = 8.69 Hz), 7.69 (q, 1H, $H_{arom6'}$, $J_o$ = 8.01 Hz, $J_m$ = 1.04 Hz), 8.15 (d, 1H, NH-G, $J$ = 7.88 Hz), 8.44 (s, 1H, Ph′NH); MS, *m/z*: 788 ($M^+$ + 1, 35), 810 ($M^+$ + 23, 100); acc. mass: ($C_{34}H_{50}N_3O_{14}S_2$) calcd: 788.2734, found: 788.2760. Anal. ($C_{34}H_{49}N_3O_{14}S_2$): C, H, N.

**Di-*tert*-butyl 4-{[4′-(*N*,*N*-Bis(2″-mesyloxyethyl)amino)-phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (15b)**, was obtained as an solid (0.80 g, 95%), mp 41−46 °C. $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.43 (s, 9H, $CO_2$-*t*-Bu), 1.70−2.05 (2m, 2H, $CH_2$CH(NH)), 2.30−2.40 (m, 2H, $CH_2CO_2$-*t*-Bu), 3.14 (s, 3H, $CH_3SO_3$), 3.15 (s, 3H, $CH_3$-$SO_3$), 3.67 (t, 4H, $NCH_2$), 3.95−4.05 (m, 1H, *CH*(NH)$CH_2$), 4.30 (t, 4H, $CH_2$OMes), 5.12 (s, 2H, $PhCH_2$), 6.76 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.05 Hz), 7.12 (d, 2H, $H_{arom2+6}$, $J$ = 7.16 Hz), 7.30 (d, 2H, $H_{arom2'+6'}$), 7.44 (d, 2H, $H_{arom3+5}$), 8.09 (d, 1H, NH-G), 9.36 (s, 1H, Ph′NH); MS, *m/z*: 788 ($M^+$ + 1, 45), 810 ($M^+$ + 23, 10);

acc. mass: ($C_{34}H_{50}N_3O_{14}S_2$) calcd: 788.2734, found: 788.2720. Anal. ($C_{34}H_{49}N_3O_{14}S_2$): C, H, N; S required 8.14, found 7.71.

**Diallyl 4-{*N*-[4′-bis(2″-mesyloxyethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamate (16b)** was obtained as a gum (0.73 g, 100%). $^1$H NMR, $\delta_H$, (ppm): 1.85−2.20 (2m, 2H, $CH_2$CH(NH)), 2.44 (t, 2H, $CH_2CO_2$, $J$ = 7.77 Hz), 3.14 (s, 9H, $CH_3SO_3$, $NCH_3$), 3.71 (t, 4H, $NCH_2$), 4.29 (t, 5H, $CH_2$OMes + $CH$(NH)$CH_2$, $J$ = 4.95 Hz), 4.54 (d, 2H, $CH_2$O allyl, $J$ = 5.40 Hz), 4.60 (d, 2H, $CH_2$O allyl, $J$ = 5.30 Hz), 4.96 (s, 2H, PhCH$_2$), 5.15−5.37 (m, 4H, $CH_2$= allyl), 5.80−6.00 (m, 2H, CH= allyl), 6.61 (d, 1H, NH-G, $J$ = 7.99 Hz), 6.75 (d, 2H, $H_{arom3'+5'}$, $J$ = 9.00 Hz), 7.08 (d, 2H, $H_{arom2'+6'}$), 7.18 (d, 2H, $H_{arom2+6}$), 7.33 (d, 2H, $H_{arom3+5}$, $J$ = 8.24 Hz), 8.61 (s, 1H, PhNH). MS $m/z$: 769 ($M^+$ + 1, 4); acc. mass: ($C_{33}H_{45}N_4O_{13}S_2$) calcd 769.2425, found 769.2456. Anal. ($C_{33}H_{44}N_4O_{13}S_2$): C, H, N, S.

**Di-*tert*-butyl 4-{*N*-[4′-bis(2″-mesyloxyethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (17b)** was obtained as a glass (2.84 g, 100%). $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.70−2.00 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.15 (s, 6H, $CH_3SO_3$), 3.17 (s, 3H, $NCH_3$), 3.73 (t, 4H, $NCH_2$), 3.90−4.05 (m, 1H, $CH$(NH)$CH_2$), 4.29 (t, 4H, $CH_2$OMes, $J$ = 5.54 Hz), 5.05 (s, 2H, PhCH$_2$), 6.77 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.90 Hz), 7.05 (d, 2H, $H_{arom3+5}$), 7.12 (d, 2H, $H_{arom2'+6'}$), 7.29 (d, 2H, $H_{arom2+6}$), 8.11 (d, 1H, NH-G, $J$ = 7.26 Hz). MS $m/z$: 801 ($M^+$, 45), 824 ($M^+$ + Na, 10); acc. mass: ($C_{35}H_{51}N_3O_{14}S_2$) calcd 801.2843, found 801.2812.

**2-Bis(2′-mesyloxyethyl)amino-*N*-*tert*-butyloxycarbonylaniline (48a)** resulted as an oil (2.01 g, 88%). Purified by preparative HPLC ($CH_2Cl_2$:AcOEt 5:1). $^1$H NMR, $\delta_H$, (ppm): 1.46 (s, 9H, $CO_2$-*t*-Bu), 3.13 (s, 6H, $CH_3SO_3$), 3.27 (t, 4H, $NCH_2$, $J$ = 5.03 Hz), 4.08 (t, 4H, $CH_2$OMes), 7.03 (t, 1H, $H_{arom4(5)}$, $J_o$ = 7.97 Hz), 7.18 (t, 1H, $H_{arom5(4)}$), 7.40 (dd, 1H, $H_{arom3}$, $J_o$ = 8.93 Hz), 7.99 (d, 1H, $H_{arom6}$, $J_o$ = 9.08 Hz), 8.13 (s, 1H, PhNH); MS, $m/z$: 453 ($M^+$ + 1, 30), 475 ($M^+$ + 23, 55), 397 ($M^+$ − t-Bu + 1, 100); acc. mass: ($C_{17}H_{29}N_2O_8S_2$) calcd: 453.1365, found: 453.1350. Anal. ($C_{17}H_{29}N_2O_8S_2$): H, N, S; C required 45.12, found 45.63%.

**4-Bis(2′-mesyloxyethyl)amino-*N*-*tert*-butyloxycarbonylaniline (48b)** was obtained after purification by preparative HPLC ($CH_2Cl_2$:AcOEt 3:1) as an oil (155 mg, 57%). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, $CO_2$-*t*-Bu), 3.13 (s, 6H, $CH_3SO_3$), 3.66 (t, 4H, $NCH_2$, $J$ = 5.67 Hz), 4.27 (t, 4H, $CH_2$OMes), 6.72 (d, 2H, $H_{arom3+5}$, $J$ = 8.93 Hz), 7.27 (d, 2H, $H_{arom2+6}$), 8.93 (s, 1H, NH). MS, $m/z$: 452 ($M^+$, 41), 396 ($M^+$ − t-Bu, 44), 357 ($M^+$ − $CH_3SO_3$, 48); acc. mass: ($C_{17}H_{28}N_2O_8S_2$) calcd: 452.1287, found: 452.1270.

**4-(*N*′-*Benzyloxycarbonyl-N*′-*methylamino)-N*,*N*-bis(2′-mesyloxyethyl)aniline (53b)** was obtained as an oil (1.96 g, 100%). $^1$H NMR $\delta_H$: 3.15 (s, 6H, $CH_3SO_3$), 3.18 (s, 3H, $NCH_3$), 3.73 (t, 4H, N$CH_2$, $J$ = 5.63 Hz), 4.30 (t, 4H, $CH_2$OMes), 5.06 (s, 2H, Ph$CH_2$), 6.77 (d, 2H, $H_{arom3+5}$, $J$ = 8.93 Hz), 7.12 (d, 2H, $H_{arom2+6}$), 7.25−7.37 (m, 5H, $H_{arom\ benzyl}$). MS $m/z$: 500 ($M^+$, 100), 523 ($M^+$ + Na, 15); acc. mass: ($C_{21}H_{28}N_2O_8S_2$) calcd 500.1287, found 500.1260.

**Diallyl4-{[4′-(*N*,*N*-Bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (18b).** A solution of **14b** (0.12 g, 0.13 mmol) and NaI (0.22 g, 1.5 mmol) in 8 mL of acetone was stirred at reflux for 4 h. The solvent was evaporated, and the residue was retaken up in 30 mL of AcOEt, washed with 20 mL of $H_2O$, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (cyclohexane: AcOEt 1:1) to afford **18b** (0.10 g, 94%) as a brown solid, mp 138−141 °C. $^1$H NMR, $\delta_H$, (ppm): 1.85−2.15 (2m, 2H, $CH_2$CH(NH)), 2.45 (t, 2H, $CH_2CO_2$, $J$ = 7.79 Hz), 3.20−3.35 (t, 4H, $NCH_2$, under $H_2O$ peak), 3.67 (t, 4H, $CH_2$I), 4.25−4.40 (m, 1H, $CH$(NH)$CH_2$), 4.55 (d, 2H, $CH_2$O allyl, $J$ = 5.43 Hz), 4.61 (d, 2H, $CH_2$O allyl, $J$ = 5.23 Hz), 5.02 (s, 2H, PhCH$_2$), 5.15−5.40 (m, 4H, $CH_2$= allyl), 5.85−6.05 (m, 2H, CH= allyl), 6.58−6.65 (m, 3H, NH-G + $H_{arom3'+5'}$), 7.28 (d, 4H, $H_{arom2+6}$ + $H_{arom2'+6'}$, $J$ = 8.44 Hz), 7.39 (d, 2H, $H_{arom3+5}$, $J$ = 8.33 Hz), 8.62 (s, 1H, PhNH), 9.29 (s, 1H, Ph′NH); MS, $m/z$: 819 ($M^+$ + 1, 100); acc. mass: ($C_{30}H_{37}N_4O_7I_2$) calcd: 819.0752, found: 819.0740. Anal. ($C_{30}H_{36}N_4O_7I_2$): C, H, N.

Using the same procedure, the following compounds have been obtained:

**Diallyl4-{[2′-(*N*,*N*-bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (18a)** as a solid, purified by preparative HPLC (eluent: cyclohexane: AcOEt, 3:1), mp 95−6 °C, (0.134 g, 68.7%). $^1$H NMR, $\delta_H$, (ppm): 1.82−2.16 (2m, 2H, $CH_2$CH(NH)), 2.45 (t, 2H, $CH_2$-$CO_2$, $J$ = 7.38 Hz), 3.21 (m, 8H, N($CH_2CH_2I)_2$), 4.28−4.34 (m, 1H, $CH$(NH)$CH_2$), 4.53−4.62 (m, 4H, $CH_2$O allyl, 5.09 (s, 2H, PhCH$_2$), 5.10−5.36 (m, 4H, $CH_2$= allyl), 5.86−6.09 (m, 2H, CH= allyl), 6.61 (d, 1H, NH-G, $J$ = 7.98 Hz), 7.04 (dt, 1H, $H_{arom4'(5')}$, $J_o$ = 7.65 Hz, $J_m$ = 1.55 Hz), 7.17 (dt, 1H, $H_{arom5'(4')}$, $J_o$ = 7.90 Hz, $J_m$ = 1.30 Hz), 7.28 (d, 2H, $H_{arom3+5}$, $J$ = 8.55 Hz), 7.38 (d, 2H, $H_{arom2+6}$), 7.65 (dd, 1H, $H_{arom6'}$, $J_o$ = 8.10 Hz, $J_m$ = 1.30 Hz), 8.51 (s, 1H, PhNH), 8.64 (s, 1H, Ph′NH); MS, $m/z$: 819 ($M^+$ + 1, 1.5), 841 ($M^+$ + Na, 6.5); acc. mass: ($C_{30}H_{36}N_4O_7I_2$Na) calcd: 841.0571, found: 841.0555. Anal. ($C_{30}H_{36}N_4O_7I_2$): H, N, I; C required 44.03, found 44.46.

**Di-*tert*-butyl 4-{[2′-(*N*,*N*-bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (21a)** resulted as a foamy solid, purified by preparative HPLC (cyclohexane:AcOEt, 3:1), (0.109 g, 67%), mp 39−40 °C. $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.10 (2m, 2H, $CH_2$CH(NH)), 2.31−2,38 (m, 2H, $CH_2$-$CO_2$), 3.20−3.25 (m, 8H, N($CH_2CH_2I)_2$), 3.90−4.10 (m, 1H, $CH$(NH)$CH_2$), 5.18 (s, 2H, PhCH$_2$), 7.05 (dt, 1H, $H_{arom5'(4')}$, $J_o$ = 7.55 Hz, $J_m$ = 1.24 Hz), 7.10 (d, 2H, $H_{arom3+5}$, $J$ = 8.44 Hz), 7.18 (t, 1H, $H_{arom4'(5')}$), 7.34 (d, 1H, $H_{arom3'}$, $J$ = 7.34 Hz), 7.43 (d, 2H, $H_{arom2+6}$), 7.93 (d, 1H, $H_{arom6'}$ $J$ = 7.57 Hz), 8.10 (d, 1H, NH-G, $J$ = 7.93 Hz), 8.57 (s, 1H, Ph′-NH). MS, $m/z$: 852 ($M^+$ + 1, 40), 874 ($M^+$ + Na, 37); acc. mass: ($C_{32}H_{44}N_3O_8I_2$) calcd 852.1218; found 852.1240. Anal. ($C_{32}H_{43}N_3O_8I_2$): C, H, N.

**Di-*tert*-butyl 4-{[4′-(*N*,*N*-bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (21b)** resulted as a gum, purified by preparative HPLC (cyclohexane:AcOEt 3:1) (0.2 g, 94%). $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.10 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$, $J$ = 7.37 Hz), 3.25−3.40 (t, 4H, $NCH_2$, under $H_2O$), 3.68 (t, 4H, $CH_2$I), 3.95−4.05 (m, 1H, $CH$(NH)$CH_2$), 5.10 (s, 2H, PhCH$_2$), 6.64 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.51 Hz), 7.10 (d, 2H, $H_{arom2+6}$, $J$ = 8.21 Hz), 7.28 (d, 2H, $H_{arom2'+6'}$), 7.42 (d, 2H, $H_{arom3+5}$), 8.07 (broad s, 1H, NH-G), 9.33 (s, 1H, Ph′-NH). MS, $m/z$: 851 ($M^+$, 92), 874 ($M^+$ + Na, 8); acc. mass: ($C_{32}H_{43}N_3O_8I_2$) calcd 851.1140; found 851.1150.

**Diallyl 4-{*N*-[4′-bis(2″-iodoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamate (25b)**, purified by column chromatography (cyclohexane:AcOEt 1:1), resulted as a solid (0.165 g, 60%), mp 138−140 °C. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.15 (2m, 2H, $CH_2$CH(NH)), 2.44 (t, 2H, $CH_2CO_2$), 3.13 (s, 3H, $NCH_3$), 3.25−3.35 (t, 4H, $NCH_2$), 3.70 (t, 4H, $CH_2$I, $J$ = 6.17 Hz), 4.25−4.40 (m, 1H, $CH$(NH)-$CH_2$), 4.54 (d, 2H, $CH_2$O allyl, $J$ = 6.27 Hz), 4.60 (d, 2H, $CH_2$O allyl, $J$ = 4.42 Hz), 4.95 (s, 2H, PhCH$_2$), 5.10−5.37 (m, 4H, $CH_2$= allyl), 5.80−6.00 (m, 2H, CH= allyl), 6.62 (d, 3H, $H_{arom3'+5'}$+NH-G, $J$ = 8.67 Hz), 7.09 (d, 2H, $H_{arom2'+6'}$), 7.18 (d, 2H, $H_{arom2+6}$), 7.33 (d, 2H, $H_{arom3+5}$, $J$ = 7.72 Hz), 8.61 (s, 1H, PhNH). MS $m/z$: 833 ($M^+$ + 1, 7), 855 ($M^+$ + Na, 14); acc. mass: ($C_{31}H_{38}N_4O_7I_2$Na) calcd 855.0728, found 855.0755. Anal. ($C_{31}H_{38}N_4O_7I_2$): H, N; C required 44.73, found 45.24%.

**Di-*tert*-butyl 4-{*N*-[4′-bis(2″-iodoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (28b)**, purified by column chromatography (cyclohexane:AcOEt 1:1), resulted as a glass (0.165 g, 79.2%), mp 40−41 °C. $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.05 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.16 (s, 3H, $NCH_3$), 3.25−3.35 (t, 4H, $NCH_2$), 3.72 (t, 4H, $CH_2$I, $J$ = 7.76 Hz), 3.90−4.05 (m, 1H, $CH$(NH)-$CH_2$), 5.05 (s, 2H, PhCH$_2$), 6.65 (d, 3H, $H_{arom3'+5'}$, $J$ = 8.86 Hz), 7.06 (d, 2H, $H_{arom3+5}$, $J$ = 8.29 Hz), 7.12 (d, 2H, $H_{arom2'+6'}$), 7.30 (d, 2H, $H_{arom2+6}$), 8.11 (d, 1H, NH-G, $J$ = 7.78 Hz). MS $m/z$: 865 ($M^+$, 57), 888 ($M^+$ + Na, 26); acc. mass: ($C_{33}H_{45}N_3O_8I_2$) calcd 865.1310, found 865.1296.

**2-Bis(2′-iodoethyl)amino-*N*-*tert*-butyloxycarbonylaniline (49a)** was obtained from the corresponding bis-mesyl derivative as an oil (0.121 g, 78.6%) and was purified by preparative HPLC (cyclohexane:AcOEt, 3:1). $^1$H NMR, $\delta_H$, (ppm): 1.46 (s, 9H, $CO_2$-*t*-Bu), 3.29 (m, 8H, N($CH_2CH_2I$)$_2$), 7.01 (dt, 1H, $H_{arom4(5)}$, $J_o = 7.97$, $J_m = 1.35$ Hz), 7.15 (t, 1H, $H_{arom5(4)}$), 7.32 (dd, 1H, $H_{arom3}$, $J_o = 7.83$, $J_m = 1.35$ Hz), 7.90 (dd, 1H, $H_{arom6}$, $J_o = 8.21$, $J_m = 1.35$ Hz), 8.25 (s, 1H, PhNH). MS, *m*/*z*: 517 ($M^+ + 1$, 40), 389 ($M^+ - HI + 1$, 55); acc. mass: ($C_{15}H_{23}N_2O_2I_2$) calcd 516.9849; found 516.9870. Anal. ($C_{15}H_{22}N_2O_2I_2$): C, H, N.

**4-Bis(2′-iodoethyl)amino-*N*-*tert*-butyloxycarbonylaniline (49b)** was obtained by the same method as a solid, mp 121−125 °C (0.25 g, 53%). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, O-*t*-Bu), 3.20−3.35 (t, 4H, $NCH_2$), 3.67 (t, 4H, $CH_2I$, $J$ = 7.57 Hz), 6.61 (d, 2H, $H_{arom3+5}$, $J$ = 8.87 Hz), 7.26 (d, 2H, $H_{arom2+6}$), 8.93 (s, 1H, NH). MS, *m*/*z*: 516 ($M^+$, 75), 460 ($M^+$ − t-Bu, 100); acc. mass: ($C_{15}H_{22}N_2O_2I_2$) calcd 515.9771; found 515.9750.

**4-(*N*′-*Benzyloxycarbonyl*-*N*′-*methylamino*)-*N*,*N*-bis(2′-iodoethyl)aniline (54b)** was obtained as a solid (0.40 g, 70.9%), mp 93−95 °C. $^1$H NMR $\delta_H$: 3.16 (s, 3H, $NCH_3$), 3.30 (t, 4H, $NCH_2$, $J$ = 5.63 Hz), 3.72 (t, 4H, $CH_2I$, $J$ = 7.67 Hz), 5.06 (s, 2H, Ph$CH_2$), 6.65 (d, 2H, $H_{arom3+5}$, $J$ = 8.78 Hz), 7.13 (d, 2H, $H_{arom2+6}$), 7.25−7.37 (m, 5H, $H_{arom\ benzyl}$). MS *m*/*z*: 564 ($M^+$, 100), 587 ($M^+$ + Na, 12); acc. mass: ($C_{19}H_{22}N_2O_2I_2$) calcd 563.9771, found 563.9750. Anal. ($C_{19}H_{22}N_2O_2I_2$): C, H, N.

**Di-*tert*-butyl 4-{[4′-(*N*,*N*-Bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (22b).** To a THF solution (15 mL) of **15b** (0.18 g, 0.23 mmol) was added LiBr (0.44 g, 5.0 mmol). After 1.5 h stirring at reflux, the solvent was evaporated, and the residue was retaken up in $CH_2Cl_2$ (25 mL) and extracted with $H_2O$ (25 mL), and the organic layer was dried ($MgSO_4$) and evaporated to dryness. Purification was achieved by preparative HPLC (cyclohexane:AcOEt 3:1), when product **22b** (0.12 g, 69%) was obtained as a solid, mp 48−50 °C. $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.10 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.55 (t, 4H, $NCH_2$), 3.72 (t, 4H, $CH_2$Br), 3.95−4.05 (m, 1H, -*CH*(NH)$CH_2$), 5.10 (s, 2H, $PhCH_2$), 6.68 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.72 Hz), 7.10 (d, 2H, $H_{arom2+6}$, $J$ = 8.00 Hz), 7.28 (d, 2H, $H_{arom2'+6'}$), 7.42 (d, 2H, $H_{arom3+5}$), 8.06 (broad s, 1H, NH-G), 9.32 (s, 1H, Ph′-NH). MS, *m*/*z*: 758 ($M^+$ + 1, 100), 713 ($M^+ - CO_2$, 40), 679 ($M^+$ + 1 − Br, 25); acc. mass: ($C_{32}H_{43}N_3O_8Br_2$) calcd 756.1495; found 756.1480. Anal. ($C_{32}H_{43}N_3O_8Br_2$): C, H, N.

Using a similar procedure the following compounds were obtained:

**Diallyl 4-{[4′-(*N*,*N*-Bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (19b)** was obtained after purification by column chromatography (cyclohexane:AcOEt 1:1) as a solid, mp 121−123 °C (0.25 g, 43%). $^1$H NMR, $\delta_H$, (ppm): 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.45 (t, 2H, $CH_2CO_2$, $J$ = 8.19 Hz), 3.55 (t, 4H, $NCH_2$, $J$ = 7.15 Hz), 3.71 (t, 4H, $CH_2$Br, $J$ = 7.19 Hz), 4.25−4.35 (m, 1H, *CH*(NH)$CH_2$), 4.54 (d, 2H, $CH_2$O allyl, $J$ = 5.41 Hz), 4.60 (d, 2H, $CH_2$O allyl, $J$ = 5.29 Hz), 5.00 (s, 2H, $PhCH_2$), 5.15−5.40 (m, 4H, $CH_2$═ allyl), 5.80−6.00 (m, 2H, CH═ allyl), 6.64 (d, 1H, NH-G, $J$ = 7.41 Hz), 6.66 (d, 2H, $H_{arom3'+5'}$, $J$ = 9.00 Hz), 7.27 (d, 4H, $H_{arom2+6}$ + $H_{arom2'+6'}$, $J$ = 8.59 Hz), 7.38 (d, 2H, $H_{arom3+5}$, $J$ = 8.62 Hz), 8.68 (s, 1H, PhNH), 9.38 (s, 1H, Ph′NH); MS, *m*/*z*: 724 ($M^+$, 3), 747 ($M^+$ + Na, 2), 645 ($M^+$ − Br, 2); acc. mass: ($C_{30}H_{36}N_4O_7Br_2Na$) calcd 745.0848, found 745.0860. Anal. ($C_{30}H_{36}N_4O_7Br_2$): C, H, N, Br.

**Diallyl 4-{[2′-(*N*,*N*-bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (19a)** was purified by column chromatography (cyclohexane:AcOEt 3:1) to yield **19a** (0.197 g, 85%) as a solid, mp 120−122 °C. $^1$H NMR, $\delta_H$, (ppm): 1.87−2.08 (2m, 2H, $CH_2$CH(NH)), 2.45 (t, 2H, $CH_2CO_2$, $J$ = 7.38 Hz), 3.30 (t, 4H, $NCH_2$, $J$ = 5.99 Hz), 3.44 (t, 5H, $CH_2$Br), 4.26−4.35 (m, 1H, *CH*(NH)$CH_2$), 4.53−4.62 (dd, 4H, $CH_2$O allyl), 5.07 (s, 2H, $PhCH_2$), 5.16−5.37 (m, 4H, $CH_2$═ allyl), 5.82−5.99 (m, 2H, CH═ allyl), 6.63 (d, 1H, NH-G, $J$ = 7.97 Hz), 7.05 (t, 1H, $H_{arom4'(5')}$, $J$ = 7.67 Hz), 7.19 (t, 1H, $H_{arom5'(4')}$), 7.26 (d, 2H, $H_{arom3+5}$, $J$ = 8.55 Hz), 7.38 (d, 2H, $H_{arom2+6}$), 7.95 (d, 1H, $H_{arom6'}$, $J$ = 8.05 Hz), 8.63 (s, 1H, PhNH), 8.66 (s, 1H, Ph′NH); MS, *m*/*z*: 725 ($M^+$ + 1, 1), 747 ($M^+$ + Na, 4), 645 ($M^+$ − HBr + 1, 1); acc. mass: ($C_{30}H_{36}N_4O_7Br_2Na$) calcd 745.0848, found 745.0830. Anal.($C_{30}H_{36}N_4O_7Br_2$) C, H, N, Br.

**Di-*tert*-butyl 4-{[2′-(*N*,*N*-bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (22a)** was obtained as a solid and purified by preparative HPLC (cyclohexane:AcOEt 3:1), (0.103 g, 86.5%), mp 32−35 °C. $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.87−2.10 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.33 (t, 4H, $NCH_2$), 3.44 (t, 4H, $CH_2$Br), 3.93−4.10 (m, 1H, *CH*(NH)$CH_2$), 5.17 (s, 2H, $PhCH_2$), 7.05−7.40 (m, 4H, $H_{arom3+5}$ + $H_{arom4'(5')}$ + $H_{arom5'(4')}$), 7.42 (d, 2H, $H_{arom2+6}$, $J$ = 8.57 Hz), 7.94 (d, 1H, $H_{arom6'}$, $J$ = 8.00 Hz), 8.10 (broad d, 1H, NH-G), 8.64 (s, 1H, Ph′-NH). MS, *m*/*z*: 758 ($M^+$ + 1, 78), 780 ($M^+$ + Na, 100), 678 ($M^+$ + 1 − HBr, 25); acc. mass: ($C_{32}H_{44}N_3O_8Br_2$) calcd 756.1495; found 756.1470. Anal. ($C_{32}H_{43}N_3O_8Br_2$·0.1toluene) C, H, N.

**Diallyl 4-{*N*-[4′-bis(2″-bromoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamate (26b)** was obtained from **16b** as a solid, (0.16 g, 64%), mp 104−107 °C. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.15 (2m, 2H, $CH_2$CH(NH)), 2.44 (t, 2H, $CH_2CO_2$), 3.13 (s, 3H, $NCH_3$), 3.56 (t, 4H, $NCH_2$, $J$ = 6.74 Hz), 3.75 (t, 4H, $CH_2$Br), 4.25−4.35 (m, 1H, *CH*(NH)$CH_2$), 4.54 (d, 2H, $CH_2$O allyl, $J$ = 5.52 Hz), 4.59 (d, 2H, $CH_2$O allyl, $J$ = 5.21 Hz), 4.95 (s, 2H, $PhCH_2$), 5.10−5.37 (m, 4H, $CH_2$═ allyl), 5.80−6.00 (m, 2H, CH═ allyl), 6.61 (d, 1H, NH-G, $J$ = 8.34 Hz), 6.68 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.89 Hz), 7.09 (d, 2H, $H_{arom2'+6'}$), 7.18 (d, 2H, $H_{arom2+6}$), 7.33 (d, 2H, $H_{arom3+5}$, $J$ = 8.26 Hz), 8.61 (s, 1H, PhNH). MS *m*/*z*: 738 ($M^+$, 5), 761 ($M^+$ + Na, 15); acc. mass: ($C_{31}H_{38}N_4O_7Br_2$) calcd 759.1005, found 759.1021. Anal. ($C_{31}H_{38}N_4O_7Br_2$): C, H, N.

**Di-*tert*-butyl 4-{*N*-[4′-bis(2″-bromoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (29b)** was obtained from **17b** as a solid, (0.67 g, 69.7%), mp 36−39 °C. $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.05 (2m, 2H, $CH_2$CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.16 (s, 3H, $NCH_3$), 3.58 (t, 4H, $NCH_2$, $J$ = 7.00 Hz), 3.77 (t, 4H, $CH_2$Br), 3.90−4.05 (m, 1H, *CH*(NH)$CH_2$), 5.05 (s, 2H, $PhCH_2$), 6.70 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.89 Hz), 7.06 (d, 2H, $H_{arom3+5}$, $J$ = 8.34 Hz), 7.12 (d, 2H, $H_{arom2'+6'}$), 7.29 (d, 2H, $H_{arom2+6}$), 8.11 (d, 1H, NH-G, $J$ = 7.86 Hz). MS *m*/*z*: 771 ($M^+$, 75); acc. mass: ($C_{33}H_{45}N_3O_8Br_2$) calcd 769.1573, found 769.1550. Anal. ($C_{33}H_{45}N_3O_8Br_2$): C, H, N.

**2-Bis(2′-bromoethyl)amino-*N*-*tert*-butyloxycarbonylaniline (50a)** was obtained from **48a** as an oil (0.79 g, 85.3%) and was purified by preparative HPLC (cyclohexane:AcOEt 3:1). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, $CO_2$-*t*-Bu), 3.30 (t, 4H, $NCH_2$, $J$ = 5.96 Hz), 3.45 (t, 4H, $CH_2$Br), 7.02 (dt, 1H, $H_{arom4(5)}$, $J_o = 7.88$, $J_m = 1.36$ Hz), 7.16 (t, 1H, $H_{arom5(4)}$), 7.36 (dd, 1H, $H_{arom3}$, $J_o = 7.85$, $J_m = 1.33$ Hz), 7.91 (dd, 1H, $H_{arom6}$, $J_o = 8.12$, $J_m = 1.28$ Hz), 8.35 (s, 1H, PhNH); MS, *m*/*z*: 423 ($M^+$ + 1, 40), 367 ($M^+$ − *t*-Bu + 1, 100), 341 ($M^+$ − Br, 80); acc. mass: ($C_{15}H_{23}N_2O_2Br_2$) calcd 421.0126, found 421.0140. Anal. ($C_{15}H_{22}N_2O_2Br_2$): H, N, Br; C required 42.68, found 43.20%.

**4-Bis(2′-bromoethyl)amino-*N*-*tert*-butyloxycarbonylaniline (50b)** was obtained by the same method, as a solid, mp 91−92 °C (0.18 g, 73.5%). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, O-*t*-Bu), 3.55 (t, 4H, $NCH_2$, $J$ = 6.93 Hz), 3.67 (t, 4H, $CH_2$Br), 6.65 (d, 2H, $H_{arom3+5}$, $J$ = 8.92 Hz), 7.27 (d, 2H, $H_{arom2+6}$), 8.92 (s, 1H, NH). MS, *m*/*z*: 422 ($M^+$, 95), 366 ($M^+$ − t-Bu, 100); acc. mass: ($C_{15}H_{22}N_2O_2Br_2$) calcd 420.0048; found 420.0060. Anal. ($C_{15}H_{22}N_2O_2Br_2$): H, N; C required 42.68, found 43.13%.

**4-(*N*′-Benzyloxycarbonyl-*N*′-methylamino)-*N*,*N*-bis(2′-bromoethyl)aniline (55b)** was obtained as an oil (0.32 g, 63.9%). $^1$H NMR $\delta_H$: 3.17 (s, 3H, $NCH_3$), 3.58 (t, 4H, $NCH_2$, $J$ = 7.12 Hz), 3.72 (t, 4H, $CH_2$Br), 5.06 (s, 2H, Ph$CH_2$), 6.70 (d, 2H, $H_{arom3+5}$, $J$ = 8.98 Hz), 7.12 (d, 2H, $H_{arom2+6}$), 7.25−7.37 (m, 5H, $H_{arom\ benzyl}$). MS *m*/*z*: 470 ($M^+$, 100), 493 ($M^+$ + Na, 7); acc. mass: ($C_{19}H_{22}N_2O_2Br_2$) calcd 648.0048, found 648.0030. Anal. ($C_{19}H_{22}N_2O_2Br_2$) H, N; C required 48.53, found 49.01%.

**Diallyl 4-{*N*-[4′-Bis(2″-chloroethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamate (27b). 16b** (0.245 g, 0.32 mmol) was dissolved in DMA (25 mL). Lithium chloride (0.21 g, 5 mmol) was added to the reaction mixture, and it was stirred for 24 h at room temperature. The solvent was evaporated, and the residue was taken up in AcOEt (50 mL) and extracted with distilled water (2 × 50 mL). The organic layer was separated, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (eluent AcOEt:cyclohexane 1:1) to afford **27b** (0.105 g, 51%) as a gum. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.20 (2m, 2H, $CH_2$CH(NH)), 2.44 (t, 2H, $CH_2CO_2$, $J$ = 8.26 Hz), 3.13 (s, 3H, $NCH_3$), 3.70 (s, 8H, N($CH_2CH_2Cl)_2$), 4.25−4.40 (m, 1H, $CH$(NH)-$CH_2$), 4.54 (d, 2H, $CH_2O$ allyl, $J$ = 5.44 Hz), 4.59 (d, 2H, $CH_2O$ allyl, $J$ = 5.35 Hz), 4.95 (s, 2H, $PhCH_2$), 5.10−5.35 (m, 4H, $CH_2$= allyl), 5.80−6.00 (m, 2H, CH= allyl), 6.61 (d, 1H, NH-G, $J$ = 8.35 Hz), 6.70 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.86 Hz), 7.08 (d, 2H, $H_{arom2'+6'}$), 7.18 (d, 2H, $H_{arom2+6}$), 7.33 (d, 2H, $H_{arom3+5}$, $J$ = 8.56 Hz), 8.61 (s, 1H, PhNH). MS *m*/*z*: 671 ($M^+$ + Na, 20); acc. mass: ($C_{31}H_{38}N_4O_7Cl_2Na$) calcd 671.2015, found 671.2037. Anal. ($C_{31}H_{38}N_4O_7Cl_2$): C, H, N Cl.

**Di-*tert*-butyl 4-{*N*-[4′-bis(2″-chloroethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (30b)** was obtained from **17b** as a solid, (0.60 g, 95.2%), mp 35−39 °C. $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.75−2.05 (2m, 2H, $CH_2$CH(NH)), 2.39 (t, 2H, $CH_2CO_2$), 3.16 (s, 3H, $NCH_3$), 3.72 (s, 8H, N($CH_2CH_2Cl)_2$), 3.90−4.05 (m, 1H, $CH$(NH)$CH_2$), 5.05 (s, 2H, $PhCH_2$), 6.72 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.96 Hz), 7.06 (d, 2H, $H_{arom3+5}$, $J$ = 8.25 Hz), 7.11 (d, 2H, $H_{arom2'+6'}$), 7.30 (d, 2H, $H_{arom2+6}$), 8.12 (d, 1H, NH-G). MS *m*/*z*: 681 ($M^+$, 28), 704 ($M^+$ + Na, 20); acc. mass: ($C_{33}H_{45}N_3O_8Cl_2$) calcd 681.2584, found 681.2560. Anal. ($C_{33}H_{45}N_3O_8Cl_2$): C, H, N, Cl.

**Diallyl 4-{[2′-(*N*,*N*-bis(2″-chloroethyl)amino)phenyl]-carbamoyloxymethyl}phenylcarbamoyl-L-glutamate (20a)** was obtained from **14a**. The product purified by HPLC (cyclohexane:AcOEt 2:1) resulted as a solid (0.054 g, 50%), mp 118−9 °C, $^1$H NMR, $\delta_H$, (ppm): 1.84−2.13 (2m, 2H, $CH_2$CH-(NH)), 2.45 (t, 2H, $CH_2CO_2$, $J$ = 8.00 Hz), 3.25 (t, 4H, $NCH_2$, $J$ = 5.80 Hz), 3.54 (t, 4H, $CH_2Cl$), 4.26−4.35 (m, 1H, $CH$(NH)-$CH_2$), 4.53−4.61 (dd, 4H, $CH_2O$ allyl, $J$ = 5.34 Hz), 5.06 (s, 2H, $PhCH_2$), 5.16−5.37 (m, 4H, $CH_2O$= allyl), 5.82−5.99 (m, 2H, CH= allyl), 6.65 (d, 1H, NH-G, $J$ = 8.00 Hz), 7.05 (t, 1H, $H_{arom4'(5')}$, $J$ = 7.00 Hz), 7.20 (t, 1H, $H_{arom5'(4')}$, $J$ = 7.90 Hz), 7.25 (d, 2H, $H_{arom3+5}$, $J$ = 8.48 Hz), 7.37 (d, 2H, $H_{arom2+6}$), 7.97 (d, 1H, $H_{arom6'}$, $J$ = 7.82 Hz), 8.64 (s, 1H, PhNH), 8.67 (s, 1H, Ph′NH); MS, *m*/*z*: 635 ($M^+$ + 1, 9), 657 ($M^+$ + Na, 18), 598 ($M^+$ − Cl, 6); acc. mass: ($C_{30}H_{36}N_4O_7Cl_2Na$) calcd 657.1859, found 657.1880. Anal.($C_{30}H_{36}N_4O_7Cl_2$): C, H, N, Cl.

**4-(*N*′-*Benzyloxycarbonyl-N*′-*methylamino)-N,N*-bis(2′-chloroethyl)aniline (56b)** was obtained as an oil (0.245 g, 62.5%). $^1$H NMR $\delta_H$: 3.17 (s, 3H, $NCH_3$), 3.72 (s, 8H, N($CH_2CH_2$-$Cl)_2$), 5.06 (s, 2H, Ph$CH_2$), 6.72 (d, 2H, $H_{arom3+5}$, $J$ = 9.01 Hz), 7.12 (d, 2H, $H_{arom2+6}$), 7.25−7.37 (m, 5H, $H_{arom\ benzyl}$). MS *m*/*z*: 380 ($M^+$, 100), 403 ($M^+$ + Na, 5); acc. mass: ($C_{19}H_{22}N_2O_2Cl_2$) calcd 380.1058, found 380.1040. Anal. ($C_{19}H_{22}N_2O_2Cl_2$) H, N; C required 59.85, found 60.26%.

**Di-*tert*-butyl 4-{[2′-(*N*,*N*-bis(2″-chloroethyl)amino)-phenyl] carbamoyloxy methyl}-phenyl-oxycarbonyl-L-glutamate (23a) and di-*tert*-butyl 4-{[2′-(*N*,*N*-(2″-mesyl-oxyethyl)(2″-chloroethyl)amino)phenyl]carbamoyloxy-methyl}phenyloxycarbonyl-L-glutamate (24a).** To a stirred solution of 0.348 g (0.44 mmol) **15a** in 10 mL of acetonitrile heated at 50−60 °C (in the bath), 0.234 g (4.0 mmol) of NaCl (finely grounded) were added at once. After 48 h the reaction was stopped, the solvent evaporated under vacuum. The oily residue containing a mixture of **23a** and **24a** was dissolved in 10 mL of AcOEt, washed, dried and evaporated again. The product was purified by preparative HPLC (cycloxexane:AcOEt 3:1). The first separated fraction afforded **23a** as a glassy solid (0.149 g, 50%), mp 40−41 °C. $^1$H NMR, $\delta_H$, (ppm): 1.40 (s, 9H, $CO_2$-*t*-Bu), 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.78−2.03 (2m, 2H, $CH_2$-CH(NH)), 2.34 (t, 2H, $CH_2CO_2$), 3.26 (t, 4H, $NCH_2$, $J$ = 6.07 Hz), 3.55 (t, 4H, $CH_2Cl$), 3.95−4.01 (m, 1H, -$CH$(NH)$CH_2$), 5.15 (s, 2H, $PhCH_2$), 7.02−7.10 (m, 3H, $H_{arom3+5}$ + $H_{arom5'(4')}$), 7.12 (q, 1H, $H_{arom4'(5')}$, $J_o$ = 7.75 Hz, $J_m$ = 1.20 Hz), 7.40 (d, 2H, $H_{arom2+6}$, $J$ = 8.61 Hz), 7.96 (d, 1H, $H_{arom6'}$, $J$ = 8.18 Hz), 8.10 (d, 1H, NH-G, $J$ = 7.91 Hz), 8.67 (s, 1H, Ph′-NH). MS, *m*/*z*: 668 ($M^+$ + 1, 100), 690 ($M^+$ + Na, 50), 632 ($M^+$ − Cl, 55); acc. mass: ($C_{32}H_{44}N_3O_8Cl_2Na$) calcd 690.2325; found 690.2350. Anal. ($C_{32}H_{43}N_3O_8Cl_2$): C, H, N.

**Di-*tert*-butyl 4-{[2′-(*N*,*N*-(2″-mesyloxyethyl)(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamate (24a)** resulted as a glassy solid (0.065 g, 20.5%), mp 36−37 °C, as the second fraction from the HPLC separation. $^1$H NMR, $\delta_H$, (ppm): 1.41 (s, 9H, $CO_2$-*t*-Bu), 1.42 (s, 9H, $CO_2$-*t*-Bu), 1.80−2.05 (2m, 2H, $CH_2$CH(NH)), 2.34 (m, 2H, $CH_2CO_2$), 3.04 (s, 6H, $CH_3SO_3$), 3.28 (t, 4H, $NCH_2$), 3.55 (t, 2H, $CH_2Cl$, $J$ = 6.02 Hz), 3.95−4.02 (m, 1H, -$CH$(NH)$CH_2$), 4.12 (t, 2H, $CH_2OMes$, $J$ = 5.34 Hz), 5.15 (s, 2H, $PhCH_2$), 7.03−7.11 (m, 3H, $H_{arom3+5}$ + $H_{arom5'(4')}$), 7.20 (t, 1H, $H_{arom4'(5')}$, $J$ = 7.75 Hz), 7.43 (d, 2H, $H_{arom2+6}$, $J$ = 8.47 Hz), 7.96 (q, 1H, $H_{arom6'}$, $J_o$ = 8.15 Hz, $J_m$ = 1.27 Hz), 8.10 (d, 1H, NH-G, $J$ = 7.87 Hz), 8.53 (s, 1H, Ph′-NH). MS, *m*/*z*: 728 ($M^+$ + 1, 30), 750 ($M^+$ + Na, 100), 691 ($M^+$ + 1-HCl, 20); acc. mass: ($C_{33}H_{46}N_3O_{11}$-SClNa) calcd 750.2439; found 750.2460. Anal. ($C_{33}H_{46}N_3O_{11}$-SCl): C, H, N.

**4-Bis(2′-chloroethyl)amino-*N*-*tert*-butyloxycarbonyl-aniline (51a) and 2-[(2′-chloroethyl)(2′-mesyloxyethyl)-amino]-*N*-*tert*-butyloxycarbonylaniline (52a)** were obtained by the same method. After HPLC separation (cyclohexane:AcOEt 1.5:1) **51a** resulted from the first fraction as an oil (0.195 g, 26.4%). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, O-*t*-Bu), 3.25 (m, 4H, $NCH_2$, $J$ = 5.85 Hz), 3.55 (t, 4H, $CH_2Cl$), 7.02 (t, 1H, $H_{arom4(5)}$, $J$ = 7.90 Hz), 7.17 (t, 1H, $H_{arom5(4)}$), 7.36 (d, 1H, $H_{arom3}$, $J$ = 7.87 Hz), 7.92 (d, 1H, $H_{arom6}$, $J$ = 8.00 Hz), 8.34 (s, 1H, PhNH); MS, *m*/*z*: 333 ($M^+$ + 1, 40), 355 ($M^+$ + Na, 3), 296 ($M^+$ − Cl, 40), 277 ($M^+$ − t-Bu + 1, 90); acc. mass: ($C_{15}H_{23}N_2O_2$-$Cl_2$) calcd 333.1137, found 333.1150. Anal. ($C_{15}H_{22}N_2O_2Cl_2$) C, H, N.

From the second fraction **52a** resulted as an oil (0.164 g, 19.1%). $^1$H NMR, $\delta_H$, (ppm): 1.45 (s, 9H, O-*t*-Bu), 3.13 (s, 3H, $CH_3SO_3$), 3.29 (t, 4H, $NCH_2$), 3.55 (t, 2H, $CH_2Cl$, $J$ = 5.76 Hz), 4.10 (t, 2H, $CH_2OMes$, $J$ = 5.08 Hz), 7.03 (t, 1H, $H_{arom4(5)}$, $J_o$ = 7.35 Hz), 7.17 (t, 1H, $H_{arom5(4)}$), 7.36 (d, 1H, $H_{arom3}$, $J_o$ = 7.76 Hz), 7.91 (dd, 1H, $H_{arom6}$, $J_o$ = 8.05 Hz), 8.35 (s, 1H, PhNH). MS, *m*/*z*: 415 ($M^+$ + 23, 25), 393 ($M^+$ + 1, 65), 356 ($M^+$ + 1 − HCl, 20); acc. mass: ($C_{16}H_{26}N_2O_5SCl$) calcd 393.1251; found 393.1260. Anal. ($C_{16}H_{26}N_2O_5SCl$): C, H, N, Cl; S required 8.16, found 7.70%.

**4-{[4′-(*N*,*N*-Bis(2″-bromoethyl)amino)phenyl]-carbamoyloxymethyl}phenylcarbamoyl-L-glutamic Acid (32b). 19b** (75 mg, 0.1 mmol) and Pd tetrakis(triphenylphosphine) (5 mg, 5 $\mu$mol) were dissolved in 4 mL of $CH_2Cl_2$. Pyrrolidine (30 $\mu$L, 0.36 mmol) was added in one portion. After 30 min stirring, the solution was diluted with AcOEt. The pyrrolidine salt of the deprotected carboxylic acid precipitated at once. The reaction mixture was partially evaporated, and the remaining solvent was diluted with AcOEt and concentrated to remove $CH_2Cl_2$ selectively. The precipitate left in the flask after discarding the solvent was washed twice with AcOEt, dried, dissolved in 8 mL of methanol, and eluted through a column loaded with 40 cm$^3$ IRC50 resin (H form) previously washed with MeOH. After evaporation the elute yield **32b** (56 mg, 84%) as a white solid, mp 105−107 °C. $^1$H NMR, $\delta_H$, (ppm): 1.70−2.10 (2m, 2H, $CH_2$CH(NH)), 2.25−2.35 (m, 2H, $CH_2CO_2$), 3.52 (t, 4H, $NCH_2$, $J$ = 7.50 Hz), 3.71 (t, 4H, $CH_2Br$), 4.10−4.25 (m, 1H, $CH$(NH)$CH_2$), 5.00 (s, 2H, $PhCH_2$), 6.48 (d, 1H, NH-G, $J$ = 7.95 Hz), 6.66 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.83 Hz), 7.28 (d, 4H, $H_{arom2+6}$ + $H_{arom2'+6'}$, $J$ = 8.41 Hz), 7.39 (d, 2H, $H_{arom3+5}$, $J$ = 8.17 Hz), 8.68 (s, 1H, PhNH), 9.38 (s, 1H, Ph′NH). MS, *m*/*z*: 665 ($M^+$ + Na, 15); acc. mass: ($C_{24}H_{28}N_4O_7Br_2Na$) calcd 665.0222, found 665.0211.

Using this deprotection procedure the following compounds were synthesized:

**4-{[4′-(*N*,*N*-Bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid (31b)** was obtained from diallyl ester **18b** by a similar procedure

but using morpholine instead of pyrrolidine as allyl scavenger. **31b** (0.045 g, 61%) resulted as a solid, mp 110−112 °C. $^1$H NMR, $\delta_H$, (ppm): 1.75−2.15 (2m, 2H, $CH_2$CH(NH)), 2.20−2.35 (m, 2H, $CH_2CO_2$), 3.25−3.35 (t, 4H, $NCH_2$, under $H_2O$), 3.67 (t, 4H, $CH_2I$), 4.15−4.30 (m, 1H, $CH$(NH)$CH_2$), 5.00 (s, 2H, $PhCH_2$), 6.48 (d, 1H, NH-G, $J$ = 8.53 Hz), 6.62 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.55 Hz), 7.28 (d, 4H, $H_{arom2+6}$ + $H_{arom2'+6'}$, $J$ = 8.38 Hz), 7.39 (d, 2H, $H_{arom3+5}$, $J$ = 8.09 Hz), 8.68 (s, 1H,PhNH), 9.38 (s, 1H, Ph′NH); MS, *m*/*z*: 738 ($M^+$, 20), 761 ($M^+$ + Na, 25); acc. mass: ($C_{24}H_{28}N_4O_7I_2Na$) calcd: 760.9945, found: 760.9960.

**4-{[2′-(*N*,*N*-Bis(2″-iodoethyl)amino)-phenyl]-carbamoyloxymethyl}-phenyl-carbamoyl-L-glutamic acid (31a)** was obtained from diallyl ester **18a** by a similar procedure using morpholine. A solid (0.100 g, 93.4%) resulted, mp 96−98 °C. $^1$H NMR, $\delta_H$, (ppm): 1.72−2.08 (2m, 2H, $CH_2$CH(NH)), 2.29 (t, 2H, $CH_2CO_2$, $J$ = 6.25 Hz), 3.16−3.26 (m, 8H, N($CH_2CH_2I$)$_2$), 4.18−4.22 (m, 1H, $CH$(NH)$CH_2$), 5.09 (s, 2H, $PhCH_2$), 6.45 (d, 1H, NH-G, $J$ = 7.98 Hz), 7.04 (dt, 1H, $H_{arom4'(5')}$, $J_o$ = 7.62, $J_m$ = 1.61 Hz), 7.17 (dt, 1H, $H_{arom5'(4')}$, $J_o$ = 7.78, $J_m$ = 1.39 Hz), 7.28 (d, 1H, $H_{arom3+5}$, $J$ = 8.60 Hz), 7.38 (d, 2H, $H_{arom2+6}$, $J$ = 8.40 Hz), 7.92 (dd, 1H, $H_{arom6'}$, $J_o$ = 8.16, $J_m$ = 1.32 Hz), 8.51 (s, 1H, NH−Ph), 8.65 (s, 1H, Ph′NH); MS, *m*/*z*: 739 ($M^+$ + 1, 30), 761 ($M^+$ + Na, 100), 611 ($M^+$ − HI + 1, 25); acc. mass: ($C_{24}H_{28}N_4O_7I_2Na$) calcd: 760.9945, found: 760.9969. Anal. ($C_{24}H_{28}N_3O_8I_2.0.8AcOEt$): C, H, N.

**4-{[2′-(*N*,*N*-Bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamic Acid (32a).** The previous described procedure using pyrrolidine led to **32a** (0.068 g, 95.6%) as a solid, mp 94−95 °C. $^1$H NMR, $\delta_H$, (ppm): 1.84−2.03 (2m, 2H, $CH_2$CH(NH)), 2.27 (t, 2H, $CH_2CO_2$, $J$ = 7.18 Hz), 3.30 (t, 4H, $NCH_2$, $J$ = 5.69 Hz), 3.42 (t, 4H, $CH_2$-Br), 4.13−4.21 (m, 1H, $CH$(NH)$CH_2$), 5.06 (s, 2H, $PhCH_2$), 6.47 (d, 1H, NH-G, $J$ = 7.84 Hz), 7.04 (dt, 1H, $H_{arom4'(5')}$, $J_o$ = 8.68, $J_m$ = 1.56 Hz), 7.18 (dt, 1H, $H_{arom5'(4')}$, $J_o$ = 7.78, $J_m$ = 1.43 Hz), 7.25 (d, 1H, $H_{arom3+5}$, $J$ = 8.64 Hz), 7.37 (d, 2H, $H_{arom2+6}$), 7.94 (dd, 1H, $H_{arom6'}$, $J$ = 8.16, $J_m$ = 1.24 Hz), 8.61 (s, 1H, NHPh), 8.69 (s, 1H, Ph′NH). MS, *m*/*z*: 645 ($M^+$ + 1, 6), 667 ($M^+$ + Na, 55), 611 ($M^+$ − Br, 16); acc. mass: ($C_{24}H_{28}N_4O_7$-$Br_2Na$) calcd: 760.9945, found: 760.9969. Anal. ($C_{24}H_{27}N_3O_8$-$Br_2$): C, H; N required 8.70, found 8.16.

**4-{[2′-(*N*,*N*-Bis(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid (33a)** was obtained as a solid (0.054 g, 69.5%), mp 93−94 °C using morpholine as allyl scavanger. $^1$H NMR, $\delta_H$, (ppm): 1.77−2.00 (2m, 2H, $CH_2$CH(NH)), 2.28 (t, 2H, $CH_2CO_2$, $J$ = 7.49 Hz), 3.25 (t, 4H, $NCH_2$, $J$ = 5.82 Hz), 3.55 (t, 4H, $CH_2Cl$), 4.14−4.22 (m, 1H, $CH$(NH)$CH_2$), 5.07 (s, 2H, $PhCH_2$), 6.48 (d, 1H, NH-G, $J$ = 7.77 Hz), 7.05 (t, 1H, $H_{arom4'(5')}$, $J_o$ = 7.56 Hz), 7.19 (t, 1H, $H_{arom5'(4')}$), 7.25 (d, 1H, $H_{arom3+5}$, $J$ = 8.53 Hz), 7.37 (d, 2H, $H_{arom2+6}$), 7.94 (d, 1H, $H_{arom6'}$, $J$ = 8.10 Hz), 8.62 (s, 1H, NH−Ph), 8.71 (s, 1H, Ph′NH). MS, *m*/*z*: 577 ($M^+$ + Na, 100); acc. mass: ($C_{24}H_{28}N_4O_7Cl_2Na$) calcd: 577.1254, found: 577.1254. Anal. ($C_{24}H_{27}N_3O_8Cl_2$·0.6$H_2O$): C, H, N.

**4-{*N*-[4′-Bis(2″-iodoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid (39b)** was obtained as a solid using pyrrolidine as allyl scavanger, (0.105 g, 77.8%), mp 103−105 °C. $^1$H NMR, $\delta_H$, (ppm): 1.75−2.00 (2m, 2H, $CH_2$CH(NH)), 2.28 (t, 2H, $CH_2CO_2$, $J$ = 7.43 Hz), 3.14 (s, 3H, $NCH_3$), 3.30 (t, 4H, $NCH_2$), 3.72 (t, 4H, $CH_2I$, $J$ = 7.64 Hz), 4.10−4.25 (m, 1H, $CH$(NH)$CH_2$), 4.96 (s, 2H, $PhCH_2$), 6.46 (d, 1H, NH-G, $J$ = 7.48 Hz), 6.64 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.85 Hz), 7.10 (d, 2H, $H_{arom2'+6'}$), 7.17 (d, 2H, $H_{arom2+6}$, $J$ = 7.49 Hz), 7.34 (d, 2H, $H_{arom3+5}$), 8.68 (s, 1H, PhNH). MS *m*/*z*: 752 ($M^+$ + 1, 10), 775 ($M^+$ + Na, 35); acc. mass: ($C_{25}H_{30}N_4O_7I_2Na$) calcd 775.0102, found 775.0088.

**4-{*N*-[4′-Bis(2″-bromoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid (40b)** was obtained as a solid using pyrrolidine as allyl scavanger, (0.10 g, 80%), mp 75−77 °C. $^1$H NMR, $\delta_H$, (ppm): 1.80−1.95 (m, 2H, $CH_2$CH(NH)), 2.28 (t, 2H, $CH_2CO_2$, $J$ = 7.33 Hz), 3.14 (s, 3H, $NCH_3$), 3.71 (s, 8H, N($CH_2CH_2Cl$)$_2$), 4.10−4.20 (m, 1H, $CH$(NH)$CH_2$), 4.95 (s, 2H, $PhCH_2$), 6.48 (d, 1H, NH-G, $J$ = 7.17 Hz), 6.68 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.74 Hz), 7.09 (d, 2H, $H_{arom2'+6'}$), 7.18 (d, 2H, $H_{arom2+6}$), 7.34 (d, 2H, $H_{arom3+5}$, $J$ = 8.45 Hz), 8.74 (s, 1H, PhNH). MS *m*/*z*: 658 ($M^+$, 8), 681 ($M^+$ + Na, 15); acc. mass: ($C_{25}H_{30}N_4O_7Br_2Na$) calcd 679.0379, found 679.0386.

**4-{*N*-[4′-Bis(2″-chloroethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenylcarbamoyl-L-glutamic acid (41 b)** was obtained as a solid using pyrrolidine as allyl scavanger, (0.075 g, 90%), mp 70−72 °C. $^1$H NMR, $\delta_H$, (ppm): 1.75−2.00 (2m, 2H, $CH_2$CH(NH)), 2.27 (t, 2H, $CH_2CO_2$), 3.14 (s, 3H, $NCH_3$), 3.71 (s, 8H, N($CH_2CH_2Cl$)$_2$), 4.10−4.20 (m, 1H, $CH$(NH)$CH_2$), 4.95 (s, 2H, $PhCH_2$), 6.49 (d, 1H, NH-G), 6.70 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.60 Hz), 7.09 (d, 2H, $H_{arom2'+6'}$), 7.18 (d, 2H, $H_{arom2+6}$), 7.34 (d, 2H, $H_{arom3+5}$, $J$ = 8.14 Hz), 8.71 (s, 1H, PhNH). MS *m*/*z*: 568 ($M^+$, 25), 591 ($M^+$ + Na, 100); acc. mass: ($C_{25}H_{30}N_4O_7Cl_2Na$) calcd 591.1389, found 591.1371.

**4-{[4′-(*N*,*N*-Bis(2″-mesyloxyethyl)amino)phenyl]carbamoyloxymethyl}phenyl-oxycarbonyl-L-glutamic Acid (34b).** Di-*tert*-butyl ester **15b** (0.10 g, 0.13 mmol) was dissolved in formic acid (5 mL) and stirred under argon at room temperature for 48 h. The formic acid was evaporated under vacuum (pump), and the residue was evaporated five times with $CH_2Cl_2$ and toluene to yield **34b** as a foamy solid (0.078 g, 89%), mp 92−95 °C. $^1$H NMR, $\delta_H$, (ppm): 1.75−2.15 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2CO_2$, $J$ = 7.81 Hz), 3.13 (s, 6H, $CH_3SO_3$), 3.67 (t, 4H, N$CH_2$, $J$ = 5.22 Hz), 4.00−4.10 (m, 1H, $CH$(NH)$CH_2$), 4.28 (t, 4H, $CH_2$OMes), 5.10 (s, 2H, $PhCH_2$), 6.75 (d, 2H, $H_{arom3'+5'}$, $J$ = 6.62 Hz), 7.11 (d, 2H, $H_{arom3+5}$, $J$ = 8.37 Hz), 7.28 (d, 2H, $H_{arom2'+6'}$), 7.42 (d, 2H, $H_{arom2+6}$), 8.04 (d, 1H, NH-G, $J$ = 6.40 Hz), 9.33 (s, 1H, Ph′NH). MS, *m*/*z*: 676 ($M^+$ + 1, 100); acc. mass: ($C_{26}H_{34}N_3O_{14}S_2$), calcd 676.1482, found: 676.1470. Anal. ($C_{26}H_{33}N_3O_{14}S_2$): C, H, N.

The following compounds were prepared by the same procedure:

**4-{[4′-(*N*,*N*-Bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (35b)** was obtained from di-*tert*-butyl ester **21b** as a solid (0.065 g, 73%), mp 117−120 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm)**:** 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2CO_2$, $J$ = 6.80 Hz), 3.20−3.40 (t, 4H, $NCH_2$, under $H_2O$), 3.72 (t, 4H, $CH_2I$), 4.00−4.10 (m, 1H, $CH$(NH)$CH_2$), 5.10 (s, 2H, $PhCH_2$), 6.64 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.77 Hz), 7.12 (d, 2H, $H_{arom2+6}$, $J$ = 8.29 Hz), 7.28 (d, 2H, $H_{arom2'+6'}$), 7.42 (d, 2H, $H_{arom3+5}$), 8.06 (d, 1H, NH-G, $J$ = 7.45 Hz), 9.35 (s, 1H, Ph′NH). MS, *m*/*z*: 740 ($M^+$ + 1, 100), 762 ($M^+$ + Na, 45), ($M^+$ − I, 30); acc. mass: ($C_{24}H_{28}N_3O_8I_2$) calcd 739.9966; found 739.9980. Anal. ($C_{24}H_{27}N_3O_8I_2$): C, H, N.

**4-{[4′-(*N*,*N*-Bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (36b)** was obtained from di-*tert*-butyl ester **22b** as a solid (0.065 g, 73%), mp 73−76 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2$-$CO_2$), 3.55 (t, 4H, $NCH_2$), 3.72 (t, 4H, $CH_2Br$), 4.00−4.15 (m, 1H, $CH$(NH)$CH_2$), 5.10 (s, 2H, $PhCH_2$), 6.69 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.55 Hz), 7.12 (d, 2H, $H_{arom2+6}$, $J$ = 8.28 Hz), 7.29 (d, 2H, $H_{arom2'+6'}$), 7.42 (d, 2H, $H_{arom3+5}$), 8.02 (d, 1H, NH-G), 9.32 (s, 1H, Ph′-NH). MS, *m*/*z*: 646 ($M^+$ + 1, 100), 668 ($M^+$ + Na, 55); acc. mass: ($C_{24}H_{28}N_3O_8Br_2$) calcd 644.0243; found 644.0230. Anal. ($C_{24}H_{27}N_3O_8Br_2$): H, N; C required 44.67, found 45.09%.

**4-{[2′-(*N*,*N*-Bis(2″-mesyloxyethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (34a)** resulted as a foamy solid (0.063 g, 99%), mp 56−57 °C. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.04 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2CO_2$, $J$ = 7.96 Hz), 3.04 (s, 6H, $CH_3SO_3$), 3.30 (t, 4H, $NCH_2$, $J$ = 5.21 Hz), 4.00−4.05 (m, 1H, $CH$(NH)$CH_2$), 4.11 (t, 4H, $CH_2$OMes), 5.13 (s, 2H, $PhCH_2$), 7.03−7.19 (m, 4H, $H_{arom3+5}$, $H_{arom4'}$, $H_{arom5'}$), 7.44 (d, 2H, $H_{arom2+6}$, $J$ = 8.56 Hz), 7.95 (d, 2H, $H_{arom6'}$, $J$ = 7.96 Hz), 8.10 (d, 1H, NH-G, $J$ = 8.10 Hz), 8.42 (s, 1H, Ph′NH). MS, *m*/*z*: 676 ($M^+$ + 1, 18), 698 ($M^+$ + 23, 10); acc. mass: ($C_{26}H_{34}N_3O_{14}S_2$), calcd 676.1482, found: 676.1460. Anal. ($C_{26}H_{33}N_3O_{14}S_2$.0.8 $HCO_2H$): C, H, N.

**4-{[2′-(*N*,*N*-Bis(2″-iodoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (35a)** was obtained from di-*tert*-butyl ester **21a** as a solid (0.096 g, 93.2%), mp 56−58 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.86−2.11 (2m, 2H, $CH_2$CH(NH)), 2.36 (t, 2H, $CH_2$-

$CO_2$, $J$ = 7.02 Hz), 3.20−3.26 (m, 8H, N($CH_2CH_2I$)$_2$), 3.97−4.10 (m, 1H, $CH$(NH)$CH_2$), 5.17 (s, 2H, $PhCH_2$), 7.05−7.18 (m, 4H, $H_{arom2+6}$ + $H_{arom4'(5')}$ + $H_{arom4'(5')}$), 7.34 (d, 1H, $H_{arom3'}$, $J$ = 7.80 Hz), 7.43 (d, 2H, $H_{arom2+6}$, $J$ = 8.40 Hz), 7.93 (d, 1H, $H_{arom6'}$, $J$ = 8.13 Hz), 8.06 (d, 1H, NH-G, $J$ = 7.99 Hz), 8.57 (s, 1H, Ph′NH). MS, *m/z*: 740 ($M^+$ + 1, 100), 762 ($M^+$ + Na, 45), ($M^+$ − I, 100); acc. mass: ($C_{24}H_{28}N_3O_8I_2$) calcd 739.9966; found 739.9990. Anal. ($C_{24}H_{27}N_3O_8I_2$+0.2$C_7H_8$): C, H, N.

**4-{[2′-(*N*,*N*-Bis(2″-bromoethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (36a)** was obtained from di-*tert*-butyl ester **22a** as a solid (0.081 g, 92%), mp 63−64 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.36 (t, 2H, $CH_2CO_2H$, $J$ = 7.33 Hz), 3.32 (t, 4H, $NCH_2$, $J$ = 5.95 Hz), 3.44 (t, 4H, $CH_2Br$), 3.97−4.10 (m, 1H, -$CH$(NH)$CH_2$), 5.16 (s, 2H, $PhCH_2$), 7.06 (t, 1H, $H_{arom4'(5')}$, $J$ = 7.56 Hz), 7.10 (d, 2H, $H_{arom3+5}$, $J$ = 7.99 Hz), 7.19 (t, 1H, $H_{arom5'(4')}$, $J$ = 7.63 Hz), 7.41 (d, 2H, $H_{arom2+6}$), 7.95 (d, 1H, $H_{arom6'}$, $J$ = 8.08 Hz), 8.03 (d, 1H, NH-G, $J$ = 7.68 Hz), 8.66 (s, 1H, Ph′NH). MS, *m/z*: 646 ($M^+$ + 1, 35), 668 ($M^+$ + Na, 50), 566 ($M^+$ − Br, 45); acc. mass: ($C_{24}H_{28}N_3O_8Br_2$) calcd 644.0243; found 644.0220. Anal. ($C_{24}H_{27}N_3O_8Br_2$+0.3$C_7H_8$): C, H, N.

**4-{[2′-(*N*,*N*-Bis(2″-chloroethyl)amino)phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (37a)** was obtained from di-*tert*-butyl ester **23a** as a solid (0.111 g, 74%), mp 55−56 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.70−2.07 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2CO_2$), 3.27 (t, 4H, $NCH_2$, $J$ = 6.00 Hz), 3.56 (t, 4H, $CH_2Cl$), 4.00−4.07 (m, 1H, -$CH$(NH)$CH_2$), 5.16 (s, 2H, $PhCH_2$), 7.02−7.12 (m, 3H, $H_{arom3+5}$, $H_{arom4'(5')}$), 7.20 (dt, 1H, $H_{arom5'(4')}$, $J_o$ = 7.76 Hz, $J_m$ = 1.47 Hz), 7.40 (d, 2H, $H_{arom2+6}$,), 7.96 (dd, 2H, $H_{arom6'}$, $J_o$ = 7.96 Hz, $J_m$ = 1.27 Hz), 8.08 (d, 1H, NH-G, $J$ = 8.08 Hz), 8.67 (s, 1H, Ph′NH). MS, *m/z*: 556 ($M^+$ + 1, 30), 578 ($M^+$ + 23, 10); acc. mass: ($C_{24}H_{28}N_3O_8Cl_2$) calcd 556.1253; found 556.1270. Anal. ($C_{24}H_{27}N_3O_8Cl_2$): C, H, N.

**4-{[2′-(*N*,*N*-(2″-Chloroethyl)(2″-mesyloxyethyl)amino)-phenyl]carbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (38a)** was obtained from di-*tert*-butyl ester **24a** as a solid (0.058 g, 94.1%), mp 61−63 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.05 (2m, 2H, $CH_2$CH(NH)), 2.36 (t, 2H, $CH_2CO_2$, $J$ = 8.01 Hz), 3.03 (s, 3H, $CH_3SO_2$), 3.28 (t, 4H, $NCH_2$, $J$ = 5.37 Hz), 3.54 (t, 2H, $CH_2Cl$, $J$ = 5.92 Hz), 3.95−4.02 (m, 1H, -$CH$(NH)$CH_2$), 4.11 (t, 2H, $CH_2OMes$), 5.14 (s, 2H, $PhCH_2$), 7.02−7.22 (m, 4H, $H_{arom3+5}$, $H_{arom4'}$, $H_{arom5'}$), 7.42 (d, 2H, $H_{arom2+6}$, $J$ = 8.40 Hz), 7.95 (d, 2H, $H_{arom6'}$, $J$ = 7.88 Hz), 8.08 (d, 1H, NH-G, $J$ = 7.98 Hz), 8.67 (s, 1H, Ph′NH). MS, *m/z*: 615 ($M^+$, 100), 638 ($M^+$ + Na, 17); acc. mass: ($C_{25}H_{30}N_3O_{11}ClS$) calcd 615.1275, found 615.1290. Anal. ($C_{25}H_{30}N_3O_{11}ClS$): C, H, N.

**4-{*N*-[4′-Bis(2″-iodoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (42b)** was obtained from di-*tert*-butyl ester **28b** as a solid (0.53 g, 83.9%), mp 70−72 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.15 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2CO_2$, $J$ = 9.00 Hz), 3.16 (s, 3H, $NCH_3$), 3.25−3.35 (t, 4H, $NCH_2$), 3.72 (t, 4H, $CH_2I$, $J$ = 7.62 Hz), 4.00−4.10 (m, 1H, $CH$(NH)$CH_2$), 5.05 (s, 2H, $PhCH_2$), 6.65 (d, 2H, $H_{arom3'+5'}$, $J$ = 8.95 Hz), 7.07 (d, 2H, $H_{arom3+5}$, $J$ = 8.46 Hz), 7.12 (d, 2H, $H_{arom2'+6'}$), 7.29 (d, 2H, $H_{arom2+6}$), 8.10 (d, 1H, NH-G, $J$ = 7.81 Hz). MS *m/z*: 753 ($M^+$, 43), 776 ($M^+$ + Na, 43); acc. mass: ($C_{25}H_{29}N_3O_8I_2$) calcd 753.0022, found 753.0044.

**4-{*N*-[4′-Bis(2″-bromoethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (43b)** was obtained from di-*tert*-butyl ester **29b** as a solid (0.437 g, 85.3%), mp 64−67 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.37 (t, 2H, $CH_2CO_2$, $J$ = 8.05 Hz), 3.16 (s, 3H, $NCH_3$), 3.58 (t, 4H, $NCH_2$, $J$ = 6.84 Hz), 3.77 (t, 4H, $CH_2Br$), 4.00−4.10 (m, 1H, $CH$(NH)$CH_2$), 5.05 (s, 2H, $PhCH_2$), 6.70 (d, 2H, $H_{arom3'+5'}$, $J$ = 9.04 Hz), 7.07 (d, 2H, $H_{arom3+5}$, $J$ = 8.50 Hz), 7.12 (d, 2H, $H_{arom2'+6'}$), 7.29 (d, 2H, $H_{arom2+6}$), 8.10 (d, 1H, NH-G, $J$ = 8.07 Hz). MS *m/z*: 659 ($M^+$, 90), 682 ($M^+$ + Na, 95); acc. mass: ($C_{25}H_{29}N_3O_8Br_2$) calcd 657.0321, found 657.0340.

**4-{*N*-[4′-Bis(2″-chloroethyl)aminophenyl]-*N*-methylcarbamoyloxymethyl}phenyloxycarbonyl-L-glutamic acid (44b)** was obtained from di-*tert*-butyl ester **30b** as a solid (0.355 g, 81.5%), mp 60−63 °C, by a similar procedure. $^1$H NMR, $\delta_H$, (ppm): 1.80−2.10 (2m, 2H, $CH_2$CH(NH)), 2.36 (t, 2H, $CH_2CO_2$, $J$ = 7.95 Hz), 3.16 (s, 3H, $NCH_3$), 3.72 (s, 8H, N($CH_2CH_2Cl$)$_2$), 3.95−4.10 (m, 1H, $CH$(NH)$CH_2$), 5.05 (s, 2H, $PhCH_2$), 6.72 (d, 2H, $H_{arom3'+5'}$, $J$ = 9.02 Hz), 7.08 (d, 2H, $H_{arom3+5}$, $J$ = 9.72 Hz), 7.11 (d, 2H, $H_{arom2'+6'}$), 7.31 (d, 2H, $H_{arom2+6}$), 8.06 (d, 1H, NH-G). MS *m/z*: 569 ($M^+$, 25), 592 ($M^+$ + Na, 30); acc. mass: ($C_{25}H_{29}N_3O_8Cl_2$) calcd 569.1352, found 569.1332.

**4-Bis(2′-mesyloxyethyl)aminoaniline Hydrobromide (57b).** To a solution containing 0.145 g (0.32 mmol) **48b** in AcOH (1 mL) was added 45% HBr in AcOH solution (2 mL), and the reaction mixture was stirred at room temperature for 10 min. The reaction mixture was added dropwise in dry $Et_2O$ under stirring. A white precipitate was formed, which was centrifugated. The pellet was resuspended in fresh $Et_2O$ (40−45 mL) and centrifugated again. The procedure was repeated five times. **57b**·2HBr was obtained as a hygroscopic solid (0.12 g, 73%).$^1$H NMR, $\delta_H$, (ppm): 3.15 (s, 6H, $CH_3SO_3$), 3.75 (t, 4H, $NCH_2$, $J$ = 5.55 Hz), 4.32 (t, 4H, $CH_2OMes$), 6.89 (d, 2H, $H_{arom3+5}$, $J$ = 9.07 Hz), 7.18 (d, 2H, $H_{arom2+6}$), 9.57 (s, 3H, $NH_3^+$). Anal. ($C_{12}H_{20}N_2O_6S_2$.1.8HBr): C, H, N.

The following compounds were obtained by the same procedure:

**4-Bis(2′-iodoethyl)aminoaniline hydrobromide (58b)** was obtained as a solid (0.105 g, 94%), mp 155−158 °C. $^1$H NMR, $\delta_H$, (ppm): 3.31 (t, 4H, $NCH_2$, $J$ = 7.72 Hz), 3.74 (t, 4H, $CH_2I$), 6.77 (d, 2H, $H_{arom3+5}$, $J$ = 8.87 Hz), 7.19 (d, 2H, $H_{arom2+6}$), 9.62 (s, 3H, $NH_3^+$). MS, *m/z*: 417 ($M^+$ + 1, 100); acc. mass: ($C_{10}H_{15}N_2I_2$) calcd 416.9325; found 416.9340. Anal. ($C_{10}H_{14}N_2I_2$.1.8HBr.0.2$CH_3COOH$): C, H, N.

**4-Bis(2′-bromoethyl)aminoaniline hydrobromide (59b·2HBr)** was obtained as a solid (0.16 g, 100%), mp > 230 °C (decomp).$^1$H NMR, $\delta_H$, (ppm): 3.59 (t, 4H, $NCH_2$, $J$ = 6.99 Hz), 3.79 (t, 4H, $CH_2Br$), 6.82 (d, 2H, $H_{arom3+5}$, $J$ = 7.87 Hz), 7.19 (d, 2H, $H_{arom2+6}$), 9.63 (s, 3H, $NH_3^+$). MS, *m/z*: 323 ($M^+$ + 1, 100); acc. mass: ($C_{10}H_{15}N_2Br_2$) calcd 320.9602; found 320.9587. Anal. ($C_{10}H_{14}N_2Br_2$·1.6HBr·0.2$CH_3COOH$): C, H, N.

**2-Bis(2′-iodoethyl)aminoaniline hydrobromide (58a)** was obtained as a solid (0.195 g, 67.8%), mp < 50 °C. MS, *m/z*: 417 ($M^+$ + 1, 65); acc. mass: ($C_{10}H_{15}N_2I_2$) calcd 416.9325; found 416.9316. Anal. ($C_{10}H_{14}N_2I_2$·HBr): C, H, N.

**2-Bis(2′-bromoethyl)aminoaniline hydrobromide (59a)** was obtained as a solid (0.102 g, 32%), mp 150−151 °C. MS, *m/z*: 323 ($M^+$ + 1, 100); acc. mass: ($C_{10}H_{15}N_2Br_2$) calcd 320.9602; found 320.9594. Anal. ($C_{10}H_{14}N_2Br_2$.HBr): C, H, N.

**2-Bis(2′-chloroethyl)aminoaniline hydrobromide (60a)** was obtained as a solid (0.124 g, 72.1%), mp 147−148 °C. $^1$H NMR, $\delta_H$, (ppm): 3.36 (t, 4H, $NCH_2$, $J$ = 6.70 Hz), 3.60 (t, 4H, $CH_2Cl$), 7.25−7.53 (m, 4H, $H_{arom}$); MS, *m/z*: 223 ($M^+$ + 1, 100), 195 ($M^+$ − Cl, 10); acc. mass: ($C_{10}H_{15}N_2Cl_2$) calcd 233.0612; found 233.0624. Anal. ($C_{10}H_{14}N_2Cl_2$.HBr): C, H, N.

The following compounds were prepared by the same method, except that the reaction time was 1.5 h instead of 10 min:

**4-Bis(2′-iodoethyl)amino-*N*-methylaniline hydrobromide (61b)** was obtained as a solid (0.19 g, 86.5%), mp 140−142 °C. $^1$H NMR, $\delta_H$, (ppm): 2.89 (s, 3H, $NCH_3$), 3.30 (t, 4H, $NCH_2$, $J$ = 7.18 Hz), 3.74 (t, 4H, $CH_2I$), 6.80 (d, 2H, $H_{arom3+5}$, $J$ = 8.90 Hz), 7.33 (d, 2H, $H_{arom2+6}$), 10.28 (s, 3H, $NH_2^+$). MS, *m/z*: 431 ($M^+$ + 1, 100), 453 ($M^+$ + Na, 2); acc. mass: ($C_{11}H_{17}N_2I_2$) calcd 430.9481; found 430.9470. Anal. ($C_{11}H_{16}N_2I_2$.2HBr): H, N; C required 22.32, found 22.78%.

**4-Bis(2′-bromoethyl)amino-*N*-methylaniline hydrobromide (62b)** was obtained as a solid (0.134 g, 90.7%), mp 191−194 °C. $^1$H NMR, $\delta_H$, (ppm): 2.89 (s, 3H, $NCH_3$), 3.59 (t, 4H, $NCH_2$, $J$ = 6.93 Hz), 3.81 (t, 4H, $CH_2Br$), 6.85 (d, 2H, $H_{arom3+5}$, $J$ = 8.79 Hz), 7.33 (d, 2H, $H_{arom2+6}$), 10.29 (s, 3H, $NH_2^+$). MS, *m/z*: 337 ($M^+$ + 1, 100); acc. mass: ($C_{11}H_{17}N_2Br_2$) calcd 334.9758; found 334.9770.

**4-Bis(2′-chloroethyl)amino-*N*-methylaniline hydrobromide (63b)** was obtained as a solid (0.088 g, 80.8%), mp 179−182 °C. $^1$H NMR, $\delta_H$, (ppm): 2.89 (s, 3H, $NCH_3$), 3.74 (s, 8H, $N(CH_2CH_2Cl)_2$), 6.87 (d, 2H, $H_{arom3+5}$, $J$ = 8.55 Hz), 7.32 (d, 2H, $H_{arom2+6}$), 10.23 (s, 3H, $NH_2^+$). MS, *m/z*: 247 ($M^+$ + 1, 100), 269 ($M^+$ + Na, 5); acc. mass: ($C_{11}H_{17}N_2Cl_2$) calcd 247.0769; found 247.0750. Anal. ($C_{11}H_{16}N_2Cl_2.2HBr$): C, H, N.

**Aqueous Half-Life Determination.** Compounds were prepared as 10 mM concentrates in MeOH (**59a**, **60a**) or in DMSO (all others) and diluted 100 fold in CPG2 assay buffer (100 mM Tris-HCl, pH 7.3; 260 $\mu$M $ZnCl_2$; 1 mL) to give 100 $\mu$M solutions. Aliquots (10 $\mu$L) were injected into a Partisphere C18 column (125 × 4.6 mm, 5 $\mu$m, Whatman) (compounds **60a**, **63b**) or a Synergi Polar RP phenyl phase column (150 × 4.6 mm, 4$\mu$m, Phenomenex) (all others) and eluted isocratically (1 mL/min) with 10 mM ammonium acetate (pH 5.0) containing percentages of methanol (65−85%) that gave retention times of 3−4 min. The eluate was monitored at 265−275 nm (**35b**, **36b**, **42b**, **43b**, **44b**) or 250 nm (all others). The amount of starting material remaining after various periods of incubation was determined either by repeat injection from a single vial (**33a**, **37a**, **39b**, **41b**, **42b**, **44b**), or by delayed injections from a new vial each time (all others). The results were expressed as fraction of starting material as a function of time, and the half-life was determined by nonlinear regression to a one-phase exponential decay, constraining the maximum to 1 and the minimum to 0 (GraphPad Prism, GraphPad Software Inc., San Diego, CA). Compounds **58b**, **59a**, **59b**, **61b**, and **62b** proved too labile for half-life determination by HPLC, and a spectrophotometric method was employed. The change in absorbance on dilution into aqueous conditions at a wavelength previously determined to give the largest difference was monitored for 3 min at a sampling rate of 100/min. The data were fitted to a rising or falling exponential by linear regression with no constraints (GraphPad Prism), and the half-life was calculated as 0.69/rate constant. It was established that this method gave similar results to the more unequivocal HPLC method.

**Enzyme Kinetics.** Reactions were set up containing CPG2 assay buffer (1 mL), CPG2 (50 mU) and prodrug (5−50 $\mu$M in steps of 5 $\mu$M) from concentrates as above. The vials were incubated at 37 °C, and the amount of prodrug remaining in the mixture was determined by HPLC as above at 0, 5, 10, 15, and 20 min post start. The rate of loss of compound in $\mu$M/min was determined by regression. The rate of chemical-only loss, calculated from the first derivative of the equation for exponential decay, was subtracted, and the kinetic parameters were derived from nonlinear regression to the Michaelis−Menten equation (GraphPad Prism).

**Cytotoxicity Determination.** The construction of WiDr cells engineered to stably express stCPG2(Q)3 or $\beta$-gal was as previously described for other cell lines. [16,22] Cells ($2 \times 10^6$) were seeded into six-well plates, producing confluent monolayers in 48 h. Compounds were dissolved in DMSO at 10 mM (**31b**, **32b**, **35b**, **36b**), 20 mM (**58b**, **59b**, **61b**, **62b**), or 50 mM (all others), immediately prior to treatment, diluted in culture medium, and added to the wells. A similar concentration of compound solution was added after an incubation of 1 h, and the cells were incubated for an additional 20 h. The cells were harvested and reseeded in quadruplicate in 96-well plates at $\sim 2 \times 10^3$/well and incubated until the control wells achieved confluence. The plates were then fixed and stained with sulforhodamine-B, and the extinction coefficient at 590 nm was determined. The results were expressed as percentage of control growth as a function of log(dose), and the $IC_{50}$ was determined by nonlinear regression to a log dose−effect sigmoid, constraining the minimum to be positive (GraphPad Prism).

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