Design, Synthesis and Biological Evaluation of Glutathione Peptidomimetics as Components of Anti-Parkinson Prodrugs

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Plethoras of CNS-active drugs fail to effect their pharmacologic response due to their in vivo inability to cross the blood−brain barrier (BBB). The classical prodrug approach to overcome this frailty involves lipophilic derivatives of the polar drug, but we herein report a novel approach by which endogenous transporters at BBB are exploited for brain drug delivery. The crucial role played by glutathione in pathogenesis of Parkinson's and the presence of its influx transporters at the basolateral membrane of BBB served as the basis for our anti-Parkinson prodrug design strategy. A metabolically stable analogue of glutathione is used as a carrier for delivery of dopamine and adamantamine. An account of successful syntheses of these prodrugs along with their transport characteristics and stability determination is discussed.

Introduction

Unfavorable physicochemical properties hinder the transport of several entities across the blood−brain barrier (BBB)[a] and thereby limit their utility as CNS-active agents. The brain microvessel endothelium is a barrier toward the passive transport of hydrophilic substances into the brain. Such obstacles to the CNS penetration of therapeutic agents have in the past been circumvented by approaches that bank on the synergy of more than one disciplines; e.g., chemistry-based (lipophilic prodrugs), biology-based approaches (carrier/receptor mediated transport), and drug delivery based (intrathecal/interstitial/olfactory delivery).[1] A prodrug approach that encompasses covalent linkage of the drug to a carrier that can get recognized by a specific transporter protein and hence get transported has garnered interest in recent years.

While the classical prodrug approach to improve membrane permeability of polar drugs uses lipophilic derivatives, increasing their passive membrane penetration, the targeted prodrug approach[2] exploits transporters that mediate transport of polar nutrients such as amino acids and peptides. Therefore, targeting a specific membrane transporter is particularly rewarding when the drug in question is polar or charged. Several examples of the application of this promising strategy have been reported in the literature. The best known example of carrier-mediated drug delivery is the transport of L-dopa, a precursor of the neurotransmitter dopamine, in the treatment of Parkinson's disease by the large neutral amino acid transporter (LNNA).[2,3] Among various membrane transporters, peptide transporters are attractive targets in prodrug design due to their broad substrate specificity[4] and high capacity.[5] The important role played by the antioxidant glutathione in the pathogenesis of Parkinson's disease[6] and presence of glutathione transporters at the BBB caught our attention.[7] Oxidative stress (reactive oxygen species such as the superoxide radical, hydroxide radical, and semiquinone radicals) has been implicated in the progressive degeneration of dopaminergic neurons, which in turn is one of the principal causes of Parkinson's disease. Glutathione, with the help of enzymes glutathione peroxidase and reductase, forms detoxification machinery against these oxidative species. We therefore sought to develop a prodrug strategy targeting glutathione transporters at BBB to deliver anti-Parkinson drugs that normally can not cross the BBB.

Levodopa (L-dopa) remains as the gold standard for the treatment of Parkinson's disease. Although L-dopa enters CNS via the large neutral amino acid transporters at BBB and is enzymatically cleaved in the brain to release dopamine, a large percentage of circulating L-dopa does not penetrate the BBB and, as a consequence, undergoes peripheral decarboxylation to generate metabolites that cause dopamine-related side effects.[8] While this study was in progress, glycoconjugates of dopamine and L-dopa were explored by Bonina and De Caprariis[9] as a means to increase their BBB permeability by carrier- mediated transport (GLUT 1). Several peptidyl prodrugs of dopamine and L-dopa have also been shown to increase the BBB permeability of L-dopa along with improved bioavailability.[5,10] In our attempts at increasing BBB penetration of dopamine, we focused on the glutathione uptake transporters that are located on the luminal side of the BBB. The broad substrate specificity displayed by these transporters provides ample opportunity for rational prodrug design.

The design of glutathione transporter targeted prodrug (Figure 1) involved three components: the carrier, glutathione (GSH), the active drug, and a suitable linker for conjugation of the carrier with the drug molecule. A significant impediment for the direct utilization of GSH as a carrier is the presence of γ-glutamyl transpeptidase (γ-GT), which would cleave the γ-glutamyl−cysteinyl peptide bond, rendering these prodrugs inactive. Incorporation of the metabolically stable urea analogue of glutathione **3**, as a carrier molecule, would be of added benefit due to its known stability against γ-GT cleavage[11,12] and retention of crucial structural features necessary for recognition by glutathione transporters. In addition, release of such an antioxidant molecule would most likely contribute in attenuating the damage by the oxidative stress generated in Parkinson's disease.

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[a] Abbreviations: BBB, blood−brain barrier; LNAA, large neutral amino acid transporter; GSH, glutathione; MDCK, Madin Darby canine kidney; γ-GT, γ-glutamyl transpeptidase.

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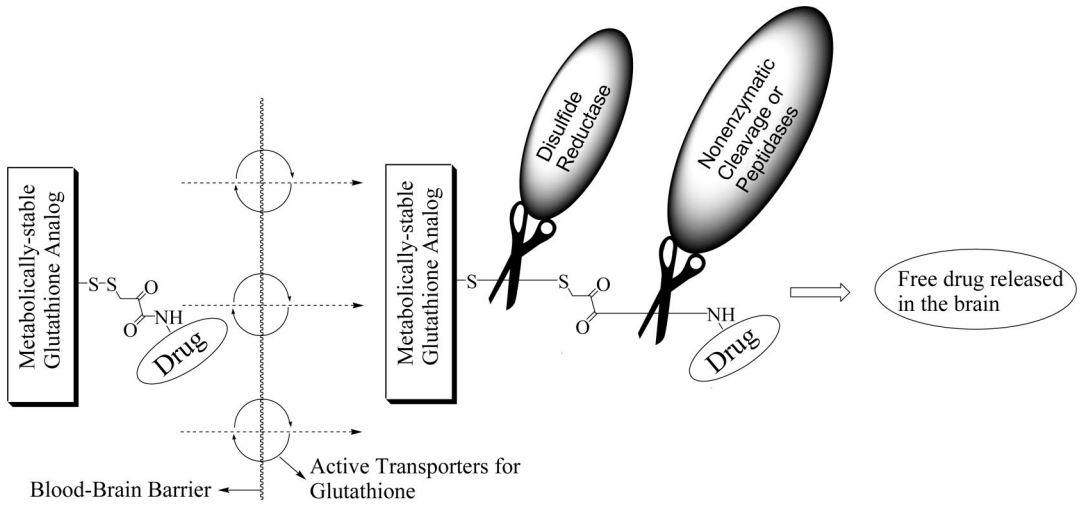


Figure 1. Prodrug design rationale.

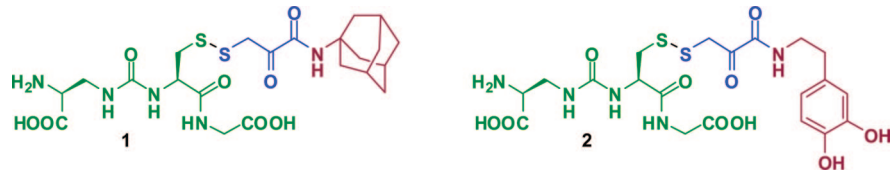


Figure 2. Structures of anti-Parkinson's prodrugs of adamantamine (**1**) and dopamine (**2**). Shown in green is the carrier, metabolically stable glutathione analogue; in blue is the linker, mercaptopyruvic acid, and in purple is the active drug moiety.

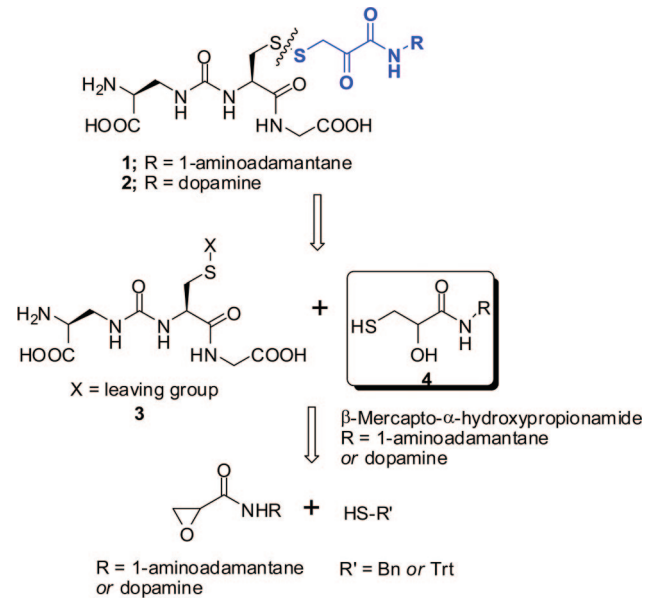
Versatility was regarded to be paramount in the structural design of these conjugates because the linkage of a pharmacologically benign or beneficial carrier to a multitude of CNS-active drugs was the ultimate goal. The success of any prodrug design is dependent on the identity of the linker between the carrier and the drug. Such a linker should fulfill two requirements: first, it should be able to release the active drug at the target site, preferably by a selective enzymatic reaction, and second, the cleavage products should be nontoxic. Because of these aspects, mercaptopyruvate, the metabolite of cysteine, was especially attractive. The carrier glutathione analogue was attached to the linker via a heterodisulfide linkage due to the known stability of this linkage in plasma and the abundance of enzyme disulfide reductase in the brain compared to other body compartments.[13] The amine functional group of dopamine/adamantamine was attached to the linker by an amide bond, which is expected to be cleaved by peptidases in the brain with reasonable stability in the plasma.

Specifically, this account includes our synthetic efforts to arrive at these anti-Parkinson's prodrugs **1** and **2** (Figure 2). The biological evaluation of these prodrugs in terms of their preliminary BBB permeability prediction and their ability to convert into an active drug form are also discussed.

Chemistry

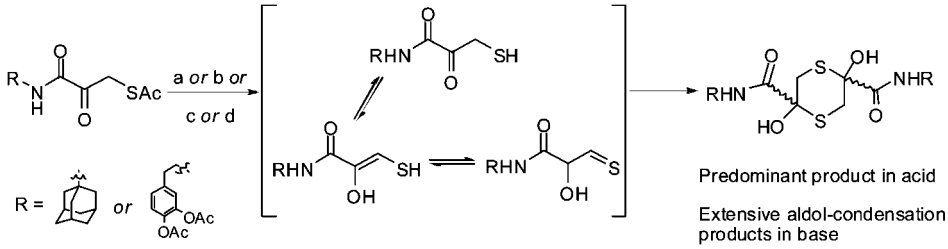
Our initial disconnections were centered around the creation of the heterodisulfide linkage adjacent to the α-ketoester, an arrangement that presented a considerable synthetic challenge. Sensitivity of this link to reductive and nucleophilic protocols led us to plan its creation toward the end of the synthesis (Scheme 1). This effectively divided our prodrugs into two fragments: the urea tripeptide **3** and the mercaptolactamides **4**. We recently have described the synthesis of a derivative of urea tripeptide **3** that bears protecting groups at its N, C, and S termini.[12]

Scheme 1. Retrosynthetic Strategy for Prodrugs **1** and **2**



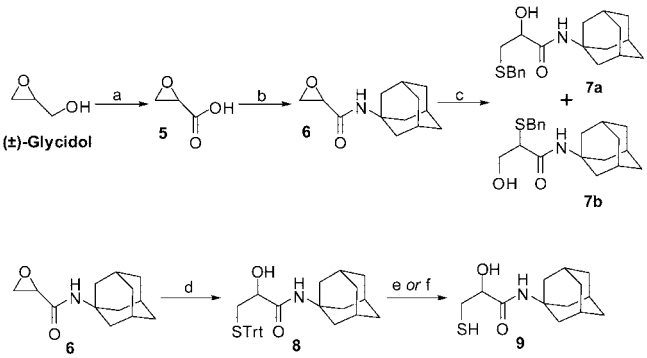
Our initial attempts for the synthesis of the mercaptopyruvamides **4** by alkylation and/or peptide coupling of commercially available 3-bromopyruvic acid were thwarted by the highly reactive α-bromoketone functionality that caused numerous side-reactions. We were unable to sequentially introduce these groups through the oxidation of a pyruvamide and subsequent α-bromination because the latter transformation could not be effected in our hands. A biomimetic route to these molecules though oxidative deamination of cysteine also was unsuccessful. Alkylation of 3-bromopyruvamides (obtained via the dimethylketal of 3-bromopyruvic acid) with thioacetate, although successful, was unfruitful because the hydrolysis of the thioamide function resulted in equilibration of aldehyde and thioal-

Scheme 2. Equilibration of Aldehyde and Thioaldehyde[a]



[a] Reagents and conditions: (a) LiOH, THF/H_2O; (b) NH_4OH, CH_3OH; (c) hydrazine hydrate; (d) 2N HCl.

Scheme 3. Synthesis of α-Hydroxy-β-mercaptopropanamide **9**[a]



[a] Reagents and conditions: (a) RuO_2, $NaIO_4$, CCl_4, CH_3CN, cat. H_2O; (b) 1-aminoadamantane, isobutyl chloroformate NMM, CH_2Cl_2, 52% over 2 steps; (c) HSBn, *n*BuLi, $BF_3 \cdot OEt_2$, THF, 56%; (d) HSTrt, *n*BuLi, BF3·OEt_2, THF, 69%; (e) $AgNO_3$, pyridine, CH_3OH, 54%; (f) Et_3SiH, CF_3COOH, CH_2Cl_2, 89%.

dehyde functional groups (Scheme 2), thereby leading to rapid degradation of the product to dithianes and other aldol-condensation polymeric products.

Building upon the knowledge garnered from these studies, we formulated a sequence of disconnections that segregated the presence of the incompatible α-bromo or a thiol and a keto functionality across intermediates.

The β-mercapto-α-hydroxypropionamide derivatives in Scheme 1 could be envisioned as being formed by the opening of the corresponding epoxyamides with a thiol nucleophile. Synthesis of such an epoxyamide started with oxidation of (*rac*)-glycidol with $RuO_2/NaIO_4$ (Scheme 3) to the oxirane carboxylic acid (**5**),[14] which was coupled to adamantamine, resulting in amide **6**. Opening of the epoxide **6** with a thiol nucleophile inadvertently revealed problems with regioselectivity. Use of benzyl mercaptan as a nucleophile resulted in two inseparable regioisomers (**7a** and **7b**) by attack at the α- and β-carbon of the carbonyl of the amide, respectively. The electron-withdrawing nature of the amide seems to override the steric factor, resulting in the two regioisomers. One way to side-step this problem was to use a sterically bulky thiol nucleophile to annul the electron-withdrawing power of the amide. As expected, trityl mercaptan afforded a single regioisomer **8** in 69% yield. Deprotection of trityl protection under Lewis or Brønsted acidic conditions resulted in thiol **9**.

These optimized reaction conditions were applied for the dopamine linker as well (Scheme 4). TBS-protected dopamine **10** was coupled to acid **5**, forming amide **11**. Regioselective opening of the epoxide **11** was achieved with trityl mercaptan, followed by Lewis acid deprotection of the trityl protection. Neutralization of the silver salt formed after trityl deprotection with silver nitrate proved tricky due to acid lability of the TBS-protecting groups. Exchanging the silver salt with an excess of another thiol-like dithiothreitol provided the solution to this problem affording the thiol **13** in 96% yield.[15]

Both the thiols **9** and **13** were then subjected to heterodisulfide formation reaction with the thiol of urea tripeptide **14**. The retrosynthetic plan in Scheme 1 depicts activation of tripeptide thiol by a suitable electrophile, followed by attack of this active intermediate by thiols **9** or **13** to arrive at the heterodisulfide linkage.

Electrophiles like iodine, mesyl chloride, or NBS led to significant formation of the undesired homodisulfide of the tripeptide thiol, which was a result of the greater electrophilicity of the activated thiol than the electrophile itself. This was not found to be the case with molecular chlorine because the S−Cl intermediate is not more electrophilic than chlorine gas and also because chlorine can be removed completely under reduced pressure. A conceptually similar but more practical solution was found in the use of the commercially available methoxycarbonyl sulfenyl chloride,[16] which gave a clean formation of the active intermediate from tripeptide thiol **14** (Scheme 5).

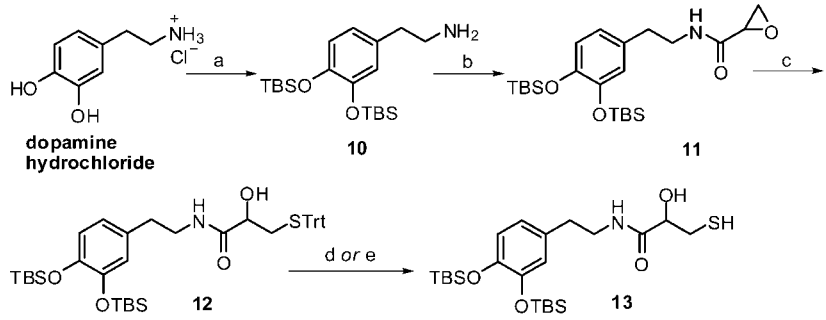
Following the methodology that we described in our previous report,[12] synthesis of tripeptide **14** was accomplished from Boc-L-asparagine. The active intermediate formed by reaction of thiol **14** with methoxycarbonyl sulfenyl chloride[16] was attacked by thiols **9** and **13** to afford heterodisulfides **15a** and **15b**. Oxidation of secondary hydroxyl in **15a** and **15b** with Dess−Martin periodinane gave prodrug precursors **16a** and **16b**. Global deprotection of adamantamine prodrug precursor **16a** using TFA gave the adamantamine prodrug **1** in 72% yield. Similarly for dopamine prodrug, precursor **16b** was subjected to TBS-deprotection followed by deprotection of N-Boc and *tert*-butyl esters using TFA to afford the dopamine prodrug **2**.

Results and Discussion

Biological Evaluation of Anti-Parkinson's Prodrugs. Our prodrug design rationale is based on two primary abilities of the prodrugs (Figure 1): (1) the ability to cross the BBB through recognition by glutathione transporters and allow their penetration into the brain, (2) the ability to release the active drug, once inside the brain. We chose to evaluate the prodrugs for the aforementioned properties in two systems: one designed to predict their BBB permeability and inhibit the transport of GSH to ascertain the utilization of same transport mechanism by both of them and, second, to examine their convertibility into active drug form.

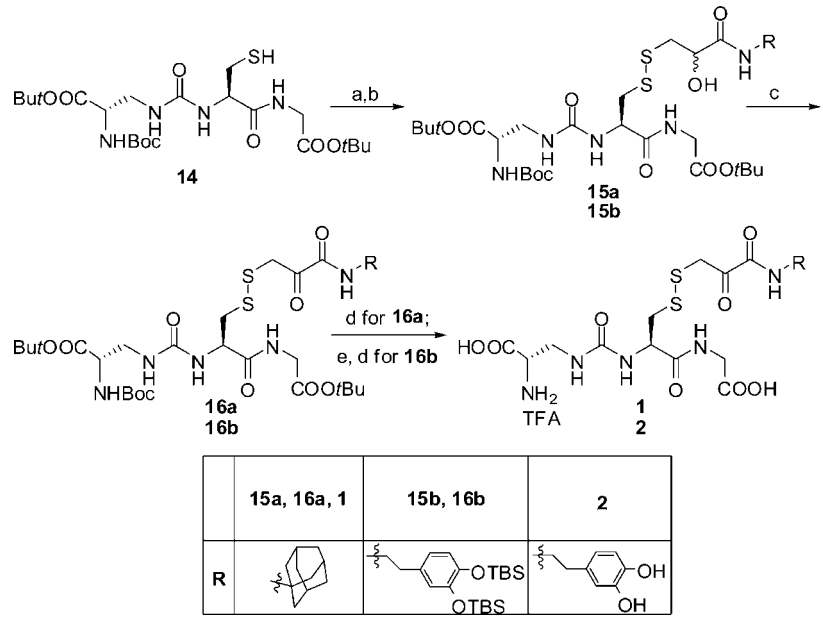
Directional Transport Studies of Prodrugs 1 and 2 across MDCK Cell Monolayer. For testing the ability of our prodrugs to cross BBB using a carrier-assisted transport mechanism, we performed an in vitro directional transport experiment using the BBB cell model, the Madin Darby canine kidney (MDCK II) cell line.[17] A simple and precise fluorimetric quantitation method was developed for measurement of the prodrugs in these experiments. The presence of a primary amino

Scheme 4. Synthesis of α-Hydroxy-β-mercaptopropanamide **13**[a]



[a] Reagents and conditions: (a) TBSCl, imidazole, CH_2Cl_2, 86%; (b) **5**, isobutyl chloroformate, NMM, CH_2Cl_2, 56%; (c) HSTrt, *n*BuLi, $BF_3 \cdot OEt_2$, THF, 65%; (d) $AgNO_3$, pyridine, trimethyl ammonium chloride, CH_3OH, 24%; (e) $AgNO_3$, pyridine, dithiothreitol, $EtOH/CH_2Cl_2$, 96%.

Scheme 5. Synthesis of Prodrugs **1** and **2**[a]



	15a, 16a, 1	15b, 16b	2
R		OTBS, OTBS	OH, OH

[a] Reagents and conditions: (a) $ClSOOCH_3$, CH_3OH; (b) **9** or **13**, Et_3N, CH_3OH; (c) Dess−Martin periodinane, CH_2Cl_2; (d) TFA/CH_2Cl_2 (1:1); (e) TBAF, THF.

group in our molecules prompted us to use fluorescamine,[18] which forms fluorescent covalent complexes with amines and allows for quantitation of the concentration of prodrugs as a function of the fluorescence emitted by the complex. Alhough this method gave a good estimation of prodrug concentration, the instability of reagent fluorescamine in its aqueous solution (in which the directional transport experiment is performed) and the low sensitivity of this method (high nanomolar range) led us to search for another similar reagent. *o*-Phthalaldehyde is another such reagent that forms fluorescent isoindoles with primary and secondary amines in presence of an added thiol reagent (like β-mercaptoethanol or *N*-acetylcysteine).[19] The stability of the *o*-phthalaldehyde in an aqueous solution, high sensitivity (high picomolar range), and reproducibility of this method made it an ideal detection method for our directional flux experiment.

To our knowledge, the MDCK cell line has never been studied for glutathione transport. It was therefore essential to first ascertain the existence of functional glutathione transporters in this cell line. In our first experiment, we studied the transport of glutathione itself through an MDCK cell monolayer, expecting to display the general trend of the transport from apical (blood) → basal (brain) > basal (brain) → apical (blood). At a concentration of 100 μM, we observed that the transport of glutathione from the apical to the basal side was approximately three times greater than the transport in the reverse direction (Data not shown). We also noted a saturation of transporters at a 1 mM concentration of glutathione.

A similar transport experiment was performed on adamantamine prodrug **1** at 100 μM concentration. In this case also, we observed that the influx of prodrug **1** into the basal compartment was much greater than its efflux (Figure 3). Furthermore, to ensure that the prodrug was not being metabolized as it crossed the MDCK cell monolayer, the integrity of prodrug **1** was studied by HPLC. The presence of single peak on the HPLC chromatogram corresponding to the intact prodrug **1** (cross-checked by doping this sample with an authentic sample of **1** to obtain a single peak as well) verified the identity of the substance transported (Figure S1, part A, Supporting Information).

Directional transport experiment with dopamine prodrug **2** displayed apical → basal transport > basal → apical transport (Figure 4). The integrity of the substance transported was verified in a manner similar to **1**. (Figure S1, part B, Supporting Information). These preliminary experiments successfully demonstrated the carrier-mediated transport of our prodrugs (**1** and **2**) in an in vitro BBB model.

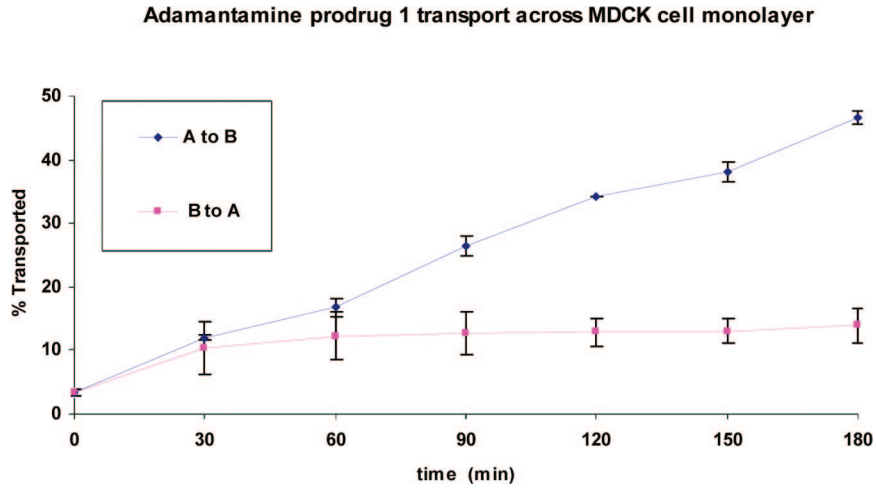


Figure 3. Results of adamantamine prodrug **1** transport experiment across MDCK cell monolayer.

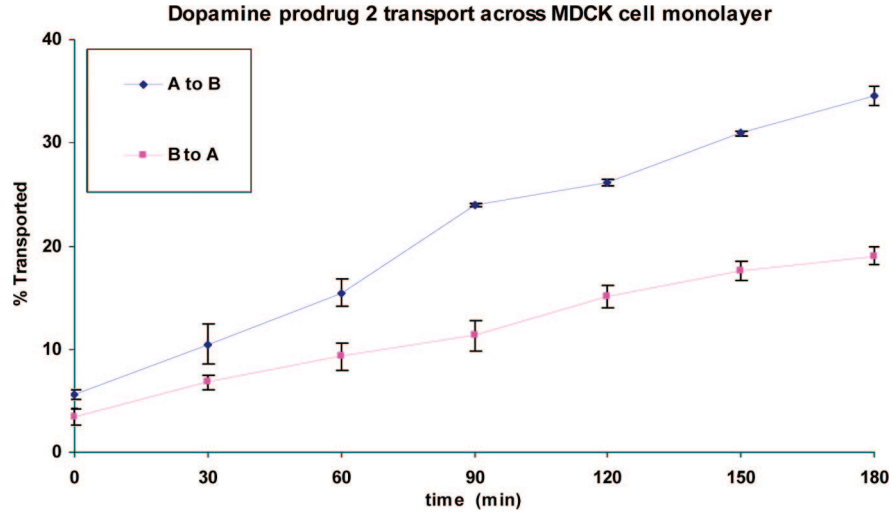


Figure 4. Results of dopamine prodrug **2** transport experiment across MDCK cell monolayer.

Inhibition of Glutathione Uptake by Glutathione-Based Prodrugs. We next examined the ability of the prodrugs to inhibit glutathione transport across MDCK cell monolayer as a means of corroborating on the same transport mechanism used by glutathione as well as prodrugs. For this, uptake of ^{3}H-labeled glutathione at a 100 nM concentration by MDCK cell monolayer was determined in the absence and presence of prodrugs (**1**, **2**; at 1000, 100, and 10 nM). ^{14}C-Mannitol was used as an internal standard to govern the tightness of the MDCK monolayer (Figure 5).

The decrease in glutathione transport observed in the presence of added prodrug (**1** or **2**) when compared to its transport in the absence of prodrugs was suggestive of the utilization of the same transport mechanism by both, glutathione and the prodrugs. Second, the negligible transport of mannitol, the internal standard, supported the fact that the observed transport of glutathione was due to utilization of the transporters and not by mere passive diffusion through leaky junctions in the MDCK cell monolayer.

In Vitro Stability Studies: Convertibility of Prodrugs into Active Drugs. To determine the stability of prodrugs in physiological media and their ability to deliver the active drug after brain uptake, prodrugs were simultaneously incubated in buffer (pH 7.0), rat plasma, and rat brain homogenate.[9] In this experiment, the prodrug **2** was incubated with these biological samples at 37 °C and aliquots were taken at various time intervals. Lack of a chromophore makes detection of adamantamine impossible by HPLC with simple UV detection. For this reason, only the dopamine prodrug **2** was studied in this experiment. The progress of the incubations was monitored by HPLC (UV detector, $\lambda = 280$ nm) analyzing the disappearance of the prodrug **2** and the formation of dopamine. The half-life of this prodrug was about 6 h in brain homogenate, while in blood plasma, it had $t_{1/2}$ of 3 h, indicating its probable breakdown by plasma peptidases. Table 1 reports the half-life ($t_{1/2}$) of prodrug **2** in the different incubation media.

The appearance of a peak corresponding to the active drug dopamine, balanced by a proportionate decrease in the intensity of the peak corresponding to the prodrug **2**, can be seen in the HPLC chromatogram (Figure S2, Supporting Information).

Conclusion

For the targeted delivery of anti-Parkinson drugs into the CNS using the glutathione transport system, the glutathionyl prodrugs **1** and **2** must show affinity for the glutathione transporter at BBB, be able to release the active drug at the target site, and possess a good stability balance between the periphery and the brain. The results of the directional transport of prodrugs and the inhibition of glutathione uptake by the prodrugs are suggestive of the utilization of the same transport mechanism to get inside the brain. Although these prodrugs fulfilled the

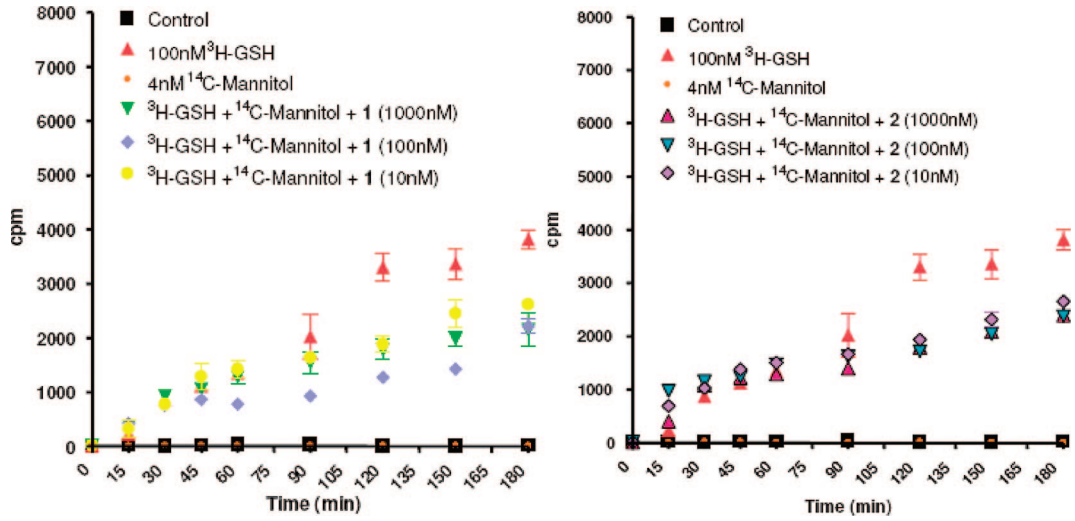


Figure 5. Inhibition of glutathione uptake across MDCK cell monolayer by prodrugs **1** (left) and **2** (right) [control = buffer; ^{3}H-GSH = ^{3}H-glutathione].

Table 1. Half-life of Prodrug **2**

	$t_{1/2}$ in h
buffer (pH 7.2)	>12
plasma	3
brain homogenate	$5^1/_2$−6

targeted-prodrug criteria with a sustained release of the active drug in brain extract, exhibition of labilily in plasma is an issue that needs to be addressed in the further development of this chemical strategy.

To conclude, these studies resulted in an efficient practical synthetic route toward new glutathione-based prodrugs. The chemistry involved in the synthesis of each component of these prodrugs (carrier, active drug, and linker) circumvents many of the potential instability problems associated with the intermediates leading to these biologically important building blocks. The biological data indeed support our hypotheses and pioneers the utilization of metabolically stable glutathione as a carrier for drug targeting into the brain.

Experimental Section

General Procedures. All commercial chemicals were used as supplied unless otherwise indicated. Dry solvents (THF, Et_2O, CH_2Cl_2, and DMF) were dispensed under argon from an anhydrous solvent system with two columns packed with neutral alumina or molecular sieves. Flash chromatography was performed with Silica-P flash silica gel (silicycle, 230−400 mesh) with indicated mobile phase. All reactions were performed under inert atmosphere of ultrapure argon with oven-dried glassware. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer. High-resolution mass data were acquired on a Bruker Bio TOF-II spectrometer capable of positive and negative ion ESI source, using PPG or PEG as internal standards.

General Procedure for Heterodisulfide Bond Formation. A methanolic solution of tripeptide **14** (0.35 mmol) was added dropwise to a solution of Cl-SCOOCH$_3$ (0.42 mmol) in MeOH (3 mL) at 0 °C over 20 min. The reaction mixture was allowed to stir at 0 °C for another 1 h and allowed to warm to room temperature. A CH$_3$OH solution of thiol **9** or **13** (1.05 mmol) was added to the above solution at once, followed by 0.70 mmol of Et_3N. The reaction mixture was stirred under argon at room temperature overnight, and the solvent was evaporated to dryness to obtain a sticky residue that was taken in EtOAc (30 mL). Organic layer was washed with 10% citric acid solution (20 mL), saturated $NaHCO_3$ solution (20 mL), and brine. EtOAc layer was separated, dried over Na_2SO_4, and evaporated to dryness. The residue obtained was dissolved in ethyl acetate and passed through a plug of silica gel with the aid of EtOAc. Combined eluent was evaporated to dryness and the residue (ESI-LRMS for **15a** 796.4 (M + Na)$^+$ and for **15b** 1026.5 (M + Na)$^+$) was carried over to the oxidation step without any further purification or characterization. Crude yields of ca. 65% were obtained in both of the cases.

To a anhydrous CH_2Cl_2 (5 mL) solution of alcohol **15a** or **15b** (0.30 mmol) at room temperature under argon, Dess−Martin periodinane reagent (0.45 mmol) was added all at once. The reaction mixture was allowed to stir for an hour (indicating completion of reaction by TLC), after which the reaction mixture was quenched with 2-propanol and evaporated to dryness to get a white solid. The solid residue was partitioned between EtOAc (20 mL) and 10 mL of saturated $NaHCO_3$ solution containing 5% sodium thiosulfate. The EtOAc layer was separated and washed with brine, dried over Na_2SO_4, and evaporated to obtain an oil that was purified by silica gel column chromatography.

Boc-γ-Gla{-Cys[3-(*N*-adamantylpyruvamide)thio]-Gly-OtBu}-OtBu (16a). Yield 70%, clear oil, hexanes/EtOAc 1:1 then 1:4, R_f 0.60 (EtOAc/hexanes, 3:2); $[\alpha]_D$ −6.4 (*c* 0.94, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11, 6.77, 5.98, 5.50, 5.35 (5s, 5H, N*H*), 4.60 (m, 1H, α-C*H*:Gla), 4.17 (m, 1H, α-C*H*:Cys), 3.90−3.78 (m, 4H, SC*H*$_2$C(=O), C*H*$_2$:Gly), 3.62−3.35 (m, 3H, β-C*H*$_2$:Gla, β-C*H*$_A$H$_B$:Cys), 2.97 (m, 1H, β-CH$_A$*H*$_B$:Cys), 2.08−1.62 (m, 15H, C*H*$_2$:Adm, C*H*:Adm), 1.43, 1.41, 1.36 (3s, 27H, C(C*H*$_3$)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.5, 171.1, 170.1, 168.7, 158.9, 158.1, 156.0 (*C*(=O)), 82.7, 82.4, 82.3 (*C*(CH$_3$)$_3$), 60.6 (α-*C*:Gla), 53.1 (α-*C*:Cys), 43.1−28.2 (β-*C*:Gla, *C*H$_2$:Gly, S*C*H$_2$C(=O), β-*C*:Cys, C(*C*H$_3$)$_3$, *C*:Adm). ESI-HRMS *m*/*z* 794.3479 (M + Na)$^+$; $C_{35}H_{57}N_5O_{10}S_2$ + Na$^+$ requires 794.3439.

Boc-γ-Gla{-Cys[3-(*N*-(*O*,*O*′-di(*tert*-butyl-dimethylsilyl)dopaminylpyruvamide)-thio]-Gly-OtBu}-OtBu (16b). Yield 73%, clear oil, hexanes/EtOAc 1:1 then 1:3, R_f 0.45 (EtOAc/hexanes, 3:2); $[\alpha]_D$ −17.3 (*c* 1.33, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18, 7.03 (2s, 2H, N*H*), 6.57−6.44 (m, 3H, Ar), 5.86, 5.44, 5.29 (3s, 3H, N*H*), 4.48 (q, *J* = 3.0 Hz, 6.3 Hz, 1H, α-C*H*:Gla), 4.04 (m, 1H, α-C*H*:Cys), 3.78−3.73 (m, 4H, SC*H*$_2$C(=O), C*H*$_2$:Gly), 3.46−3.13 (m, 5H, β-C*H*$_2$:Gla, β-C*H*$_A$H$_B$:Cys, CH$_2$C*H*$_2$NH), 2.85−2.82 (m, 1H, β-CH$_A$*H*$_B$:Cys), 2.56 (t, *J* = 3.6 Hz, 2H, C*H*$_2$CH$_2$NH), 1.28, 1.27, 1.24 (3s, 27H, C(C*H*$_3$)$_3$), 0.79 (s, 18H, SiC(C*H*$_3$)$_3$), 0.00 (s, 12H, SiC*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.1, 175.1, 174.1, 172.7, 163.9, 161.9, 159.9 (*C*(=O)), 150.9, 149.7, 135.1, 125.5, 125.2 (*C*$_{Ar}$), 86.6, 86.3, 84.1 (*C*(CH$_3$)$_3$), 64.4 (α-*C*:Gla), 57.1 (α-*C*:Cys), 46.6, 46.2, 45.2, 44.9, 38.6, 36.7 (β-*C*:Gla, *C*H$_2$:Gly, β-*C*:Cys, S*C*H$_2$C(=O), CH$_2$C*H*$_2$NH, C*H*$_2$CH$_2$NH), 33.7, 32.4, 32.1, 32.0, 30.0 (C(*C*H$_3$)$_3$), 22.5, 22.4 (Si*C*(CH$_3$)$_3$), 0.0 (Si*C*H$_3$). ESI-

HRMS *m/z* 1024.4648 $(M + Na)^+$; $C_{45}H_{79}N_5O_{12}S_2Si_2 + Na^+$ requires 1024.4597.

TFA.H_2N-γ-Gla{-Cys[3-(*N*-adamantylpyruvamide)thio]-Gly-OH}-OH (1). The protected tripeptide **16a** (80 mg, 0.10 mmol) was treated with a mixture TFA and CH_2Cl_2 (1:1 v/v, 6 mL) at room temperature for 4 h. Concentration of the reaction mixture followed by trituration with freshly distilled diethyl ether afforded a white solid. Recrystallization of the solid from a aqueous ethanol gave analytically pure **1** (53 mg, 77%). R_f 0.45 (butanol/acetic acid/H_2O, 12:5:3); $[\alpha]_D$ −5.7 (*c* 0.65, 1N HCl). ^{1}H NMR (300 MHz, $CDCl_3$/CF_3COOD) δ 4.53 (m, 1H, α-C*H*:Cys), 4.32 (m, 1H, α-C*H*:Gla), 4.16−3.82 (m, 4H, SCH_2C(=O), CH_2:Gly), 3.52−3.41 (m, 2H, β-CH_2:Gla), 3.21−2.98 (m, 2H, β-CH_2:Cys), 2.21−1.70 (m, 15H, CH_2:Adm, C*H*:Adm). ^{13}C NMR (75 MHz, $CDCl_3$/CF_3COOD) δ 195.1, 174.3, 172.6, 169.8, 159.5, 158.4 (*C*(=O)), 53.4 (α-*C*:Cys), 51.4 (α-*C*:Gla), 44.6 (β-*C*:Gla), 42.8 (*C*H_2:Gly), 41.2−29.3 (S*C*H_2C(=O), β-*C*:Cys, *C*:Adm). ESI-HRMS *m/z* 560.1871 $(M + H)^+$; $C_{22}H_{33}N_5O_8S_2 + H^+$ requires 560.1843. Reverse phase HPLC was run on Varian Microsorb column (C18, 5 μm, 4.6 mm × 250 mm) using two solvent systems with 0.5 mL/min flow rate and detected at 220/254 nm. Solvent system 1: 0.04 M TEAB (triethylammonium bicarbonate) in water/70% acetonitrile in water = 1/1, t_R = 5.39 min, purity = 98.57%. Solvent system 2: 0.04 M TEAB in water/70% acetonitrile in water = 20 − 100% B linear, t_R = 6.21 min, purity = 96.34%.

TFA·H_2N-γ-Gla{-Cys[3-(*N*-(*O*,*O*′-di(*tert*-butyl-dimethylsilyl)-dopaminylpyruvamide)thio]-Gly-OH}-OH (2). To a solution of **16b** (95 mg, 0.09 mmol) in THF (5 mL) was added commercial TBAF (1 M solution in THF, 0.18 mL, 0.18 mmol) at 0 °C. After the mixture was stirred at room temperature for 30 min, the reaction was quenched by adding H_2O. The resulting mixture was diluted with EtOAc. The layers were separated; the organic layer was washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by silica gel flash chromatography to obtain the TBS-deprotected precursor tripeptide (64 mg, 87% yield) that was used directly in the subsequent reaction. The TBS-deprotected tripeptide obtained above (60 mg, 0.07 mmol) was dissolved in a mixture of TFA and CH_2Cl_2 (1:1 v/v, 5 mL) at room temperature and stirred for 4 h. The reaction mixture was then concentrated and the residue was triturated with ether and EtOAc to get product as fluffy solid. Recrystallization of this solid from a water/ethanol mixture gave analytically pure **2** (53 mg, 77% yield). R_f 0.3 (butanol/acetic acid/H_2O, 12:7:3); $[\alpha]_D$ −10.3 (*c* 0.36, 1N HCl). ^{1}H NMR (300 MHz, $CDCl_3$/CF_3COOD) δ 6.76−6.54 (m, 3H, Ar), 4.57 (m, 1H, α-C*H*:Cys), 4.24 (m, 1H, α-C*H*:Gla), 3.98−3.81 (m, 4H, SCH_2C(=O), CH_2:Gly), 3.52−3.34 (m, 4H, β-CH_2:Gla, CH_2CH_2NH), 3.12−2.89 (m, 2H, β-CH_2:Cys), 2.69 (m, 2H, C$H_2$$CH_2$NH). ^{13}C NMR (75 MHz, $CDCl_3$/CF_3COOD) δ 195.4, 174.6, 172.4, 170.7, 160.5, 158.2 (*C*(=O)), 147.2, 146.8, 133.5, 123.5, 121.8 (C_{Ar}), 54.4 (α-*C*:Cys), 49.9 (α-*C*:Gla), 45.9 (β-*C*:Gla), 42.4 (*C*H_2:Gly), 41.6, 39.3, 37.7, 36.9 (β-*C*:Cys, S*C*H_2C(=O), CH_2CH_2NH, C$H_2$$CH_2$NH).ESI-HRMS *m/z* 562.1298 $(M + H)^+$; $C_{20}H_{27}N_5O_{10}S_2 + H^+$ requires 562.1272. Reverse phase HPLC was run on Varian Microsorb column (C18, 5 μm, 4.6 mm × 250 mm) using two diverse solvent systems with 0.5 mL/min flow rate and detected at 220/254 nm. Solvent system 1: 0.04 M TEAB (triethylammonium bicarbonate) in water/70% acetonitrile in water = 2/3, t_R = 7.06 min, purity = 95.43%. Solvent system 2: 0.04 M TEAB in water/70% acetonitrile in water = 20−100% B linear, t_R = 7.53 min, purity = 98.43%.

In Vitro Directional Transport Experiment Using MDCK Cell Monolayer. The influence of the blood brain barrier (BBB) on permeabilities of prodrugs was determined by MDCK cell monolayers growing on a permeable support. MDCK cells (Madin−Darby canine kidney cells) were seeded into 6-well Transwell inserts in the presence of a suitable growth medium (DMEM supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL)) at 3.0×10^5 cells per well and placed in the CO_2 TC incubator to grow for 3 days. The cells were grown until they reached confluency, which was evaluated by visual observation under a microscope. In our directional transport experiment, the upper compartment of the Transwell was designated as the apical (A) and the lower compartment designated was the basal (B) side.

For the experiment, the medium was aspirated and the cells were washed and preincubated with the assay buffer for 30 min. The assay buffer was then replaced with assay buffer containing glutathione/prodrug **1** or **2** in the donor side, i.e., the apical side (1.5 mL), to determine flux from the apical to the basal side or the basal side (2.6 mL) to determine flux from the basal to the apical side. Drug-free fresh assay buffer was placed on the receiver side. The experimental wells were incubated on an orbital shaker at 37 °C for the duration of the experiment (180 min) except while drawing samples and replacing the assay buffer. Then 100 μL samples were drawn from the receiver side at 0, 30, 60, 90, 120, 150, and 180 min and replaced with drug free fresh assay buffer. Similarly, 100 μL samples were drawn from the donor side at 0 and 180 min and replaced with the assay buffer containing glutathione.

All the aliquots were analyzed by fluorimetric assay based on using *o*-phthalaldehyde (OPA). OPA reagent solution (complete) was made by addition of 20 μL of β-mercaptoethanol to 5 mL of OPA reagent solution (incomplete) immediately prior to its use. Then 15 μL of each aliquot was mixed with 150 μL of OPA reagent solution (complete) in a Costar 96-well black opaque plate. Samples were allowed to incubate for 2 min with moderate shaking at room temperature and the fluorescence intensity was measured using a SynergyHT fluorescence plate reader with a 360 nm, 40 nm bandwidth, excitation filter, and a 460 nm, 40 nm bandwidth, emission filter. The sensitivity setting was at 45, and the data was collected from the top. The percentage transport across the monolayer was determined by dividing the fluorescence reading at various time intervals with the fluorescence measured at the start of the experiment. The integrity of glutathione/prodrug **1** or **2** transported across MDCK cell monolayer was verified by HPLC.

Assay buffer: NaCl (7.13 g), $NaHCO_3$ (2.10 g), glucose (1.8 g), HEPES (2.38 g), KCl (0.224 g), $MgSO_4$ (0.295 g), $CaCl_2$ (0.206 g), K_2HPO_4 (0.070 g) in 1000 mL H_2O adjusted to pH 7.4.

HPLC system: Varian ProStar 210; UV detection: 220/254 nm; column: Varian Microsorb C18, 5 μm, 4.6 mm × 150 mm; solvent system: linear gradient 20−100% solvent B [solvent *A* = 0.04 M TEAB (triethylammonium bicarbonate) in water; solvent *B* = 70% acetonitrile in water]; flow rate: 0.5 mL/min; retention time (t_R): prodrug **1** 6.20 min; prodrug **2** 7.52 min.

In Vitro Blood and Brain Stability Studies. Plasma. Blood was drawn from a rat though a heart puncture after anesthetization. Ten μL of 0.5 M EDTA was added per mL of blood. Samples were centrifuged at 14000 RPM for 15 min to separate plasma, placed on ice, and used immediately.

Brain Homogenate. The brain was removed and homogenized in cold 0.1 M phosphate buffer of pH 7.2 with a Dounce tissue homogenizer (1:5 w/v). Samples were then placed on ice and used immediately.

Procedure for the Stability Studies. 100 μL of 20 mM compound in phosphate buffer was added to 2 mL of buffer (phosphate buffer, pH 7.2), plasma, or brain homogenate and gently vortexed. Samples were incubated at 37 °C and 200 μL aliquots were removed every half hour. Then 200 μL of acetonitrile was added to each aliquot and vortexed. Samples were centrifuged for 15 min to remove proteins and the supernatants were analyzed by HPLC.

HPLC System: Waters Delta 600; UV detection: 220/254 nm; column: Atlantis DC18, 5 μm, 4.6 mm × 150 mm; solvent system: linear gradient 5−50% solvent B [solvent *A* = 0.1% heptafluorobutyric acid (HFBA) in water; solvent *B* = acetonitrile]; flow rate: 1.0 mL/min; retention time (t_R): dopamine 11.2 min; prodrug **2** 12.2 min.

Inhibition of Glutathione Uptake by Prodrugs 1 and 2. Procedure same as in the directional transport experiment. Here, the transport of [Glycine-2-^{3}H]-glutathione from the apical to the basal direction at 100 nM concentration was challenged with 0, 10, 100, and 1000 nM concentrations of prodrugs **1** or **2**. In each well, D-[1-^{14}C]-mannitol (4 nM) was added as an internal standard. Then

100 μL aliquots were taken at 15 min intervals for the first hour and every 30 min thereafter up to 3 h from the basal (bottom) chamber and replaced with assay buffer to keep the solution volume constant. The aliquots taken during the experiment were combined with Ecolite scintillation cocktail and the radioactivity was measured with Beckman Coulter LS 6500 scintillation counter. The percentage transport of glutathione across the monolayer was determined by dividing the radioactivity reading at various time intervals with the radioactivity measured at the start of the experiment.

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Supporting Information Available: Experimental details for the synthesis of **5**−**14** and **18**−**26**, in vitro BBB permeability assay and in vitro stability assay of prodrugs. This material is available free of charge via the Internet at http://pubs.acs.org.

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