

# DESIGN AND SYNTHESIS OF TRPV1 ANTAGONISTS; PROBING THE D-REGION BINDING SITE USING AMIDOALKYL SUBSTITUENTS.

Victoria D. Held (David Rusterholz, Advisor)  
Department of Chemistry,  
University of Wisconsin - River Falls

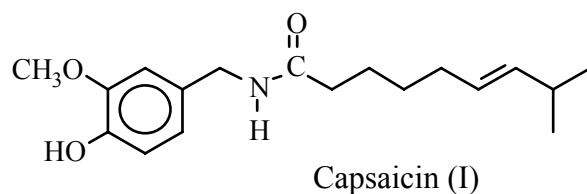
## Abstract

Transient receptor potential vanilloid subfamily 1 (TRPV1) is a membrane bound ion channel that mediates the pain response elicited by capsaicin, resiniferatoxin, and similar drugs. Structure activity studies of compounds that bind to TRPV1 indicate several binding sites, namely: 1) an aromatic "A" region, 2) a polar "B" region, and 3) a hydrophobic "C" region. A fourth region has recently been proposed based on the structure of resiniferatoxin. This "D" region is thought to be responsible for the increased potency of the drug. A new series of potential TRPV1 antagonists was designed incorporating molecular features intended to interact with the known A, B, and C binding sites. In order to investigate the interaction of the D binding region, amidoalkyl groups were incorporated into the target structures. The synthesis of several members of the target series was accomplished using a convergent synthetic strategy. Ultimately these compounds will be tested for their effectiveness as TRPV1 antagonists.

## Introduction

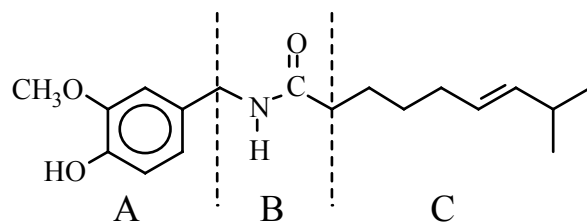
Capsaicin (**I**) is the component in chili peppers that makes them pungent. Capsaicin produces its pungent sensation by stimulating a class of receptors called transient receptor vanilloid subfamily 1 (TRPV1).<sup>1</sup> In mammals, TRPV1 receptors are found primarily in unmyelinated nerve cells involved in pain sensation known as nociception, but they are also found in many other body organs including the smooth muscle tissue of the gastrointestinal tract and epithelial cells that line the bladder.<sup>2</sup>

Capsaicin has long been known for its pungent properties, but what is less well known is its ability to desensitize nociceptive nerve cells to provide pain relief. This has led to the



employment of capsaicin in several commercial pain-relieving drugs. Medications that use capsaicin usually are prepared in the form of a cream and have been used in the topical treatment of pain associated with diabetic neuropathy, arthritis, and postherpetic neuralgia. There is also evidence that a nasal spray containing capsaicin may be able to provide relief from migraine headaches.<sup>3</sup> Upon application, these medications produce an initial stinging sensation that is followed by desensitization.

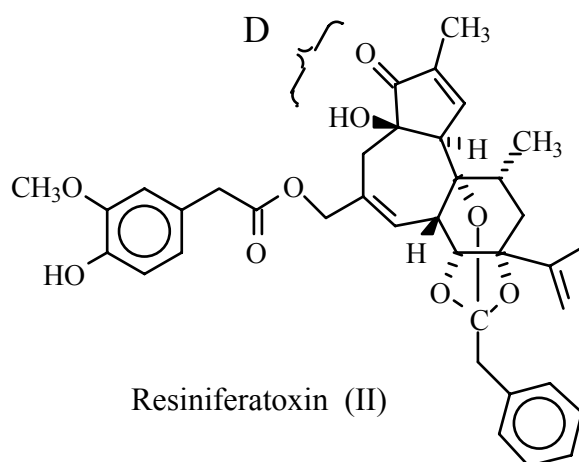
In nociceptive nerve fibers, TRPV1 receptors are nonselective cation channels, and when activated cause an influx of ions, chiefly calcium, into the cell. In these cells the TRPV1 receptors are believed to be located on the inner side of the cell membrane.<sup>4</sup> The influx of positive charge is thought to trigger an action potential that communicates the nociceptive signal to the central nervous system resulting in the sensation of pain. Extended exposure to capsaicin causes a long lasting, high concentration of intracellular  $\text{Ca}^{+2}$  that results in death of the nociceptive nerve cell and permanent loss of TRPV1.



Past efforts to create a therapeutic agent have focused on separating the desired desensitizing effect of capsaicin from its pungent properties. There is little known about how TRPV1 operates, but structure-function studies are slowly discovering the protein's design. Some of the first to report the biological effects of TRPV1 agonist analogs were Szolcsanyi and Jansco-Gabor.<sup>5</sup> These workers considered capsaicin to have three regions (A, B, and C). The A region is an aromatic ring attached to a polar B region. The C region is chiefly comprised of a long hydrophobic chain. Within the A region, the ring substituent pattern that produced the optimal agonist effect was a 3-methoxy-4-hydroxy pattern. The similarity of this

substitution pattern to the natural product vanillin has led to the broad designation of capsaicin-like substances as “vanilloids”. Several lines of evidence suggest that the 4-hydroxy substituent is important due to its ability to hydrogen bond. For example, replacing the 4-hydroxy group with a methoxy or a 3,4-methylenedioxy group lowered the resulting compound’s agonist properties.<sup>6</sup> It has been noted that for optimal agonistic properties, the B region must contain a group that can both donate and accept a hydrogen bond.<sup>7</sup> A thiourea group in the B region has proved to greatly increase the potency of the resulting compound. It is believed that the C region must be hydrophobic and moderate in size (eight to ten carbons). It is possible that this hydrophobic C region allows the compound to diffuse through the membrane of the cell effectively. If the compound were too hydrophobic, it would tend to remain in the lipid bilayer and potentially not be able to pass through to the inside where it binds to TRPV1.

Although capsaicin interacts with TRPV1, it does not possess practical pharmaceutical properties. It is metabolized quickly in the body, and because of its lipophilic nature it has high non-specific binding and a low affinity for TRPV1. These challenges led investigators to consider another naturally found TRPV1 agonist. The phorbol ester resiniferatoxin (II) also activates the TRPV1 receptor, but it is over 1000 times more potent than capsaicin.<sup>8</sup> The A, B, and C features can be identified within the complex structure of resiniferatoxin. To account for the high potency, a fourth region has been proposed to contribute to receptor binding. The 3-keto and 4-hydroxy groups are believed to comprise this fourth “D” region.

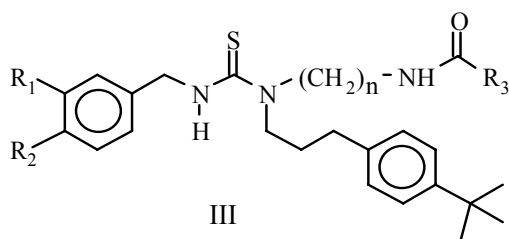


The first studies aimed at delineating capsaicin agonist structure-activity relationships (SAR) did not result in useful therapeutic agents because all of the new analogs caused an initial pain response. Since TRPV1 has been identified and cloned,<sup>9</sup> investigators have focused on creating TRPV1 antagonists. Compounds that can mimic capsaicin's affinity for the TRPV1 receptor, but have low efficacy might provide the desired analgesic benefits without the undesirable burning sensation.

While not all types of pain are caused by vanilloids, a number of painful conditions are believed to be caused, at least in part, by the body's own endogenous vanilloid-like substances. A TRPV1 antagonist would only prove useful if the stimuli involved in the pain response were due to these endogenous vanilloids. The design of a TRPV1 antagonist requires the inclusion of structural features similar to capsaicin or resiniferatoxin to promote TRPV1 affinity, but differences that are great enough to result in no or low agonist efficacy. Therefore, the structures of TRPV1 antagonists are similar to those of agonists, but with slight variations. One group created a compound that incorporates this approach with a 2,2-dimethylpropanoyl ester in the D region to mimic the 3-keto-4-hydroxy group in resiniferatoxin.<sup>10</sup> Like TRPV1 agonists, a thiourea for the B region has been found to be optimal.<sup>11</sup> Others have found that halogenation of the A region conferred antagonistic properties.<sup>12</sup>

### Statement of the Problem

The goal of the research described herein was to synthesize compounds (**III**) intended to be TRPV1 antagonists. These compounds contain A, B, C, and D regions that mimic the structural features of capsaicin and resiniferatoxin to varying degrees. In structure **III** the R1



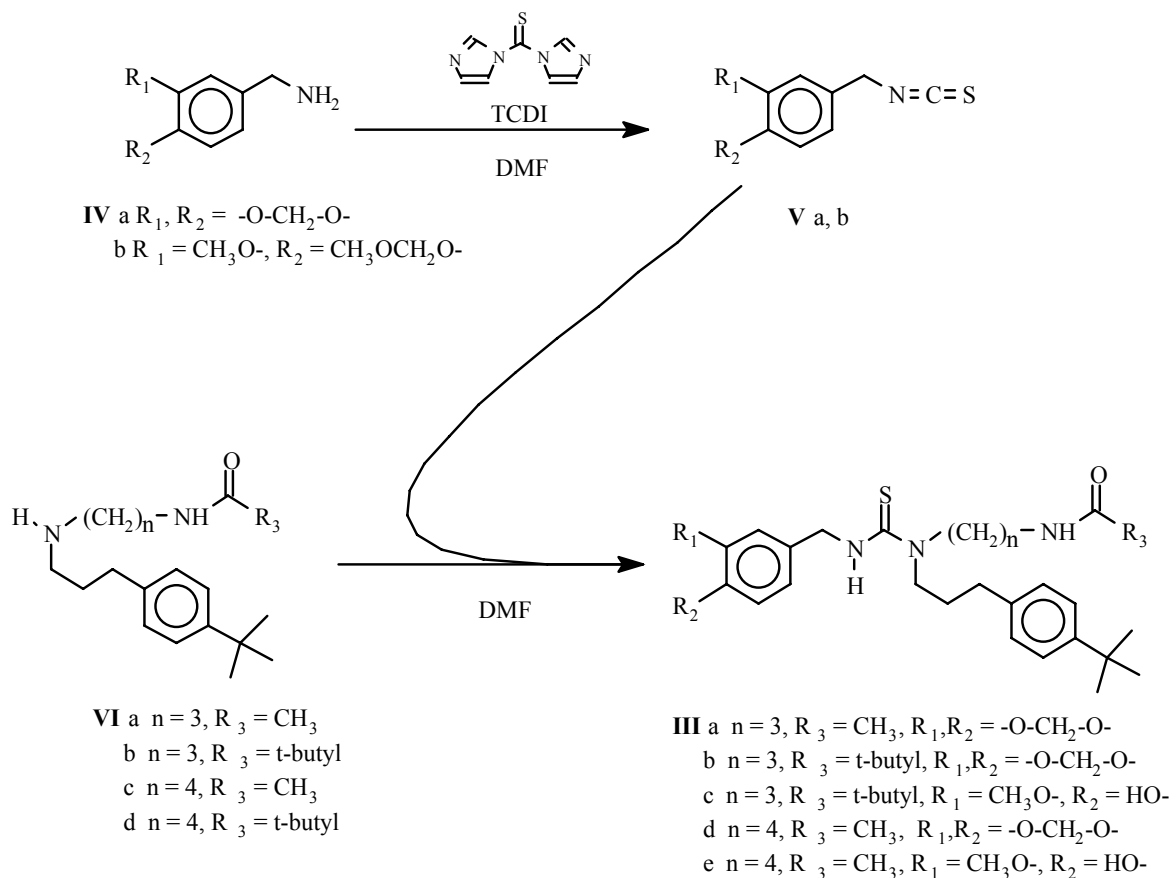
and R2 substituents in the A region are a 3,4-methylenedioxy group or the vanilloid 4-hydroxy-3-methoxy substituent pattern. The B region consists of a thiourea group. A *t*-butyl group attached to a benzene ring comprises the C region. A three-carbon chain attaches the C and B regions. The D region is an amide with R3 being a methyl or a *t*-butyl group. The optimal distance for interaction with the TRPV1 receptor of the D region from the B region is not known, but is estimated to be between two and four carbons. Therefore the number of carbons separating the D region from the B region was also varied ( $n = 2, 3, \text{ and } 4$ ).

Few attempts have been made by medicinal chemists to build structures that provide for interactions with the proposed D region binding site. This research is intended to investigate the use of an amide functional group separated from the B region by various distances as a possible binding moiety.

## Discussion

The synthetic approach to the target compounds **III** has been to attach the C region of the target compound to the D region and then attach the A region through the formation of the thiourea B region. Scheme 1 shows the final steps of this pathway. Compounds **IVa,b** were reacted with *N,N*-thiocarbonyldiimidazole (TCDI) in DMF to give **Va,b**. The crude products were purified using flash chromatography. The IR (infrared) spectra of **V** showed the characteristic peaks at  $2095\text{ cm}^{-1}$  and  $2185\text{ cm}^{-1}$  due to the isothiocyanate group. Compounds **Va,b** were reacted with **VIa-d**, followed by deprotection when necessary, to give the target compounds **IIIa-e**. Flash chromatography was used to purify the products that were obtained in 40-97% yield.

The intermediate **VI** structures that possess the C and D regions of the final target compounds were prepared as shown in Scheme 2. The commercially available *p-t*-butylbenzaldehyde was reacted with malonic acid in pyridine to give **VII** in excellent yield. Hydrogenation of **VII** gave **VIII** in good yield and this product in turn was esterified in acidic methanol to give **IX**. Compound **IX** was reacted with various diaminoalkanes ( $n = 2, 3, \text{ or } 4$ ) in 30% water. The amount of water used in this step was very important. If too little water were used, the product formed a cyclic tetrahydropyrimidine compound, but if too much water were used the reaction would not occur.<sup>13</sup> The resulting products, **X**, were not soluble in water and only slightly soluble in ether. As a result, three layers formed during the extraction

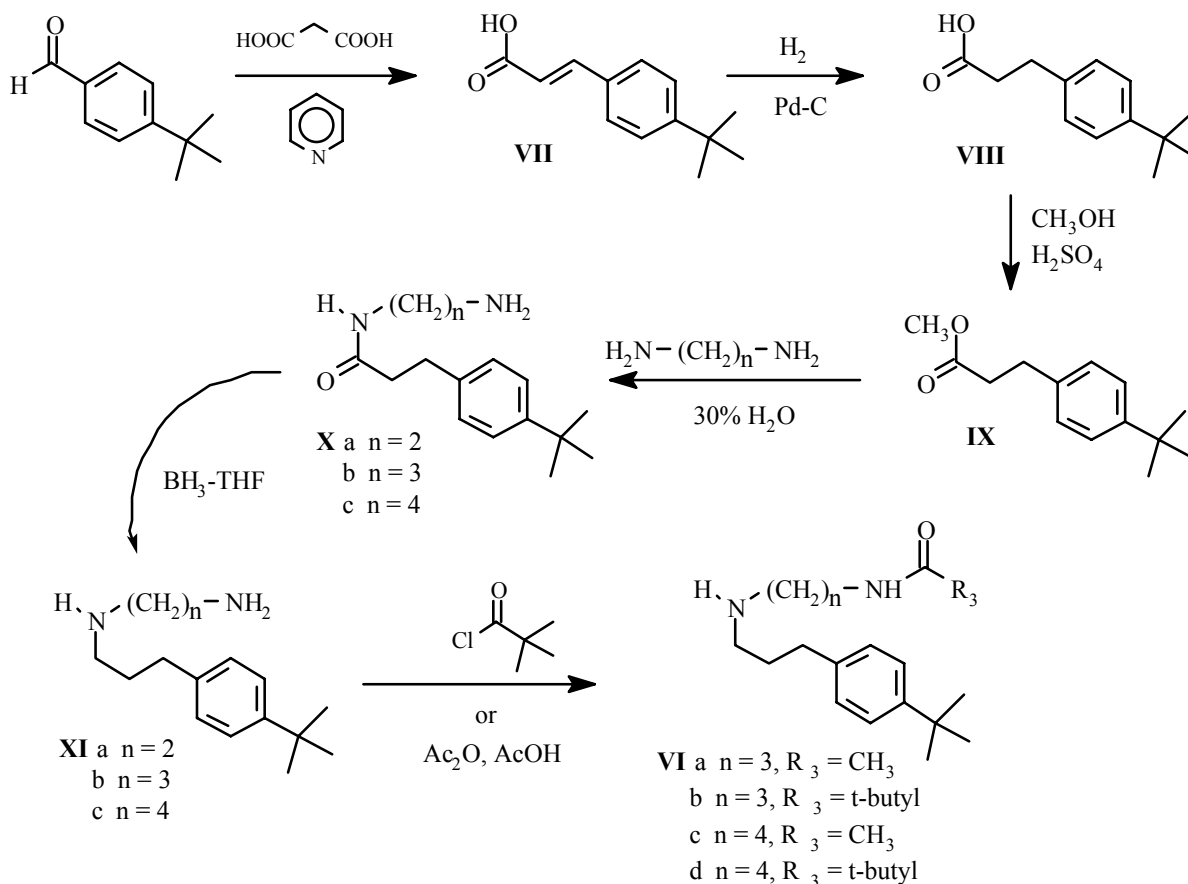


Scheme 1. Final Steps in the Synthesis of Target Structures **III**.

stage. The middle layer was determined to be the desired product. The ether layer also contained a small amount of product, so the two organic layers were combined. The products were initially yellow oils, but as they were dried by concentration from ethanol *in vacuo* they converted to waxy solids. The products **X** ( $n = 2, 3,$  and  $4$ ) were obtained in quantitative, 97%, and 92% yields respectively.

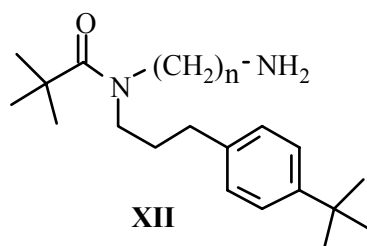
Borane THF was used for the reduction of **X**. Five equivalents of borane were used because the amide required three equivalents of borane<sup>14</sup> and neutralization of the amine within **X** required an additional equivalent. The fifth equivalent of borane was used to

neutralize any water in the reaction solvent. The yields of **XI** (n = 2, 3 and 4) were 67%, quantitative, and 86% respectively.



Scheme 2. Preparation of Combined C and D Region Structures, **VI**.

The reaction of **XI** in its free base form with 2,2-dimethylpropanoyl chloride yielded a mixture of both **VI** and **XII**. To prevent **XII** from forming, **XI** was converted to its HCl salt. This allowed the acidity of the amines to determine where the acid chloride would react. Using this strategy, high yields of **VI** virtually free of **XII** were obtained.



The HCl salts of **XI** (n=2, 3, or 4) proved to be troublesome to work with. They were hygroscopic and the difficulty in preparing them prompted a shift to pursuing the acetamides (R<sub>3</sub> = CH<sub>3</sub>) instead of the 2,2-dimethylpropanoylamides (R<sub>3</sub> = t-butyl). Therefore, compounds **XI** as the free bases were reacted with acetic anhydride in acetic acid to give **VI**(a and c) (R<sub>3</sub> = CH<sub>3</sub>). Acylation was observed to occur almost exclusively on the primary amine.

### Conclusion

Following the sequences of reactions shown in Schemes I and II, target compounds **IIIa-e** were successfully prepared and characterized. When biological testing has been completed it is hoped that the usefulness of an amidoalkyl substituent will be revealed as a binding moiety for the TRPV1 receptor.

### Methods

<sup>1</sup>H NMR spectra were obtained using a Bruker DPX200 NMR spectrometer and were recorded at 200MHz using TMS as the internal standard. IR spectra were obtained using a Mattson Genesis II FTIR. Melting points were obtained using a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, IN. Where analyses are indicated by symbols of the elements, the analytical results obtained were within +/- 0.4% of the calculated values. High-resolution mass spectra were obtained from the University of Minnesota Mass Spectrometry Facility.

#### p-t-Butylcinnamic acid – **VII**

A mixture of 20.36 g (0.1255 mol) of *p-t*-butylbenzaldehyde (Aldrich), 26.12 g (0.251 mol) of malonic acid, 250 mL of pyridine, and 2.66 g (0.00313 mol) of piperidine was heated in a steam bath with occasional stirring for four hours during which time the mixture dissolved completely. After cooling to room temperature the mixture was poured into 260 mL of concentrated HCl on 1000 mL of ice. The white solid that formed was removed by filtration and washed with water. The white solid was digested in approximately 1 L of water and filtered a second time. The product was dried overnight in air to give a yield of 24.39 g (95.16%). mp 201.5-202°C, Lit.<sup>15</sup> mp 201-204°C.

#### 3-(4'-t-Butylphenyl)propanoic acid – **VIII**

To a 500 mL hydrogenation bottle was added 7.00 g (0.0343 mol) of **VII** along with 0.15 g of 10% palladium on activated carbon and 80 mL of absolute ethanol. The mixture was pressurized to 59 psig and shaken for 24 hours. The mixture was filtered through celite and the residue was washed with absolute ethanol. The filtrate was filtered and washed again. After drying by concentration *in vacuo* the product was a white solid. Yield 6.88 g (98.29%). mp 105-108°C, Lit.<sup>16</sup> mp 116-117°C.

**Methyl 3-(4'-t-butylphenyl)propanoate – IX**

In a 300 mL round bottom flask 5.00 g (0.0242 mol) of **VIII** was dissolved in 100 mL of methanol. Three drops of concentrated H<sub>2</sub>SO<sub>4</sub> was added and the mixture was refluxed overnight. The flask was cooled and the mixture was concentrated *in vacuo* until 10 mL remained. Diethyl ether (75 mL) was added and the solution was washed with 50 mL of 5% NaHCO<sub>3</sub> followed by 50 mL of H<sub>2</sub>O. The diethyl ether layer was dried, filtered, and concentrated *in vacuo* to give a yellow oil. After a few minutes the oil crystallized in a yield of 6.26 g (quant.) mp 39-41°C, Lit.<sup>17</sup> mp 40°C.

**N-(2'-Aminoethyl)-3-(4''-t-butylphenyl)propanamide – Xa**

In a 100 mL round bottom flask 2.90 g (0.013 mol) of **IX**, 2.37 g (0.0395 mol) of 1,2-ethylenediamine, and 2.62 g of water were heated and stirred in an oil bath at 70°C. After three hours the solution was transferred into a 250 mL separatory funnel and a mixture of 60 mL of water and 20 mL of saturated NaCl was added. A white precipitate formed and was extracted with 40 mL of diethyl ether. When the diethyl ether was added, three layers formed in the separatory funnel. The bottom layer was discarded while the top two layers were diluted with methanol and concentrated *in vacuo*. The product was obtained as a yellow oil in the amount of 1.83 g (55%).

**N-(3'-Aminopropyl)-3-(4''-t-butylphenyl)propanamide – Xb**

In a 100 mL round bottom flask 3.50 g (0.0159 mol) of **IX**, 4.71 g (0.0636 mol) of 1,3-propanediamine, and 2.02 g of water were added. The mixture was heated and stirred in an oil bath at 70°C. After three hours the solution was transferred into a 250 mL separatory funnel and 80 mL of water was added. A white precipitate formed and was extracted with 2x40mL of diethyl ether. When the diethyl ether was added, three layers formed in the separatory funnel. The middle layer and the diethyl ether layers were combined, washed with water, and concentrated *in vacuo* to give a yellow oil in the amount of 4.03 g (97%).

**N-(4'-Aminobutyl)-3-(4''-t-butylphenyl)propanamide – Xc**

To a 100 mL round bottom flask was added 3.82 g (0.0174 mol) of **IX**, 6.12 g (0.0694 mol) of 1,4-butanediamine, and 2.62 g of water. The mixture was heated and stirred in an oil bath at 70°C. After three hours the solution was transferred into a 250 mL separatory funnel and 80 mL of water was added. A white precipitate formed and was extracted with 4x40mL with diethyl ether. When the diethyl ether was added, three layers formed in the separatory funnel. The bottom layer was discarded while the middle and the diethyl ether layers were combined, washed with water, dried, filtered, and concentrated *in vacuo* to give a yellow oil in the amount of 4.44 g (92%).

**N-(2'-Aminoethyl)-3-(4''-t-butylphenyl)propanamine – XIa**

In a 500 mL round bottom flask 5.31 g (0.021 mol) of **Xa** was suspended in 50 mL of dry THF. The solution was stirred, placed in an ice bath, and put under nitrogen. To the flask was slowly added 107 mL (0.107 mol) of 1M BH<sub>3</sub>·THF using a pressure-equalizing addition funnel. The nitrogen was turned off and the solution was refluxed for two hours. Nitrogen flow was resumed and after cooling in an ice bath, 17.8 mL of 6M HCl (0.107mol) was added

dropwise. The solution was allowed to stir for 20 minutes before being made basic by the addition of a NaOH solution. The solution was extracted with 3x40mL of diethyl ether. The diethyl ether layers were combined, washed with water, dried, filtered, and concentrated *in vacuo*. The product was obtained as a white solid in a yield of 3.84 g (67%).

#### N-(3'-Aminopropyl)-3-(4''-t-butylphenyl)propanamine – **XIb**

In a 250 mL round bottom flask 4.03 g (0.015 mol) of **Xb** was suspended in 40 mL of dry THF. The solution was stirred, placed in an ice bath, and put under nitrogen. To the flask was slowly added 77 mL (0.077 mol) of 1M BH<sub>3</sub>·THF. The nitrogen was turned off and the solution was refluxed for 1.5 hours. Nitrogen flow was resumed and after cooling in an ice bath, 13 mL of 6M HCl (0.077 mol) was added drop wise. The solution was allowed to stir for 20 minutes before being made basic by the addition of a NaOH solution. The solution was extracted with 3x40mL of diethyl ether. The diethyl ether layers were combined, washed with water, dried, filtered, and concentrated *in vacuo* to give a clear oil in the amount of 4.52 g (quant.). The crude product was converted to the HCl salt with 1M HCl · diethyl ether. The salt was recrystallized with boiling hot isopropanol. The hygroscopic crystals were filtered with difficulty and dried in a vacuum oven to give a waxy solid in the amount of 1.70 g (34%).

#### N-(4'-Aminobutyl)-3-(4''-t-butylphenyl)propanamine – **XIc**

In a 250 mL round bottom flask 3.45 g (0.012 mol) of **Xc** was suspended in 40 mL of dry THF. The solution was stirred, placed in an ice bath, and put under nitrogen. To the flask was slowly added 62 mL (0.062 mol) of 1M BH<sub>3</sub>·THF. The nitrogen was turned off and the solution was refluxed for 1.5 hours. Nitrogen flow was resumed and after cooling in an ice bath, 10.3 mL of 6 M HCl (0.062mol) was added dropwise. The solution was allowed to stir for 20 minutes before being made basic by the addition of a NaOH solution. The solution was extracted with 3x40mL of diethyl ether. The diethyl ether layers were combined, washed with water, dried, and filtered. The filtrate was concentrated *in vacuo* to give a white solid. Yield 3.31 g (96%). The white solid was converted to the HCl salt with 1M HCl · diethyl ether. The salt was recrystallized with boiling isopropanol. The resulting waxy solid was obtained in the amount of 0.44 g (15%). mp (dec. at 234°C).

#### N-[3-(N'-[3'-(4''-t-Butylphenyl)propyl]amino)propyl]acetamide – **VIa**

To a 100 mL round bottom flask was added 4.09 g (0.016 mol) of **XIb** dissolved in 40 mL of acetic acid followed by 1.85 g (0.018 mol) of acetic anhydride. The solution was stirred overnight, diluted with 100 mL of water, and made basic by adding 6M NaOH. The solution was extracted with 5x30mL of ethyl acetate. The combined organic layers were washed with saturated NaCl, dried, and concentrated *in vacuo* to give the product as a brown oil in the amount of 4.05 g (85%).

#### N-[3'-(N'-[3''-(4'''-t-Butylphenyl)propyl]amino)propyl]-2,2-dimethylpropanamide – **VIb**

In a 100 mL round bottom flask 1.38 g (0.0043 mol) of **XIb** · HCl was suspended in chloroform and while stirring in an ice bath, 0.50 g (0.0043 mol) of trimethylacetylchloride dissolved in two mL of chloroform was added dropwise. To the solution was slowly added 0.43 g (0.0043 mol) of triethylamine dissolved in two mL of chloroform. The solution was

stirred overnight. The solution was diluted with 4 mL of chloroform and washed with 0.5 M NaOH, dried, and concentrated *in vacuo* to give a yellow oil in the amount of 1.42 g (81%). The crude product was converted to its HCl salt with 1M HCl · diethyl ether and recrystallized from boiling isopropanol. The crystals were obtained in the amount of 0.97 g (55%). mp 151-153°C.

#### N-[4'-(N'-[3''-(4'''-t-Butylphenyl)propyl]amino)butyl]acetamide – **VIc**

To a 100 mL round bottom flask was added 2.30 g (0.008 mol) of **XIc** dissolved in 20 mL of acetic acid followed by 1.27 g (0.0096 mol) of acetic anhydride. The solution was stirred overnight, diluted with 100 mL of water, and made basic by adding 6M NaOH. The solution was extracted with 5x30mL of ethyl acetate. The combined organic layers were washed with saturated NaCl, dried, and concentrated *in vacuo* to give the products as a brown oil in the amount of 1.87 g (70%).

#### N-[4'-(N'-[3''-(4'''-t-Butylphenyl)propyl]amino)butyl]-2,2-dimethylpropanamide – **VIId**

In a test tube 0.20 g (0.6 mmol) of **XIc** · HCl (n=4) was suspended in chloroform. While stirring in an ice bath, 0.507 g (0.0006 mol) of trimethylacetylchloride dissolved in two mL of chloroform and 0.06 g (0.006 mol) of triethylamine dissolved in two mL of chloroform were added. The solution was stirred overnight. The solution was diluted with chloroform and washed with 0.5 M NaOH, dried, and concentrated *in vacuo* to give a yellow oil. Yield 0.30 g (quant.).

#### 3,4-Methylenedioxybenzyl isothiocyanate – **Va**

In a 50 mL round bottom flask was placed 2.93 g (0.017 mol) of thiocarbonyldiimidazole (TCDI) dissolved in 15 mL of DMF. To the flask was slowly added 2.0 g (0.013 mol) of **IVa** in 5 mL of DMF. The mixture was stirred at room temperature for 24 hours, diluted with 100 mL of water, and extracted with 4x40mL of ethyl acetate. The ethyl acetate layers were combined and washed with 50 mL of water followed by 50 mL of saturated NaCl. The organic solution was dried, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography using 33:67 ethyl acetate/hexanes to give 0.55 g of a yellow oil (22%).

#### 3-Methoxy-4-methoxymethoxybenzyl isothiocyanate – **Vb**

In a 50 mL round bottom flask was added 1.33 g (0.00746 mol) of TCDI and 20 mL of toluene. The solution was heated to completely dissolve the TCDI and the solution turned an orange-gold color. While in an ice bath a solution of 0.74 g (0.00373 mol) of **IVb**, 0.89 g (0.01119 mol) of pyridine, and 5 mL of toluene was added dropwise. The reaction mixture was stirred for one hour at room temperature and for an additional hour at 100°C. The solution was diluted with 200 mL of H<sub>2</sub>O, washed with 4x40mL of ethyl acetate, dried, filtered, and concentrated *in vacuo*. A precipitate formed when the resulting oil was dissolved in 50:50 ethyl acetate and hexanes. The precipitate was determined with TLC to contain a small amount of product that was not recovered. The 50:50 solution was purified by flash chromatography to obtain the product in the amount of 0.61 g (68.5%). mp 47°C.

N-(3-(4'-t-Butylphenyl)propyl)-N-(3''-(acetamido)propyl)-N'-(3,4-methylenedioxyphenyl)methyl thiourea – **IIIa**

In a 25 mL vial 0.53 g (0.05 mol) of **VIa** was dissolved in 5 mL of dry DMF. To the solution was added 0.30 g (0.0015 mol) of **Va** and the solution was stirred overnight. The reaction mixture was heated to 75°C for one hour, cooled, and diluted with 80 mL of water. The white precipitate was extracted with 4x40mL of ethyl acetate. The combined organic layers were washed with saturated NaCl solution, dried, and concentrated *in vacuo* to give a yellow oil in the amount of 0.71 g (97%). Mass spectrum FAB HRMS m/z 484.2646 [M+H]<sup>+</sup> (C<sub>27</sub>H<sub>38</sub>N<sub>3</sub>O<sub>3</sub>S requires 484.2628).

N-(3-(4'-t-Butylphenyl)propyl)-N-(3''-(2,2-dimethylpropanamido)propyl)-N'-(3,4-methylenedioxyphenyl)methyl thiourea– **IIIb**

In a vial was placed 0.23 g (0.0012 mol) of **VI·HCl**, 0.43 g (0.0012 mol) of **Va**, 5 mL of DMF, and 0.13 g (0.1177 mol) of triethylamine. The solution was stirred for 24 hours at room temperature. The solution was transferred to a 250 mL separatory funnel and diluted with 80 mL of water. A brown precipitate formed and the solution was extracted with 4x40mL of ethyl acetate. The ethyl acetate layers were dried, filtered, and concentrated *in vacuo*. The crude product was purified using flash chromatography with 40:60 ethyl acetate/hexanes as the solvent to give an oil in a yield of 0.43 g (68%). Anal. (C<sub>30</sub>H<sub>43</sub>N<sub>3</sub>O<sub>3</sub>S) %C, Theor. 68.53, Found 68.32; %H, Theor. 8.24, Found 8.22; %N, Theor. 7.99, Found 7.93; %S, Theor. 6.10, Found 6.08.

N-(3-(4'-t-Butylphenyl)propyl)-N-(3''-(2,2-dimethylpropanamido)propyl)-N'-(3-methoxy-4-hydroxyphenyl)methyl thiourea – **IIIc**

In a vial was placed 0.34 g (0.00104 mol) of **VIb**, 0.25 g (0.00104 mol) of **Vb**, and 5 mL of dry DMF. The solution was stirred for 72 hours, heated to 65°C for one hour, and diluted with 80 mL of water. A white precipitate formed. Saturated NaCl was added to the aqueous layer and the solution was extracted with 5x30mL of ethyl acetate. The ethyl acetate layers were washed with water, dried, and concentrated *in vacuo*. The product was obtained as an oil in the amount of 0.69 g (quant.). The product was dissolved in 5 mL of ethanol and 0.75 mL of concentrated HCl. The mixture was stirred for one hour, concentrated *in vacuo*, dissolved in ethyl acetate, and dried overnight. The solution was filtered and evaporated to obtain the product in the amount of 0.20 g (36%). mp 115-117°C. Anal. (C<sub>30</sub>H<sub>45</sub>N<sub>3</sub>O<sub>3</sub>S) %C, Theor. 68.27, Found 68.31; %H, Theor. 8.59, Found 8.75; %N, Theor. 7.96, Found 7.95; %S, Theor. 6.08, Found 6.13.

N-(3-(4'-t-Butylphenyl)propyl)-N-(4''-(acetamido)butyl)-N'-(3,4-methylenedioxyphenyl)methyl thiourea – **III d**

In a vial 0.53 g (0.05 mol) of **VIc** was dissolved in 5 mL of dry DMF. To the solution was added 0.30 g (0.0015 mol) of **Va** and the solution was stirred overnight. The reaction mixture was heated to 65°C for one hour, cooled, and diluted with 80 mL of water. The white precipitate was extracted with 4x40mL of ethyl acetate. The combined organic layers were washed with a saturated NaCl solution, dried, and concentrated *in vacuo* to give a crude yield of 0.48g (66%). The red oil was flash chromatographed using ethyl acetate as the solvent to

give the product as a colorless glass in the amount of 0.29 g (40%). Mass spectrum FAB HRMS  $m/z$  498.2786  $[M+H]^+$  ( $C_{28}H_{40}N_5O_3S$  requires 498.2785).

N-(3-(4'-t-Butylphenyl)propyl)-N-(4''-(acetamido)butyl)-N'-(3-methoxy-4-hydroxyphenyl)methyl thiourea – **IIIe**

In a vial 0.47 g (0.0014 mol) of **VIc** was dissolved in 5 mL of dry DMF. To the solution was added 0.30 g (0.0015 mol) of **Vb** and the solution was stirred overnight. The reaction mixture was heated to 65°C for one hour, cooled, and diluted with 80 mL of water. The white precipitate was extracted with 4x40mL of ethyl acetate. The combined organic layers were washed with saturated NaCl solution, dried, and concentrated *in vacuo* to give a crude yield of 79%. The clear oil was flash chromatographed using ethyl acetate as the solvent to give the product as a yellow oil in the amount of 0.29 g (43%). The product was dissolved in 5 mL of ethanol and 0.75 mL of concentrated HCl. The mixture was stirred for one hour, concentrated *in vacuo*, dissolved in ethyl acetate, and dried overnight. The solution was filtered and concentrated *in vacuo*, to obtain the product in the amount of 0.23 g (34%).

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