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# Synthesis and Biological Studies of Novel Neurotensin(8–13) Mimetics

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**Abstract**—Novel neurotensin (NT) (8–13) ($Arg^8$-$Arg^9$-$Pro^{10}$-$Tyr^{11}$-$Ile^{12}$-$Leu^{13}$) mimetics **3**, **4** were designed by adopting all intrinsic functional groups of the native neurotensin(8–13) and using a substituted indole as a template to mimic the pharmacophore of NT(8–13). Biological studies at subtype 1 of the NT receptor showed that **3** has a 55 and 580 nM binding affinity at rat and human neurotensin receptors, respectively. As a comparison, compounds **5** and **6** were also synthesized. The binding difference between **3**, **4** and **5**, **6** argues the importance of the carboxylic group in achieving higher potency NT(8–13) mimetics.


## Introduction

Neurotensin (NT),[1] a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu), is widely distributed in the central and peripheral systems.[2] It acts as a neuromodulator and is associated with numerous physiological functions.[3] More interestingly, NT has extremely potent antinociceptive activity[4] and may play a major role in the pathogenesis of schizophrenia.[5] It is, thus, possible that potent NT analogues could be used as effective therapeutic agents for treating schizophrenia and other diseases.

Peptidic analogues, often serve as powerful tools to elucidate structure–activity relationships,[6] are limited for use as drugs because of their susceptibility to peptidase degradation, poor oral bioavailability, and their difficulty in crossing the blood–brain barrier. Development of non-peptidic NT analogues has, therefore, been a continuing interest.[7,8] Structure–activity studies on NT show that its C-terminal hexapeptide NT(8–13) ($Arg^8$-$Arg^9$-$Pro^{10}$-$Tyr^{11}$-$Ile^{12}$-$Leu^{13}$) is equipotent to or even more potent than the native NT(1–13) in binding to the NT receptor, while maintaining the same biological and pharmacological properties.[9] Furthermore, the two guanidino groups of $Arg^8$-$Arg^9$, the hydroxyphenyl group of $Tyr^{11}$, the side chains of $Ile^{12}$-$Leu^{13}$ and the carboxylic group of $Leu^{13}$ are the functional groups of NT(8–13).[7a] Therefore, the C-terminal hexapeptide NT(8–13) was used as a model in our design of non-peptidic NT(8–13) analogues. Subsequently, we applied a multiple template approach with the help of computer modelling.[10] The strategy of the multiple template approach was to convert a vast number of conformers of a peptide into a comparatively small number of partially flexible molecules that would altogether mimic the conformation of the native peptide. Each of these partially flexible molecules is constructed of an aromatic ring as a template that is substituted with several alkylene chains with functional groups residing at the chain ends. The aromatic ring, as a template, and the alkylene chains, as spacers, were rationally designed to govern the steric positions of the functional groups to render the effective interaction of the functional groups with the receptor. According to the multiple template approach, we first prepared a series of partial non-peptidic NT(8–13) analogues (e.g., **1**, Scheme 1)[11] with a substituted indole as template mimicking $Arg^8$-$Arg^9$-$Pro^{10}$. Later, we described three non-peptidic NT(8–13) mimetics with improved potency. In these non-peptidic mimetics, various substituted pyrroles, were used as templates to substitute for $Arg^8$-$Arg^9$-$Pro^{10}$ and 1-aminoadamantane was introduced to mimic $Ile^{12}$-$Leu^{13}$ (e.g., **2**, Scheme 1).[12] We herein report the synthesis and some biochemical studies of four novel NT(8–13)

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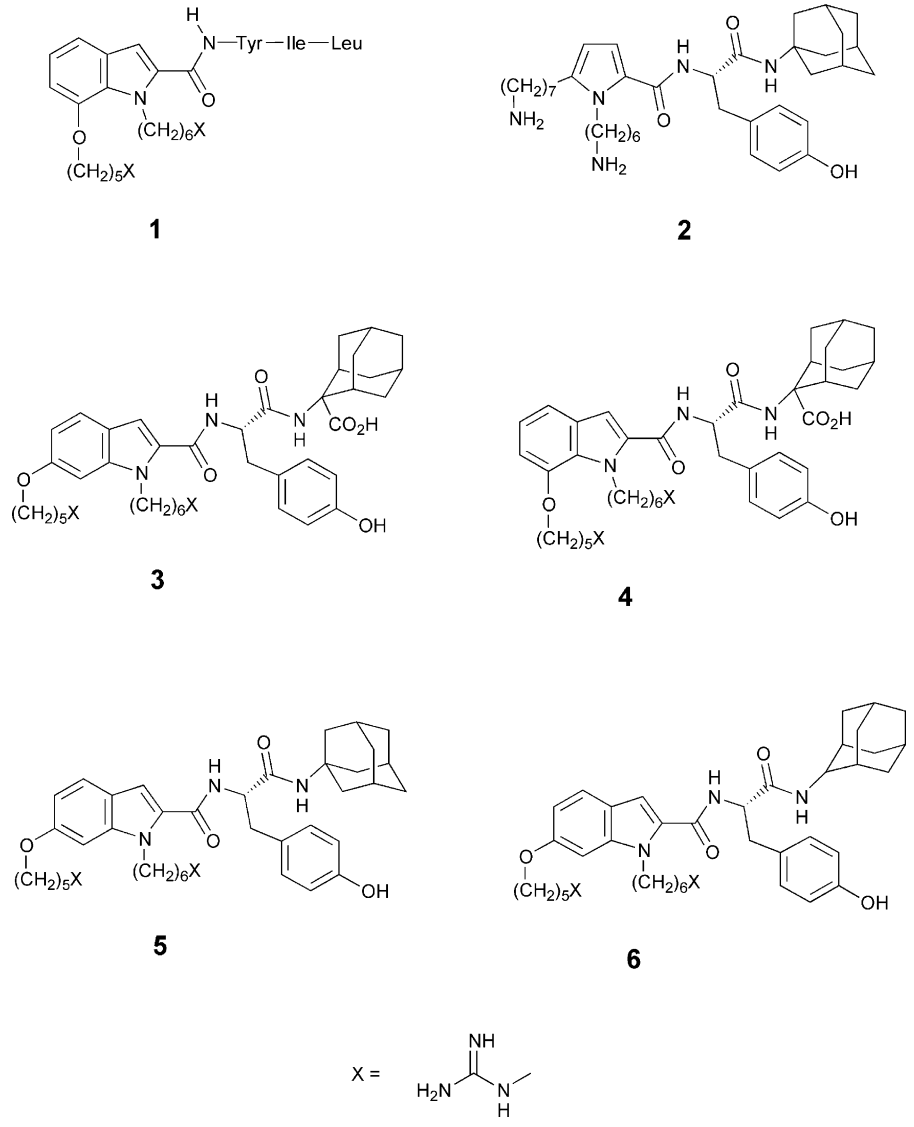



**Scheme 1.**

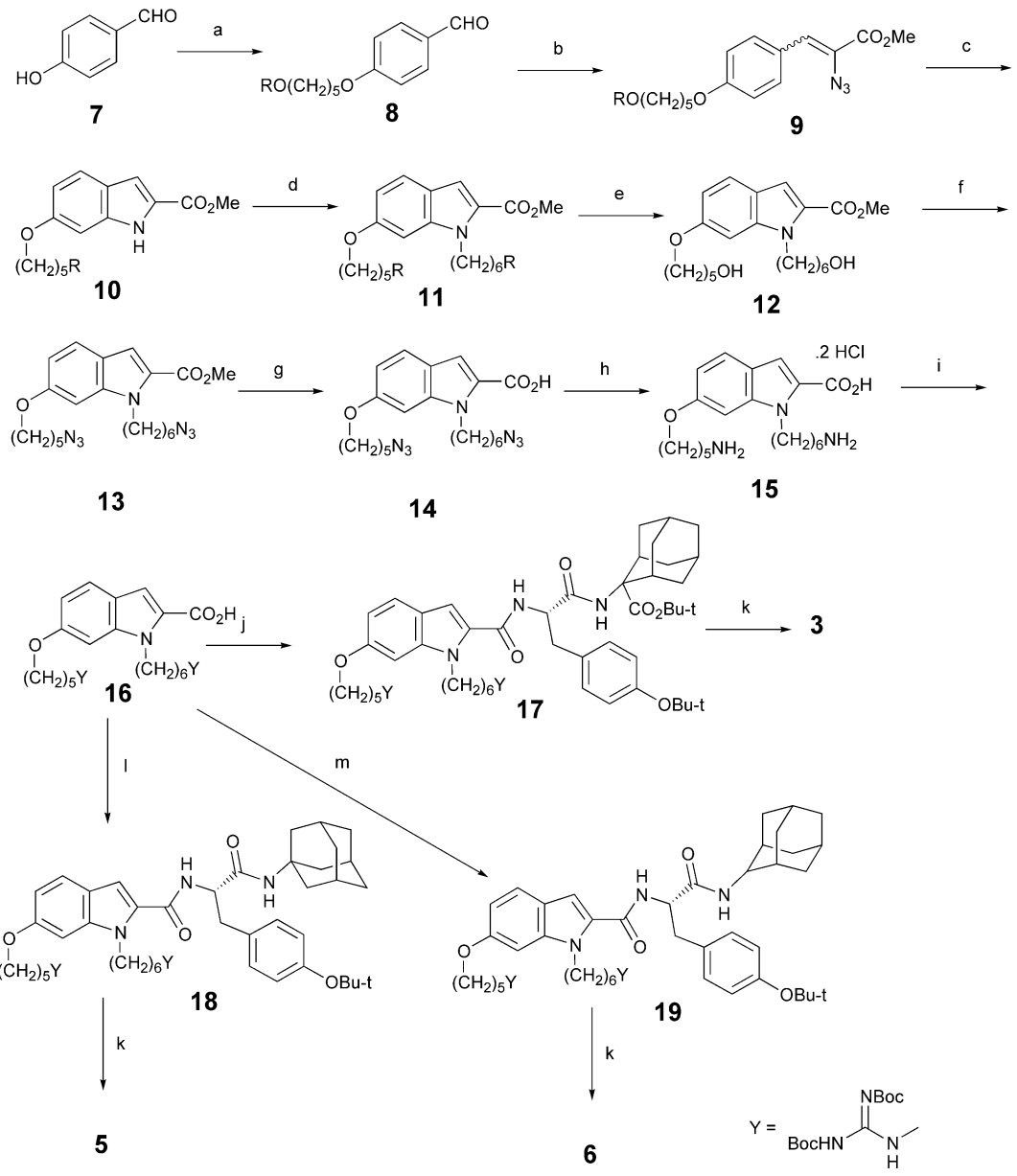
mimetics (**3**–**6**) designed by capitalizing on the findings from our previous research (Scheme 1).

Based on the results from our previous studies, we used 1-(6-guanidinohexyl)-6-(5-guanidinopentyloxy)-indole-2-carbonyl (for **3**, **5** and **6**) and 1-(6-guanidinohexyl)-7-(5-guanidinopentyloxy)-indole-2-carbonyl (for **4**) to replace the $Arg^{8}$-$Arg^{9}$-$Pro^{10}$ fragment.[11] Tyrosine was kept in these mimetics. 1-Adamantylamino (for **5**) and 2-adamantylamino (for **6**) were used to mimic $Ile^{12}$-$Leu^{13}$, because of the lipophilic property of the adamantyl backbone. In addition to this property of the adamantyl backbone, a carboxylic group was further introduced into mimics **3** and **4** by using 2-aminoadamantane-2-carboxylic acid, since the importance of the C-terminal carboxylic group to the binding potency was proven by previous structure–activity studies using peptidic analogues.[13]

## Chemistry

Mimetics **3**, **5** and **6** were synthesized through a common intermediate **16** (Scheme 2).

Alkylation of *p*-hydroxybenzaldehyde **7** with 5-(*tert*-butyldimethylsilyoxy)pentanol using Mitsunobu reaction gave alkyloxybenzaldehyde **8**. Treatment of **8** with methyl azidoacetate in the presence of MeONa yielded the azidoacrylate **9**, which was converted into indole **10** by refluxing in toluene. Alkylation of **10** with 6-(*tert*-butyldimethylsilyoxy)hexyl iodide led to 1,2,6-tris-substituted indole **11**. The two *tert*-butyldimethylsilyoxy moieties in **11** were transformed into two azido groups in **13**, first by reacting with TBAF in THF, then with $HN_3$, $PPh_3$ and DEAD in $CH_2Cl_2$. Saponification of **13** with 2 N KOH afforded diazido acid **14**, which after being subjected to catalytic hydrogenation in the presence of concentrated hydrochloric acid afforded **15** in the bishydrochloride salt form. Treatment of **15** with *N*,*N*′-bis-(*tert*-butyloxycarbonyl)-*S*-methylisothiourea and Triton-B in methanolic solution in DMSO provided the intermediate **16**. Coupling of **16** with **27**, **28**, and **29** promoted by DCC and HOBT in DMF generated **17**, **18**, and **19**, respectively. Deprotection of **17**–**19** with trifluoroacetic acid and methylene chloride (1:1), followed by purification using reverse-phase HPLC yielded




**Scheme 2.** Reagents for: (a) $TBDMSO(CH_2)_5OH$, $PPh_3$, DEAD, 93%; (b) $N_3CH_2CO_2Me$, NaOMe, MeOH, 58%; (c) Toluene, reflux, 67%; (d) $TBDMSO(CH_2)_6I$, $K_2CO_3$, acetonitrile, 85%; (e) TBAF, THF, 86%; (f) $N_3H$, DEAD, $CH_2Cl_2$, 90%; (g) 2N KOH aq, EtOH, 99%; (h) $H_2$, Pd/C, concd HCl/aq, MeOH/ethyl acetate, 97%; (i) *N*,*N*′-di-*tert*-Butoxycarbonyl-*s*-methyl-isothiourea, Triton-B, DMSO, 67%; (j) **27**, DCC, HOBT, DMF, 67%; (k) $TFA/CH_2Cl_2$; (l) **28**, DCC, HOBT, DMF, 88%; (m) **29**, DCC, HOBT, DMF, 97%.

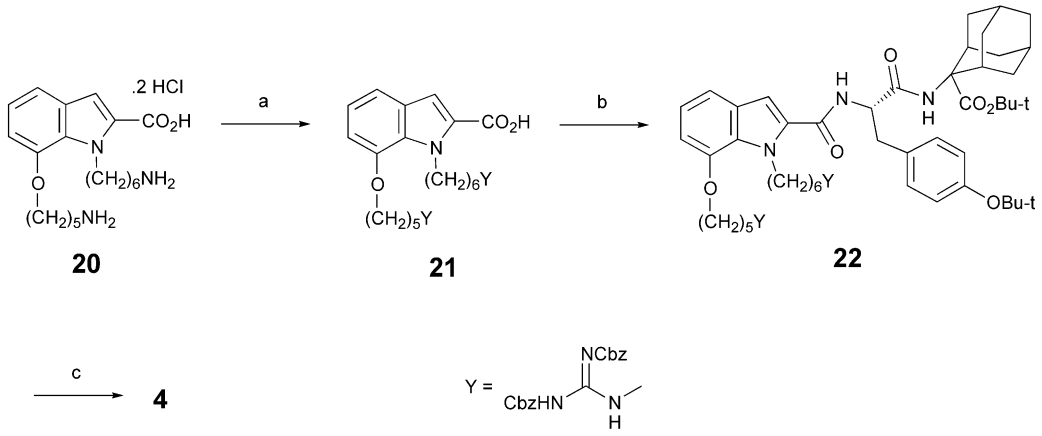
**3**, **5**, and **6**. Mimic **4** was derived from intermediate **21** that was prepared similarly to the synthesis of **16** by reaction of (**20**)[11] with *N*, *N*′-bis-benzyloxycarbonyl-*S*-methylisothiourea (Scheme 3). Coupling of **21** with **27** was carried out in the same way as for the preparation of **17** to give **22**. Deprotection of **22**, first by catalytic hydrogenation in the presence of Pd/C and AcOH in methanol, subsequently by treating with TFA in $CH_2Cl_2$, afforded mimic **4** after purification on reverse-phase HPLC.

Preparation of **27** started from 2-amino-adamantyl-2-carboxylic acid (**23**)[14] (Scheme 4).

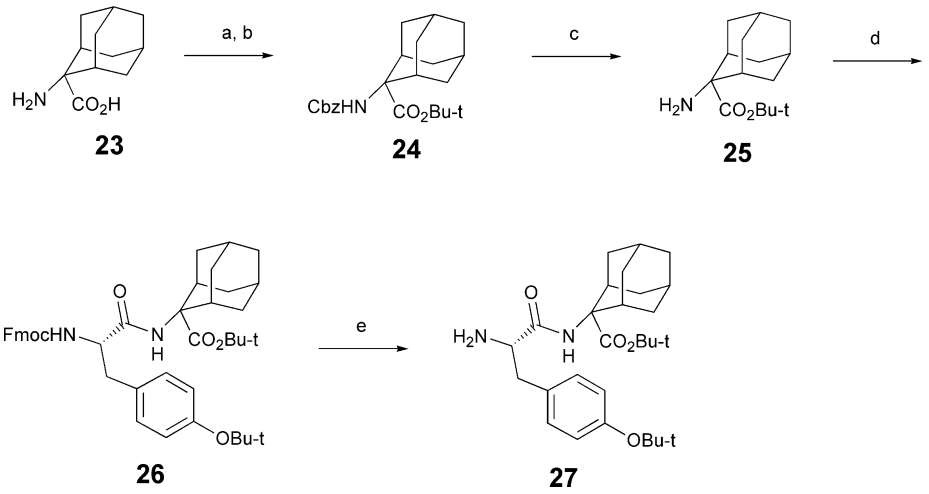
Benzyloxycarbonylation of (**23**)[15] followed by treatment with $(t\text{-BuO})_2CHNMe_2$ in toluene at 110 °C led to **24**.[16] Removal of the benzyloxycarbonyl group was performed under catalytic hydrogenation condition resulted in **25**. Coupling of **25** with *N*-Fmoc-*O*-*tert*-butyl-tyrosine using DCC and HOBT yielded **26**, which was converted into **27** by treating with 5% of piperidine in THF. Compound **29** was prepared according to the method for the synthesis of **28** as described in our previous paper.[17]

## Biological results and discussion

Mimics **3**–**6** were tested for their ability to compete for [$^3$H]neurotensin binding at the human and rat NT receptors, subtype 1, by the use of methods previously described.[18] The sources of these receptors were membranal preparations from two CHO-K1 cell lines, stably expressing these receptors. The results are listed in table 1 along with those for NT(1–13) and NT(8–13).




**Scheme 3.** Reagents for: (a) *N*,*N*′-di-*tert*-butoxycarbonul-*s*-methyl-isothiourea, Triton-B, DMSO, 47%; (b) **27**, DCC, HOBT, DMF, 88%; (c) TFA/$CH_2Cl_2$.




**Scheme 4.** Reagents for: (a) Cbz-Cl, 2N KOH aq, 23%; (b) *N*,*N*-Dimethylformamide di-*tert*-butyl acetal, toluene, 49%; (c) $H_2$, Pd/C, AcOH, MeOH, 91%; (d) *N*-Fmoc-*O*-*tert*-butyl-tyrosine, DCC, HOBT, DMF, 92%; (e) 5% piperidine in THF, 85%.

Among all four mimetics, mimics **3** and **4** were equipotent at the human neurotensin receptor with a $K_d$ of 580 nM. However, mimic **3** ($K_d = 55$ nM) was about twice as potent as mimic **4** ($K_d = 101$ nM ) at the rat neurotensin receptor. Comparison of mimics **3** ($K_d = 580$ and 55 nM at human and rat NT receptors, respectively) and **4** ($K_d = 580$ and 101 nM at human and rat NT receptors, respectively) with **5** ($K_d = 2100$ and 450 nM at human and rat NT receptors, respectively) and **6** ($K_d = 2100$ and 2700 nM at human and rat NT receptors, respectively) argued the importance of the carboxylic acid group for the binding affinity, both at human and rat neurotensin receptors, as has been established from studies with the peptidic NT analogues.[7a] The potency difference resulting from the carboxylic acid for these mimetics is, however, far less than that for peptidic analogues. This lack of a distinguishing potency difference could suggest that the bulky adamantyl skeleton hinders the carboxylic acid group from reaching the optimal binding site of the receptors. Mimics **3**, **4**, and **5** were about 5- to 10- fold more potent at rat than at the human NT receptors. Interestingly, mimic **6** appeared to be slightly more potent at the human than at the rat NT receptor. The little steric and lipophilic difference between 1-adamantyl (in mimic **5**) and 2-adamantyl (in mimic **6**) may explain the same binding affinity between mimic **5** and mimic **6** at the human NT receptor. Surprisingly, mimic **5** is about five times as potent as mimic **6** when binding at rat receptor. Similarly, the steric difference between mimics **3** and **4**, caused by shifting the 5-guanidinopentyloxy group from

**Table 1.** Binding potencies of NT(1–13), NT(8–13) and mimetics **3**–**6** at human and rat NT receptors, subtype 1, expressed in CHO-K1 membranes[a]

| Compound | $K_d$ (nM) at human NT receptor | $K_d$ (nM) at rat NT receptor |
|---|---|---|
| NT(1–13) | 1.70±0.06 (95) | 2.10±0.09 (60) |
| NT(8–13) | 0.16±0.01 (4) | 0.14±0.01 (3) |
| **3** | 580±50 (4) | 55±10 (4) |
| **4** | 580±40 (3) | 101±3 (3) |
| **5** | 2100±200 (5) | 450±20 (3) |
| **6** | 2100±200 (3) | 2700±100 (3) |

[a]$K_d$ is equilibrium dissociation constant. Values are geometric mean ±SEM. *n* values are in parentheses.

the 6 (in mimic **3**) to the 7 (in mimic **4**) position at the indole part, resulted in nearly no affinity change at the human NT receptors. However, this caused the binding affinity at the rat NT receptor to be lowered by one-half.

In a large series of peptide analogues of NT(8–13) studies, most compounds had equal affinity for the rat as compared to the human NT receptors, subtype 1.[6b] A few compounds had higher affinity for the rat receptor than for the human receptor and none had higher affinity for the human receptor over that for the rat receptor.[6b] We recently identified the third outer loop of the NT receptor, subtype 1, as the binding site for NT(8–13) and its analogues.[6c,d] The larger binding pocket in the rat receptor, therefore more accommodative to ligand size, may explain why compounds bind with higher affinity to this receptor than to the human receptor. [6d]

Mimics **3, 5** and **6** were further examined for their effect on phosphatidylinositol turnover (PI) in intact CHO-K1 cell expressing either human or rat NT receptors (subtype 1). The NT receptor is coupled to PI turnover. In each case, compounds were tested at a final concentration of 0.1 mM. Our results indicated that mimic **3** behaved like an antagonist at both the rat and human NT receptors. Mimics **5** and **6** were not antagonists at the rat receptor. Interestingly, the evidence suggests that mimic **5** was a partial agonist.

## Conclusion

In summary, we have reported the synthesis and binding activity of compounds **3**, **4**, **5**, and **6** as novel NT(8–13) mimetics. They were designed with the intention of using the intrinsic functional groups of NT(8–13). The steric orientation of these functional groups was controlled by a bis-alkylene substituted indole as the template. The affinity difference between **3**, **4**, and **5**, **6** confirmed the important contribution of the carboxylic group to the binding affinity. However, the lengthy spacers in these compounds make these compounds too flexible to be entropically favorable upon binding. The findings could aid in our subsequent effort to pursue more potent NT(8–13) mimetics by designing compounds that better mimic the pharmacophore of NT(8–13) with approriate rigidity.

## Experiment

Tetrahydrofuran was distilled from sodium benzophenone ketyl prior to use. DMSO and DMF were dried with $CaH_2$. Methylene chloride was distilled from $P_2O_5$ prior to use. Solvents used for chromatography were purchased in 5-gal drums, redistilled from an all-glass apparatus, and stored in glass bottles. Silica gel 60 (Merck, 230–400 mesh ASTM for flash chromatography) was used for column chromatography. TLC was performed on Merck silica gel 60F-254 (0.25 mm, precoated on glass). Other reagents were used as supplied by the Aldrich Chemical Co. NMR spectra were taken on a Bruker AC-300 (300 MHz for $^1H$ and 75.5 MHz for $^{13}C$) instrument. Chemical shifts are reported in δ units with reference to $Me_4Si$ (δ = 0.00 ppm) for $^1H$ or $CDCl_3$ (δ = 77.0 ppm) and DMSO-$d_6$ (δ = 39.5 ppm) for $^{13}C$ as internal standards. *J* Values were recorded in Hz. Mass spectra were obtained on a Finnigan MAT-900 instrument. Low- and high-resolution mass data were collected by employing EI at 70 eV with PFK reference or by using ESI with a reference material of PEG 400. Melting points were determined in open capillary tubes on a Gallenkamp capillary melting point apparatus and are uncorrected.

**4-[5-(*tert*-Butyldimethylsilyloxy)pentyloxy]benzaldehyde (8).** To a solution of 4-hydroxybenzaldehyde (1.4 g, 11.5 mmol), 5-(*tert*-butyldimethylsilyloxy)pentanol (2.2 g, 10 mmol) and triphenylphosphine (3.1 g, 12 mmol) in dry $CH_2Cl_2$ (40 mL) was added diethyl azidodicarboxylate (DEAD) (2.1 g, 12 mmol) at 0 °C under $N_2$ with stirring. Stirring was continued for 30 min at the same temperature, then 1 h at room temperature. The reaction mixture was poured into $H_2O$ (40 mL), extracted with $CH_2Cl_2$ (2 × 40 mL). The combined extracts were washed with saturated $NaHCO_3$ (40 mL), brine (50 mL) and dried ($MgSO_4$). Solvent was removed to give a residue, which was chromatographed on a silica gel column, eluting with 5% of ethyl acetate/hexanes, to afford **8** (3.0 g, 93%) as a light yellow oil; ν max (neat)/$cm^{-1}$: 1694, 1601, 1256, 1098; $^1H$ NMR (300 MHz, $DCCl_3$) δ 9.88 (s, 1H), 7.83 (d, *J* = 8.7 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H), 4.05 (t, *J* = 6.5 Hz, 2H), 3.65 (t, *J* = 6.2 Hz, 2H), 1.91–1.81 (m, 2H), 1.65–1.50 (m, 4H), 0.90 (s, 9H), 0.05 (s, 6H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 190.7, 164.2, 131.9, 129.8, 114.7, 68.3, 62.9, 32.4, 28.8, 25.9, 22.3, 18.3,−5.3; LRMS (EI) 265 ($M^+-C_4H_9$); HRMS (EI) *m*/*z* calculated for $C_{14}H_{21}O_3Si$ ($M^+-C_4H_9$) 265.1259, found 265.1397.

**Methyl 2-azido-3-{4-[5-(*tert*-butyldimethylsilyloxy)pentyloxy]phenyl}propenoate (9).** Sodium metal (0.5 g, 21.7 mmol) was added in portions into anhydrous methanol (40 mL) under $N_2$. The resultant sodium methoxide solution was cooled to −5 °C and a mixture of the aldehyde **8** (2.0 g, 6.2 mmol) and methyl azidoacetate (2.1 g, 18.3 mmol) in methanol (5 mL) was added to it dropwise. The mixture was stirred at 0 °C for 1 h, then overnight at room temperature. It was then quenched with cold $H_2O$ (50 mL) and extracted with ether (3 × 60 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The oil was chromatographed on silica gel, using 5% of ethyl acetate/hexanes as eluent, to yield **9** (1.5 g, 58%) as a light yellow oil; $\nu_{max}$ (neat)/$cm^{-1}$: 2122, 1715, 1703, 1602, 1252, 1097; $^1H$ NMR (300 MHz, $DCCl_3$) δ 7.78 (d, *J* = 8.9 Hz, 1H), 6.93–6.85 (m, 2H), 4.00 (t, *J* = 6.5 Hz, 2H), 3.90 (s, 3H), 3.64 (t, *J* = 6.3 Hz, 1H), 1.86–1.78 (m, 2H), 1.61–1.48 (m, 4H), 0.90 (s, 9H), 0.05 (s, 6H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 164.2, 160.1, 132.4, 127.8, 125.8, 125.7, 122.9, 114.4, 103.0, 67.9, 62.9, 52.7, 32.5, 29.0, 28.9, 25.9, 22.3, 18.3, −5.3; LRMS (EI) 419 ($M^+$); HRMS (EI) *m*/*z* calculated for $C_{21}H_{33}N_3O_4Si$ ($M^+$) 419.2260, found 419.2276.

**Methyl 6-[5-(*tert*-butyldimethylsilyloxy)pentyloxy]indole-2-carboxylate (10).** A solution of **9** (1.37 g, 3.26 mmol) in dry toluene (15 mL) was refluxed under $N_2$ for 8 h. Solvent was removed under reduced pressure. Crude product that was crystallized from ethyl acetate and hexane to yield **10** (0.86 g, 67%) as a white powder, mp 105.5–106.9 °C; ν max (KBr)/$cm^{-1}$: 3322, 1686, 1631, 1252, 1101; $^1$H NMR (300 MHz, $DCCl_3$) δ 8.74 (s, 1H), 7.54 (d, $J$ = 9.2 Hz, 1H), 7.14 (d, $J$ = 1.8 Hz, 1H), 6.86–6.82 (m, 2H), 4.00 (t, $J$ = 6.5 Hz, 2H), 3.92 (s, 3H), 3.65 (t, $J$ = 6.2 Hz, 2H), 1.94–1.81 (m, 2H), 1.68–1.50 (m, 4H), 0.90 (s, 9H), 0.06 (s, 6H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 162.4, 158.4, 138.0, 125.9, 123.3, 121.7, 112.8, 109.2, 94.4, 68.2, 63.0, 51.8, 32.5, 29.0, 26.0, 22.4, 18.4, −5.3; LRMS (EI) 391 ($M^+$); HRMS (EI) $m/z$ calculated for $C_{21}H_{33}NO_4Si$ ($M^+$) 391.2178, found 391.2183.

**Methyl 1-[6-(*tert*-butyldimethylsilyloxy)hexyl]-6-[5-(*tert*-butyldimethylsilyloxy)pentyloxy]indole-2-carboxylate (11).** A mixture of **10** (790 mg, 2.0 mmol), 6-(*tert*-butyldimethylsilyloxy)hexyl iodide (820 mg, 2.4 mmol), $K_2CO_3$ (552 mg, 4 mmol), and 18-crown-6 (30 mg) in acetonitrile (20 mL) was stirred at 70 °C for 12 h under $N_2$. After being cooled to room temperature, the mixture was poured into $H_2O$ (50 mL), and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$), and evaporated under reduced pressure to give a light yellow oil. Chromatography of the oil on a silica gel column, eluting with 5% of ethyl acetate/hexanes, yielded **11** (1.1 g, 85%) as a white solid, 40.2–42.1 °C; $\nu_{max}$ (neat)/$cm^{-1}$: 1713, 1624, 1470, 1221, 1105; $^1$H NMR (300 MHz, $DCCl_3$) δ 7.52 (d, $J$ = 8.7 Hz, 1H), 7.23 (s, 1H), 6.81 (dd, $J$ = 2.0 and 8.7 Hz, 1H), 6.74 (s, 1H), 4.48 (t, $J$ = 7.6 Hz, 2H), 4.03 (t, $J$ = 6.4 Hz, 2H), 3.88 (s, 3H), 3.65 (t, $J$ = 6.2 Hz, 2H), 3.58 (t, $J$ = 6.6 Hz, 2H), 1.92–1.76 (m, 4H), 1.65–1.46 (m, 6H), 1.42–1.35 (m, 4H), 0.89 (s, 9H), 0.88 (s, 9H), 0.06 (s, 6H), 0.03 (s, 6H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 162.3, 158.1, 140.1, 126.0, 123.4, 120.3, 112.1, 111.0, 93.3, 68.2, 63.1, 63.0, 51.3, 44.6, 32.7, 32.6, 30.4, 29.1, 26.8, 25.9, 25.6, 22.4, 18.3, −5.3; LRMS (EI) 605 ($M^+$); HRMS (EI) $m/z$ calculated for $C_{33}H_{59}NO_5Si_2$ ($M^+$) 605.3931, found 605.3939.

**Methyl 1-(6-hydroxyhexyl)-6-(5-hydroxypentyloxy)indole-2-carboxylate (12).** To a solution of **11** (1.1 g, 1.7 mmol) in THF (10 mL) was added tetrabutylammonium fluoride (5.1 mL, 1 M in THF) at room temperature with stirring. Stirring was continued for 90 min. The mixture was poured into saturated $NH_4Cl$ aq (50 mL), and then extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with $H_2O$ (2 × 50 mL), brine (50 mL), and dried ($MgSO_4$). Solvent was removed under reduced pressure to give a yellow oil, which was subjected to silica gel column chromatography, eluting with 80% of ethyl acetate/hexanes, to afford **12** (550 mg, 86%) as a light yellow oil; $\nu_{max}$ (neat)/$cm^{-1}$: 3364, 1711, 1622, 1495, 1209; $^1$H NMR (300 MHz, $DCCl_3$) δ 7.53 (d, $J$ = 8.7 Hz, 1H), 7.23 (s, 1H), 6.81 (dd, $J$ = 2.0 and 8.7 Hz, 1H), 6.75 (s, 1H), 4.49 (t, $J$ = 7.5 Hz, 2H), 4.05 (t, $J$ = 6.5 Hz, 2H), 3.88 (s, 3H), 3.70 (t, $J$ = 6.3 Hz, 2H), 3.62 (t, $J$ = 6.5 Hz, 2H), 1.93–1.74 (m, 4H), 1.72–1.50 (m, 8H), 1.49–1.35 (m, 4H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 162.3, 157.9, 140.1, 125.9, 123.3, 120.2, 112.1, 111.0, 93.3, 68.1, 62.5, 51.3, 44.3, 32.4, 32.3, 30.1, 28.9, 26.5, 25.3, 22.3; LRMS (EI) 377 ($M^+$); HRMS (EI) $m/z$ calculated for $C_{21}H_{33}NO_5$ ($M^+$) 377.2202, found 377.2211.

**Methyl 1-(6-azidohexyl)-6-(5-azidopentyloxy)indole-2-carboxylate (13).** To a solution of **12** (520 mg, 1.38 mmol), triphenylphosphine (868 mg, 3.3 mmol) in dry $CH_2Cl_2$ (20 mL) was added $HN_3$ (2.8 mL, 1.5 in $CH_2Cl_2$) and followed by DEAD (624 mg, 3.6 mmol) by the drop at 0 °C with stirring. Stirring was continued for 30 min at room temperature. The mixture was poured into $H_2O$ (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The oil was chromatographed on a silca gel column, eluting with 10% of ethyl acetate/hexanes, to yield **13** (533 mg, 90%) as a yellow oil; $\nu_{max}$ (neat)/$cm^{-1}$: 2097, 1711, 1622, 1495, 1209; $^1$H NMR (300 MHz, $DCCl_3$) δ 7.53 (d, $J$ = 8.7 Hz, 1H), 7.23 (s, 1H), 6.81 (d-d, $J$ = 2.1 and 8.8 Hz, 1H), 6.74 (s, 1H), 4.04 (t, $J$ = 6.2 Hz, 2H), 3.88 (s, 3H), 3.33 (t, $J$ = 6.7 Hz, 2H), 3.24 (t, $J$ = 6.8 Hz, 2H), 1.94–1.56 (m, 10H), 1.46–1.37 (m, 4H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 162.3, 157.9, 140.0, 126.0, 123.4, 120.3, 112.0, 111.0, 93.3, 67.9, 51.32, 51.3, 44.4, 30.1, 28.8, 28.6, 28.59, 26.4, 23.4; LRMS (EI) 427 ($M^+$); HRMS (EI) $m/z$ calculated for $C_{21}H_{29}N_7O_3$ ($M^+$) 427.2331, found 427.2340.

**1-(6-azidohexyl)-6-(5-azidopentyloxy)indole-2-carboxylic acid (14).** A solution of **13** (450 mg, 1.05 mmol) in methanol (4 mL), THF (6 mL) and 4N KOH. aq (2 mL) was stirred for 12 h at room temperature. The solution was acidified with 20% of HCl to pH = 4 and extracted with ethyl acetate (3 × 40 mL). The combined extract was washed with brine (50 mL), dried ($MgSO_4$), and filtered through a very short silica gel column. Solvent was removed to give pure **14** (430 mg, 99%) as a light yellow powder: mp 54.5–55.8 °C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3073 (br), 2095, 1669, 1622, 1240; $^1$H NMR (300 MHz, $DCCl_3$) δ 7.56 (d, $J$ = 8.8 Hz, 1H), 7.42 (s, 1H), 6.82 (d, $J$ = 8.9 Hz, 1H), 6.73 (s, 1H), 4.51 (t, $J$ = 7.0 Hz, 2H), 4.05 (t, $J$ = 6.2 Hz, 2H), 3.33 (t, $J$ = 6.4 Hz, 2H), 3.25 (t, $J$ = 6.7 Hz, 2H), 1.95–1.53 (m, 10H), 1.51–1.32 (m, 4H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 166.6, 158.5, 140.8, 125.1, 123.8, 120.4, 113.4, 112.5, 93.2, 68.0, 51.4, 44.5, 30.1, 28.9, 28.72, 28.7, 26.4, 26.3, 23.5; LRMS (ESI) 414 ($M + H^+$); HRMS (ESI) $m/z$ calculated for $C_{20}H_{28}N_7O_3$ ($M + H^+$) 414.2253, found 414.2253.

**1-{[6-*N*, *N*′-bis-(*tert*-Butyloxycarbonyl)guanidino]hexyl}-6-{[5-*N*, *N*′-bis(*tert*-butyloxycarbonyl)-guanidino]pentyloxy}-indole-2-carboxylic acid (16).** To a solution of **14** (470 mg, 1.13 mmol) in MeOH (5 mL), ethyl acetate (5 mL) and concentrated HCl (0.2 mL) was added Pd/C (10%, 50 mg). The mixture was stirred under an atmosphere of $H_2$ for 12 h at room temperature, then filtered through Celite. The filtrate was evaporated and dried under vacuum to give **15** (474 mg, 97%) as a light yellow powder, which was directly used in the preparation of **16**. Thus, to a solution of **15** (474 mg, 1.1 mmol), *N*,*N*′-bis-*tert*-butyloxycarbonyl-*S*-methyl-isothiourea (696

mg, 2.4 mmol) in DMSO (15 mL) was added Triton-B (40% in MeOH, 1.5 mL). The solution was stirred at rt for 72 h under $N_2$, then poured into cold water, acidified with 20% HCl to pH = 4. Next, it was extracted with ethyl acetate (3 × 30 mL). The combined extracts were washed with water (50 mL), brine (50 mL), dried ($Na_2SO_4$), and concentrated under reduced pressure. The crude product was purified by column chromatography, eluting with 5% of methanol/chloroform, to afford the desired product **16** (660 mg, 71%) as a light yellow powder, mp 97.4–99.5 °C; $\nu_{max}$ (KBr)/cm$^{-1}$: 3331, 3138 (br), 1723, 1634, 1138; $^1$H NMR (300 MHz, $DCCl_3$) δ 11.49 (s, 1H), 8.37 (t, $J$ = 4.9 Hz, 1H), 8.31 (t, $J$ = 5.0 Hz, 1H), 7.54 (d, $J$ = 8.8 Hz, 1H), 7.37 (s, 1H), 6.80 (dd, $J$ = 1.9 and 8.7 Hz, 1H), 6.72 (s, 1H), 4.51 (t, $J$ = 7.0 Hz, 2H), 4.02 (t, $J$ = 6.2 Hz, 2H), 3.49–3.34 (m, 4H), 1.92–1.30 (m, 50H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 165.7, 163.4, 158.2, 156.1, 153.2, 140.4, 125.7, 123.5, 120.3, 112.2, 93.2, 83.0, 82.9, 79.2, 67.9, 44.3, 40.9, 40.8, 30.0, 28.9, 28.7, 28.2, 28.0, 26.5, 26.4, 23.4; LRMS (ESI) 846 (M + H$^+$); HRMS (ESI) $m/z$ calculated for $C_{42}H_{68}N_7O_{11}$ (M + H$^+$) 846.4976, found 846.5048.

***N*-{(2*S*)-1-[2-(*tert*-Butyloxycarbonyl)adamantyl-2-amino]-3-(4-*tert*-butyloxyphenyl)-1-oxo-propan-2-yl} 1-{6-[*N*,*N*′-bis-(*tert*-butyloxycarbonyl)guanidino]-hexyl}-6-{[5-(*N*,*N*′-bis-(*tert*-butyloxycarbonyl)guanidino]pentyloxy}indole-2-carboxamide (17).** A solution of **16** (101 mg, 0.14 mmol), *N*-2-[(2-*tert*-butyloxycarbonyl)-2-adamantyl] (2*S*)-2-amino-3-(4-*tert*-butyloxyphenyl)propanamide (54 mg, 0.12 mmol), DCC (30 mg, 0.15 mmol) and HOBT (20 mg, 0.15 mmol) in DMF (8 mL) was stirred at room temperature for 12 h under $N_2$. The mixture was then poured into cold water (30 mL), and then extracted with EtOAc (3 × 30 mL). The combined extracts were washed with $H_2O$ (40 mL), brine (40 mL), dried ($Na_2SO_4$), and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel, using 30% of ethyl acetate/hexanes as eluent, to give **17** (105 mg, 67%) as a light yellow oil; $\nu_{max}$ (neat)/cm$^{-1}$: 3331, 1721, 1640, 1159; $^1$H NMR (300 MHz, $DCCl_3$) δ 11.52 (s, 1H), 11.50 (s, 1H), 8.35 (t, $J$ = 4.8 Hz, 1H), 8.28 (t, $J$ = 4.7 Hz, 1H), 7.45 (d, $J$ = 8.7 Hz, 1H), 7.26–7.23 (m, 2H), 6.96 (d, $J$ = 8.3 Hz, 2H), 6.79 (dd, $J$ = 1.8 and 8.7 Hz, 1H), 6.75–6.63 (m, 3H), 6.24 (s, 1H), 4.80–4.71 (m, 1H), 4.43 (t, $J$ = 7.8 Hz, 2H), 4.03 (t, $J$ = 6.2 Hz, 2H), 3.54–3.45 (m, 2H), 3.45–3.35 (m, 2H), 3.20 (d-d, $J$ = 5.9 and 13.9 Hz, 1H), 3.06 (dd, $J$ = 8.2 and 13.9 Hz, 1H), 2.37 (s, 2H), 2.16–2.03 (m, 2H), 2.00–1.28 (m, 78H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 170.9, 169.3, 163.3, 162.2, 157.5, 156.1, 154.4, 153.3, 139.5, 131.7, 129.9, 129.5, 124.4, 122.8, 120.3, 111.8, 105.2, 93.6, 83.0, 80.5, 79.2, 78.4, 68.0, 63.9, 54.4, 49.1, 44.6, 40.9, 40.8, 37.8, 37.1, 33.9, 33.7, 33.0, 32.9, 32.7, 30.3, 29.0, 28.8, 28.3, 28.1, 27.9, 26.8, 26.7, 26.4, 25.6, 24.9, 23.5; LRMS (ESI) 1298 (M + H$^+$); HRMS (ESI) $m/z$ calculated for $C_{70}H_{108}N_9O_{14}$ (M + H$^+$) 1298.8015, found 1298.8007.

***N*-[(2*S*)-1-Adamantylamino-3-(4-*tert*-butyloxyphenyl)-1-oxo-propan-2-yl] 1-{6-[*N*,*N*′-bis-(*tert*-butyloxycarbonyl)-guanidino]hexyl}-6-{[5-(*N*,*N*′-bis-(*tert*-butyloxy-carbonyl)guanidino]pentyloxy}indole-2-carboxamide (18).** This was prepared similarly to the preparation of **17** in 88% yield as a light yellow oil; $\nu_{max}$ (neat)/cm$^{-1}$: 3329, 1723, 1634, 1159; $^1$H NMR (300 MHz, $DCCl_3$) δ 11.51 (s, 1H), 11.49 (s, 1H), 8.35 (t, $J$ = 4.9 Hz, 1H), 8.28 (t, $J$ = 4.8 Hz, 1H), 7.46 (d, $J$ = 8.7 Hz, 1H), 7.21 (d, $J$ = 8.3 Hz, 2H), 6.97 (d, $J$ = 8.3 Hz, 2H), 6.88 (d, $J$ = 7.7 Hz, 1H), 6.83–6.74 (m, 2H), 5.12 (s, 1H), 4.63–4.42 (m, 2H), 4.03 (t, $J$ = 6.2 Hz, 2H), 3.55–3.45 (m, 2H), 3.44–3.35 (m, 2H), 3.23 (dd, $J$ = 5.1 and 13.5 Hz, 1H), 2.93 (dd, $J$ = 9.3 and 13.5 Hz, 1H), 2.02 (s, 3H), 1.95–1.30 (m, 71H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 169.3, 163.6, 161.9, 157.4, 156.1, 156.0, 154.3, 153.3, 153.2, 139.4, 131.9, 130.0, 129.9, 124.4, 122.7, 120.4, 111.6, 105.1, 93.6, 83.0, 79.1, 78.4, 68.0, 55.1, 52.1, 44.4, 41.4, 40.9, 40.8, 38.5, 36.2, 30.3, 29.3, 29.0, 28.9, 28.8, 28.3, 28.0, 26.7, 23.5; LRMS (ESI) 1198 (M + H$^+$); HRMS (ESI) $m/z$ calculated for $C_{65}H_{100}N_9O_{12}$ (M + H$^+$) 1198.7491, found 1198.7536.

***N*-[(2*S*)-1-(2-adamantylamino)-3-(4-*tert*-butyloxyphenyl)-1-oxo-propan-2-yl] 1-{6-[*N*, *N*′-bis-(*tert*-butyloxycarbonyl)-guanidino]hexyl}-6-{[5-(*N*, *N*′-bis-(*tert*-butyloxy-carbonyl)-guanidino]pentyloxy}indole-2-carboxamide (19).** This was prepared similarly to the preparation of **17** in 97% yield as a light yellow oil; $\nu_{max}$ (neat)/cm$^{-1}$: 3329, 1723, 1642, 1155, 1134; $^1$H NMR (300 MHz, $DCCl_3$) δ 11.51 (s, 1H), 11.49 (s, 1H), 8.34 (t, $J$ = 4.8 Hz, 1H), 8.27 (t, $J$ = 4.7 Hz, 1H), 7.46 (d, $J$ = 8.7 Hz, 1H), 7.21 (d, $J$ = 8.3 Hz, 2H), 6.95 (d, $J$ = 8.3 Hz, 2H), 6.83–6.72 (m, 3H), 6.18 (d, $J$ = 8.0 Hz, 1H), 4.80–4.70 (m, 1H), 4.56–4.48 (m, 2H), 4.08–3.95 (m, 3H), 3.53–3.42 (m, 2H), 3.42–3.32 (m, 2H), 3.26 (dd, $J$ = 5.6 and 13.8 Hz, 1H), 3.05 (dd $J$ = 8.6 and 13.8 Hz, 1H), 1.95–1.31 (m, 73H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 169.7, 163.6, 162.2, 157.5, 156.1, 154.4, 153.33, 153.3, 139.5, 131.7, 129.8, 124.4, 122.8, 120.3, 111.8, 105.2, 93.6, 83.0, 79.1, 78.4, 68.0, 54.8, 53.5, 44.5, 40.9, 40.8, 37.8, 37.4, 37.0, 36.95, 31.8, 31.7, 30.3, 29.0, 28.9, 28.8, 28.3, 28.1, 27.0, 26.7, 23.5; LRMS (ESI) 1198 (M + H$^+$); HRMS (ESI) $m/z$ calculated for $C_{65}H_{100}N_9O_{12}$ (M + H$^+$) 1198.7491, found 1198.7546.

**1-[6-(*N*,*N*′-bis-benzyloxycarbonyl)guanidino]hexyl-7-{[5-(*N*,*N*′-bis-benzyloxycarbonyl)guanidino]pentyloxy}indole-2-carboxylic acid (21).** This was prepared similarly to the preparation of **16** in 47% yield as a light yellow oil; $\nu_{max}$ (neat)/cm$^{-1}$: 3337, 3130 (br), 1728, 1644, 1574, 1211; $^1$H NMR (300 MHz, $DCCl_3$) δ 11.74 (s, 1H), 8.35 (t, $J$ = 5.1 Hz, 1H), 8.30 (t, $J$ = 5.0 Hz, 1H), 7.41–7.24 (m, 22H), 6.99 (t, $J$ = 7.9 Hz, 1H), 6.68 (d, $J$ = 7.7 Hz, 1H), 5.16 (s, 2H), 5.12 (s, 6H), 4.88 (t, $J$ = 7.4 Hz, 2H), 4.08 (t, $J$ = 6.3 Hz, 2H), 3.47 (q, $J$ = 6.3 Hz, 2H), 3.39 (q, $J$ = .65 Hz, 2H), 1.95–1.50 (m, 10H), 1.45–1.30 (m, 4H); $^{13}$C NMR (75.5 MHz, $DCCl_3$) δ 165.8, 163.6, 156.0, 155.9, 153.83, 153.8, 147.2, 136.7, 134.6, 129.7, 128.7, 126.6, 120.8, 115.1, 112.9, 105.8, 68.1, 68.0, 67.7, 67.1, 46.7, 41.1, 40.9, 32.0, 28.9, 28.8, 28.7, 26.5, 26.2, 23.5; LRMS (ESI) 982 (M + H$^+$); HRMS (ESI) $m/z$ calculated for 1/2 of $C_{54}H_{61}N_7O_{11}$ (M + 2H$^+$) 491.7214, found 491.7200.

***N*-{(2*S*)-1-[2-(*tert*-butyloxycarbonyl)adamantyl-2-amino]-3-(4-*tert*-butyloxyphenyl)-1-oxo-propan-2-yl}1-[6-(*N*,*N*′-bis-benzyloxycarbonyl)guanidino]hexyl-7-{[5-(*N*,*N*′-bis-benzyloxycarbonyl)guanidino]pentyloxy}indole-2-carboxamide (22).** This was prepared similarly to the preparation of **17** in 88% yield as a light yellow oil; $\nu_{max}$

(neat)/$cm^{-1}$: 3339, 1730, 1644, 1260; $^1H$ NMR (300 MHz, $DCCl_3$) δ 11.74 (s, 1H), 11.72 (s, 1H), 8.34 (t, $J=5.2$ Hz, 1H), 8.26 (t, $J=5.0$ Hz, 1H), 7.40–7.24 (m, 22H), 7.13 (d, $J=8.0$ Hz, 1H), 7.0–6.95 (m, 3H), 6.75 (d, $J=7.4$ Hz, 1H), 6.66–6.62 (m, 2H), 6.20 (s, 1H), 5.15–5.10 (m, 8H0, 4.85–4.70 (m, 3H), 4.07 (t, $J=6.4$ Hz, 2H), 3.46 (q, $J=6.5$ Hz, 2H), 3.37 (q, $J=6.9$ Hz, 2H), 3.19 (dd, $J=6.0$ and 13.9 Hz, 1H), 3.05 (dd, $J=8.2$ and 13.8 Hz, 1H), 2.36 (s, 2H), 2.16–2.02 (m, 2H), 2.0–1.49 (m, 20H), 1.45–1.25 (m, 22H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 170.8, 169.2, 163.6, 162.3, 155.9, 155.8, 154.4, 153.8, 153.7, 147.1, 136.73, 136.7, 134.5, 131.6, 131.2, 129.8, 128.6, 128.3, 128.0, 127.8, 126.8, 124.3, 120.7, 114.3, 105.3, 105.0, 80.5, 78.3, 68.1, 68.0, 67.6, 67.0, 63.9, 54.4, 48.9, 46.8, 41.1, 40.8, 37.7, 37.0, 33.8, 33.6, 32.9, 32.8, 32.6, 32.3, 28.9, 28.7, 28.6, 27.9, 26.74, 26.7, 26.4, 25.5, 24.9, 23.4; LRMS (ESI) 1434 ($M+H^+$); HRMS (ESI) $m/z$ calculated for 1/2 of $C_{82}H_{101}N_9O_{14}$ ($M+2H^+$) 717.8734, found 717.8742.

***N*-{(2*S*)-1–2-[(2-hydroxycarbonyl)adamantyl]amino-3-(4-hydroxyphenyl)-1-oxo-propan-2-yl} 1-(6-guanidinohexyl)-6-(5-guanidinopentyloxy)indole-2-carboxamide bis(trifluroacetate) salt (3).** A solution of **17** (50 mg, 0.04 mmol) in TFA (2 mL) and $CH_2Cl_2$ (3 mL) was stirred for 90 min at room temperature. Solvent was then removed under reduced pressure. The crude product was purified on reverse phase HPLC (detector wave-length setting at 220 nm) on a Vydak $C_8$ (15–20 μm particle size, 250 mm L × 22 mm ID) column using gradient elution, starting with 90% of buffer A (0.1% TFA in $H_2O$) and then linearly increasing the concentration of buffer B (80% of $CH_3CN$ in buffer A) to 90% in 60 min at a flow rate of 8 mL/min, to give the desired product **3** as its bis(trifluoroacetate) salt; $\nu_{max}$ (KBr)/$cm^{-1}$: 3391, 3204, 1665, 1202; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 12.14 (s, 1H), 9.16 (s, 1H), 8.38 (d, $J=8.6$ Hz, 1H), 7.78 (s, 1H), 7.65–7.56 (m, 1H), 7.55–7.49 (m, 2H), 7.48–6.70 (m, 12H), 6.72 (d, $J=9.0$ Hz, 1H), 6.63 (d, $J=8.1$ Hz, 2H), 4.68–4.61 (m, 1H), 4.49–4.30 (m, 2H), 4.01 (t, $J=5.9$ Hz, 2H), 3.18–2.81 (m, 6H), 2.09–1.95 (m, 4H), 1.85–1.31 (m, 20H), 1.30–1.12 (m, 4H); LRMS (FAB) 786 ($M+H^+$); HRMS (FAB) $m/z$ calculated for $C_{42}H_{60}N_9O_6$ ($M+H^+$) 786.4666, found 786.4712.

***N*-{(2*S*)-1–2-[(2-hydroxycarbonyl)adamantyl]amino-3-(4-hydroxyphenyl)-1-oxo-propan-2-yl} 1-(6-guanidinohexyl)-7-(5-guanidinopentyloxy)indole-2-carboxamide bis(trifluroacetate) salt (4).** To a solution of **22** (40 mg, 0.03 mmol) in MeOH (4 mL) and AcOH (1 mL) was added Pd/C (10%, 10 mg). The mixture was stirred under $H_2$ atmosphere for 12 h at room temperature and filtered through Celite. Solvent was evaporated to give a light yellow residue, which was dissolved in TFA (2 mL) and $CH_2Cl_2$ (2 mL). The resulting solution was stirred for 90 min. Solvent was removed under reduced pressure. The crude product was purified on reverse-phase HPLC as for **3** to give **4** in its bis(trifluoroacetate) salt. LRMS (ESI) 786 ($M+H^+$); HRMS (ESI) $m/z$ calculated for 1/2 of $C_{42}H_{61}N_9O_6$ ($M+2H^+$) 393.7372, found 393.7347.

***N*-{(2*S*)-1-(1-adamantylamino)-3-(4-hydroxyphenyl)-1-oxo-propan-2-yl} 1-(6-guanidinohexyl)-6-(5-guanidinopentyloxy)indole-2-carboxamide bis(trifluoroacetate) salt (5).** This was prepared similarly to the preparation of **3**. $\nu_{max}$ (KBr)/$cm^{-1}$: 3397, 1671, 1204; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 9.17 (s, 1H), 8.22 (d, $J=8.5$ Hz, 1H), 7.75 (t, $J=5.4$ Hz, 1H), 7.66 (t, $J=5.3$ Hz, 1H), 7.55–6.85 (m, 14H), 6.73 (dd, $J=1.7$ and 8.7 Hz, 1H), 6.63 (d, $J=8.4$ Hz, 2H), 4.56–4.42 (m, 3H), 4.02 (t, $J=6.0$ Hz, 2H), 3.18–3.08 (m, 2H), 3.07–2.96 (m, 2H), 2.95–2.76 (m, 2H), 2.01 (s, 3H), 1.93 (s, 6H), 1.85–1.34 (m, 16H), 1.32–1.12 (m, 4H); $^{13}C$ NMR (75.5 MHz, DMSO-$d_6$) δ 170.6, 161.6, 156.8, 156.6, 155.7, 138.7, 130.4, 130.2, 128.3, 122.4, 119.7, 114.7, 111.2, 105.3, 93.7, 67.6, 55.1, 50.8, 43.4, 41.0, 40.7, 40.6, 38.7, 36.0, 30.0, 28.8, 28.4, 28.3, 25.8, 24.0, 22.9, 21.3; LRMS (ESI) 742 ($M+H^+$); HRMS (ESI) $m/z$ calculated for 1/2 of $C_{41}H_{61}N_9O_4$ ($M+2H^+$) 371.7421, found 371.7395.

***N*-[(2*S*)-1-(2-adamantylamino)-3-(4-hydroxyphenyl)-1-oxo-propan-2-yl] 1-(6-guanidinohexyl)-6-(5-guanidinopentyloxy)indole-2-carboxamide bis(trifluoroacetate) salt (6).** This was prepared similarly to the preparation of **3** as a white powder. $\nu_{max}$ (KBr)/$cm^{-1}$: 3432, 1662, 1204; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 9.18 (s, 1H), 8.40 (d, $J=8.4$ Hz, 1H), 7.81–7.70 (m, 2H), 7.64 (t, $J=5.2$ Hz, 1H), 7.55–6.82 (m, 13H), 6.73 (dd, $J=1.4$ and 8.8 Hz, 1H), 6.64 (d, $J=8.3$ Hz, 2H), 4.73–4.64 (m, 1H), 4.52–4.28 (m, 2H), 4.01 (t, $J=6.0$ Hz, 2H), 3.83 (d, $J=6.7$ Hz, 1H), 3.19–3.10 (m, 2H), 3.10–3.00 (m, 2H), 3.00–2.85 (m, 2H), 2.08–1.33 (m, 24H), 1.31–1.13 (m, 4H); $^{13}C$ NMR (75.5 MHz, DMSO-$d_6$) δ 170.8, 161.7, 156.8, 156.6, 155.7, 138.7, 130.2, 130.0, 128.2, 122.4, 119.7, 114.7, 111.2, 105.4, 93.8, 67.6, 54.7, 53.0, 43.4, 40.2, 37.0, 36.7, 36.6, 36.4, 31.3, 31.0, 30.9, 29.8, 28.3, 28.2, 26.7, 25.8, 25.7, 22.8; LRMS (ESI) 742 ($M+H^+$); HRMS (ESI) $m/z$ calculated for 1/2 of $C_{41}H_{61}N_9O_4$ ($M+2H^+$) 371.7423, found 371.7430.

**2-*N*-Benzyloxycarbonyl-2-*tert*-butyloxycarbonyl-adamantane (24).** To a solution of 2-*N*-benzyloxycarbonyl-2-hydroxycarbonyl-adamantane (490 mg, 1.49 mmol) in toluene (10 mL) was added *N*,*N*-dimethylformamide di-*tert*-butyl acetal (1.3 g, 6.3 mmol) at 110 °C under $N_2$ with stirring. Stirring was continued at the same temperature for 10 h. The reaction mixture was cooled to room temperature and poured into saturated $NaHCO_3$ (40 mL), and then extracted with ethyl acetate (3 × 40 mL). The combined extracts were washed with brine (50 mL), dried ($Na_2SO_4$), and evaporated under reduced pressure to give a residue. Chromatography of the residue on silica gel, eluting with 10% of ethyl acetate/hexanes, yielded **24** (282 mg, 49%) as a white solid, mp 120.7–122.0 °C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3316, 1726, 1694, 1512; $^1H$ NMR (300 MHz, $DCCl_3$) δ 7.36–7.26 (m, 5H), 5.06 (s, 2H), 4.90 (s, 1H), 2.45 (s, 2H), 2.12–1.94 (m, 4H), 1.88–1.60 (m, 8H), 1.39 (s, 9H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 171.1, 154.4, 136.6, 128.3, 128.0, 127.9, 80.4, 66.3, 63.6, 37.7, 33.9, 32.7, 27.7, 26.8, 26; LRMS (ESI) 386 ($M+H^+$); HRMS (ESI) $m/z$ calculated for $C_{23}H_{32}NO_4$ ($M+H^+$) 386.2331, found 386.2337.

**2-*tert*-Butyloxycarbonyl-2-amino-adamantane (25).** A mixture of **24** (260 mg, 0.68 mmol), 10% Pd/C (50 mg), AcOH (0.8

mL) in methanol (25 mL) was stirred at rt under $H_2$ for 12 h. The mixture was filtered through Celite and washed with ethyl acetate (50 mL). Solvent was removed from the combined organic part to give **25** (155 mg, 91%) as a white solid, mp 96.2–97.8 °C; ν max (KBr)/$cm^{-1}$: 3378, 1713, 1246; $^1H$ NMR (300 MHz, $DCCl_3$) δ 2.38–2.15 (m, 2H), 2.15–1.98 (m, 2H), 1.98–1.45 (m, 21H); LRMS (ESI) 252 ($M+H^+$); HRMS (ESI) *m/z* calculated for $C_{15}H_{26}NO_2$ ($M+H^+$) 252.1963, found 252.1970.

***N*-2-[(2-*tert*-Butyloxycarbonyl)adamantyl]2(*S*)-2-*N*-Fmoc-3-(4-*tert*-butyloxyphenyl)propanamide (26).** A solution of *N*-Fmoc-*O*-*tert*-butyl-tyrosine (189 mg, 0.36 mmol), **25** (76 mg, 0.30 mmol), DCC (120 mg, 0.54 mmol) and HOBt (70 mg, 0.48 mmol) in DMF (5 mL) was stirred under $N_2$ at room temperature for 12 h. The mixture was poured into saturated $NaHCO_3$ (20 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with $H_2O$ (2 × 40 mL), brine (40 mL), dried ($Na_2SO_4$), and evaporated under reduced pressure to give a residue. Chromatography of the residue on a silica gel column, eluting with 20% of ethyl acetate/hexanes, afforded **26** (200 mg, 92%) as a white solid, mp 78.0–80.0 °C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3306, 1738, 1665, 1537, 1507, 1254, 1159; $^1H$ NMR (300 MHz, $DCCl_3$) δ 7.75 (d, $J=7.5$ Hz, 2H), 7.55 (d, $J=7.3$ Hz, 2H), 7.39 (t, $J=7.4$ Hz, 2H), 7.30 (t, $J=7.3$ Hz, 2H), 7.25–7.12 (m, 2H), 6.92 (d, $J=8.3$ Hz, 2H), 5.88 (s, 1H), 5.49 (s, 1H), 4.42–4.29 (m, 2H), 4.18 (t, $J=7.0$ Hz, 1H), 3.16–2.90 (m, 2H), 2.27 (s, 2H), 2.13–2.06 (m, 2H), 1.79 (s, 2H), 1.75–1.40 (m, 17H), 1.31 (s, 9H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 170.8, 169.2, 155.9, 154.4, 143.6, 141.2, 131.4, 129.8, 127.7, 127.0, 125.0, 124.2, 119.9, 80.5, 78.3, 67.1, 63.9, 56.2, 47.0, 37.8, 37.7, 33.6, 32.9, 32.8, 32.6, 32.5, 28.7, 27.9, 26.7, 26.3; LRMS (ESI) 693 ($M+H^+$); HRMS (ESI) *m/z* calculated for $C_{43}H_{53}N_2O_6$ ($M+H^+$) 693.3930, found 693.3930.

***N*-2-[(2-*tert*-Butyloxycarbonyl)adamantyl] [2(*S*)-2-amino-3-(4-*tert*-butyloxyphenyl)]propanamide (27).** A solution of **26** (195 mg, 0.28 mmol) in piperidine (0.5 mL) and THF (10 mL) was stirred for 90 min at room temperature under $N_2$. Solvent was removed under reduced pressure to give a solid residue, which was chromatographed on silica gel column, eluting first with 50% of ethyl acetate/hexanes and then 85% of ethyl acetate/ethanol, to yield **27** (110 mg, 90%) as a white solid; mp 60.5–62.0 °C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3370, 3324, 1740, 1674, 1505, 1161; $^1H$ NMR (300 MHz, $DCCl_3$) δ 7.68 (s, 1H), 7.13 (d, $J=8.4$ Hz, 2H), 6.93 (d, $J=8.4$ Hz, 2H), 3.54 (dd, $J=3.8$ and 9.5 Hz, 1H), 3.19 (dd, $J=3.8$ and 13.8 Hz, 1H), 2.63 (dd, $J=9.6$ and 13.8 Hz, 1H), 2.48 (s, 2H0, 2.18–2.06 (m, 2H), 1.92–1.60 (m, 10H), 1.47 (s, 9H), 1.39 (s, 2H), 1.33 (s, 9H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 172.4, 171.3, 154.1, 132.5, 129.6, 124.2, 80.2, 78.2, 62.8, 56.2, 39.9, 37.8, 33.7, 33.1, 33.1, 32.6, 32.5, 28.7, 27.9, 26.8, 26.5; LRMS (ESI) 471 ($M+H^+$); HRMS (ESI) *m/z* calculated for $C_{28}H_{43}N2O_4$ ($M+H^+$) 471.3222, found 471.3250.

***N*-2-Adamantyl [(2*S*)-2-amino-3-(*tert*-butyloxyphenyl)]-propanamide (29).** This was prepared similarly to the preparation of **27** as a white solid, mp 166.5–168.4 °C; $\nu_{max}$ (KBr)/$cm^{-1}$: 3349, 1655, 1512, 1163; $^1H$ NMR (300 MHz, $DCCl_3$) δ 7.68 (d, $J=8.1$ Hz, 1H), 7.08 (d, $J=8.2$ Hz, 2H), 6.91 (d, $J=8.3$ Hz, 2H), 4.06–4.01 (m, 1H), 3.61–3.54 (m, 1H), 3.19–3.10 (m, 1H), 2.73 (dd, $J=8.6$ and 13.7 Hz, 1H), 1.91–1.54 (m, 14H), 1.32 (s, 9H); $^{13}C$ NMR (75.5 MHz, $DCCl_3$) δ 173.0, 154.2, 132.6, 129.7, 124.2, 78.3, 56.4, 52.5, 40.3, 37.5, 37.1, 37.0, 32.0, 31.9, 31.8, 28.8, 27.2, 27.1; LRMS (ESI) 393 ($M+H^+$); HRMS (ESI) *m/z* calculated for $C_{23}H_{35}N_2O_2$ ($M+H^+$) 371.2698, found 371.2714.

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