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Novel inhibitors of lysine specific demethylase 1 as epigenetic modulators

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NOVEL INHIBITORS OF LYSINE SPECIFIC DEMETHYLASE 1 AS EPIGENETIC MODULATORS

by

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Approved by:

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Advisor Date

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DEDICATION

This work is dedicated to my brother Sam, and everyone else who has helped me find my way.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the generous support of The Department of Pharmaceutical Sciences at Wayne State University.

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CHAPTER 1 BACKGROUND

The Histone Code

Chromatin architecture is a key determinant in the regulation of gene expression, and this architecture is strongly influenced by post–translational modifications of histones.[1, 2] Histone protein tails contain lysine residues that interact with the negative charges on the DNA backbone. These lysine-containing tails, consisting of up to 40 amino acid residues, protrude through the DNA strand, and act as a site for post-translational modification of chromatin, allowing alteration of higher order nucleosome structure.[3] Multiple post-translational modifications of histones mediate remodeling of chromatin, with acetylation being the best characterized process.[4] Transcriptional repression is associated with specific CpG island DNA methylation and recruitment of histone deacetylases (HDACs) to gene promoters that cooperate in the silencing of specific genes.[5, 6] Normal mammalian cells exhibit an exquisite level of control of chromatin architecture by maintaining a balance between histone acetyltransferase (HAT) and HDAC activity.[7]

In cancer, CpG island DNA promoter hypermethylation in combination with other chromatin modifications, including decreased activating marks and increased repressive marks on histone proteins 3 and 4, have been associated with the silencing of tumor suppressor genes.[8] The important role of promoter CpG island methylation and its relationship to covalent histone modifications has recently been reviewed.[9] As was mentioned above, the N-terminal lysine tails of histones can undergo numerous posttranslational modifications, including phosphorylation, ubiquitination, acetylation and methylation.[4, 10, 11] Lysine methylation on histones can signal transcriptional activation or repression, depending on the specific lysine residue involved.[12-14] All known histone lysine methyltransferases contain a conserved SET methyltransferase domain, and it has been shown that aberrant methylation of histones due to SET domain deregulation is linked to carcinogenesis.[15] Histone methylation, once thought to be

an irreversible process, has recently been shown to be a dynamic process regulated by the addition of methyl groups by histone methyltransferases and removal of methyl groups from mono- and dimethyllysines by lysine specific demethylase 1 (LSD1), and from mono-, di, and trimethyllysines by specific Jumonji C (JmjC) domain-containing demethylases.[10, 11, 16, 17] Additional demethylases in the JmjC demethylase class are continuing to be identified.[18, 19] Recent evidence suggests that LSD1 is required for maintenance of global DNA methylation,[20] indicating that the LSD1-mediated demethylation is a general mechanism for transcriptional control.

Lysine Specific Demethylase

Relevance

Studies suggest that LSD1 hyperactivity plays an important role in the development of cancer by promoting aberrant silencing of tumor suppressor genes. LSD1 co-localizes with the androgen receptor in normal human prostate and in prostate tumors,[21] where it interacts with the androgen receptor in vitro and in vivo, and stimulates androgen-receptor-dependent transcription. Conversely, knockdown of LSD1 protein levels in these tumors abrogates androgen-induced transcriptional activation and cell proliferation.[21] It has been suggested that LSD1 is a prognostic marker in prostate cancer.[22] High levels of LSD1, nuclear expression of the FHL2 co-activator, high Gleason score and grade, and very strong staining of nuclear p53 correlate significantly with relapse of prostate carcinoma during follow-up. Thus LSD1 and nuclear FHL2 may serve as novel biomarkers predictive for aggressive prostate carcinogenesis and point to a role of LSD1 and FHL2 in constitutive activation of AR-mediated growth signals.[23] In neuroblastoma, siRNA knockdowns of LSD1 decreased cellular growth, induced expression of differentiation-associated genes, and increased histone 3 lysine 4 (H3K4) methylation. LSD1 inhibition using monoamine oxidase inhibitors resulted in an increase of global H3K4 methylation and growth inhibition of neuroblastoma cells in vitro, and reduced neuroblastoma xenograft growth in vivo. Thus, LSD1 is involved in maintaining the undifferentiated, malignant

phenotype of neuroblastoma cells, and inhibition of LSD1 reprograms the transcriptome of neuroblastoma cells and inhibits neuroblastoma xenograft growth.[24] These data provide a clear indication that LSD1 has emerged as a valid antitumor target. Notably, Huang et al. demonstrated that his novel LSD1 inhibitors work synergistically with the current clinical chemotherapeutic agent 5-azacytadine to inhibit tumor growth better than when the agents are used independently. [25]

History

LSD1 was identified in part because its C-terminal domain shares significant sequence homology with the amine oxidases acetylpolyamine oxidase (APAO) and spermine oxidase (SMO).[10, 26] Several groups have identified amines, guanidines or similar analogues that act as selective inhibitors of these amine oxidases.[26-32] Bi et al. reported the synthesis of a novel series of (bis)guanidines and (bis)biguanides[33] that are potent antitrypanosomal agents in vitro, with IC_{50} values as low as 90 nM. Because of their structural similarity to guanidine-based inhibitors of APAO and SMO, studies were undertaken to determine whether these agents were inhibitors of LSD1, and whether this inhibition had any influence on selected chromatin marks in tumor cells. Nine of the 13 compounds tested were found to inhibit LSD1 activity by >50% at 1 μM, with compounds **1c** and **2d** producing the most dramatic effects.[34] The most potent inhibitor, **2d**, exhibited non-competitive kinetics at concentrations up to 2.5 mM. A 48 hr exposure of HCT116 human colon carcinoma cells to increasing concentrations of analogue **2d** produced significant global increases in both monomethyl H3K4 (H3K4me1) and dimethyl H3K4 (H3K4me2), while not affecting global dimethyl histone3 lysine 9 (H3K9me2) levels, which is a known repressive mark. These compounds also induced the re-expression of multiple, aberrantly silenced genes important in the development of colon cancer, including members of the secreted frizzle-related proteins (SFRPs) and the GATA family of transcription factors. SFRPs have been proposed as tumor suppressor factors based on it's loss in patient tumors.[35] GATA transcription factors have been found to regulate tumor differentiation and suppress tumor dissemination.[36]

Physical Properties and Active Site

The X-ray structure and mechanism of LSD1 have been reported.[37] The LSD1 polypeptide chain (MW 110 kDa) tertiary structure contains three distinct structural domains: the tower domain, the SWIRM domain and the FAD binding/oxidase domain. The tower domain, connected directly to the catalytic site, is thought to be a platform for the binding of transcriptional factors in the CoRest[38, 39] complex. Binding of CoRest to LSD1 enhances the ability of LSD1 to demethylate histone lysine residues.[40] In addition to CoRest, LSD1 binds to the protein factor BHC80, which inhibits the demethylation process, and to the androgen receptor, which has been proposed to alter LSD1 substrate specificity such that it demethylates mono- and dimethyl histone 3 lysine 9 and acts as a transcriptional activator.[21] The LSD1 SWIRM domain[41] is typical of other such domains found in proteins involved in chromatin remodeling,[42] and is potentially the site of DNA binding. The oxidase domain has two functional lobes, one for binding the FAD cofactor, and another for substrate binding. The FAD binding/oxidase domain is intimately bound to the SWIRM domain through a series of hydrophobic interactions, and point mutations that disrupt these interactions greatly reduce catalytic activity. The LSD1 active site binds the substrate lysine residue, as well as ~20 adjacent amino acids residues in the histone tail. LSD1 catalysis is an FAD-dependent oxidative demethylation that most likely proceeds through a protonated imine intermediate.[43] The K_m for H3K4me2 has been determined to be 30 mM[10] .

Existing work on inhibition of LSD1

Histone 3 dimethyllysine 4 (H3K4me2) is a transcription-activating chromatin mark that is found at gene promoters.[44, 45] LSD1 catalyzes the oxidative demethylation of H3K4me1 and H3K4me2; this transformation is an enzymatic reaction associated with transcriptional repression.[10] Demethylation of this mark by LSD1 may prevent expression of tumor suppressor genes important in human cancer.[34] Thus, LSD1 has emerged as a target for the development of a new class of antitumor drugs that act as epigenetic modulators.[46]

A few existing classes of compounds have been shown to act as inhibitors of LSD1. The active site structure of LSD1 has significant sequence homology to monoamine oxidases A and B (MAO A and B), and to N^1-acetylpolyamine oxidase (APAO) and spermine oxidase (SMO).[10, 47, 48] It has been shown that classical MAO inhibitors phenelzine and tranylcypromine inhibit demethylation by the recombinant LSD1/CoRest complex, and increase global levels of H3K4me2 in the P19 embryonal carcinoma cell line.[47, 48] The synthetic substrate analogue aziridinyl-K4H3$_{1-21}$ reversibly inhibited LSD1 with an IC_{50} of 15.6 mM, while propargyl-K4H3$_{1-21}$ produced time-dependent inactivation with a K_i of 16.6 mM.[43] Propargyl-K4H3$_{1-21}$ was later shown to inhibit LSD1 through formation of a covalent adduct with the enzyme-bound FAD flavin cofactor.[48, 49] McCafferty et al. described the synthesis of a series of trans-2-arylcyclopropylamine analogues that inhibit LSD1 with K_i values between 188 and 566 mM.[50] These analogues were 1-2 orders of magnitude more potent against MAO A and MAO B, with the exception of one. Ueda et al. identified small molecule tranylcypromine derivatives that are selective for LSD1 over MAO-A and MAO-B,[51] and Binda et al. described similar tranylcypromine analogues that exhibited selectivity between LSD1 and the newly identified histone demethylase LSD2.[52] Taken together, these data suggest that potent and selective inhibitors for the homologous flavin-dependent amine oxidase LSD1 can be designed and synthesized.

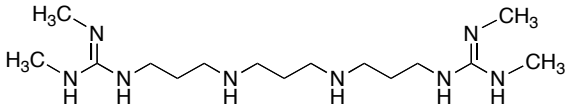
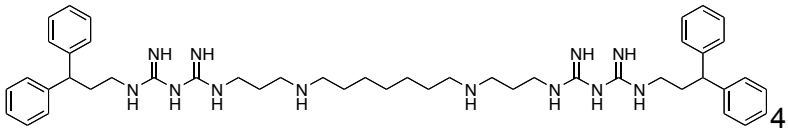
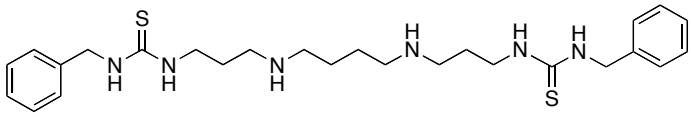
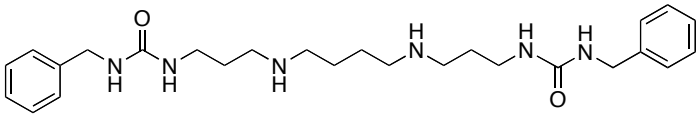
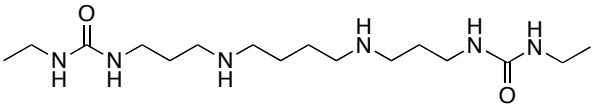
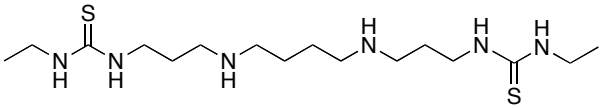
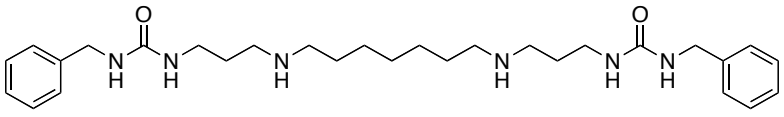
Because of the promising cellular effects of **2d**, the synthesis and evaluation of additional analogues was proposed. To access a library of more diverse analogues related to **2d**, the published syntheses of Bi et al.[33] was adapted to produce a series of 31 isosteric (bis)alkylureas or (bis)alkylthioureas **3**-**31** (Table 2.1) and these analogues were evaluated for the ability to inhibit LSD1 and induce increases in global H3K4me2 in vitro. The central hypothesis of the research described in this thesis is that compounds that inhibit LSD1 can be identified and developed for the treatment of human cancer. The work described within, includes structure activity relationship (SAR) experiments designed to optimize the structure of the two

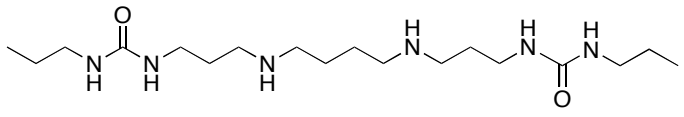
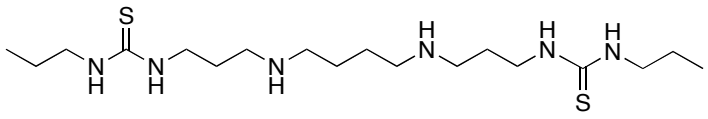
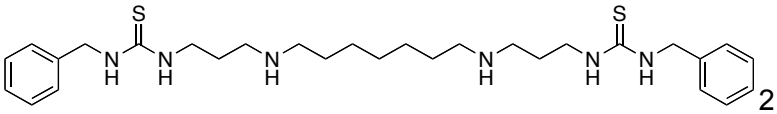
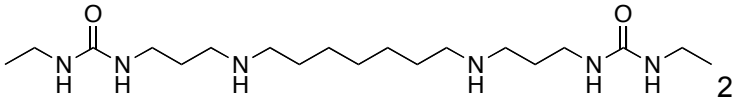
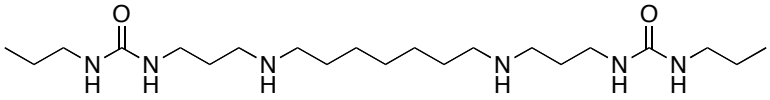
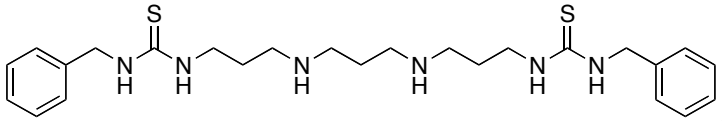
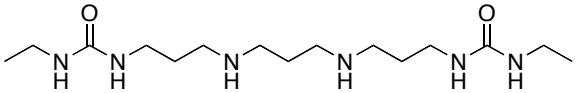
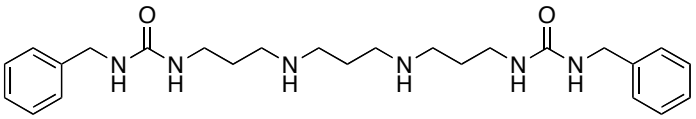
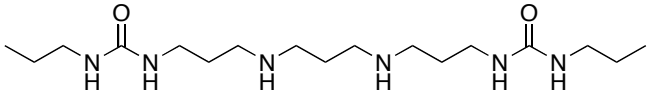
lead inhibitors of LSD1 (bis)guanidine **1c** and (bis)biguanide **2d**. In the current work, four different structural classes of analogues were examined: symmetrically and unsymmetrically alkylated polyamino(bis)guanidines, polyamino(bis)ureas, and polyamino(bis)thioureas.

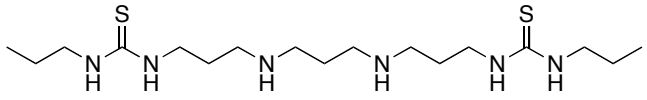
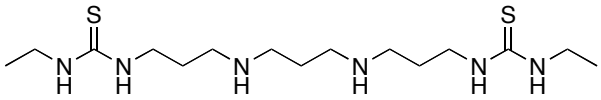
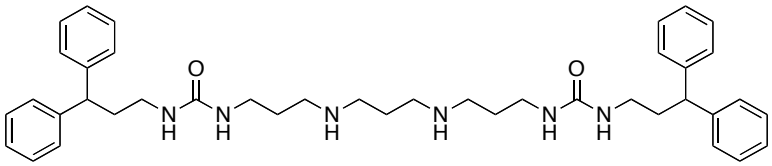
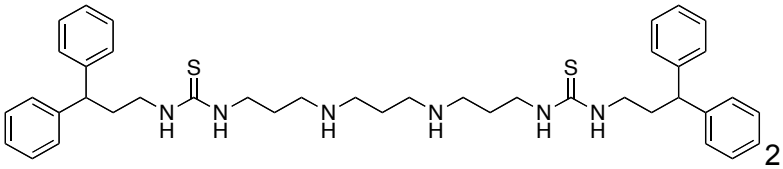
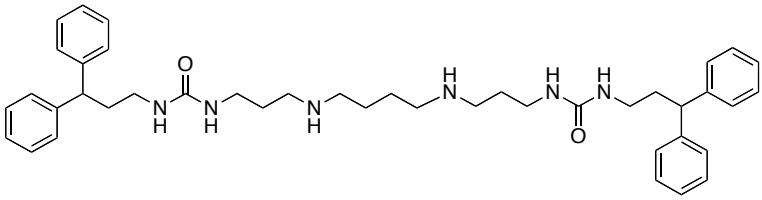
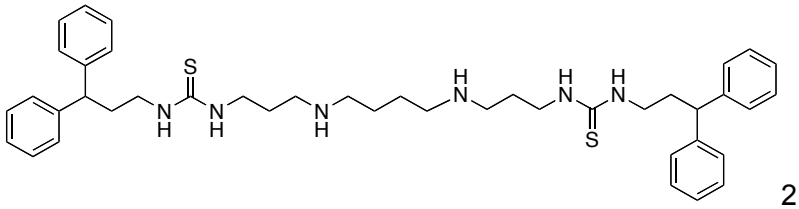
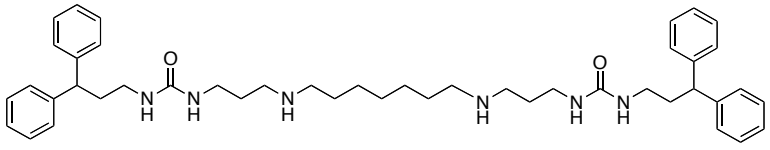
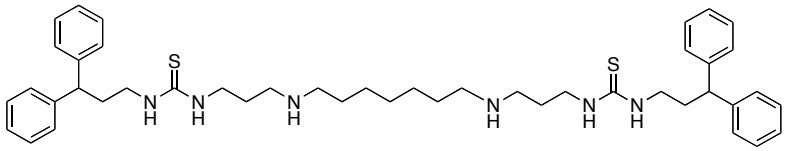
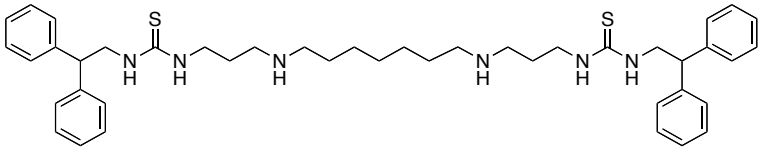
CHAPTER 2 CHEMISTRY

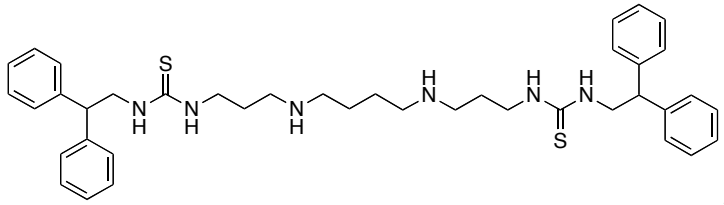
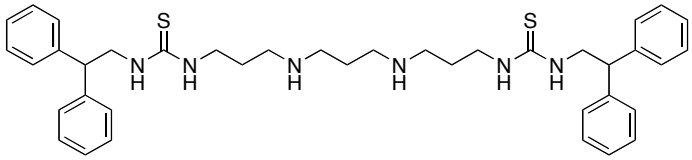
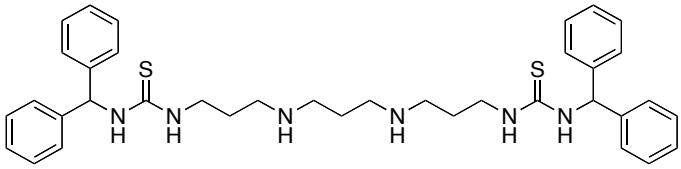
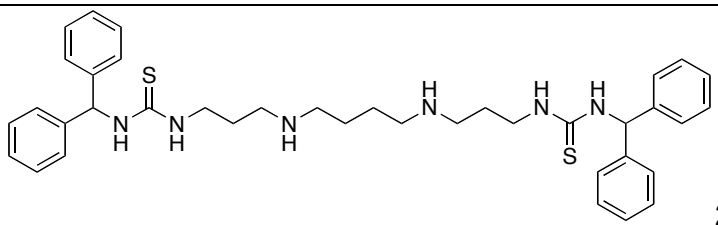
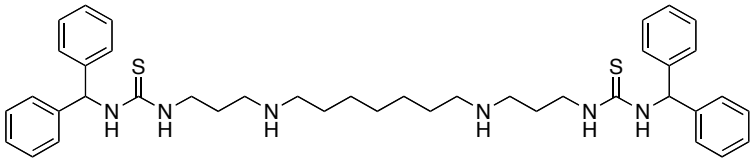
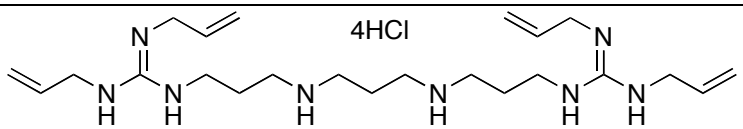
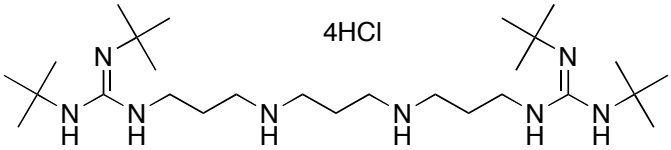
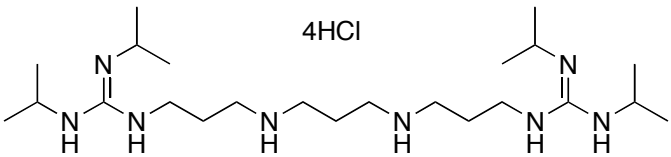
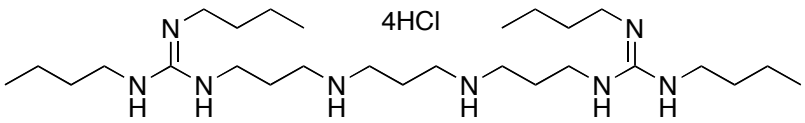
2.1 Polyamino(bis)ureas and Polyamino(bis)thioureas

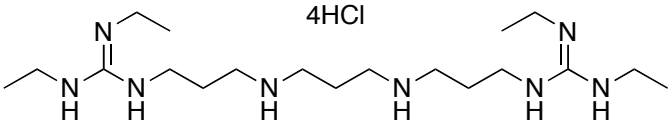
Table 2.1 Structures and effect of 1c, 2d, and 3-35 on LSD1 activity in vitro. Percent of LSD1 activity remaining was determined following treatment with 10 μM of each test compound as determined by the luminol-dependent chemiluminescence method (NA = not active).

Structure	Compound	% LSD1 Activity Remaining
 4 HCl	**1c**	**NA**
 4 HCl	**2d**	**17**
 2 HCl	**3**	**75**
 2 HCl	**4**	**50**
 2 HCl	**5**	**49**
 2 HCl	**6**	**40**
 2 HCl	**7**	**NA**

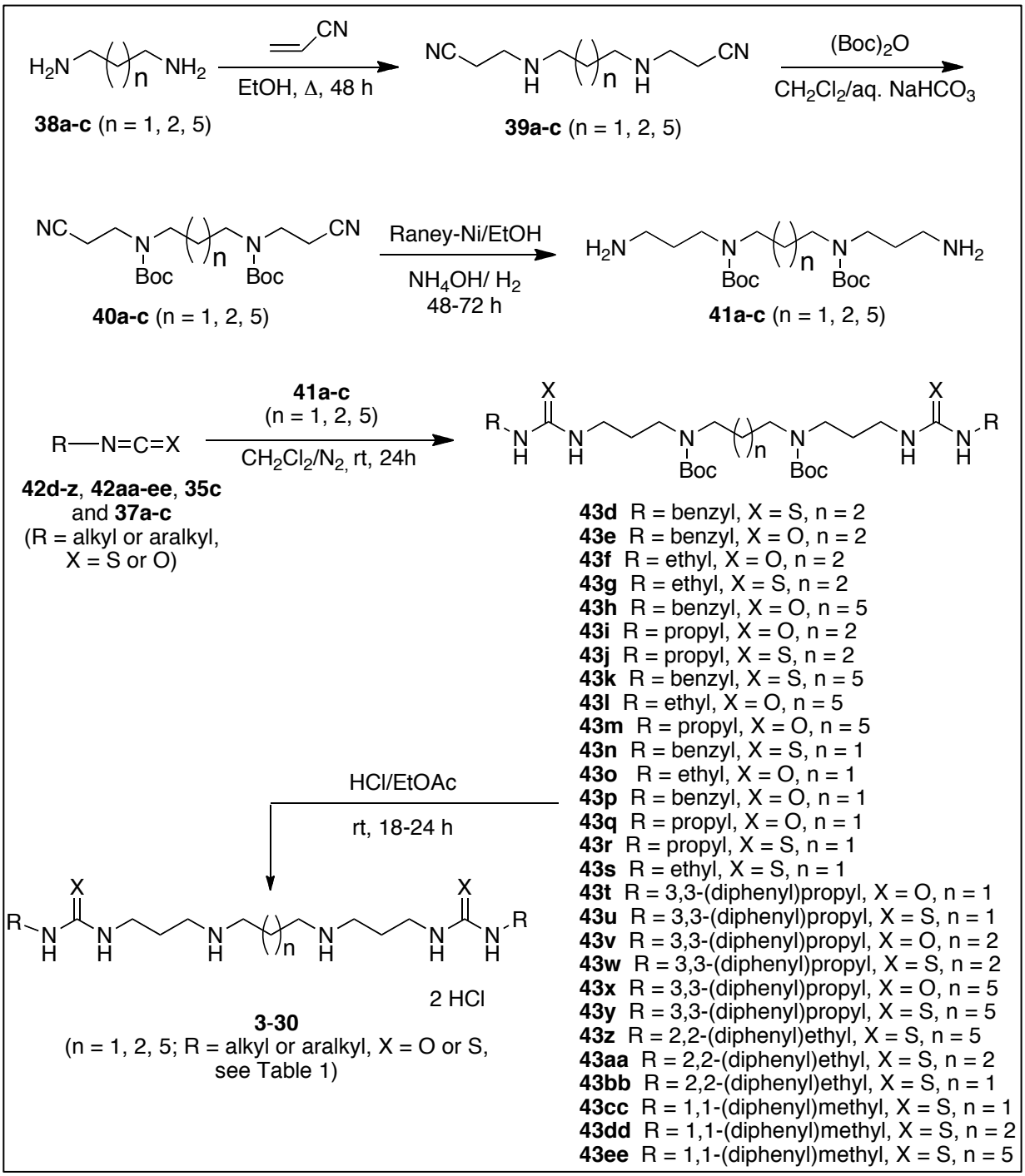
 2 HCl	**8**	**79**
 2 HCl	**9**	**90**
 2 HCl	**10**	**36**
 2 HCl	**11**	**96**
 2 HCl	**12**	**92**
 2 HCl	**13**	**52**
 2 HCl	**14**	**66**
 2 HCl	**15**	**60**
 2 HCl	**16**	**51**

 2 HCl	**17**	**NA**
 2 HCl	**18**	**36**
 2 HCl	**19**	**93**
 2 HCl	**20**	**92**
 2 HCl	**21**	**75**
 2 HCl	**22**	**89**
 2 HCl	**23**	**52**
 2 HCl	**24**	**77**
 2 HCl	**25**	**20**

 2 HCl	**26**	**17**
 2 HCl	**27**	**25**
 2 HCl	**28**	**51**
 2 HCl	**29**	**34**
 2 HCl	**30**	**29**
 4HCl	**31**	**NA**
 4HCl	**32**	**NA**
 4HCl	**33**	**NA**
 4HCl	**34**	**NA**

	**35**	**NA**

Scheme 2.1 Synthesis of Polyamino(bis)ureas and Polyamino(bis)thioureas **3-30**



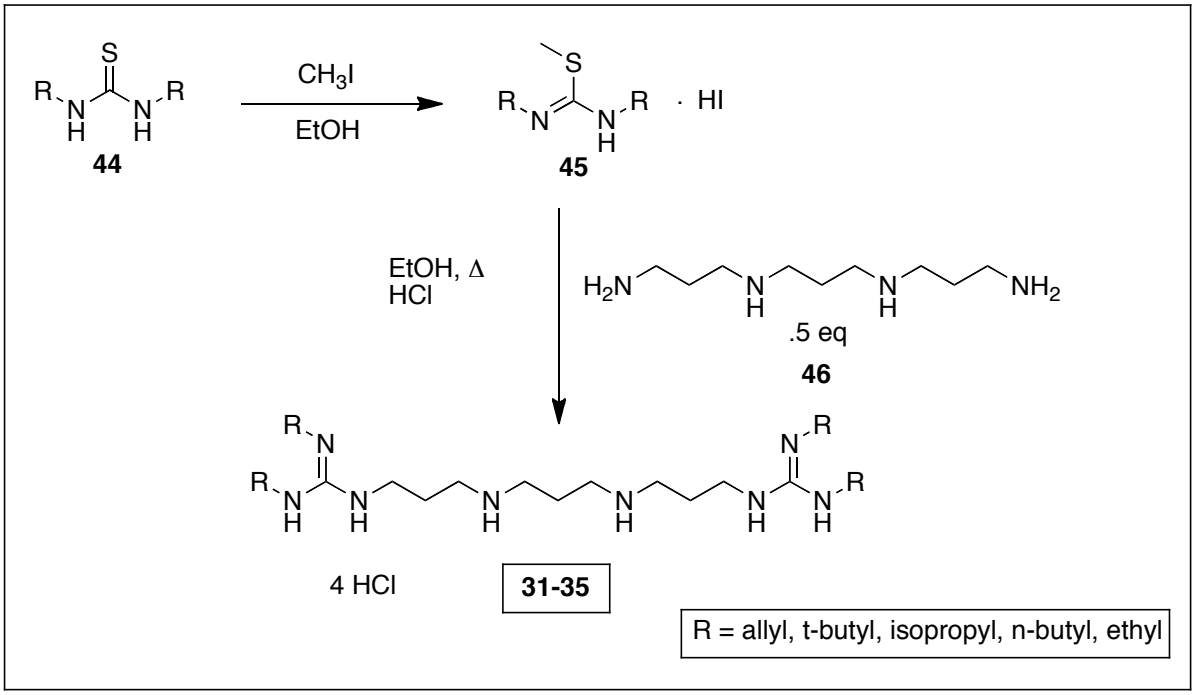
Preparation of compounds **3-30** depended on the commercial availability of the appropriate isocyanates and isothiocyanates. To access a library of isosteric urea and thiourea analogues related to **1c** and **2d**, we employed our previously published synthesis[33] of precursor

molecules **41a-c**, as shown in Scheme 2.1. The appropriate diamine **38a**, **38b** or **38c** was (bis)cyanoethylated (acrylonitrile, EtOH, reflux) to afford the corresponding (bis)cyano intermediates **39a-c**. The central nitrogens in **39a-c** were then N-Boc protected ($(Boc)_2O$, CH_2Cl_2/Aq. $NaHCO_3$)[53] to form **40a-c**, and the cyano groups were reduced (Raney Ni) to yield the desired diamines **41a-c**.[33, 54] Compounds **41a-c** were then reacted with the appropriate isocyanates or isothiocyanates **42d-z**, **42aa-ee**, **35c** and **37a-c**[55] to produce the corresponding protected (bis)ureas or (bis)thioureas **43d-z** and **43aa-ee**, followed by acid removal of the N-Boc protection groups (HCl in EtOAc)[53] to afford the desired urea or thiourea products **3**-**30**.

Importantly, the syntheses described in Scheme 2.1 can be adapted to produce a wide variety of analogues with chemical diversity in the length of the alkyl chains, and in the terminal alkyl- or aralkyl substituents.

2.2. Symmetrically Alkylated Polyamino(bis)guanidines 31-35

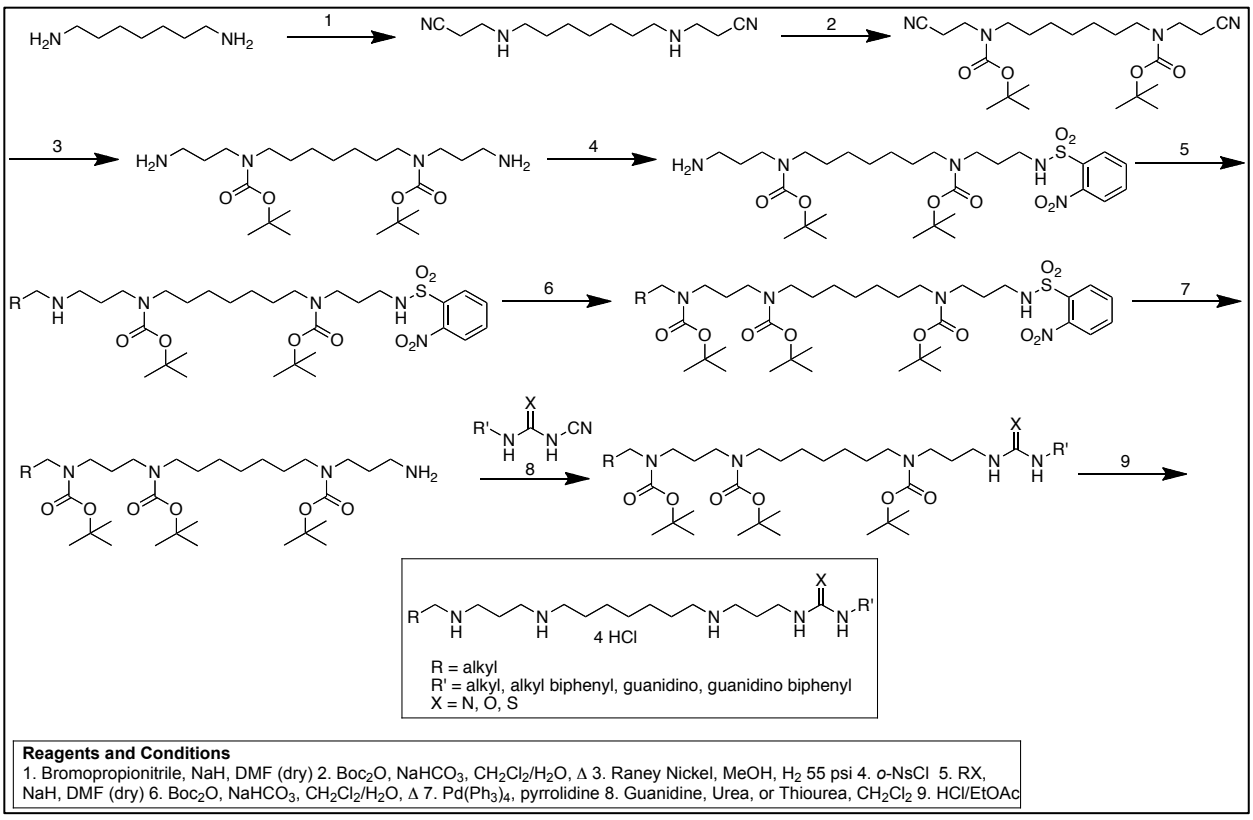
Scheme 2.2 Synthesis of symmetrically alkylated polyamino(bis)guanidines **31-35**



Using a procedure adapted by Percival et al.[56] S-Methyl-N,N'-dialkylisothiuronium iodides **45** were prepared by the interaction of the commercially available N,N'-dialkylthioureas **44** and methyl iodide in ethyl alcohol. All of the S-Methyl-N,N'-dialkylisothiuronium iodides **45** were recovered in crystalline form. Compounds **31-35** were prepared by refluxing .5 eq of commercially available N,N'-bis(3-aminopropyl)-1,3-propanediamine **46** and S-Methyl-N,N'-dialkylisothiuronium iodides **45** in ethyl alcohol. Compounds **31-35** were recovered in crystalline form.

2.3 Unsymmetrically Alkylated Polyamino(bis)guanidines, Polyamino(bis)ureas and Polyamino(bis)thioureas

Scheme 2.3 Synthesis of unsymmetrically alkylated polyamino(bis)guanidines, polyamino(bis)ureas and polyamino(bis)thioureas



We have begun to optimize reaction conditions for the synthesis of the unsymmetrical analogues outlined in Scheme 2.3. We have successfully isolated gram quantities of the intermediate following step 4.

CHAPTER 3 BIOLOGICAL EVALUATION

3.1 Polyamino(bis)ureas and Polyamino(bis)thioureas

As previously observed, compound **2d** at 10 μM reduced LSD1 activity by 82.9%. Among the 31 urea and thiourea isosteres **3**-**30**, six compounds were essentially inactive (i.e. produced <20% inhibition), while 11 analogues (ureas **4** and **5**, thioureas **6**, **10**, **18**, **25**, **26**, **27**, **29,** and **30**) reduced LSD1 activity by 50% or greater at 10 μM concentration (Table 2.1)**.**

The three most effective LSD1 inhibitors, compounds **25**-**27**, were chosen for additional studies as outlined below. Subsequent experiments were conducted in the Calu-6 human anaplastic non-small cell lung carcinoma line because it has a highly reproducible response to epigenetic modulation, and because it is known that various tumor suppressor genes are silenced in this line. In order for synthetic analogues to be effective at the cellular level, any observed decreases in cellular LSD1 activity should be accompanied by an increase in global H3K4me1 and H3K4me2 content. Thus, the ability of compounds **25**, **26** and **27** to produce increases in global H3K4me1 and H3K4me2 levels was measured as previously described. The results of these studies are shown in Figure 3.1. At 24 hours, analogues **25** and **27** produced significant increases in both H3K4me1 (Figure 3.1, Panel A) and H3K4me2 (Figure 3.1, Panel B), while analogue **26** induced a significant increase in H3K4me1, but decreased the relative amount of H3K4me2. A similar pattern was observed at 48 hours (Figure 3.1, Panels C and D). Compound **25** produced the most dramatic increases in H3K4me1 and H3K4me2 at both 24 and 48 hours. The reduction in H3K4me2 and corresponding increase in H3K4me1 by **26** at both 24 and 48 hours cannot be readily explained, and is the subject of continuing investigation. However, this anomalous finding seems to correlate with the observed cytotoxicity of **26** (Figure 3.3).

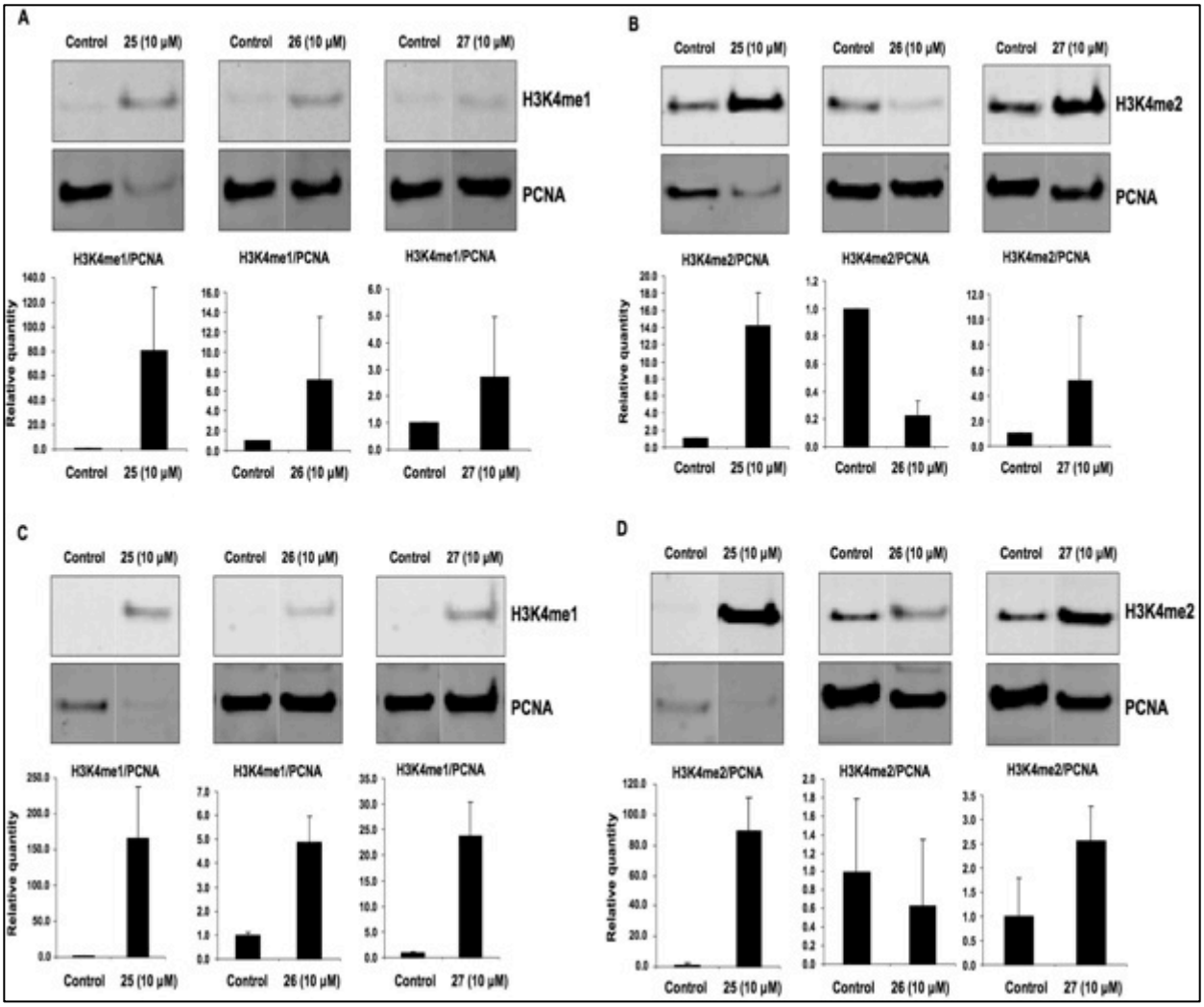


Figure 3.1 Effect of compounds **25**−**27** on the expression of global H3K4me1 and H3K4me2. Calu-6 human anaplastic nonsmall cell lung carcinoma cells were treated with a 10 μM concentration of **25**, **26**, or **27** for 24 h (A,B) or 48 h (C,D) as described in Materials and Methods. (A,C) Global H3K4me1 expression and (B,D) global H3K4me2 expression. Proliferating cell nuclear antigen (PCNA) was used as a loading control. Shown are Western blot images from a single representative experiment performed in triplicate. Relative protein expression levels were determined by quantitative Western Blot analysis using the Odyssey infrared detection system shown as bar graphs. The results represent the mean of three treatments ± SD. The protein expression level for control samples was set to a value of 1.

These data strongly suggest that intracellular inhibition of LSD1 by **25**-**27** leads to significant increases in methylation at the H3K4 chromatin mark. It is noteworthy that in HCT116 human colon tumor cells, compounds **25**-**27** all produced at least a 2-fold increase in global H3K4me2, with the most effective analogue being compound **25**.

The ability of compounds **25**-**27** to induce the re-expression of aberrantly silenced tumor suppressor genes *in vitro* was next measured using the Calu-6 human lung carcinoma cell line. The tumor suppressor genes SFRP2 and GATA4 were chosen because they are known to be under expressed in human lung cancer, and because they are thought to play a role in tumorigenesis when silenced. Thus, the genes coding for these proteins are well-documented LSD1 targets. Cells were treated for 24 hours with either a 5 or 10 μM concentration of **25**, **26** or **27**, after which the levels of secreted frizzle-related protein 2 (SFRP2), a soluble modulator of Wnt signaling, and the zinc-finger transcription factor GATA4, were determined by quantitative PCR (qPCR). The results of these studies are shown in Figure 3.2. All three compounds produced increases in SFRP2 expression that appeared to be dose dependent for **25** and **27** (Figure 3.2 A). Compound **27** produced the largest increase in SFRP2 expression at 10 μM (4.8-fold increase). Compounds **25** and **26** did not produce significant increases in GATA4 levels at 5 and 10 μM (Figure 3.2 B), and compound **27** induced a 1.3-fold increase in GATA4 mRNA at 10 μM, and had no significant effect at 5 μM (Figure 3.2 B). The increase in GATA4 mRNA caused by 10 μM **27** is reproducible, but is not statistically significant ($P > 0.05$).

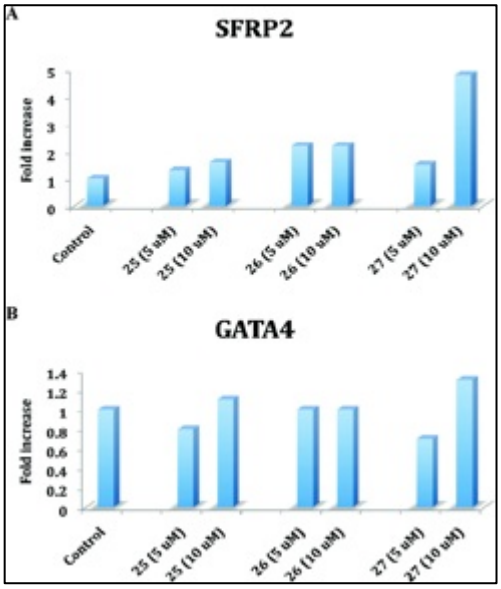


Figure 3.2 Effect of compounds **25−27** on the re-expression of secreted frizzle-related protein 2 (SFRP2, (A)) and the transcription factor GATA4 ((B)) mRNA. Calu-6 human anaplastic

nonsmall cell lung carcinoma cells were treated with either a 5 or 10 μM concentration of **25**, **26**, or **27** for 24 h. cDNA was then synthesized from mRNA, amplified and measured by qPCR. Each data point is the average of three determinations that differed in all cases by 5% or less.

The (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reduction assay was used to determine the effects of compounds **25**-**27** on cell viability in the Calu-6 cell line. Cells were treated with increasing concentrations of each test compound for 96 hours prior to measurement of cell viability, and growth inhibition (GI_{50}) values were then determined from the resulting dose-response curve. Compounds **25**, **26** and **27** produced moderate reduction in cell viability, with GI_{50} values of 10.3, 38.3 and 9.4 μM, respectively (Figure 3.3).

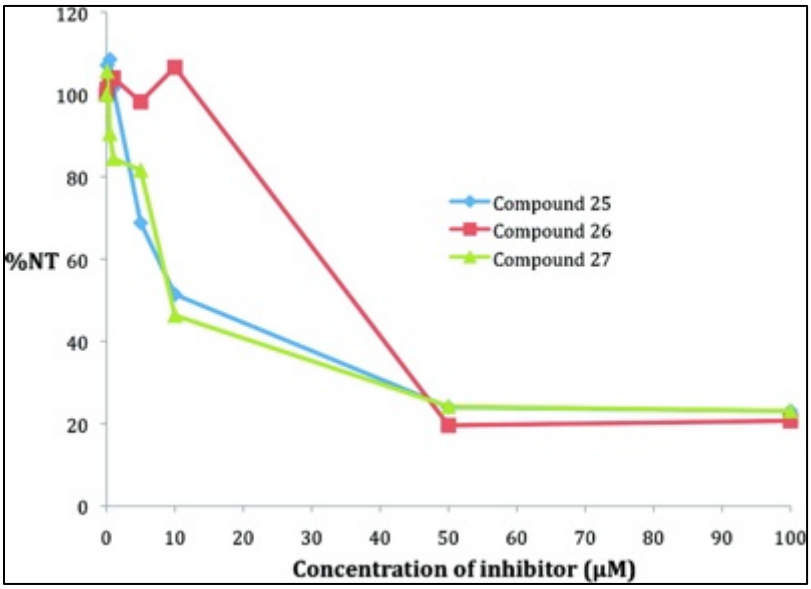


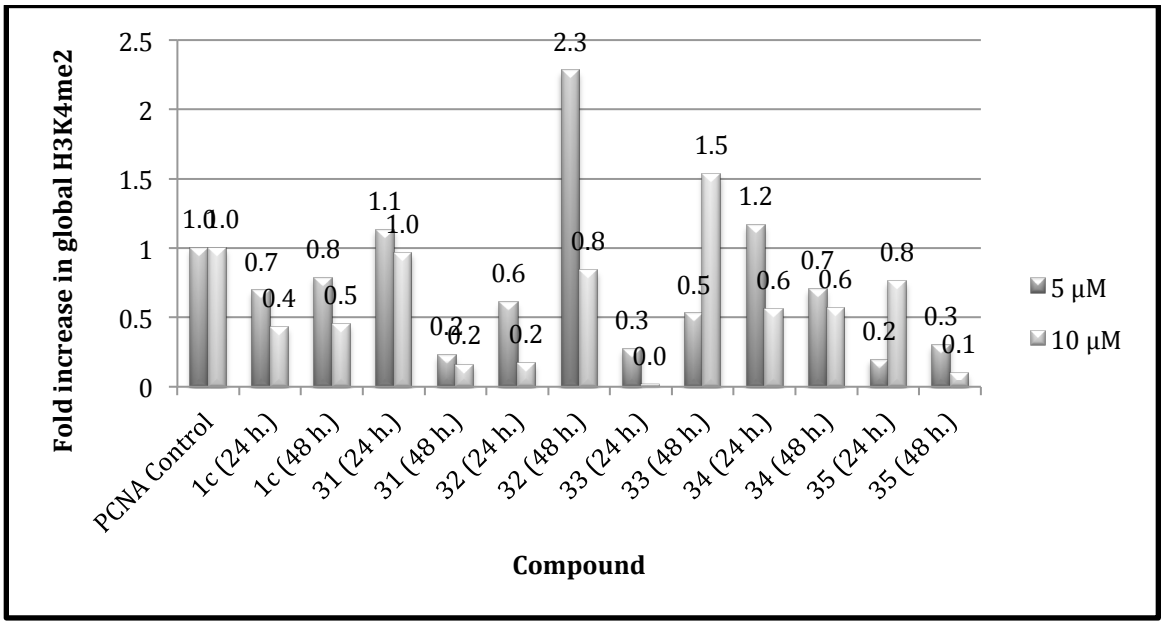
Figure 3.3 Effect of compounds **25−27** on Calu-6 human anaplastic nonsmall cell lung carcinoma cell viability as measured by standard MTS assay. Cells were treated with increasing concentrations of each test compound for 96 h prior to measurement of cell viability. %NT refers to the percent of viable cells remaining at time *T* (96 h) as compared to the number of cells seeded, N_0. Each data point is the average of three determinations that differed in all cases by 5% or less.

3.2 Symmetrically Alkylated Polyamino(bis)guanidines 31-35

Based on the activity of lead compound **1c**, analogues **31-35** were synthesized and evaluated (Table 2.1). The LSD1 inhibitory activity of these compounds was unremarkable, and

had only modest effects on global H3K4me2 levels. After 48 h. exposure, 5 μM compound **32** produced a 2.3 fold increase in the level of H3K4me2 in the KG1a hematopoietic cell line, but not in HL60 human promyelocytic leukemia cells. Compound **33** caused a 1.6-fold increase in H3K4me2 in the KG1a line at 10 μM, and did not affect H3K4me2 levels in the HL60 line. Compounds **31**, **34**, and **35** had no effect in either cell line (Figure 3.4).

A. KG1a Hematopoietic cell line



B. HL60 human promyelocytic leukemia cells

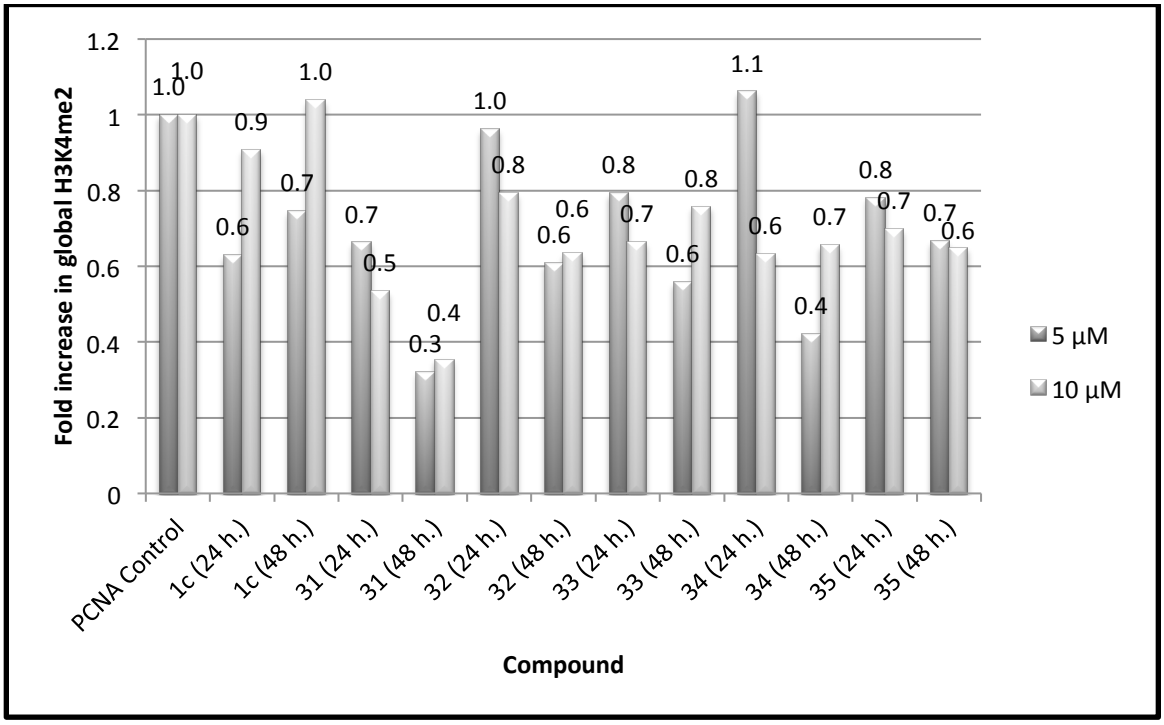


Figure 3.4 Effect of compounds **1c** and **31-35** on the expression of global H3K4me2. KG1a and HL60 cells (A,B) were treated with 5 and 10 µM concentration of compound for 24 h. and 48 h. as described in Materials and Methods. Proliferating cell nuclear antigen (PCNA) was used as a loading control. Relative protein expression levels were determined by quantitative Western Blot analysis using the Odyssey infrared detection system shown as bar graphs.

CHAPTER 4 DISCUSSION

4.1 Polyamino(bis)ureas and Polyamino(bis)thioureas

Compounds **3**-**30** were synthesized using pathways that are facile and relatively inexpensive, and that can be used to introduce chemical diversity into the resulting urea and thiourea analogues, thus making them suitable for generation of a library of related ureas and thioureas. Our initial series of guanidine and biguanide derivatives [34] represented the first novel small molecule inhibitors of LSD1 with potential for development as therapeutic agents. The current studies suggest that replacement of the imine NH functionality of the terminal guanidine in **1c** with oxygen or sulfur is an allowable isosteric change, and active analogues in both the urea and thiourea series were identified (Table 2.1). However, the sulfur isosteric replacement is likely more acceptable, since the 8 best LSD1 inhibitors (**6**, **10**, **18**, **25-27, 29,** and **30**) were all thioureas. A more bulky aromatic substituent on the terminal nitrogen, as in **25-27**, **29** and **30** appears to impart greater activity than the smaller alkyl or benzyl substituents found in **6**, **10** and **18**. There did not appear to be predictable differences in activity between analogues with 3, 4 or 7 carbon central chains, suggesting that this parameter may not have a great influence on inhibition of the enzyme. This is especially apparent among **25**-**27** (terminal N-substituent = 2,2-diphenylethyl), which have 7, 4 and 3 carbon central regions, respectively, but vary in activity by less than 5%. However, by contrast, among compounds **28**-**30** (terminal N-substituent = 1,1-diphenylmethyl), inhibitory potency did appear to be proportional to the length of the internal carbon chain. Additional analogues will need to be synthesized and evaluated to generate a more accurate set of structure/activity relationships for this series of compounds.

The inhibitory effects of **25**-**27** on LSD1 (Table 2.1), combined with the observed methylation levels at the H3K4 chromatin mark (Figure 3.1 A-D) strongly suggest that LSD1 is inhibited in the Calu-6 tumor cell line, resulting in increases in the substrates H3K4me1 and H3K4me2. The anomolous reduction in H3K4me2 at 24 and 48 hours caused by **26** are unexpected, and have yet to be explained. In addition, the effects of **25**-**27** on other histone

demethylases, including LSD2,[57] the Jumonji C (JmjC) domain-containing demethylases[10, 11, 16, 17] and the recently discovered JmjC demethylasee PHF8 [58, 59] need to be determined.

Compounds **25**-**27** were next evaluated for the ability to induce the re-expression of SFRP2 and GATA4 mRNA, as determined by qPCR from treated Calu-6 human lung carcinoma cells. In the case of SFRP2, all three analogues induced increases of the protein between 1.3- and 4.8-fold (Figure 3.2). These increases appeared to be dose-dependent, except in the case of **26**, which induced same level of SFRP2 expression at both 5 and 10 μM. The order of potency in this regard was **27** > **26** > **25**. Compound **27** produced 1.3-fold increase in GATA4 expression at 10 μM that was not statistically significant, and **25**-**27** at all other concentrations produced no effect on GATA4 mRNA. The observed increases in SFRP2 re-expression following treatment with **25**-**27**, and the increase in GATA4 re-expression induced by 10 μM **27**, are consistent with the previously reported effects of the parent compound **2d** [34]. The disparity in the ability of **25**-**27** to induce SFRP2 expression, but not GATA4 expression, suggests that LSD1 inhibition may have variable effects at different gene promoters.

As discussed above, compounds **25**-**27** proved to be only moderately cytotoxic in the Calu-6 non-small cell lung carcinoma line in vitro. Compounds **25** and **27** produced the most prominent reduction in cell viability, exhibiting GI_{50} values of 10.3 and 9.4 μM, respectively. These values are comparable to the GI_{50} value for other epigenetic modulators, such as the polyaminohydroxamic acid and polyaminobenzamide HDAC inhibitors developed in our laboratory [60, 61], and the parent compound **2d**. In addition, these GI_{50} values are in the range of the histone deacetylase (HDAC) inhibitor MS-275, as measured in three colon tumor cell lines [62]. Compound **26** was significantlty less cytotoxic, exhibiting a GI_{50} value of 38.3 μM. Our data suggests that decreases in H3K4me2 at 24 and 48 hours and/or minimal effects on the re-expression of SFRP2 and GATA4 by **26** could account for this reduced cytotoxicity. It is important to note that epigenetic modulators such as those mentioned above are generally used in combination with traditional cytotoxic agents, and serve to restore the ability of transformed

cells to undergo apoptosis [63]. As such, cytotoxicity is less of an issue, as long as the compound produces epigenetic effects in tumor cells that can be exploited by traditional cytotoxic agents. We have recently shown that the LSD1 inhibitor **2d** alone has little effect in vivo on tumor cell growth in an HCT116 human colon carcinoma mouse xenograft model, but acts synergistically to limit tumor growth in combination with the DNA methyltransferase inhibitor 5-azacytidine [25]. Additional studies are now being conducted to determine whether isosteres of **2d** such as **25**-**27** also produce a synergistic effect on tumor cell growth in vivo. Additional biological studies, as well as the synthesis and evaluation of additional LSD1 inhibitors in this and other compound libraries, is an ongoing effort in our laboratory.

4.2 Symmetrically Alkylated Polyamino(bis)guanidines 31-35

Based on the activity of lead compound **1c**, we synthesized and evaluated analogues **31-35** (Table 2.1). The LSD1 inhibitory activity of these compounds was unremarkable, and had only modest effects on global H3K4me2 levels (Figure 3.4). Analogues of **1c** in which all 4 terminal nitrogens were alkylated had low activity against purified LSD1. It is possible that electrostatic binding to the enzyme requires one unsubstituted heteroatom on the terminal guanidine. We prepared a second batch of **1c** for additional studies, and found that it did not produce the same results as the original sample, which was at least 3 years old. Thus there is some question about the structure of **1c**, in that it may be a metabolite producing the effect rather than the parent compound. This hypothesis is supported by our results from compounds **31-35**. However, a separate report indicates that **1c** produces the same effects on H3K4me2 that we observed, albeit in another cell system [64]. This manuscript does not provide details on the source or chemical identity of the **1c** used in their study.

4.3 Unsymmetrically Alkylated Polyamino(bis)guanidines, Polyamino(bis)ureas and Polyamino(bis)thioureas

We have developed an extensive library of LSD1 inhibitors that display promise as anti-tumor agents, however, we have only a limited scope of structure activity relationships (SAR) of

this class of compounds. To help elucidate the pharmacophore of our existing compounds, we have begun to optimize reaction conditions for the synthesis of the unsymmetrical analogues outlined in Scheme 2.3. We have successfully isolated gram quantities of the intermediate following step 4 in Scheme 2.3. We expect this class of compounds will provide structural insight on features that are determinant to ligand affinity. Specifically, we expect to determine if two functionalized moieties appended to a polyamine backbone is critical to LSD1 inhibitory activity. The goal is to ultimately use SAR to develop agents of this class with increased antitumor activity.

CHAPTER 5 EXPERIMENTAL

5.1 Methods

5.1.1 Synthesis

All reagents and dry solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), or Acros Chemical (Chicago, IL) and were used without further purification except as noted below. Pyridine was dried by passing it through an aluminum oxide column and then stored over KOH. Triethylamine was distilled from potassium hydroxide and stored in a nitrogen atmosphere. Methanol was distilled from magnesium and iodine under a nitrogen atmosphere and stored over molecular sieves. Methylene chloride was distilled from phosphorus pentoxide, and chloroform was distilled from calcium sulfate. Tetrahydrofuran was purified by distillation from sodium and benzophenone. Dimethyl formamide was dried by distillation from anhydrous calcium sulfate and was stored under nitrogen. Preparative scale chromatographic procedures were carried out using E. Merck silica gel 60, 230−440 mesh. Thin layer chromatography was conducted on Merck precoated silica gel 60 F-254. Ion exchange chromatography was conducted on Dowex 1 × 8−200 anion exchange resin. Compounds **41a−c** were synthesized as previously described.

All ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 mHz spectrometer, and all chemical shifts are reported as δ values referenced to TMS or DSS. Infrared spectra were recorded on a Jasco FT-IR spectrophotometer and are referenced to polystyrene. In all cases, ^{1}H NMR, ^{13}C NMR, and IR spectra were consistent with assigned structures. Mass spectra were recorded on a Kratos MS 80 RFA (EI and CI) or Kratos MS 50 TC (FAB) mass spectrometer. Prior to biological testing, target molecules **3−30** were determined to be 95% pure or greater by HPLC chromatography using an Agilent series 1100 high-performance liquid chromatograph fitted with a C18 reversed-phase column.

5.1.2 Expression, Purification, and Demethylase Assay of Recombinant Proteins

Full-length human LSD1 cDNA was subcloned into the pET15b bacterial expression vector (Novagen, Madison, WI) in frame with an N-terminal 6× HIS-tag and transformed into the $BL_{21}(DE_3)$ strain of *Escherichia coli*. Following selection, expression and purification of recombinant LSD1 protein were performed as previously described. Briefly, expression of LSD1-HIS protein was induced by 1 mM IPTG for 6 h at 25 °C. The HIS-tagged protein was purified using Ni-NTA affinity purification resin and column as recommended by the manufacturer (Qiagen, Valencia, CA). Bound protein was eluted by imidazole and the eluate was dialyzed in PBS at 4 °C. Enzymatic activity of LSD1 was examined using luminol-dependent chemiluminescence to measure the production of H_2O_2, as previously described. In brief, LSD1 activity was assayed in 50 mM Tris, pH 8.5, 50 mM KCl, 5 mM MgCl, 5 nmol luminol, and 20 μg/mL horseradish peroxidase with the indicated concentrations of H3K4me2 (1−21 aa) peptide as substrate. The integral values were calibrated against standards containing known concentrations of H_2O_2, and the activities expressed as pmols H_2O_2/mg protein/min.

5.1.3 Western Blotting

Cytoplasmic and nuclear fractions were prepared for Western blot analysis using the NE-PER nuclear and cytoplasmic extraction kit (Pierce, Rockford, IL). Primary antibodies against H3K4me1 and H3K4me2 were from Millipore. The pCNA monoclonal antibody was purchased from Oncogene Research Products (Cambridge, MA). Dye-conjugated secondary antibodies were used for quantification of Western blot results using the Odyssey infrared detection system and software (LI-COR Biosciences, Lincoln, NE).

5.1.4 RNA Isolation and qPCR

RNA was extracted using TRIzol reagents (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized using SuperScript III reverse transcriptase with an oligo(dT) primer (Invitrogen). qPCR was performed using the following primers: *SFRP2* sense, 5′AAG CCT GCA AAA ATA AAA ATG ATG; *SFRP2* antisense, 5′TGT AAA TGG TCT TGC TCT TGG TCT

(annealing at 57.4 °C); *GATA4*sense, 5′GGC CGC CCG ACA CCC CAA TCT; *GATA4* antisense, 5′ ATA GTG ACC CGT CCC ATC TCG (annealing at 64 °C). qPCR was performed in a MyiQ single color real-time PCR machine (Bio-Rad, Hercules, CA) with GAPDH as an internal control.

5.1.5 Determination of Cell Viability

Calu-6 human anaplastic nonsmall cell lung carcinoma cells were maintained in culture using RPMI medium plus 10% fetal bovine serum. For the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) reduction assay, 4000 cells/well were seeded in 100 µL medium in a 96-well plate and the cells were allowed to attach at 37 °C in 5% CO_2 for one day. The medium was aspirated and cells were treated with 100 µL of fresh medium containing appropriate concentrations of each test compound. The cells were incubated for 4 days at 37 °C in 5% CO_2. After 4 days, 20 µL of the MTS reagent solution (Promega CellTiter 96 Aqueous One Solution cell proliferation assay) was added to the medium. The cells were incubated for another 2 h at 37 °C under 5% CO_2 environment. Absorbance was measured at 490 nm on a microplate reader equipped with SOFTmax PRO 4.0 software to determine the cell viability.

Synthetic H3K4me2 peptides were purchased from Millipore (Billerica, MA). Calu-6 cells were maintained in RPMI medium, supplemented with 10% fetal bovine serum (Gemini Bio-Products, Woodland, CA) and grown at 37 °C in 5% CO_2 atmosphere.

5.2 Synthetic Procedures and Spectral Analysis

5.2.2 (Bis)urea and (Bis)thioureas

3,3-Diphenylpropylisocyanate (35c)

A 4.24 g (0.020 mol) portion of 3,3-diphenylpropylamine was dissolved in 90 mL of dry toluene in a 250 mL round-bottomed flask under a nitrogen atmosphere, and triphosgene (2.98 g, 0.010 mol) was added to the reaction mixture. The reaction mixture was heated under reflux for 5 h and then cooled to room temperature, at which time an additional 0.5 g of triphosgene was

added. The reaction was then stirred for an additional 18 h at room temperature. During this time, the formation of product was monitored by TLC using hexane:ethyl acetate (3:1). When the reaction was complete, activated charcoal (0.50 g) was carefully added into reaction mixture to decolorize the solution, which was stirred for 30 min and filtered. The filtrate was concentrated under reduced pressure to give a light pale-yellow semisolid. A 100 mL portion of*n*-hexane/ethyl ether(1:1 ratio) was then added, and the mixture was stirred for 15 min. The solution was filtered and concentrated to afford 4.23 g of viscous material. The crude product was purified by flash chromatography on silica gel eluted with dichloromethane to furnish 3,3-diphenylpropylisocyanate **35c** as a white solid (1.31 g, 28% yield). ^{1}H NMR ($CDCl_3$): δ 7.38−7.10 (m, 10H, Ar-H), 4.09 (t, 1H, *J* = 7.2 Hz, $CHPh_2$), 3.27 (t, 2H, *J* = 6.4 Hz, CH_2NCS), 2.36 (m, 2H, CH_2CH_2). ^{13}C NMR ($CDCl_3$): δ 143.69, 128.94, 128.01, 126.85 (Ar-C), 48.14, 41.51, 36.87 (CH and CH_2).

General Procedure for Preparation of Isothiocyanates 37a−c

3,3-Diphenylpropylisothiocyanate (37c)

In a 250 mL round-bottomed flask under a nitrogen atmosphere, 3,3-diphenylpropylamine **34c**(2.10 g, 0.010 mol) was dissolved in 40 mL of freshly distilled THF, 3.64 g (5.0 mL, 0.036 mol) of triethylamine was added, and the mixture was cooled to 5 °C in an ice bath. Carbon disulfide (0.76 g, 0.96 mL, 0.10 mol) was then added to the reaction mixture via syringe over 20 min. Following addition of carbon disulfide, the mixture was stirred an additional 30 min, warmed to room temperature, and allowed to stir a further 2 h. A ^{1}H NMR of an aliquot (after removing the solvent in vacuo) indicated that conversion to the dithiocarbamate salt **36c** was complete. ^{1}H NMR (DMSO-d_6): δ 8.46 (t, 1H, NH), 7.34−7.12 (m, 8H, Ar-H), 7.06 (t, 2H, Ar-H), 3.94 (t, 1H, $CHPh_2$), 3.34 (m, 2H, CH_2NCS), 3.04 (q, 6H, NCH_2CH_3), 2.24 (m, 2H, CH_2CH_2), 1.20 (t, 6H, NCH_2CH_3).

The reaction mixture from above was recooled in an ice bath, 2.38 g of tosyl chloride (0.012 mol) was added, and the reaction mixture was allowed to stir for 30 min at 5 °C. It was then

warmed to room temperature and stirred for an additional 3 h. The solvent was removed in vacuo, the reaction was partitioned between 40 mL of 1.0 N HCl and 150 mL of Et_2O, and the two-phased mixture was stirred for 10 min. The organic layer was separated and the aqueous layer was extracted with a 100 mL portion of Et_2O. The combined organic layers was dried over Na_2SO_4 and concentrated to produce a viscous oil that solidified during vacuum drying. The product was purified by flash chromatography on silica gel (eluted with CH_2Cl_2) to give **37c** as a white solid (1.48 g, 53% based on **34c**, TLC R_f: 0.45 (*n*-hexane/EtOAc, 9:1). ^{1}H NMR ($CDCl_3$): δ 7.32−7.19 (m, 10H, Ar-H), 4.08 (t, 1H, *J* = 8.0 Hz, $CHPh_2$), 3.44 (t, 2H, *J* = 6.8 Hz, CH_2NCS), 2.41 (m, 2H, CH_2CH_2). ^{13}C NMR ($CDCl_3$): δ 143.17, 129.08, 127.97, 126.99 (Ar-C), 48.12, 43.66, 35.69 (CH and CH_2).

1,1-Diphenylmethylisothiocyanate (37a)

Isothiocyanate **37a** was prepared from 1,1-diphenylethylamine **34a** and carbon disulfide using the procedure described above for the synthesis of **37c**. The product was isolated as a white solid in 70% yield. TLC R_f: 0.90 (*n*-hexane/$MeCO_2Et$, 4:1). ^{1}H NMR ($CDCl_3$): δ 7.40−7.31 (m, 10H, Ar-H), 5.99 (s, 1H, $CHPh_2$). ^{13}C NMR ($CDCl_3$): δ 139.43, 129.18, 128.57, 126.85 (Ar-C), 64.82 (CH).

2,2-Diphenylethylisothiocyanate (37b)

Isothiocyanate **37b** was prepared from 1,1-diphenylethylamine **34a** and carbon disulfide using the procedure described above for the synthesis of **37c**. The product was isolated as a white solid in 87% yield. ^{1}H NMR (DMSO-d_6): δ 7.36−7.29 (m, 8H, Ar-H), 7.24−7.20 (t, 2H, *J* = 7.2 Hz, Ar-H), 4.45 (t, 1H, *J* = 8.0 Hz, $CHPh_2$), 4.34 (d, 2H, *J* = 7.6 Hz, CH_2NCS). ^{13}C NMR (DMSO-d_6): δ 1 41.64, 129.31, 128.53, 127.67 (Ar-C), 51.18, 48.95 (CH and CH_2).

General Procedure for Preparation of N-Boc Protected (Bis)thioureas

1,12-Bis-{3-[1-(benzyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43d)

In a 100 mL round-bottom flask, a 0.3 g portion of 4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diaza-1,12-diaminododecane **41b** (0.0008 mol) was dissolved in 20 mL of HPLC grade CH_2Cl_2 under a nitrogen atmosphere and the mixture was cooled to 0 °C. A solution of benzylisothiocyanate (240 mg, 0.0016 mol) in 5 mL of CH_2Cl_2 was then added dropwise with stirring, and the reaction mixture was allowed to stir at room temperature for 5 h. During this time, the formation of product was monitored by TLC (CH_2Cl_2/MeOH/NH_4OH 89:10:1). After completion of the reaction, the CH_2Cl_2 was removed under reduced pressure to produce a viscous colorless oil. The crude product was purified by flash chromatography on silica gel eluted with CH_2Cl_2/MeOH/NH_4OH (94.5:5:0.5 followed by 89:10:1) to furnish pure **43d** (0.46 g, 88% yield) as viscous oil. R_f: 0.46 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.31 (m, 10H, Ar-H), 6.31 (b, 2H, NH), 4.55 (bs, 4H, NCH_2), 3.54 (bs, 4H, NCH_2), 3.20 (bs, 4H, NCH_2), 3.10 (bs, 4H, NCH_2), 1.65 (bs, 4H, CH_2CH_2), 1.46 (bs, 4H, CH_2CH_2), 1.38 (s, 18H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(ethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43g)

Compound **43g** was prepared from 375 mg of **41b** (375 mg, 0.0009 mol) and ethylisothiocyanate according to procedure described above for the synthesis of **43d** to afford **43g** (512 mg, 95%) as viscous oil. R_f: 0.52 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.40 (b, 2H, NH), 6.00 (b, 2H, NH), 3.56 (m, 4H, NCH_2), 3.34 (b, 4H, NCH_2), 3.26 (b, 4H, NCH_2), 3.12 (b, 4H, NCH_2), 1.71 (b, 4H, CH_2CH_2), 1.50 (bs, 4H, CH_2CH_2), 1.40 (s, 18H, $C(CH_3)_3$), 1.20 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 80.36 ($[CH_3]_3C$), 46.95, 43.34, 41.19, 38.12, 28.63, 27.31, 26.16 (CH_2), 14.28 (CH_3).

1,12-Bis-{3-[1-(propyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43j)

Compound **43j** was prepared from 260 mg of **41b** (0.0007 mol) and *n*-propylisothiocyanate according to procedure described above for the synthesis of **43d** to afford **43j** (380 mg, 96%) as viscous oil. R_f: 0.51 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ3.50−3.36 (b, 8H, NCH_2), 3.28−3.20 (m, 8H, NCH_2), 3.26 (b, 4H, NCH_2) 1.78 (b, 4H, CH_2CH_2), 1.52 (bs, 4H,

CH_2CH_2), 1.46 (s, 18H, $C[CH_3]_3$), 0.73 (t, 6H, J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ80.36 ($[CH_3]_3C$), 46.95, 43.34, 41.19, 38.12, 28.63, 27.31, 26.16 (CH_2), 14.28 (CH_3).

1,15-Bis-{3-[1-(benzyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43k)

Compound **43k** was prepared from 220 mg of **41c** (0.0005 mol) and benzylisothiocyanate according to procedure described above for the synthesis of **43d** to afford **43k** (360 mg, 96%) as viscous oil. ^1H NMR ($CDCl_3$): δ 7.39−7.30 (m, 10H, Ar-H), 4.76 (b, 4H, CH_2Ph), 3.46 (b, 4H, NCH_2), 3.18 (m, 8H, NCH_2), 1.52 (b, 4H, CH_2CH_2), 1.54 (b, 4H, CH_2CH_2), 1.44 (s, 18H, $C(CH_3)_3$), 1.28 (b, 6H, CH_2CH_2).

1,11-Bis-{3-[1-(benzyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43n)

Compound **43n** was prepared from 291 mg of **41a** (0.0008 mol) and benzylisothiocyanate according to the procedure described above for **43d** to afford **43n** (373 mg, 73%) as viscous oil.R_f: 0.87 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.35 (m, 10H, Ar-H), 4.58 (bs, 4H, N-CH_2), 3.58 (bs, 4H, N-CH_2), 3.21 (b, 4H, N-CH_2), 3.10 (b, 4H, N-CH_2), 1.72 (b, 6H, CH_2CH_2), 1.40 (s, 18H, $C[CH_3]_3$).

1,11-Bis-{3-[1-(propyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43r)

Compound **43r** was prepared from 291 mg of **41a** (0.0008 mol) and *n*-propylisothiocyanate according to the procedure described above for **43d** to afford **43r** (379 mg, 86%) as viscous oil.R_f: 0.57 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.29 (bs, 1H, NH), 6.44 (s, 2H, NH), 3.43 (bs, 4H, N-CH_2), 3.01−3.15 (b, 12H, N-CH_2), 1.61 (bs, 6H, CH_2CH_2), 1.47 (m, J = 7.2 Hz, 4H, CH_2CH_3), 1.31 (s, 18H, $C[CH_3]_3$), 0.81 (t, J = 7.2 Hz, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(*n*-ethyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43s)

Compound **43s** was prepared from 291 mg of **41a** (0.0008 mol) and ethylisothiocyanate according to the procedure described above for **43d** to afford **43s** (347 mg, 83%) as viscous oil.R_f: 0.72 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.24 (bs, 2H, NH), 6.22 (bs, 2H, NH), 3.49 (bs, 4H, CH_2N), 3.29 (bs, 4H, N-CH_2), 3.20 (b, 4H, N-CH_2), 3.07 (b, 4H, N-CH_2), 1.62−1.74 (b, 6H, CH_2CH_2), 1.37 (s, 18H, C[CH_3]$_3$), 1.14 (t, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43u)

Compound **43u** was prepared from 155 mg of **41a** (0.0004 mol) and **37c** according to procedure described above for the synthesis of **43d** to afford **43u** (290 mg, 81%) as a white solid. R_f: 0.44 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.29−7.15 (m, 22H, Ar-H, and NH), 5.88 (b, 2H, NH), 4.04 (t, 2H, J = 7.6 Hz, $CHPh_2$), 3.53 (b, 4H, NCH_2), 3.28 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 3.12 (b, 8H, NCH_2), 2.36 (q, 4H, J = 8.0 Hz, NCH_2), 1.70 (m, 2H, CH_2CH_2), 1.47 (b, 4H, CH_2CH_2), 1.40 (s, 20H, C[CH_3]$_3$).

1,12-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43w)

Compound **43w** was prepared from 161 mg of **41b** (0.0004 mol) and **37c** according to procedure described above for the synthesis of **43d** to afford **43w** (322 mg, 89%) as a white solid. R_f: 0.52 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.25−7.16 (m, 22H, Ar-H, and NH), 5.88 (b, 2H, NH), 4.02 (t, 2H, J = 8.0 Hz, $CHPh_2$), 3.17 (b, 8H, NCH_2), 3.09 (b, 4H, NCH_2), 2.37 (q, 4H, J = 7.6 Hz, CH_2CH), 1.76−1.65 (m, 8H, CH_2CH_2), 1.41 (s, 18H, C[CH_3]$_3$).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43y)

Compound **43y** was prepared from 178 mg of **41c** (0.0004 mol) and **37c** according to procedure described above for the synthesis of **43d** to afford **43y** (305 mg, 80%) as a white solid. R_f: 0.57 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.28−7.15 (m, 20H, Ar-H), 5.88 (b, 2H, NH), 4.02 (t, 2H, J = 8.0 Hz, $CHPh_2$), 3.54 (b, 4H, NCH_2), 3.28 (b, 4H, NCH_2), 3.23 (b, 4H,

NCH_2), 3.08 (t, 4H, *J* = 7.2 Hz, NCH_2), 2.36 (q, 4H, *J* = 7.6 Hz, CH_2CH), 1.69 (bs, 4H, CH_2CH_2), 1.50 (b, 4H, CH_2CH_2), 1.40 (s, 18H, $C[CH_3]_3$), 1.28 (m, 6H, CH_2CH_2).

1,15-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43z)

Compound **43z** was prepared from 223 mg of **41c** (0.0005 mol) and **37b** according to procedure described above for the synthesis of **43d** to afford **43z** (288 mg, 79%) as a white solid. R_f: 0.68 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.31−7.19 (m, 20H, Ar-H), 5.75 (b, 2H, NH), 4.28 (b, 2H, $CHPh_2$), 4.02 (b, 4H, NCH_2), 3.54 (b, 4H, NCH_2), 3.25 (b, 4H, NCH_2), 3.09 (t, 4H, *J* = 7.2 Hz, NCH_2), 1.69 (bs, 4H, CH_2CH_2), 1.49 (b, 4H, CH_2CH_2), 1.40 (bs, 18H, $C[CH_3]_3$), 1.24 (m, 6H, CH_2CH_2).

1,12-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43aa)

Compound **43aa** was prepared from 161 mg of **41b** (0.0004 mol) and **37b** according to procedure described above for the synthesis of **43d** to afford **43aa** (295 mg, 84%) as a white solid. R_f: 0.60 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.32−7.20 (m, 20H, Ar-H), 5.77 (b, 2H, NH), 4.29 (b, 2H, $CHPh_2$), 4.02 (b, 4H, NCH_2), 3.56 (bs, 4H, NCH_2), 3.26 (bs, 4H, NCH_2), 3.12 (bs, 4H, NCH_2), 1.70 (b, 4H, CH_2CH_2), 1.48 (b, 4H, CH_2CH_2), 1.41 (s, 18H, $C[CH_3]_3$).

1,11-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43bb)

Compound **43bb** was prepared from 193 mg (0.0005 mol) of **41b** and **37b** according to procedure described above for the synthesis of **43d** to afford **43bb** (350 mg, 80%) as a white solid. R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.32−7.20 (m, 20H, Ar-H), 5.77 (bs, 2H, NH), 4.29 (bs, 2H, $CHPh_2$), 4.02 (bs, 4H, NCH_2), 3.56 (bs, 4H, NCH_2), 3.26 (bs, 4H, NCH_2), 3.12 (t, 4H, *J*= 7.2 Hz, NCH_2), 1.71 (b, 4H, CH_2CH_2), 1.41 (b, 20H, CH_2 and $C[CH_3]_3$).

1,11-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,8-[N-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43cc)

Compound **43cc** was prepared from 192 mg of **41a** (0.0005 mol) and **37a** according to procedure described above for the synthesis of **43d** to afford **43cc** as a white solid (350 mg, 83%), R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.34−7.27 (m, 20H, Ar-H), 6.43 (d, 2H, *J* = 5.2 Hz, NCH), 6.02 (b, 2H, NH), 3.52 (d, 4H, *J* = 5.2 Hz, NCH_2), 3.06 (m, 8H, NCH_2), 1.66 (bs, 6H, CH_2CH_2), 1.36 (bs, 18H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43dd)

Compound **43dd** was prepared from 201 mg of **41b** (0.0005 mol) and **37a** according to procedure described above for the synthesis of **43d** to afford **43dd** (380 mg, 89%) as white solid. R_f: 0.60 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.40 (b, 2H, NH), 7.34−7.27 (m, 20H, Ar-H), 6.43 (d, 2H, *J* = 5.2 Hz, NCH), 6.02 (b, 2H, NH), 3.52 (d, 4H, *J* = 5.2 Hz, NCH_2), 3.06 (bs, 8H, NCH_2), 1.63 (m, 4H, CH_2CH_2), 1.42 (bs, 4H, CH_2CH_2), 1.36 (s, 18H, $C[CH_3]_3$).

1,15-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43ee)

Compound **43ee** was prepared from 223 mg of **41c** and **37a** according to procedure described above for the synthesis of **43d** to afford **43ee** (408 mg, 91%) as a white solid. R_f: 0.77 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.45 (b, 2H, NH), 7.33−7.26 (m, 20H, Ar-H), 6.41 (d, 2H, *J* = 2.8 Hz, NCH), 6.03 (b, 2H, NH), 3.51 (m, 4H, NCH_2), 3.04 (m, 8H, NCH_2), 1.54 (bs, 4H, CH_2CH_2), 1.45 (b, 4H, CH_2CH_2), 1.35 (bs, 18H, $C[CH_3]_3$), 1.23 (m, 6H, CH_2CH_2).

General Procedure for Preparation of N-Boc Protected (Bis)ureas

1,12-Bis-{3-[1-(benzyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43e)

In a 100 mL round-bottom flask, a 0.35 g portion of 4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-1,12-diamino-diazadodecane **41b** (0.0009 mol) was dissolved in 20 mL of HPLC grade CH_2Cl_2 under a nitrogen atmosphere and the mixture was cooled to 0 °C. A solution of benzylisocyanate

(0.235 g, 0.0018 mol) in 5 mL of CH_2Cl_2 was then added dropwise with stirring, and the reaction mixture was allowed to stir at room temperature for 24 h. During this time, the formation of product was monitored by TLC (CH_2Cl_2:MeOH:NH_4OH 89:10:1). When the starting material had been consumed, the CH_2Cl_2 was removed under reduced pressure to afford a viscous colorless material. The crude product was purified by flash chromatography on silica gel eluted with CH_2Cl_2:MeOH:NH_4OH (97:2.5:0.5 followed by 94.5:5.0:0.5) to furnish pure **43e** (0.50 g, 86% yield) as viscous oil. R_f: 0.54 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 3.20−3.02 (m, 16H, NCH_2), 1.64 (b, 4H, CH_2CH_2), 1.48 (b, 4H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.11 (t, 6H, J = 6.4 Hz, CH_3).

1,12-Bis-{3-[1-(ethyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43f)

Compound **43f** was prepared from 368 mg of **41b** (0.0009 mol) and ethylisocyanate according to the procedure described above for **43e** to afford **43f** (480 mg, 96%) as viscous oil. R_f: 0.54 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 3.20−3.02 (m, 16H, NCH_2), 1.64 (b, 4H, CH_2CH_2), 1.48 (b, 4H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.11 (t, 6H, J = 6.4 Hz, CH_3).

1,15-Bis-{3-[1-(benzyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43h)

Compound **43h** was prepared from 230 mg of **41b** (0.0005 mol) and benzylisocyanate according to the procedure described above for **43e** to afford **43h** (350 mg, 96%) as viscous oil. R_f: 0.50 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 7.30 (m, 10H, Ar-H), 4.29 (s, 4H, CH_2Ph), 3.21−3.15 (m, 8H, NCH_2), 3.12 (t, 4H, J = 7.2 Hz, NCH_2), 1.70 (bs, 4H, CH_2CH_2), 1.50 (bs, 4H, CH_2CH_2), 1.44 (s, 18H, $C[CH_3]_3$), 1.32 (bs, 6H, CH_2CH_2).

1,12-Bis-{3-[1-(propyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43i)

Compound **43i** was prepared from 260 mg of **41c** (0.0005 mol) and *n*-propylisocyanate according to the procedure described above for **43e** to afford **43i** (356 mg, 94%) as viscous oil. R_f: 0.54 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 3.22 (m, 8H, NCH_2), 3.09 (t,

4H, *J* = 6.4 Hz, NCH_2), 3.05 (t, 4H, *J* = 7.6 Hz, NCH_2), 1.70 (b, 4H, CH_2CH_2), 1.50 (m, 8H, CH_2CH_2), 1.45 (s, 18H, $C[CH_3]_3$), 0.90 (t, 6H, *J* = 7.6 Hz, CH_3).

1,15-Bis-{3-[1-(benzyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43l)

Compound **43l** was prepared from 225 mg of **41c** (0.0005 mol) and ethylisocyanate according to the procedure described above for **43e** to afford **43l** (280 mg, 94%) as viscous oil. R_f: 0.37 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 3.28−3.12 (m, 8H, NCH_2), 3.10−3.06 (m, 8H, NCH_2), 1.68 (b, 4H, CH_2CH_2), 1.54 (b, 4H, CH_2CH_2), 1.44 (s, 18H, $C[CH_3]_3$), 1.30 (b, 6H, CH_2CH_2), 1.08 (t, 6H, *J* = 7.2 Hz, CH_2CH_2).

1,15-Bis-{3-[1-(propyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43m)

Compound **43m** was prepared from 225 mg of **41c** (0.0005 mol) and propylisocyanate according to the procedure described above for **43e** to afford **43m** (280 mg, 92%) as viscous oil. R_f: 0.35 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR (CD_3OD): δ 3.25−3.16 (m, 8H, NCH_2), 3.09−3.02 (m, 8H, NCH_2), 1.68 (b, 4H, CH_2CH_2), 1.52 (b, 8H, CH_2CH_2), 1.44 (s, 18H, $C[CH_3]_3$), 1.30 (b, 6H, CH_2CH_2), 0.90 (t, 6H, *J* = 7.2 Hz, CH_2CH_2).

1,11-Bis-{3-[1-(ethyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43o)

Compound **43o** was prepared from 287 mg of **41a** (0.0007 mol) and ethylisothiocyanate according to the procedure described above for **43e** to afford **43o** (245 mg, 62%) as viscous oil,R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 5.59 (bs, 1H, NH), 4.60 (bs, 1H, NH), 3.08−3.31 (m, 16H, N-CH_2), 1.58−1.78 (m, 6H, CH_2CH_2), 1.43 (s, 18H, $C[CH_3]_3$), 1.10 (t, *J* = 7.2 Hz, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(benzyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43p)

Compound **43p** was prepared from 302 mg of **41a** (0.0008 mol) and benzylisothiocyanate according to the procedure described above for **43e** to afford **43p** (485 mg, 95%) as viscous oil,R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.10−7.30 (m, 10H, Ar-H), 4.20

(bs, 4H, $PhCH_2$), 2.98−3.20 (m, 12H, N-CH_2), 1.65 (p, 2H, CH_2CH_2), 1.55 (p, *J* = 6.4 Hz, 4H, CH_2CH_2), 1.39 (s, 18H, $C[CH_3]_3$).

1,11-Bis-{3-[1-(*n*-propyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43q)

Compound **43q** was prepared from 291 mg of **41a** (0.0008 mol) and *n*-propylisothiocyanate according to the procedure described above for **43e** to afford **43q** (359 mg, 91%) as viscous oil.R_f: 0.63 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 5.60 (bs, 1H, NH), 4.70 (bs, 1H, NH), 3.05−3.28 (m, 16H, N-CH_2), 1.60−1.78 (m, 6H, CH_2CH_2), 1.47 (m, *J* = 7.2, 4H, CH_2CH_3), 1.42 (s, 18H, $C[CH_3]_3$), 0.88 (t, *J* = 7.2 Hz, 6H, CH_2CH_3).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,8-[*N*-(*tert*-butyl)oxycarbonyl)]-4,8-diazaundecane (43t)

Compound **43t** was prepared from 194 mg (0.0005 mol) of **41a** and **35c** according to the procedure described above for **43e** to afford **43t** (420 mg, 98%) as a viscous oil. R_f: 0.58 (CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^{1}H NMR ($CDCl_3$): δ 7.29−7.15 (m, 20H, Ar-H), 5.50 (b, 2H, NH), 3.96 (t, 2H, *J* = 8.0 Hz, $CHPh_2$), 3.25 (t, 4H, *J* = 6.4 Hz, NCH_2), 3.10 (b, 12H, NCH_2), 2.23 (q, 4H, *J*= 7.2 Hz, NCH_2), 1.72 (b, 2H, CH_2CH_2), 1.61 (b, 4H, CH_2CH_2), 1.42 (s, 18H, $C[CH_3]_3$).

1,12-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,9-[*N*-(*tert*-butyl)oxycarbonyl)]-4,9-diazadodecane (43v)

Compound **43v** was prepared from 193 mg of **41b** (0.0005 mol) and **35c** according to the procedure described above for **43e** to afford **43v** (386 mg, 92%) as viscous oil. ^{1}H NMR ($CDCl_3$): δ 7.29−7.13 (m, 22H, Ar-H, and NH), 5.50 (b, 2H, NH), 3.96 (t, 2H, *J* = 8.0 Hz, $CHPh_2$), 3.25 (t, 4H, *J*= 6.4 Hz, NCH_2), 3.10 (m, 12H, NCH_2), 2.24 (b, 4H, CH_2CH_2), 1.60 (b, 4H, CH_2CH_2), 1.42 (s, 22H, CH_2CH_2 and $C[CH_3]_3$).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,12-[*N*-(*tert*-butyl)oxycarbonyl)]-4,12-diazapentadecane (43x)

Compound **43x** was prepared from 158 mg of **41c** (0.0004 mol) and **35c** according to the procedure described above for **43e** to afford **43x** (310 mg, 95%) as viscous oil. R_f: 0.50

(CH_2Cl_2/MeOH/NH_4OH, 89:10:1). ^1H NMR ($CDCl_3$): δ 7.30−7.12 (m, 20H, Ar-H), 5.50 (b, 2H, NH), 4.40 (b, 2H, NH), 3.97 (t, 2H, *J* = 7.2 Hz, $CHPh_2$), 3.25 (t, 4H, *J* = 6.4 Hz, NCH_2), 3.10 (bs, 12H, NCH_2), 2.26 (q, 4H, *J* = 8.0 Hz, CH_2CH_2), 1.60 (bs, 4H, CH_2CH_2), 1.42 ((s, 18H, $C[CH_3]_3$), 1.24 (bs, 6H, CH_2CH_2).

General Procedure for Cleavage of N-Boc Protecting Group

1,12-Bis-{3-[1-(benzyl)thioureado]}-4,9-diazadodecane (3)

In a 100 mL round-bottom flask, a 0.4 g portion of **43d** (402 mg, 0.0006 mol) was dissolved in 30 mL of HPLC grade EtOAc under a nitrogen atmosphere, and 4.0 mL of a 1.0 M solution of HCl in EtOAc was added. The reaction mixture was allowed to stir at room temperature for 48 h, during which time the formation of product was monitored by TLC (CH_2Cl_2/MeOH/NH_4OH 89:10:1 or 78:20:2). The product precipitated as a white crystalline solid during the course of the reaction. When completion of the reaction was confirmed by TLC, the solvent was removed under reduced pressure to produce a white powder. The solid product was stirred with 30 mL of fresh EtOAc, and the solvent was decanted. The solid so obtained was vacuum-dried to give pure **3** as a white solid (315 mg, 95% yield). An analytical sample was obtained by purification on silica gel (CH_2Cl_2:MeOH:NH_4OH 89:10:1). ^1H NMR (CD_3OD): δ 7.32−7.10 (m, 10H, Ar-H), 4.67 (s, 4H, CH_2Ph), 3.71 (t, 4H, *J* = 5.6 Hz, NCH_2), 3.01 (bs, 8H, NCH_2), 1.95 (m, 4H, CH_2CH_2), 1.78 (bs, 4H, CH_2CH_2). MS (CI *m*/*z*) calcd for $C_{26}H_{40}N_6S_2$ [$M^{+\cdot}$] = 500.28; found 501.4 [M^+H].

1,12-Bis-{3-[1-(benzyl)ureado]}-4,9-diazadodecane (4)

Compound **4** was prepared from 480 mg (0.0007 mol) of **43e** according to procedure described above for the synthesis of **3** to afford 370 mg (94%) of **4** as a white solid. ^1H NMR (D_2O): δ 7.32 (m, 4H, Ar-H), 7.26 (m, 6H, Ar-H), 4.22 (s, 4H, CH_2Ph), 3.16 (t, 4H, *J* = 6.4 Hz, NCH_2), 2.88 (t, 4H,*J* = 7.2 Hz, NCH_2), 2.81 (bs, 4H, NCH_2), 1.25 (p, 4H, *J* = 6.4 and 7.2 Hz, CH_2CH_2), 1.57 (m, 4H, CH_2CH_2). ^{13}C NMR (D_2O): δ 160.89 (C═O), 139.83, 129.00, 127.45, 126.97 (Ar-C), 46.96, 45.12, 43.66, 36.44, 26.71, 22.92 (CH_2).

1,12-Bis-{3-[1-(ethyl)ureado]}-4,9-diazadodecane (5)

Compound **5** was prepared from 448 mg (0.0008 mol) of **43f** according to procedure described above for the synthesis of **3** to afford 330 mg (96%) of **5** as a white solid. ^{1}H NMR (D_2O): δ 3.15 (t, 4H, *J* = 5.6 Hz, N-CH_2), 3.05−2.98 (m, 12H, NCH_2), 1.79 (p, 4H, *J* = 7.2 Hz, CH_2CH_2), 1.71 (bs, 4H, CH_2CH_2), 1.01 (t, 6H, *J* = 7.2 Hz, CH_3). ^{13}C NMR (D_2O): δ 160.88 (C═O), 47.06, 45.31, 36.67, 35.27, 26.70, 23.06 (CH_2), 14.63 (CH_3).

1,12-Bis-{3-[1-(ethyl)thioureado]}-4,9-diazadodecane (6)

Compound **6** was prepared from 470 mg (0.0008 mol) of **43g** according to procedure described above for the synthesis of **3** to afford 314 mg (87%) of **6** as a white solid. ^{1}H NMR (D_2O): δ 3.51 (bs, 4H, NCH_2), 3.31 (bs, 4H, NCH_2), 3.06 (bs, 4H, NCH_2), 1.93 (p, 4H, *J* = 6.4 Hz, CH_2CH_2), 1.75 (bs, 4H, CH_2CH_2), 1.12 (t, 6H, *J* = 6.0 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ 154.38, 153.98 (C═O), 47.13, 45.00, 40.92, 26.13, 23.15 (CH_2), 13.49 (CH_3).

1,15-Bis-{3-[1-(benzyl)ureado]}-4,12-diazapentadecane (7)

Compound **7** was prepared from 320 mg (0.0005 mol) of **43h** according to procedure described above for the synthesis of **3** to afford 250 mg (95%) of **7** as a white solid. ^{1}H NMR (D_2O): δ 7.35 (m, 4H, Ar-H), 7.28 (m, 6H, Ar-H), 4.25 (s, 4H, CH_2Ph), 3.18 (t, 4H, *J* = 5.6 Hz, NCH_2), 2.88 (t, 4H,*J* = 7.2 Hz, NCH_2), 2.81 (t, 4H, *J* = 8.0 Hz, NCH_2), 1.76 (p, 4H, *J* = 7.2 Hz, CH_2CH_2), 1.53 (m, 4H, CH_2CH_2), 1.26 (bs, 6H, CH_2CH_2). ^{13}C NMR (D_2O): δ 160.91 (C═O), 139.83, 129.01, 127.48, 126.99 (Ar-C), 47.78, 44.97, 43.68, 36.47, 27.87, 26.71, 25.64, 25.59 (CH_2).

1,12-Bis-{3-[1-(*n*-propyl)ureado]}-4,9-diazadodecane (8)

Compound **8** was prepared from 330 mg (0.0006 mol) of **43i** according to procedure described above for the synthesis of **3** to afford 228 mg (90%) of **8** as a white solid. ^{1}H NMR (D_2O): δ 3.14 (t, 4H, *J* = 6.4 Hz, NCH_2), 3.00−2.95 (m, 12H, NCH_2), 1.79 (p, 4H, *J* = 6.4 Hz, CH_2CH_2), 1.70 (bs, 4H, CH_2CH_2), 1.40 (q, 4H, *J* = 6.4 Hz, CH_2CH_2), 0.79 (t, 6H, *J* = 7.2 Hz, CH_3). ^{13}C NMR (D_2O): δ 160.98 (C═O), 47.05, 45.31, 42.03, 36.67, 26.70, 23.05, 22.82 (CH_2), 10.77 (CH_3).

1,12-Bis-{3-[1-(*n*-propyl)thioureado]}-4,9-diazadodecane (9)

Compound **9** was prepared from 350 mg (0.0006 mol) of **43j** according to procedure described above for the synthesis of **3** to afford 240 mg (86%) of **9** as a white solid. ^{1}H NMR (D_2O): δ 3.59 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 3.07−3.00 (m, 8H, NCH_2), 1.92 (p, 4H, *J* = 7.2 and 6.4 Hz, CH_2CH_2), 1.75 (b, 4H, CH_2CH_2), 1.57−1.48 (m, 4H, CH_2CH_2), 0.85 (t, 6H, *J* = 7.2 Hz, CH_3).

1,15-Bis-{3-[1-(benzyl)thioureado]}-4,12-diazapentadecane (10)

Compound **10** was prepared from 340 mg (0.0005 mol) of **43k** according to procedure described above for the synthesis of **3** to afford 214 mg (77%) of **10** as a white solid. ^{1}H NMR (D_2O): δ 7.37−7.30 (m, 10H, Ar-H), 4.58 (b, 4H, CH_2Ph), 3.58 (b, 4H, NCH_2), 3.10−2.80 (m, 8H, NCH_2), 1.85 (b, 4H, CH_2CH_2), 1.59 (b, 4H, CH_2CH_2), 1.32 (b, 6H, CH_2CH_2).

1,15-Bis-{3-[1-(ethyl)ureado]}-4,12-diazapentadecane (11)

Compound **11** was prepared from 255 mg (0.0004 mol) of **43l** according to procedure described above for the synthesis of **3** to afford 178 mg (89%) of **11** as a white solid. ^{1}H NMR (D_2O): δ 3.16 (t, 4H, *J* = 7.2 Hz, NCH_2), 3.08 (q, 4H, *J* = 7.6 Hz, NCH_2), 2.99 (m, 8H, NCH_2), 1.79 (p, 4H, *J* = 7.2Hz, CH_2CH_2), 1.62 (bs, 4H, CH_2CH_2), 1.32 (s, 6H, CH_2CH_2), 1.02 (t, 6H, *J* = 7.2 Hz, CH_3). ^{13}C NMR (D_2O): δ 160.92 (C═O), 47.079, 45.15, 36.67, 35.25, 27.89, 26.69, 25.66 (CH_2), 14.65 (CH_3).

1,15-Bis-{3-[1-(*n*-propyl)ureado]}-4,12-diazapentadecane (12)

Compound **12** was prepared from 255 mg (0.0004 mol) of **43m** according to procedure described above for the synthesis of **3** to afford 180 mg (89%) of **12** as a white solid. ^{1}H NMR (D_2O): δ 3.16 (t, 4H, *J* = 5.6 Hz, NCH_2), 2.99 (m, 12H, NCH_2), 1.79 (p, 4H, *J* = 7.2 Hz, CH_2CH_2), 1.62 (m, 4H, CH_2CH_2), 1.42 (q, 4H, *J* = 6.4 Hz, CH_2CH_2), 1.32 (bs, 6H, CH_2CH_2), 0.81 (t, 6H, *J* = 7.2 Hz, CH_3)).^{13}C NMR (D_2O): δ 161.03 (C═O), 47.79, 45.14, 42.00, 36.67, 27.89, 26.71, 25.65, 22.86 (CH_2), 10.77 (CH_3).

1,11-Bis-{3-[1-(benzyl)thioureado]}-4,8-diazaundecane (13)

Compound **13** was prepared from 373 mg (0.0005 mol) of **43n** according to procedure described above for the synthesis of **3** to afford 302 mg (99%) of **13** as white solid. ^{1}H NMR

(DMSO-d_6): δ 9.09 (bs, 2H, NH), 8.21 (t, 2H, NH), 8.00 (bs, 2H, NH), 7.20−7.32 (m, 10H, Ar-H), 4.64 (bs, 4H, N-CH_2), 3.48 (bs, 4H, N-CH_2), 2.97 (bs, 4H, N-CH_2), 2.87 (bs, 4H, N-CH_2), 2.02 (p, 2H, CH_2CH_2), 1.86 (p, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 128.92, 127.91, 127.45 (Ar-C), 47.43, 45.30, 44.60, 41.33, 26.37, 23.01 (CH_2).

1,11-Bis-{3-[1-(ethyl)ureado]}-4,8-diazaundecane (14)

Compound **14** was prepared from 245 mg (0.0005 mol) of **43o** according to procedure described above for the synthesis of **3** to afford 178 mg (96%) of **14** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.14 (bs, 2H, NH), 6.00 (bs, 4H, NH), 2.88−3.08 (m, 12H, CH_2N), 2.82 (bs, 4H, CH_2N), 2.02 (bs, 2H, CH_2CH_2), 1.71 (bs, 4H, CH_2CH_2), 0.95 (t, J = 7.2 Hz, 6H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 159.16 (C═O), 45.25, 44.51, 36.84, 34.80, 27.51, 22.98 (CH_2), 16.34 (CH_3).

1,11-bis-{3-[1-(benzyl)ureado]}-4,8-diazaundecane (15)

Compound **15** was prepared from 485 mg (0.0007 mol) of **43p** according to procedure described above for the synthesis of **3** to afford 364 mg (99%) of **15** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.21 (bs, 6H, NH), 7.17−7.30 (m, 10H, Ar-H), 4.19 (s, 4H, N-CH_2), 3.08 (bs, 4H, N-CH_2), 2.94 (bs, 2H, N-CH_2), 2.82 (bs, 4H, N-CH_2), 2.02 (b, 2H, CH_2CH_2), 1.74 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.24 (C═O), 141.49, 128.89, 127.63, 127.20 (Ar-C), 45.27, 44.53, 43.57, 37.02, 27.45, 22.93 (CH_2).

1,11-Bis-{3-[1-(*n*-propyl)ureado]}-4,8-diazaundecane (16)

Compound **16** was prepared from 359 mg (0.0006 mol) of **43q** according to procedure described above for the synthesis of **3** to afford 303 mg (99%) of **16** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.24 (bs, 6H, NH), 2.82−3.06 (m, 16H, N-CH_2), 2.04 (b, 2H, CH_2CH_2), 1.73 (b, 4H, CH_2CH_2), 1.33 (m, J = 7.2 Hz, 4H, CH_2CH_3), 0.80 (t, J = 7.2 Hz, 4H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 159.29 (C═O), 45.22, 44.52, 41.88, 36.97, 27.39, 23.78, 22.92 (CH_2), 12.04 (CH_3).

1,11-Bis-{3-[1-(*n*-propyl)ureado]}-4,8-diazaundecane (17)

Compound **17** was prepared from 379 mg (0.0006 mol) of **43r** according to procedure described above for the synthesis of **3** to afford 317 mg (99%) of **17** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.46 (b, 2H, NH), 9.16 (b, 2H, NH), 7.82 (b, 2H, NH), 2.85−3.90 (b, 16H, N-CH_2), 1.84 (b, 2H, CH_2CH_2), 1.59 (b, 4H, CH_2CH_2), 1.44 (m, 4H, CH_2CH_3), 0.85 (t, 6H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 45.30, 44.58, 26.37, 22.92, 22.71, 22.00 (CH_2), 12.10 (CH_3).

1,11-Bis-{3-[1-(ethyl)thioureado]}-4,8-diazaundecane (18)

Compound **18** was prepared from 347 mg (0.0006 mol) of **43s** according to procedure described above for the synthesis of **3** to afford 282 mg (99%) of **18** as white solid. ^{1}H NMR (DMSO-d_6): δ 9.10 (bs, 2H, NH), 7.78 (bs, 2H, NH), 7.70 (bs, 2H, NH), 3.43 (bs, 4H, N-CH_2), 3.32 (bs, 4H, N-CH_2), 2.97 (bs, 4H, N-CH_2), 2.86 (bs, 4H, N-CH_2), 2.02 (b, 2H, CH_2CH_2), 1.83 (b, 4H, CH_2CH_2), 1.02 (t, J = 7.2 Hz, 6H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 45.27, 44.58, 38.83, 31.99, 26.38, 22.98 (CH_2), 15.12 (CH_3).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,8-diazaundecane (19)

Compound **19** was prepared from 400 mg (0.0005 mol) of **43t** according to procedure described above for the synthesis of **3** to afford 290 mg (86%) of **19** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.10 (bs, 4H, NH), 7.27−7.21 (m, 16H, Ar-H), 7.18−7.10 (m, 4H, Ar-H), 3.96 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.02 (t, 4H, J = 6.4 Hz, NCH_2), 2.92 (b, 4H, NCH_2), 2.84 (t, 4H, J = 7.2 Hz, NCH_2), 2.79 (bs, 4H, NCH_2), 2.09 (q, 4H, J = 8.0 Hz, CH_2CH_2), 1.99 (m, 2H, CH_2CH_2), 1.69 (m, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.22 (CO), 145.50, 129.08, 128.28, 126.72 (Ar-C), 48.51, 45.25, 44.49, 38.77, 36.88, 36.07, 27.45, 22.95 (CH and CH_2).

1,11-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,8-diazaundecane (20)

Compound **20** was prepared from 260 mg (0.0003 mol) of **43u** according to procedure described above for the synthesis of **3** to afford 205 mg (92%) of **20** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.10 (b, 4H, NH), 7.91 (b, 2H, NH), 7.32−7.14 (m, 20H, Ar-H), 6.10 (b, 2H, NH), 4.04 (t, 2H, J = 7.6 Hz, $CHPh_2$), 3.45 (b, 4H, NCH_2), 3.24 (b, 4H, NCH_2), 2.98 (b, 4H, NCH_2), 2.88 (b, 4H, NCH_2), 2.61 (m, 4H, CH_2CH_2), 2.04 (m, 2H, CH_2CH_2), 1.85 (m, 4H, CH_2CH_2). ^{13}C

NMR (DMSO-d_6): δ 145.36, 129.11, 128.31, 126.78 (Ar−C), 48.62, 45.29, 44.60, 42.80, 41.02, 34.97, 26.34, 22.96 (CH and CH_2).

1,12-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,9-diazadodecane (21)

Compound **21** was prepared from 370 mg (0.42 mmol) of **43v** according to procedure described above for the synthesis of **3** to afford 285 mg (90%) of **21** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.00 (bs, 4H, NH), 7.21−7.12 (m, 20H, Ar-H, and NH), 3.96 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.02 (t, 4H,J = 6.4 Hz, NCH_2), 2.84 (t, 4H, J = 6.4 Hz, NCH_2), 2.79 (b, 12H, NCH_2), 2.09 (q, 4H, J = 7.2 Hz, CH_2CH_2), 1.69 (t, 4H, J = 6.4 Hz, CH_2CH_2), 1.63 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.27 (C═O), 145.49, 129.08, 128.28, 126.73 (Ar-C), 48.49, 46.50, 45.10, 38.76, 36.88, 36.07, 27.46, 23.23 (CH and CH_2). MS (EI m/z) calculated for $C_{42}H_{56}N_6O_2$ [$M^{+\cdot}$] = 676.45; found 677.40 [M^+H].

1,12-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,9-diazadodecane (22)

Compound **22** was prepared from 260 mg (0.0003 mol) of **43w** according to procedure described above for the synthesis of **3** to afford 205 mg (92%) of **22** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.02 (bs, 4H, NH), 8.02 (b, 2H, NH), 7.30−7.12 (m, 22H, Ar-H, and NH), 4.03 (t, 2H, J = 7.6 Hz, $CHPh_2$), 3.43 (bs, 4H, NCH_2), 3.23 (bs, 4H, NCH_2), 2.85 (b, 8H, NCH_2), 2.24 (m, 4H, CH_2CH_2), 1.84 (b, 4H, CH_2CH_2), 1.67 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 145.34, 129.14, 128.31, 126.79 (Ar-C), 48.58, 46.59, 45.13, 41.18, 34.93, 26.29, 23.26 (CH and CH_2).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)ureado]}-4,12-diazapentadecane (23)

Compound **23** was prepared from 290 mg (0.0003 mol) of **43x** according to procedure described above for the synthesis of **3** to afford 225 mg (88%) of **23** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.94 (bs, 4H, NH), 7.27−7.21 (m, 16H, Ar-H), 7.13−7.10 (m, 4H, Ar-H), 3.96 (t, 2H, J = 7.2 Hz, $CHPh_2$), 3.02 (t, 4H, J = 6.9 Hz, NCH_2), 2.84 (t, 4H, J = 7.2 Hz, NCH_2), 2.77 (bs, 8H, NCH_2), 2.09 (d, 4H, J = 7.2 Hz, CH_2CH_2), 1.69 (t, 4H, J = 6.4 Hz, CH_2CH_2), 1.56 (bs, 4H, CH_2CH_2), 1.21 (bs, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 159.35 (C═O), 145.49, 129.07,

128.28, 126.72 (Ar-C), 48.49, 47.24, 45.09, 38.75, 36.82, 36.07, 28.55, 27.48, 26.37, 25.91 (CH and CH_2).

1,15-Bis-{3-[1-(3,3-diphenylpropyl)thioureado]}-4,12-diazapentadecane (24)

Compound **24** was prepared from 287 mg (0.0003 mol) of **43y** according to procedure described above for the synthesis of **3** to afford 230 mg (92%) of **24** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.87 (bs, 4H, NH), 7.89 (bs, 4H, NH), 7.32−7.25 (m, 16H, Ar-H), 7.18−7.14 (m, 4H, Ar-H), 4.10 (b, 2H, $CHPh_2$), 3.44 (b, 4H, NCH_2), 3.23 (b, 4H, NCH_2), 2.87 (m, 8H, NCH_2), 2.25 (d, 4H, J = 7.6 Hz, CH_2CH_2), 1.83 (t, 4H, J = 7.2 Hz, CH_2CH_2), 1.68 (m, 4H, CH_2CH_2), 1.28 (b, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 145.37, 129.11, 128.30, 126.70 (Ar-C), 48.61, 47.30, 45.15, 41.42, 34.93, 28.58, 26.41, 25.94 (CH and CH_2).

1,15-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,12-diazapentadecane (25)

Compound **25** was prepared from 260 mg (0.0003 mol) of **43z** according to procedure described above for the synthesis of **3** to afford 201 mg (90%) of **25** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.91 (bs, 3H, NH), 7.70 (b, 1H, NH), 7.52 (b, 1H, NH), 7.26 (bs, 16H, Ar-H), 7.16 (bs, 4H, Ar-H), 4.36 (b, 2H, $CHPh_2$), 4.04 (b, 4H, NCH_2), 3.45 (b, 4H, NCH_2), 2.78 (b, 8H, NCH_2), 1.78 (b, 4H, CH_2CH_2), 1.58 (b, 4H, CH_2CH_2), 1.25 (b, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 181.50 (C═S), 143.36, 129.16, 128.62, 127.07 (Ar-C), 50.44, 48.78, 47.30, 45.09, 28.59, 26.40, 26.25, 25.91(CH_2).

1,12-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,9-diazadodecane (26)

Compound **26** was prepared from 280 mg (0.0003 mol) of **43aa** according to procedure described above for the synthesis of **3** to afford 214 mg (89%) of **26** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.05 (b, 4H, NH), 7.79 (b, 2H, NH), 7.53 (bs, 2H, NH), 7.28 (bs, 16H, Ar-H), 7.14 (m, 4H, Ar-H), 4.36 (bs, 2H, $CHPh_2$), 4.02 (bs, 4H, NCH_2), 3.42 (bs, 4H, NCH_2), 2.81 (b, 8H, NCH_2), 1.80 (bs, 4H, CH_2CH_2), 1.66 (bs, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.29 (C═S), 143.39, 129.16, 128.63, 127.07 (Ar-C), 50.46, 48.76, 46.57, 45.09, 41.21, 26.25, 23.16 (CH and CH_2).

1,11-Bis-{3-[1-(2,2-diphenylethyl)thioureado]}-4,8-diazaundecane (27)

Compound **27** was prepared from 330 mg (0.0004 mol) of **43bb** according to procedure described above for the synthesis of **3** to afford 220 mg (79%) of **27** as a white solid. ^{1}H NMR (DMSO-d_6): δ 9.13 (b, 4H, NH), 7.77 (bs, 2H, NH), 7.50 (bs, 2H, NH), 7.27 (bs, 16H, Ar-H), 7.16 (bs, 4H, Ar-H), 4.35 (bs, 2H, $CHPh_2$), 4.04 (b, 4H, NCH_2), 3.66 (bs, 4H, NCH_2), 3.42 (bs, 4H, NCH_2), 2.94 (bs, 4H, NCH_2), 2.80 (bs, 4H, NCH_2), 2.01 (b, 2H, CH_2CH_2), 1.79 (bs, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.20 (C═S), 143.38, 129.17, 128.63, 127.07 (Ar-C), 50.45, 48.68, 46.24, 44.57, 41.05, 26.28, 22.98 (CH and CH_2).

1,11-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,8-diazaundecane (28)

Compound **28** was prepared from 335 mg (0.0004 mol) of **43cc** according to procedure described above for the synthesis of **3** to afford 227 mg (80%) of **28** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.90 (b, 4H, NH), 8.29 (b, 2H, NH), 7.40−7.22 (m, 20H, Ar-H), 6.72 (b, 2H, CH), 4.56 (b, NH), 3.52 (b, 4H, NCH_2), 2.97 (m, 8H, NCH_2), 2.02 (b, 2H, CH_2), 1.87 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.22 (C═S), 143.43, 129.08, 127.89, 127.56 (Ar-C), 61.28 (CH), 45.37, 44.59, 41.34, 26.33, 23.00 (CH_2).

1,12-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,9-diazadodecane (29)

Compound **29** was prepared from 354 mg (0.0004 mmol) of **43dd** according to procedure described above for the synthesis of **3** to afford 262 mg (87%) of **29** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.95 (b, 4H, NH), 8.30 (bs, 2H, NH), 7.30 (m, 20H, Ar-H), 6.72 (b, 2H, $CHPh_2$), 3.51 (b, 4H, NCH_2), 2.88 (b, 8H, NCH_2), 1.87 (b, 4H, CH_2CH_2), 1.66 (b, 4H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.26 (C═S), 143.42, 129.08, 127.89, 127.57 (Ar-C), 61.30 (CH), 46.60, 45.22, 41.42, 26.38, 23.28 (CH_2).

1,15-Bis-{3-[1-(1,1-diphenylmethyl)thioureado]}-4,12-diazapentadecane (30)

Compound **30** was prepared from 390 mg (0.0004 mol) of **43ee** according to procedure described above for the synthesis of **3** to afford 298 mg (89%) of **30** as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.90 (b, 4H, NH), 8.35 (b, 2H, NH), 7.30 (bs, 20H, Ar-H), 6.73 (bs, 2H, $CHPh_2$),

3.51 (bs, 4H, NCH_2), 2.89 (bs, 4H, NCH_2), 2.81 (bs, 4H, NCH_2), 1.87 (bs, 4H, CH_2CH_2), 1.60 (bs, 4H, CH_2CH_2), 1.26 (b, 6H, CH_2CH_2). ^{13}C NMR (DMSO-d_6): δ 183.29 (C═S), 143.45, 129.06, 127.89, 127.55 (Ar-C), 61.30 (CH), 47.32, 45.23, 41.42, 28.59, 26.41, 25.93 (CH_2).

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ABSTRACT

NOVEL INHIBITORS OF LYSINE SPECIFIC DEMETHYLASE 1 AS EPIGENETIC MODULATORS

by

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The recently discovered enzyme lysine-specific demethylase 1 (LSD1) plays an important role in the epigenetic control of gene expression, and aberrant gene silencing secondary to LSD1 over expression is thought to contribute to the development of cancer. We recently reported a series of (bis)guanidines and (bis)biguanides that are potent inhibitors of LSD1, and induce the re-expression of aberrantly silenced tumor suppressor genes in tumor cells in vitro. We now report a new series of isosteres that are also potent inhibitors of LSD1. These compounds induce increases in methylation at the histone 3 lysine 4 (H3K4) chromatin mark, a specific target of LSD1, in Calu-6 lung carcinoma cells. In addition, these analogues increase cellular levels of secreted frizzle-related proteins (SFRP) 2 and 5, and transcription factor GATA4. These compounds represent an important new series of epigenetic modulators with the potential for use as antitumor agents.

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