Diamine and Triamine Analogs and Derivatives as Inhibitors of Deoxyhypusine Synthase: Synthesis and Biological Activity

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Deoxyhypusine synthase catalyzes the initial step in the posttranslational formation of the amino acid hypusine [N^{ϵ}-(4-amino-2-hydroxybutyl)lysine] in eukaryotic initiation factor 5A (eIF-5A). eIF-5A and its hypusine modification are believed to be essential for cell growth. A number of compounds related to diamines and triamines were synthesized and tested as inhibitors of this enzyme. The findings indicate that the long chain triamines **2a** and **2b** and their guanyl derivatives **3a**, **3b**, **4a**, and **4b** exert inhibition by binding to enzyme through only a portion of their structures at any one time. The inhibition exhibited by *N*-ethyl-1,7-diaminoheptane **20** and its guanyl derivative **21** supports this notion and is evidence for participation of the secondary amino group in binding to enzyme. There is preliminary evidence that amidino and isothiuronium groups may also serve as basic centers for binding to enzyme. Few of the compounds tested here were comparable in inhibitory potency to 1-guanidino-7-aminoheptane (GC_7) the most effective known inhibitor of deoxhypusine synthase, and none proved nearly as efficient as GC_7 in inhibiting the enzyme in Chinese hamster ovary cells. Hence, unlike the antiproliferative effect of GC_7, for which there is evidence of cause by interference with deoxhypusine synthase catalysis (Park, M. H.; Wolff, E. C.; Lee, Y. B.; Folk, J. E. *J. Biol. Chem. 269*, **1994**, 27827–27832), the effective growth arrest exerted by several of the newly synthesized compounds cannot be attributed to inhibition of hypusine synthesis.

Introduction

The enzyme deoxyhypusine synthase catalyzes the initial step in the posttranslational production of the amino acid hypusine [N^{ϵ}-(4-amino-2-hydroxybutyl)lysine].[1,2] This step, the transfer of the 4-aminobutyl moiety of the polyamine spermidine to the ϵ-amino group of a lysine residue to form the deoxyhypusine [N^{ϵ}-(4-aminobutyl)lysine] residue, occurs at a single position in only one cellular protein, the eukaryotic translation initiation factor 5A (eIF-5A, older nomenclature eIF-4D).[3,4] Despite recent evidence that eIF-5A, unlike other translation initiation factors, is not involved in general protein synthesis,[5] there is substantial evidence that eIF-5A, which is abundant in all eukaryotic cells examined, and its hypusine modification are essential for cell proliferation.[3,4]

Several recent findings are consistent with a supposition that control of hypusine production provides a means of regulating cell growth. These include arrest of normal, as well as cancer, cell multiplication in culture[6,7] and supression of proliferation of smooth muscle cells derived from human primary atherosclerotic and restenotic coronary arteries[8] through inhibition of hypusine biosynthetic enzymes. Hence, inhibitors designed to target these enzymes specifically, by promoting cellular depletion of hypusine, may prove of value as novel antiproliferative drugs. In view of a recent report implicating eIF-5A in human immunodeficiency virus type 1 (HIV-1) replication as a Rev transactivator protein,[9] inhibitors of hypusine biosynthetic enzymes may also bear potential as anti HIV-1 agents.

A report that the fungicide guazatine, a guanylated polyamine derivative, inhibits deoxyhypusine synthase[10] prompted us to prepare several other guanylated amines and to test their inhibitory activities toward the enzyme.[11] Of the guanylated diamines and polyamines examined, 1-guanidino-7-aminoheptane (GC_7) competed most effectively with spermidine for binding to the enzyme *in vitro* and proved to be a remarkable inhibitor of the enzyme in Chinese hamster ovary(CHO) cells, essentially abolishing production of deoxyhypusine at concentrations as low as 1 μM in the medium.[6] The observation that this inhibitor curtailed cell growth without depleting cellular spermidine or eIF-5A precursor protein[6] is consistent with a critical role of hypusine in cell proliferation.

The aims of the present study were to delineate further the structural requirements for selective deoxyhypusine synthase inhibition, to establish the basis for this inhibition, and possibly to find novel, more potent drugs for control of cellular proliferation.

Chemistry

The synthesis of the triamines and their guanylated derivatives is outlined in Scheme 1. Triamines **2a–e** were prepared, with some modifications, by a general procedure for conversion of primary amines to secondary and tertiary amines with Raney nickel.[12] Synthesis of **2b** was conducted by refluxing the diamine with catalyst in dry benzene as outline previously for **2a**.[13] The 4-, 5-, and 6-carbon chain diamines reacted rapidly in the presence of Raney nickel, but produced little triamine in any case. Triamines **2c–e**, therefore, were prepared through the monoacetyl derivatives **1c–e** produced by treatment of the diamines with less than equivalent amounts of ethyl acetate at 100 °C as described for the preparation of *N*-acetyl-1,3-diaminopropane.[14] The action of Raney nickel on **1c–e** provided the *N,N'*-diacetyltriamines which were hydrolyzed directly to **2c**,

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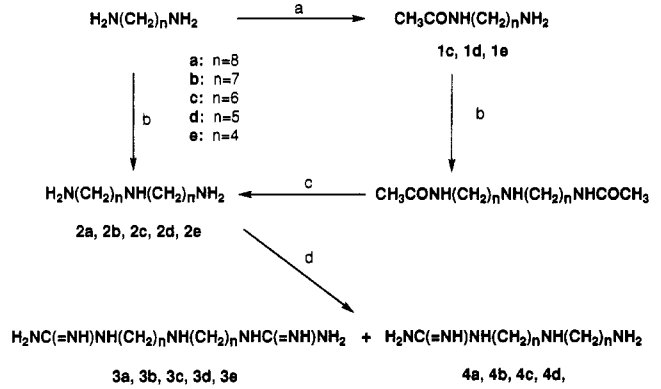
Scheme 1[a]



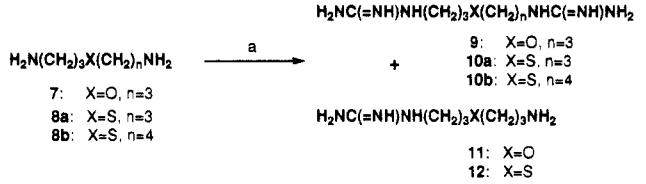
[a] Reagents: (a) $CH_3COOC_2H_5$, 100 °C; (b) Raney Ni, C_6H_6, Δ; (c) 2 N HCl, Δ; (d) $(CH_3SC(=NH)NH_2)_2{\cdot}H_2SO_4$, H_2O, Δ.

2d, and **2e**. Reaction of **2b–e** with excess aqueous *S*-methylisothiuronium sulfate yielded the guazatine homologs **3b–e**; reaction with an equivalent amount of reagent yielded the monoguanylated triamines **4a–e** which, with the exception of **4e**, were readily separable from contaminating bisguanylated triamines. Because we were unable to purify **4e** from this reaction mixture, it was synthesized by an alternative procedure outlined in Scheme 2 which precludes contamination by **3e**. In this preparation the intermediate **5**, in which one of the primary amino groups of the triamine is blocked, was produced from 4-(*p*-nitrobenzamido)butyraldehyde[15] and 1,4-diaminobutane by reductive amination. Guanylation of **5** produced the blocked monoguanylated triamine **6** which, upon deprotection by acid hydrolysis, gave **4e**.

Synthesis of the guanylated derivatives of analogs of spermidine and norspermidine in which oxygen or sulfur substitutes for the secondary nitrogen is shown in Scheme 3. These analogs, **7**,[16] **8a**,[17] and **8b**,[17] upon treatment with excess aqueous S-methylisothiuronium sulfate gave the bisguanylated derivatives **9**, **10a**, and **10b**; reaction with 1 equiv of the reagent provided the monoguanylated derivatives **11** and **12**, which were readily separable chromatographically from contaminating **9** and **10a**, respectively.

Aminooxy analogs of spermidine, in which the aminooxy group occupies a terminal position, have been prepared.[18] Scheme 4 illustrates our method for synthesis of the aminooxy analog of spermidine, where oxygen replaces a carbon adjacent to the central nitrogen. Reaction of the oxyamine- blocked putrescine analog **13**[18] with benzyloxycarbonyl chloride produced the fully blocked analog **14** which was, without isolation, selectively deblocked with HCl in 2-propanol to give the amino-protected 1-amino-3-(aminooxy)propane **15**. Reduction of the aldoxime formed between **15** and 3-azidopropanal[19] in methanol using $NaBH_3CN$ was conducted at pH 3 in order to cause very rapid reduction[20] and thus afforded the *N*-monoalkyl O-substituted hydroxylamine **16** in good yield. Reduction of the azido group in **16** to a primary amino group was accomplished with triphenylphosphine in aqueous THF.[21] The product of this reaction, **17**, upon treatment with 30% HBr in glacial acetic acid, was deprotected to yield **18**, the aminooxy analog of spermidine in which oxygen replaces the methylene on the butylamine side adjoining the central nitrogen.

Scheme 3[a]



[a] Reagents: (a) $(CH_3S(=NH)NH_2)_2{\cdot}H_2SO_4$, H_2O, Δ.

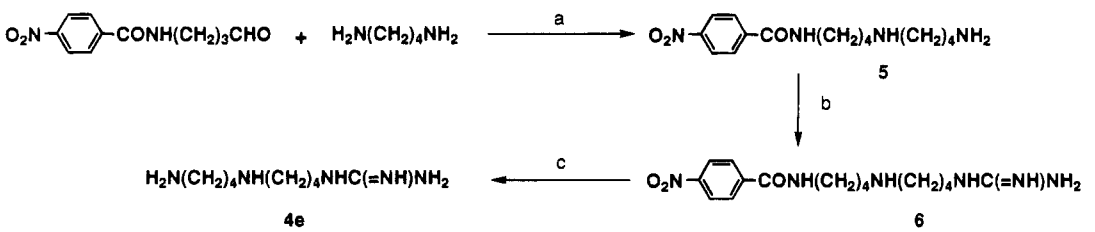
Schemes 5 and 6 outline procedures for preparation of two derivatives of the effective deoxyhypusine synthase inhibitor, GC_7, in each of which the primary amino group of GC_7 is replaced by a secondary amino group. For synthesis of the *N*-ethyl derivative (Scheme 5) 7-bromoheptanenitrile was reacted with ethylamine to give the *N*-ethylamino nitrile **19**. Upon treatment with diborane–tetrahydrofuran complex, **19** was reduced to the *N*-monoethyldiamine, **20**. Guanylation of **20** afforded the desired product **21**. The *N*-cyanoethyl derivative, **23**, was prepared (Scheme 6) by guanylation of the mono-*N*-cyanoethyldiamine **22**, produced from diaminoheptane through its reaction with 1 equiv of acrylonitrile.

All of the newly synthesized compounds, except **1c–e** (see Experimental Section), are listed in Table 1 together with some of their physical properties.

The diamidine dihydrochloride salts $[H_2NC(=NH)(CH_2)_nC(=NH)NH_2{\cdot}2HCl$, $n = 5–10$, **24a–f**, respectively] were prepared by the general procedure of Pinner[22] through the formation of the imidoethyl esters from the dinitriles and ethanol in the presence of anhydrous hydrogen chloride gas, followed by their treatment with anhydrous ammonia in ethanol. Melting points of compounds **24a**,[23,24] **24b**,[24] **24c**,[24] **24d**,[24,25] and **24f**[23] were found to be in agreement with those reported. That for 1,9-diamidinononane dihydrochloride **24e** was measured as 130–131 °C; no melting point was found in the literature for this compound. The identities of **24a–e** were verified by NMR, MS, and elemental analysis (C, H, N, Cl).

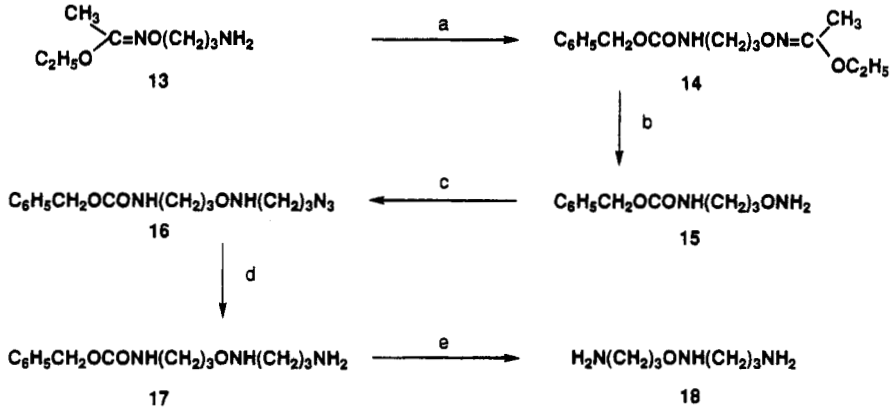
The diisothiuronium dihydrobromides $[H_2NC(=NH)S(CH_2)_nSC(=NH)NH_2{\cdot}2HBr$, $n = 4–8$, **25a–e**, respectively] were prepared analogously to literature procedures[24,26,27] by the reaction of the corresponding di-

Scheme 2[a]



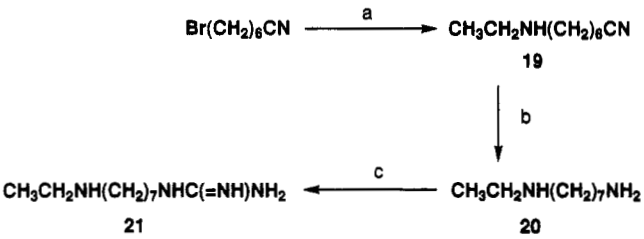
[a] Reagents: (a) $NaBH_3CN$, MeOH; (b) $CH_3OC(=NH)NH_2{\cdot}H_2SO_4$, MeOH; (c) 6 N HCl, Δ.

Scheme 4[a]



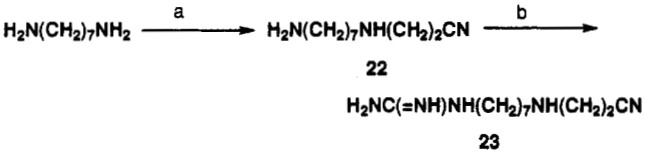
[a] Reagents: (a) $C_6H_5CH_2OCOCl$, NaOH, H_2O; (b) concentrated HCl, iPrOH; (c) $N_3(CH_2)_2CHO$, $NaBH_3CN$, MeOH; (d) $P(C_6H_5)_3$, THF, H_2O; (e) HBr/AcOH.

Scheme 5[a]



[a] Reagents: (a) $CH_3CH_2NH_2$, CH_3OH; (b) (1) BH_3·THF Δ, (2) 6 N HCl; (c) $CH_3OC(=NH)NH_2·H_2SO_4$, MeOH.

Scheme 6[a]



[a] Reagents: (a) CH_2=CHCN, EtOH; (b) $CH_3OC(=NH)NH_2·H_2SO_4$, MeOH.

bromides with thiourea in ethanol at reflux temperature. The identity of each of these compounds was verified by melting point, NMR, MS and elemental analysis (C, H, N, S).

Results and Discussion

Table 2 lists the triamines, their analogs, and derivatives and Table 3 the diamine analogs and derivatives tested as inhibitors of deoxyhypusine synthase. In earlier studies, competitive-type inhibition of this enzyme by compounds structurally related to spermidine suggested that these compounds bind to deoxyhypusine synthase at the site normally occupied by this polyamine substrate. Because the compounds studied here also bear structural resemblance to spermidine, those that inhibit are assumed to do so by competing with spermidine for its site of binding. Among the compounds of Table 2 is the fungicide called guazatine (1,17-diguanidino-9-azaheptadecane, compound **3a**) which was reported to inhibit a polyamine oxidase from oat leaf that catalyzes the cleavage of spermidine between its 5-position carbon and secondary amino groups to produce 1,3-diaminopropane and Δ^1-pyrroline.[28] For this reason guazatine was tested as, and found to be, an inhibitor of deoxyhypusine synthase,[10] which catalyzes cleavage of spermidine at the same site.[2,29] This finding led to the development of several inhibitors of the enzyme that are more effective than guazatine, e.g., GC_7 and GC_8[11] (Table 3). It was subsequently learned, however, that the sample of guazatine tested was actually a commercial formulation, also termed guazatine, containing **3a**, together with a mixture of guanidines derived from 1,8-diaminooctane and oligomers thereof.[30] Since 1,8-diaminooctane and its guanylated derivatives all displayed more effective inhibition than did the guazatine sample,[11] we chose to test pure **3a** and related compounds. It is apparent (Tables 2 and 3) that **3a** exerts a greater inhibition on deoxhypusine synthase than does the bisguanylated 1,8-diaminooctane GC_8G but significantly less than does the monoguanyldiamine GC_8. The same relationship is seen between **3b** (the **3a** homolog with seven methylene groups on each side of its central nitrogen) and GC_7G and GC_7. Furthermore, each of the 7-carbon-chained compounds is a more potent inhibitor than its respective 8-carbon-chained homolog. It seems logical to conclude that all of these compounds bind to the enzyme in a similar fashion. Furthermore, because GC_8 and GC_7 comprise only about one-half of the structures of **3a** and **3b**, respectively, it is likely that binding of the latter two involves only about one-half of their total structures at any one time. Consistent with this notion are the reduced levels of inhibition by the monoguanidines, **4a** and **4b**, as compared to their bis counterparts, **3a** and **3b**, for which the effective concentrations of the alkylguanidine portions are twice those of **4a** and **4b**. The alkyl primary amine sides of **4a** and **4b** would be expected to contribute little to inhibition since 1,8-diaminooctane is a weak inhibitor compared to GC_8,[11] as is 1,7-diaminoheptane compared to GC_7.[11] Because earlier studies showed that for a compound to function as an effective deoxyhypusine synthase inhibitor two of its basic groups (amino and/or guanidino) must be oriented at a strategic distance apart,[11] it would appear likely that the secondary amino groups of **3a**, **3b**, **4a**, and **4b**, and probably also of **2a** and **2b**, are involved in binding. That this is indeed the case is evident from the finding that **20** and **21** serve as inhibitors. The comparatively low inhibitory potency of **21** is somewhat surprising in light of the effective degrees of inhibition seen with **3b** and **4b**. Compound **21** is comprised of the putatively most inhibitory part of **4b**, with an ethyl group replacing the 7-aminoheptyl group. The degree to which inhibition

Table 1. Physical Properties of Products $[XNH(CH_2)_nY(CH_2)_mZ]$

compd	X	*n*	Y	*m*	Z	yield % (method)	mp (°C) or bp/Torr	recryst[a] solvent	formula	mol wt	anal.
2b	H	7	NH	7	NH_2	22 (A)	135–40/0.15		$C_{14}H_{33}N_3$	243.4	*b*
2c	H	6	NH	6	NH_2	59 (B)	136–40/0.22		$C_{12}H_{29}N_3$	215.4	*b*
2d	H	5	NH	5	NH_2	32 (B)	116–22/0.6		$C_{10}H_{25}N_3$	187.3	*b*
2e	H	4	NH	4	NH2	35 (B)	120/0.65		$C_8H_{21}N_3$	159.3	*b*
3b	$H_2NC(=NH)$	7	NH	7	$NHC(=NH)NH_2$	55 (C)	280–5 dec	I/II	$C_{16}H_{37}N_7 \cdot 1.5H_2SO_4$	474.6	C, H, N, S
3c	$H_2NC(=NH)$	6	NH	6	$NHC(=NH)NH_2$	76 (C)	232–4	I/II	$C_{14}H_{33}N_7 \cdot 1.5H_2SO_4$	446.6	C, H, N, S
3d	$H_2NC(=NH)$	5	NH	5	$NHC(=NH)NH_2$	61 (C)	210–2	II	$C_{12}H_{29}N_7 \cdot 1.5H_2SO_4$[c]	436.5	C, H, N, S
3e	$H_2NC(=NH)$	4	NH	4	$NHC(=NH)NH_2$	78 (C)	178–82 dec	II	$C_{10}H_{25}N_7 \cdot 1.5H_2SO_4$[c]	408.5	C, H, N, S
4a	$H_2NC(=NH)$	8	NH	8	NH_2	22 (D)	amorph		$C_{17}H_{39}N_5 \cdot H_2SO_4$	411.6	C, H, N, S
4b	$H_2NC(=NH)$	7	NH	7	NH_2	40 (D)	amorph		$C_{15}H_{35}N_5 \cdot 3HCl$[c]	412.9	C, H, N, Cl
4c	$H_2NC(=NH)$	6	NH	6	NH_2	40 (D)	amorph		$C_{13}H_{31}N_5 \cdot 3HCl$	366.8	C, H, N, Cl
4d	$H_2NC(=NH)$	5	NH	5	NH_2	38 (D)	amorph		$C_{11}H_{27}N_5 \cdot 3HCl$	338.8	C, H, N, Cl
4e	$H_2NC(=NH)$	4	NH	4	NH_2	73 (G)	290 dec	I/II	$C_9H_{23}N_5 \cdot 1.5H_2SO_4$[d]	420.5	C, H, N
5	$O_2NC_6H_5CO$	4	NH	4	NH_2	65 (E)	oil		$C_{15}H_{24}N_4O_3$	308.4	*b*
6	$O_2NC_6H_5CO$	4	NH	4	$NHC(=NH)NH_2$	49 (F)	275 dec	II/III	$C_{16}H_{26}N_6O_3 \cdot H_2SO_4$	448.5	C, H, N, S
9	$H_2NC(=NH)$	3	O	3	$NHC(=NH)NH_2$	40 (C)	305 dec	I/II	$C_8H_{20}N_6O \cdot H_2SO_4$	314.4	C, H, N, S
10a	$H_2NC(=NH)$	3	S	3	$NHC(=NH)NH_2$	64 (C)	305–7 dec	I/II	$C_8H_{20}N_6S \cdot H_2SO_4$	330.4	C, H, N, S
10b	$H_2NC(=NH)$	3	S	4	$NHC(=NH)NH_2$	32 (C)	265–8 dec	I/II	$C_9H_{22}N_6S \cdot H_2SO_4$	344.5	C, H, N, S
11	$H_2NC(=NH)$	3	O	3	NH_2	38 (D)	oil		$C_7H_{18}N_4O \cdot 2HCL$	247.2	C, H, N, Cl
12	$H_2NC(=NH)$	3	S	3	NH_2	50 (D)	oil		$C_7H_{18}N_4S \cdot 2HCl$[c]	281.2	C, H, N, Cl, S
15	$C_6H_5CH_2OCO$	1	CH_2	1	ONH_2	74 (H)	191 dec	IV	$C_{11}H_{16}N_2O_3 \cdot HCl$	260.7	C, H, N, Cl
16	$C_6H_5CH_2OCO$	3	ONH	3	N_3	50 (I)	oil		$C_{14}H_{21}N_5O_3$	307.3	*b*
17	$C_6H_5CH_2OCO$	3	ONH	3	NH_2	79 (J)	oil		$C_{14}H_{23}N_3O_3$	281.4	*b*
18	H	3	ONH	3	NH_2	59 (K)	142–4 dec	IV/V	$C_6H_{17}N_3O \cdot 3HBr$	389.9	C, H, N, Br
19	CH_3CH_2	2	CH_2	3	CN	94 (L)	80–80.5	I/VI/VII	$C_9H_{18}N_2 \cdot HBr$	235.2	*b*
20	H	7	NH	2	H	57 (M)	193–5		$C_9H_{22}N_2 \cdot 2HCl$	231.2	*b*
21	$H_2NC(=NH)$	7	NH	2	H	52 (F)	174–8	I	$C_{10}H_{24}N_4 \cdot H_2SO_4$[c]	316.4	C, H, N
22	H	7	NH	2	CN	49 (N)	193–4	III/V	$C_{10}H_{21}N_3 \cdot 2HCl$	256.2	*b*
23	$H_2NC(=NH)$	7	NH	2	CN	58 (F)	108–11	II/III	$C_{11}H_{23}N_5 \cdot H_2SO_4$[e]	359.4	C, H, N, S

[a] I, anhydrous ethanol; II, water; III, methanol; IV, 95% ethanol; V, ether; VI, chloroform; VII, benzene. [b] Microanalysis was not performed. [c] Monohydrate. [d] Tetrahydrate. [e] Dihydrate.

Table 2. Triamines and Their Analogs and Derivatives: Deoxyhypusine Synthase Inhibition *in Vitro* and Cellular Effects

	structure $[R'HN(CH_2)_nX(CH_2)_mNHR'']$						percent of control in cells						
							hypusine formed		spermidine uptake		protein synthesis		
compd	R′	n	X	m	R″	*in vitro* IC_{50}, μM	at 3 μM	at 10 μM	at 3 μM	at 10 μM	at 3 μM	at 10 μM	growth inhibition[f]
2a[a]	H	8	NH	8	H	116 ± 7							+++
3a[a]	$H_2NC(=NH)$	8	NH	8	$C(=NH)NH_2$	3.5 ± 0.33	100	100		95	100	84	++
4a	H	8	NH	8	$C(=NH)NH_2$	13 ± 2.3	36	27		80	20	22	++++
2b	H	7	NH	7	H	63 ± 7							+++
3b	$H_2NC(=NH)$	7	NH	7	$C(=NH)NH_2$	0.08 ± 0.01	90	74		95	88	67	+++
4b	H	7	NH	7	$C(=NH)NH_2$	0.3 ± 0.01	34	22		65	24	20	++++
2c	H	6	NH	6	H	>1000							++
3c	$H_2NC(=NH)$	6	NH	6	$C(=NH)NH_2$	40 ± 12	96	100		95	93	87	++
4c	H	6	NH	6	$C(=NH)NH_2$	74 ± 19	61	48		80	45	35	+++
2d	H	5	NH	5	H	>1000							+
3d	$H_2NC(=NH)$	5	NH	5	$C(=NH)NH_2$	38 ± 7	100	100		95	100	100	++
4d	H	5	NH	5	$C(=NH)NH_2$	100 ± 24		92		74	80	65	+++
2e	H	4	NH	4	H	556 ± 102							−
3e	$H_2NC(=NH)$	4	NH	4	$C(=NH)NH_2$	3.2 ± 0.4	100	100		85	100	100	++
4e	H	4	NH	4	$C(=NH)NH_2$	0.16 ± 0.02	7	3	64	28	88	93	+++
2f[b]	H	3	NH	3	H	41 ± 4							++
3f[c]	$H_2NC(=NH)$	3	NH	3	$C(=NH)NH_2$	182 ± 9	98	100		95	80	75	−
4f[c]	H	3	NH	3	$C(=NH)NH_2$	1.2 ± 0.3	50	23	76	34	77	75	++
7[d]	H	3	O	3	H	13 ± 2	100	100		100	100	85	+
9	$H_2NC(=NH)$	3	O	3	$C(=NH)NH_2$	8 ± 1	100	100		100	100	100	+
11	H	3	O	3	$C(=NH)NH_2$	0.7 ± 0.2	76	20		95	98	92	++
8a[e]	H	3	S	3	H	83 ± 9	100	93		100	86	81	++
10a	$H_2NC(=NH)$	3	S	3	$C(=NH)NH_2$	21 ± 2	100	74		81	100	60	++
12	H	3	S	3	$C(=NH)NH_2$	1.9 ± 0.3	50	24		100	40	25	+++
8b[e]	H	3	S	4	H	235 ± 23							+
10b	$H_2NC(=NH)$	3	S	4	$C(=NH)NH_2$	48 ± 2.5							++
18	H	3	ONH	3	H	385 ± 109							+

[a] Preparation in ref 13. [b] Purchased from Aldrich Chemical Co. [c] Preparation in ref 11. [d] Preparation in ref 16. [e] Preparation in ref 17. [f] The symbol defines the range of compound within which protein in treated cells is reduced to one-half the level of that in control cells at 72 h. The symbols ++++, +++, ++, +, and − represent <0.5, 0.5–1, >1–10, >10–100, and >100 μM, respectively.

can be influenced by the nature of radicals of the secondary amine is evident upon comparison of the IC_{50} values of **4b**, **21**, and **23**. The striking difference in inhibition seen with **21** and **23** must be attributed to differences in the structure of the ethyl and cyanoethyl radicals and possibly relates to the base-weakening influence of the cyano group.[31] Clearly, the comparisons made here show that, in compounds in which the two basic groups are the most satisfactorily oriented for inhibition; i.e., those with 7- and 8-carbon chains, a

Table 3. Diamine Analogs and Derivatives: Deoxyhypusine Synthase Inhibition *in Vitro* and Cellular Effects

compd	structure R	structure *n*	structure R′	*in vitro* IC_{50}, μM	percent of control in cells: hypusine formed at 3 μM	hypusine formed at 10 μM	spermidine uptake at 3 μM	spermidine uptake at 10 μM	protein synthesis at 3 μM	protein synthesis at 10 μM	growth inhibition[g]
				[$RHN(CH_2)_nNHR'$]							
GC_8G[a]	$H_2NC(=NH)$	8	$C(=NH)NH_2$	16.1 ± 3.3							
GC_8[a]	H	8	$C(=NH)NH_2$	0.55 ± 0.08							
GC_7G[a]	$H_2NC(=NH)$	7	$C(=NH)NH_2$	3.4 ± 0.7							
GC_7[a]	H	7	$C(=NH)NH_2$	0.03 ± 0.005	0[f]		100		62	50	++++
GC_6G[a]	$H_2NC(=NH)$	6	$C(=NH)NH_2$	112 ± 27							
GC_6[b]	H	6	$C(=NH)NH_2$	132 ± 19							
GC_5G[c]	$H_2NC(=NH)$	5	$C(=NH)NH_2$	6.8 ± 0.6							
GC_5[d]	H	5	$C(=NH)NH_2$	29 ± 5							
GC_4G[e]	$H_2NC(=NH)$	4	$C(=NH)NH_2$	179 ± 37							
GC_4[e]	H	4	$C(=NH)NH_2$	156 ± 37							
20	CH_3CH_2	7	H	275 ± 91							−
21	CH_3CH_2	7	$C(=NH)NH_2$	9 ± 1	76	36	95		34	28	++
23	$NCCH_2CH_2$	7	$C(=NH)NH_2$	96 ± 20							++
				[$R(CH_2)nR'$]							
24a	$H_2NC(=NH)$	5	$C(=NH)NH_2$	>1000							+
24b	$H_2NC(=NH)$	6	$C(=NH)NH_2$	>1000							+
24c	$H_2NC(=NH)$	7	$C(=NH)NH_2$	120 ± 10							++
24d	$H_2NC(=NH)$	8	$C(=NH)NH_2$	>1000							++
24e	$H_2NC(=NH)$	9	$C(=NH)NH_2$	>1000							++
24f	$H_2NC(=NH)$	10	$C(=NH)NH_2$	>1000							++
25a	$H_2NC(=NH)S$	4	$SC(=NH)NH_2$	>1000							++
25b	$H_2NC(=NH)S$	5	$SC(=NH)NH_2$	>1000							++
25c	$H_2NC(=NH)S$	6	$SC(=NH)NH_2$	780 ± 271							+++
25d	$H_2NC(=NH)S$	7	$SC(=NH)NH_2$	>1000							++
25e	$H_2NC(=NH)S$	8	$SC(=NH)NH_2$	>1000							++

[a] Preparation in ref 11. [b] Preparation in ref 6. [c] Prepared by the method outlined for GC_6G in ref 11. Anal. ($C_7H_{18}N_6{\cdot}H_2SO_4$) C, H, N. [d] Prepared by the method outlined for GC_7 in ref 11. Anal. ($C_6H_{16}N_4{\cdot}H_2SO_4{\cdot}0.5H_2O$) C, H, N, S. [e] Purchased from Sigma Chemical Co. [f] At 1 μM the hypusine formed was 3% of the control. [g] The symbol defines the range of compound within which protein in treated cells is reduced to one-half the level of that in control cells at 72 h. The symbols ++++, +++, ++, +, and − represent <0.5, 0.5−1, >1−10, >10−100, and >100 μM, respectively.

guanidino group, and a primary amino group provide the most effective combination.

For this reason it seems quite likely that the 3- and 4-carbon-chained polyamine derivatives, **3e**, **4e**, **3f**, and **4f**, inhibit by binding to enzyme through their primary amino and/or guanidino groups. Clearly, the monoguanidino compounds, **4e** and **4f**, are more potent inhibitors than their bisguanidino counterparts, **3e** and **3f**. Supporting the notion that the primary, and not the secondary, amino group participates in binding in these cases are the facts that 1-guanidino-3-aminopropane does not inhibit deoxyhypusine synthase[11] and that 1-guanidino-4-aminobutane, GC_4, gives poor inhibition. Thus, in contrast to the long-chained polyamine derivatives, **3a**, **4a**, **3b** and **4b**, each of which binds to enzyme by proper orientation of a guanidino group and the secondary amino group, the 3-and 4-carbon-chained triamine derivatives each appear to bind in a mode involving their two terminal basic groups.

The manner in which the 5- and 6-carbon-chained polyamine derivatives, **3c**, **4c**, **3d**, and **4d**, bind to enzyme is uncertain. It is clear, however, that they compete only poorly with spermidine for its site of binding.

The excellent enzyme inhibitory property of GC_7,[11] its potent antiproliferative activity in cells,[6] and its simple structure prompted us to compare structurally related compounds as potential inhibitors. Since it was discovered earlier that even a small group branched from the methylene chain of a polyamine significantly lowered or abolished its inhibitory property,[11] we chose to compare the guanidino derivatives of triamine analogs in which the central nitrogen was simply replaced by oxygen, sulfur, or a methylene. The terminal basic centers of **3f**, **4f**, **9**, **11**, **10a**, and **12** are each located three carbon atoms away from their central atoms and are, therefore, little influenced by inductive effects of the central atoms.[31] A further resemblance in these compounds is that the maximum extendable distance between their basic centers varies by less than 5%. Although the *mono*guanidino triamine analogs **4f**, **11**, and **12** display similarily effective inhibition, that given by the *mono*guanidino diamine GC_7 is far more potent. Likewise, the bisguanidino diamine GC_7G inhibits more efficiently than do **3f**, **9**, and **10a**, the bisguanidino triamine analogs. The comparatively poor inhibition seen with **3f** is notable. The bisguanidino derivative of spermidine which contains one additional methylene in its chain is a significantly better inhibitor than **3f**.[11] The opposite is the case, however, with the sulfur analog **10b**, with a IC_{50} value double that of **10a**.

Aminooxy analogs of putrescine and spermidine have proven useful as inhibitors of certain enzymes envolved in polyamine metabolism[32,33] and probably function as such by forming stable oximes with the enzyme-bound pyridoxal phosphate.[34] An aminooxy analog of spermidine with oxygen replacing the carbon next to the primary amino group in position 8 was found not to act as an inhibitor of deoxyhypusine synthase.[11] In Table 2 it may be seen that **18**, an aminooxy analog of spermidine in which oxygen replaces a carbon next to the central nitrogen, does function as an inhibitor, albeit a poor one. This poor inhibition, like that seen with **8b**, probably results from features unrelated to the basic strengths of its terminal amino groups.

It seems certain from the information collected thus far that the effectiveness of inhibition depends on the types of organic bases, i.e. primary amine, secondary

amine, or guanidine, in compounds of related structure. In order to determine if compounds containing bases other than those mentioned above can function as deoxyhypusine synthase inhibitors we tested a series of α,ω straight-chained diamidines **24a**–**f** and a series of α,ω straight-chained diisothioureas **25a**–**e** (Table 3). An alkylamidine has fewer equivalent electronic structures than an alkylguanidine. Its cation is doubly, rather than triply, degenerate, and perhaps for this reason, it is a weaker base, intermediate in strength between a guanidine and an amine. Due to the base-weakening effect of its sulfur atom, an *S*-alkylthiuronium is an even weaker base. It can be seen in Table 3 that one compound only in each series, i.e., **24c** and **25c**, displayed an IC_{50} value of less than 1 mM. The fact that the inhibition given by these straight-chained compounds, although poor, is in each case predicated on the distance between their basic centers is evidence for a mechanism of this inhibition like that of the other basic compounds studied here. Thus it appears that amidines and isothioureas are able to function like amines and guanidines as basic centers, albeit much less effective ones, in deoxyhypusine synthase inhibition.

A comparison of the IC_{50} values for the compounds in Tables 2 and 3 shows that GC_7 is the most effective inhibitor of deoxyhypusine synthase *in vitro*. Of the newly synthesized compounds, only **3b**, **4b**, **4e**, and **11** displayed IC_{50} values for enzyme inhibition of 1 μM or less, and of these, only **4e** caused a significant reduction in [^{3}H]hypusine formed in CHO cells. Deoxyhypusine is rapidly converted to hypusine in cells through catalysis by deoxyhypusine hydroxylase.[35] Since no accumulation of [^{3}H]deoxyhypusine was seen in the cells in these experiments, the reduction in radioactivity in the hypusine fraction could normally be taken as a measure of deoxyhypusine synthase inhibition, as was done in earlier similar studies.[11] It should be noted, however, that **4e** also caused a reduction in the cellular uptake of [^{3}H]spermidine. This reduction, in turn, causes (1) a decrease in the specific radioactivity of the cellular spermidine pool, (2) an underestimation of the hypusine level and, consequently, (3) an overestimation of the degree of inhibition. This is not the case with GC_7 which at 3 μM, a level that provides complete inhibition of hypusine biosynthesis, caused little reduction in spermidine uptake. The inhibition exhibited by **3b** *in vitro* is almost as potent as that of GC_7. The inability of **3b** to prevent hypusine biosynthesis in CHO cells may result from its failure to enter the cells in sufficient amount to exert its inhibitory effect.

Wide variations in inhibition of cell growth and of protein synthesis in cells are seen with the compounds in Tables 2 and 3. However, in no case, with the exception of GC_7 and possibly of **4e**, can the effects be related to inhibition of cellular deoxyhypusine synthase. Interestingly, several compounds, namely **4a**, **4b**, **4c**, **12**, and **21**, displayed quite pronounced inhibition of protein biosynthesis. The reduction in [^{3}H]hypusine seen with each of these compounds may have been largely due to a decreased biosynthesis of eIF-5A precursor protein and, consequently, to loss of protein substrate for deoxyhypusine synthase, rather than to inhibition of the enzyme *per se*. In contrast, the moderate reduction in hypusine formation seen with **11** cannot be accounted for in this way and probably results from enzyme inhibition.

In summary, a number of new compounds derived from triamines and from diamines were synthesized and, along with some related known compounds, were tested *in vitro* as inhibitors of deoxyhypusine synthase. The findings identify several groups, in addition to primary amino and guanidino groups, that can serve as basic centers in compounds that compete for spermidine binding on this enzyme and provide a partial explanation for wide variations in the inhibitory potency of some of the compounds of similar structure. A number of the compounds were tested in CHO cells for effects on hypusine formation, protein synthesis, and cell growth. Although reductions in labeling of hypusine were observed with several of the newly synthesized compounds, in most cases this appears to be largely a result of reduced [^{3}H]spermidine transport or reduced eIF-5A precursor protein synthesis, rather than inhibition of cellular deoxyhypusine synthase. In contrast to the antiproliferative effect of GC_7, for which there is substantial evidence for cause by intracellular inhibition of deoxyhypusine synthase,[6] the arrest of cell growth by some of the new compounds cannot be attributed to suppression of the activity of this enzyme. The overall cellular effects of these compounds are under investigation.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus in open capillary tubes and are uncorrected. TLC was performed on Merck silica gel 60 F254 analytical plates, visualized with UV, tolidine–Cl_2, ninhydrin, and/or Sakaguchi. Flash chromatography was conducted on Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded on a Bruker AC-250 spectrometer at 250 MHz. Chemical shifts are expressed relative to internal DMSO at 2.5 ppm (DMSO-d_6) or to HOD at 4.8 ppm (D_2O). Mass spectra were obtained on a JEOL SX 102 spectrometer (FAB) and on a Finnigan MAT 4600 spectromer (CI).

1-Acetamido-6-aminohexane (1c). A mixture of 15.34 g (0.132 mol) of 1,6-diaminohexane and 3.88 g (0.044 mol) of ethyl acetate was heated at 100 °C in a sealed tube for 24 h. The mixture was distilled to afford 5.44 g (78% based on ethyl acetate) of **1c**: bp 175–7 °C/0.27 Torr; MS (FAB, thioglycerol (t-glyc)) 159 (M + H); R_f 0.5 (7/1/2, CH_2Cl_2/CH_3OH/concentrated NH_4OH (lower layer)). Similarly prepared were **1d** and **1e**: bp 124–7 °C/ 0.7 Torr and 115–20 °C/0.6 Torr, respectively.

1,15-Diamino-8-azapentadecane (2b). Method A. To a freshly prepared suspension of Raney nickel (10 g) in 125 mL of benzene, from which the water was removed by azeotropic distillation,[12] was added 25 g (0.195 mol) of 1,7-diaminoheptane. The mixture was heated under reflux for 4 h, cooled, and decanted to remove the nickel. The nickel was washed with benzene. After removal of benzene from the combined reaction mixture and wash, the residue was distilled to give 2.6 g (11%) of **2b**: bp 135–140 °C/0.15 Torr; ^{1}H NMR (DMSO-d_6) δ 1.24 (m, 12 H), 1.33 (m, 8 H), 2.44 (t, 4 H), 2.52 (t, 4 H); MS (FAB, t-glyc) 244 (M + H); R_f 0.57 (2/2/1, CH_2Cl_2/CH_3OH/concentrated NH_4OH).

1,13- Diamino-7-azatridecane (2c). Method B. To a suspension of Raney nickel (2 g) in 25 mL of benzene, from which the water was removed as above, was added 5 g (0.032 mol) of **1c**. The mixture was heated under reflux for 7 h, at which time evolution of NH_3 had essentially ceased. Upon cooling, the mixture became gelatinous. Enough ethanol was added to render the product soluble. The nickel was removed by decantation and washed with a benzene/ethanol mixture. Removal of solvents from the combined reaction mixture and wash provided the crude N^1,N^{13}-diacetyl-1,13-diamino-7-azatridecane as an oil. To this oil was added 50 mL of 2 N HCl

and the mixture was heated under reflux for 2 h. After removal of HCl under vacuum, the residue was dissolved in a small amount of water. The solution was basified to pH 10 with solid NaOH and extracted with CH_2Cl_2 (5 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. Distillation of the resulting oil afforded 2.0 g (59%) of **2c**: bp 136–40 °C/0.22 Torr; ^{1}H NMR (DMSO-d_6) δ 1.26 (m, 8H), 1.33 (m, 8H), 2.43 (t, 4H), 2.49 (t, 4H); MS (FAB, t-glyc) 216 (M + H); R_f 0.52 (2/2/1, CH_2Cl_2/CH_3OH/concentrated NH_4OH). Similarly prepared were **2d** and **2e**.

1,15-Diguanidino-8-azapentadecane Sesquisulfate (3b). Method C. A mixture of 1.2 g (5 mmol) of **2b** and 1.53 g (11 mmol) of *S*-methylisothiuronium sulfate in 3 mL of water was heated under reflux for 1 h. To the cooled mixture was added 3 N H_2SO_4 (1.66 mL, 5 mmol), and the solvent was removed under vacuum. Recrystallization of the solid residue from 40% ethanol yielded 1.3 g (55%) of **3b**: mp 280–285 °C; ^{1}H NMR (D_2O) δ 1.23 (m, 12H), 1.44 (m, 4H), 1.52 (m, 4H), 2.87 (t, 4H), 3.03 (t, 4H); MS (FAB, t-glyc) 328 (M + H), 426 (M + H + H_2SO_4). Anal. ($C_{16}H_{37}N_7$·1.5 H_2SO_4) C, H, N, S. Similarly prepared were **3c**, **3d**, **3e**, **9**, **10a**, and **10b**.

1-Guanidino-17-amino-9-azaheptadecane Sulfate (4a). Method D. A mixture of 0.34 g (1.25 mmol) of 1,17-diamino-9-azaheptadecane[13] and 0.174 g (1.25 mmol) of *S*-methlisothiuronium sulfate in 1 mL of water was heated under reflux for 1 h. To the cooled mixture was added 3 N H_2SO_4 (0.83 mL, 2.5 mmol) and the solvent was removed under vacuum. The residue was flash chromatographed (2/2/1, CH_2Cl_2/CH_3OH/concentrated NH_4OH) to yield 0.114 g (22%) of **4a** as an amorphorous solid: R_f 0.23; ^{1}H NMR (D_2O) δ 1.22 (m, 16H), 1.45 (m, 8H), 2.65 (m, 6H), 3.03 (dt, 2H); MS (FAB, t-glyc) 314 (M + H), 412 (M + H + H_2SO_4). Anal. ($C_{17}H_{39}N_5$·H_2SO_4) C, H, N, S. Similarly prepared were **4b**, **4c**, **4d**, **11**, and **12**. However, these could not be satisfactorily separated from contaminating **3b**, **3c**, **3d**, **9**, and **11a**, respectively, by flash chromatography. Therefore, each was purified by ion exchange chromatography on a 1 × 16 cm column of Dowex 50 × 2 (H^+, 200–400 mesh) using for elution a logarithmic gradient generated from water in a 100 mL constant volume chamber by displacement with 2.5 N HCl.

1-Amino-9-(*p*-nitrobenzamido)-5-azanonane (5). Method E. A solution of 1.18 g (5 mmol) of (*p*-nitrobenzamido)-butyraldehyde[15] in 10 mL of methanol was added slowly to a solution of 1.76 g (20 mmol) of 1,4-diaminobutane in 30 mL of methanol followed by the addition of 1.5 g of $NaBH_3CN$ in small portions. The pH of the mixture was adjusted to 6 with methanolic HCl. After 18 h, excess reducing agent was destroyed by addition of concentrated HCl and the reaction mixture was taken to dryness under vacuum. The residue was dissolved in a small amount of water. After basifying to pH 10 with solid NaOH, the mixture was extracted with $CHCl_3$ (4 × 25 mL). The combined extracts were dried ($MgSO_4$), concentrated, and flash chromatographed (7/2/1, CH_2Cl_2/CH_3OH/concentrated NH_4OH (lower layer)) to yield 1 g (65%) of **5** as a yellow oil: R_f 0.23; ^{1}H NMR (DMSO-d_6) δ 1.35 (m, 4H), 1.43 (m, 2H), 1.53 (m, 2H), 2.46 (m, 6H), 3,27 (q, 2H), 8.06 (2s, 2H), 8.30 (2s, 2H); MS (CI, CH_4) 309 (M + H).

1-Guanidino-9-(*p*-nitrobenzamido)-5-azanonane Sulfate (6). Method F. To 0.31 g (1 mmol) of **5** in 2 mL of methanol was added 1 mL of triethylamine and a solution of 0.258 g (1.5 mmol) of *O*-methylisourea sulfate in 2 mL of water. After standing for 18 h, the reaction mixture was concentrated and flash chromatographed (2/2/1, CH_2Cl_2/CH_3OH/concentrated NH_4OH) to afford, after recrystallization from water/methanol, 0.22 g (49%) of **6**: R_f 0.21; mp 275 °C dec; ^{1}H NMR (D_2O) δ 1.62 (m, 8H), 2.95 (q, 4H), 3.08 (t, 2H), 3.33 (t, 2H), 7.80 (2s, 2H), 8.21 (2s, 2H); MS (FAB, t-glyc) 351 (M + H). Anal. ($C_{16}H_{26}N_6O_3$·H_2SO_4) C, H, N, S. Similarly prepared were **21** and **23**, except that the compounds were obtained directly from the reaction mixtures without purification by flash chromatography.

1-Guanidino-9-amino-5-azanonane Sesquisulfate (4e). Method G. A 0.09 g (0.2 mmol) portion of **6** in 2 mL of 6 N HCl was heated in a sealed tube at 110° for 18 h. After cooling, the precipitated material was removed by filtration and washed with water. The combined filtrate and washings, to which 3 N H_2SO_4 (0.066 mL, 0.2 mmol) was added, was evaporated. The crystalline product was recrystallized from water/ethanol to give 0.061 g (73%) of **4e**: mp 290 °C dec; ^{1}H NMR (D_2O) δ 1.62 (m, 8H), 2.93 (m, 6H), 3.09 (t, 2H); MS (FAB, t-glyc) 202 (M + H), 300 (M + H + H_2SO_4). Anal. ($C_9H_{23}N_5$·1.5H_2SO_4·4H_2O) C, H, N.

***N*^{1}-(Benzyloxycarbonyl)-1-amino-3-(aminooxy)propane Hydrochloride (15). Method H.** To a solution of 4.8 g (0.03 mol) of 1-amino-3-[[(1′-ethoxyethylidene)amino]oxy]-propane (**13**)[18] in 30 mL of water at ice bath temperature were added 5.61 g (0.033 mol) of benzyloxycarbonyl chloride and 30 mL of N NaOH portionwise over a period of 30 min with vigorous stirring. Stirring was continued for 1 h while the reaction mixture was allowed to warm to room temperature. The resulting oil was extracted with $CHCl_3$ (3 × 25 mL), and the combined extracts were washed with water (100 mL) and dried over Na_2SO_4. The oil that remained after removal of solvent was dissolved in 45 mL of 2-propanol and to this solution was added 3 mL of concentrated HCl. The precipitate formed was removed after 15 min and washed well with 2-propanol. Recrystallization from 95% ethyl alcohol afforded 5.75 g (74%) of **15**: mp 191 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 2H), 3.06 (q, 2H), 3.99 (t, 2H), 5.01 (S, 2H), 7.35 (S, 5H); MS (FAB, t-glyc) 225 (M + H). Anal. ($C_{11}H_{16}N_2O_3$·HCl) C, H, N, Cl.

***N*^{1}-(Benzyloxycarbonyl)-*N*^{3}-(3-azidopropyl)-1-amino-3-(aminooxy)propane (16). Method I.** A solution of 0.99 g (0.01 mol) of 3-azidopropanal[19] in 4 mL of methanol was added slowly to a solution of 2.6 g (0.01 mol) of **15** in 30 mL of methanol to which a tiny amount of solid methyl orange indicator had been added. The pH of the mixture was maintained at ~3 (yellow color) by the addition of 10 N NaOH. When the pH no longer changed, $NaBH_3CN$ (1.5 g) was added in small portions while the pH was maintained at ~3 (pink color) by the addition of 2 N HCl in methanol. About 30 min after the last addition of reducing agent the pH no longer changed. After 18 h, excess reducing agent was destroyed by addition of concentrated HCl and the solvents were evaporated. The residue was dissolved in 15 mL of water and the solution basified to pH 10 with solid NaOH and extracted with CH_2Cl_2 (5 × 25 mL). The combined extracts were dried ($MgSO_4$), concentrated and flash chromatographed (75/2/1, CH_2Cl_2/CH_3OH/concentrated NH_4OH(lower layer)) to afford 1.54 g (50%) of **16** as an oil: R_f 0.36; ^{1}H NMR (DMSO-d_6) δ 1.64 (m, 4H), 2.79 (q, 2H), 3.02 (q, 2H), 3.37 (t, 2H), 3.54 (t, 2H), 5.00 (s, 2H), 7.34 (s, 5H); MS (CI, CH_4) 308 (M + H).

***N*^{1}-(Benzyloxycarbonyl)-*N*^{3}-(3-aminopropyl)-1-amino-3-(aminooxy)propane (17). Method J.** To 0.615 g (2 mmol) of **16** in 10 mL of THF containing 0.04 mL (2.22 mmol) of water was added 0.558 g (2.24 mmol) of triphenylphosphine. After stirring for 18 h, the mixture was concentrated and flash chromatographed (12/2/4, CH_2Cl_2/CH_3OH/concentrated NH_4OH (lower layer)) to give 0.447 g (79%) of **17** as an oil: R_f 0.32; ^{1}H NMR (DMSO-d_6) δ 1.57 (m, 4H), 3.02 (m, 4H), 3.39 (t, 2H), 3.54 (t, 2H), 5.00 (s, 2H), 7.34 (s, 5H); MS (FAB, t-glyc) 282 (M + H).

***N*^{3}-(3-Aminopropyl)-1-amino-3-(aminooxy)propane Trihydrobromide (18). Method K.** A mixture of 0.422 g (1.5mmol) of **17** and 1 mL of 30% (wt %) hydrogen bromide in glacial acetic acid was stirred under anhydrous conditions. After 45 min, 10 mL of dry ether was added. The gum remaining after decanting the liquids solidified upon trituration with ether. The material was dissolved in 95% ethyl alchol and precipitated with ether to afford 0.345 g (59%) of **18** as a cream-colored solid: mp 142–4 °C dec; ^{1}H NMR (D_2O) δ 1.98 (m, 4H), 3.01 (t, 4H), 3.32 (dt, 2H), 4.09 (dt, H); MS (FAB, t-glyc) 148 (M + H). Anal. ($C_6H_{17}N_3O$·3HBr) C, H, N, Br.

***N*^{7}-Ethyl-7-aminoheptanenitrile Hydrobromide (19). Method L.** A mixture of 2.76 g (14.5 mmol) of 7-bromoheptanenitrile and 3 mL of a 70% aqueous solution of ethylamine in 10 mL of methanol was stirred at room temperature for 100 h. The reaction mixture was concentrated to give an oil which crystallized upon drying under vacuum. Recrystallization from a mixture of ethanol, chloroform, and benzene afforded 3.2 g (94%) of **19**: mp 80–85 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t

3H), 1.34 (m, 4H), 1.57 (m, 4H), 2.48 (t, 2H), 2.87 (m, 4H), 8.43 (s, 1H); MS (CI, NH_3) 155 (M + H).

N^1-Ethyl-1,7-diaminoheptane Dihydrochloride (20). Method M. A suspension of 1.65 g (10.7 mmol) of **19** in 30 mL of THF was added slowly with stirring to 70 mL of 1 M borane in THF under nitrogen at room temperature. The mixture was brought to reflux and maintained there for 2.5 h. After cooling, 50 mL of 6 N HCl was added slowly and the bulk of the THF was removed. Following addition of 10 mL more of 6 N HCl, the mixture was heated at reflux for 2 h. After cooling, the solid was removed by filteration and the filtrate was evaporated. The resulting solid was washed with 60 mL of acetone to yield 1.25 g (51%) of **20**: mp 193–5 °C; ^{1}H NMR (D_2O) δ 1.12 (t, 3H), 1.24 (m, 6H), 1.52 (m, 6H), 2.81~2.97 (m, 6H); MS (FAB, t-glyc) 159 (M + H).

N^1-(3-Cyanoethyl)-1,7-diaminoheptane Dihydrochloride (22). Method N. To a solution of 1.3 g (10mmol) of 1,7-diaminoheptane in 5 mL of ethanol was added, dropwise with stirring at 0 °C, 0.663 mL (10 mmol) of acrylonitrile. After standing overnight, the reaction mixture was concentrated. The oily residue was dissolved in a small amount of water and applied to a 2.5 × 12 cm column of CM-Sephadex G-25 (NH_4^+ form, Pharmacia). Elution was conducted using a linear gradient of 400 mL from 0 to 2.22 M in NH_4OH. The fractions eluted between ~1 and 1.8M NH_4OH which contained the desired product were combined and evaporated. All traces of NH_3 were removed under high vacuum and a solution of the residue in water was adjusted to pH ~2 with 6 N HCl. Evaporation followed by recrystallization of the product from methanol/ether afforded 1.2 g (49%) of **22**: mp 193–4 °C; MS (FAB, t-glyc) 184 (M + H).

***In Vitro* Enzyme Assays.** Rat testes deoxyhypusine synthase was prepared essentially as outlined.[2] The enzyme was assayed by a published procedure.[36] The assay mixtures included, in a total volume of 0.02 mL, 0.2 M glycine·NaOH buffer, 1 μM eIF-5A precursor protein (prepared by overexpression of a human eIF-5A cDNA in *Escherichia coli*), 1 mM dithiothreitol, 0.5 mM NAD^+, 2.4 μM [1,8-^{3}H]spermidine, 25 μg of bovine serum albumin, and 3–15 units of enzyme. Incubations were conducted at pH 9.5 and 37 °C for 1 h. Labeled deoxhypusine was measured after its ion exchange chromatographic separation from acid hydrolysates of the precipitated protein fractions. Test compounds were dissolved in water and their solutions, adjusted to pH ~ 9, were added to assay mixtures prior to addition of enzyme. Compounds were tested for inhibition at concentrations from 0.1 to 1000 μM. IC_{50} values (50% inhibitory concentrations) were calculated by fitting the data to the equation, $IC_{50} = I[(100 - x)/x]$, where I = inhibitor concentration and x = the percentage inhibition,[11] using the KaleidaGraph Program.

Cell Studies. Methods for culture of Chinese hamster ovary (CHO) cells and for the determination of hypusine and protein synthesis in these cells are given elsewhere.[6] Spermidine uptake was measured as the total amount of radioactivity in cells after their growth for 18 h in the presence of 5 μCi/mL of the ^{3}H-labeled polyamine. Values for hypusine formed, spermidine uptake, and new protein formed at 18 h after addition of labeled compound are recorded in Tables 2 and 3 as percentage of the corresponding values in untreated cells, i.e., percentage of control. The level of protein in control CHO cells increased ~16-fold over a period of 72 h. The relative amount of protein in treated cells as measured by the bicinchoninic acid method[37] at 72 h was taken as a crude and simple estimate of growth inhibition. The symbols recorded under cell growth inhibition in Tables 2 and 3 define the range of compound within which protein is reduced to one-half the level of that of the control cells: ++++, <0.5 μM; +++, 0.5–1.0 μM; ++, >1.0–10 μM; +, >10–100 μM; −, >100 μM.

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