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Branched-chain and Unsaturated 1,7-Diaminoheptane Derivatives as Deoxyhypusine Synthase Inhibitors

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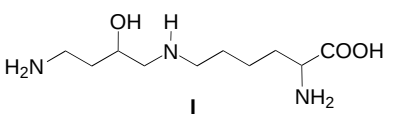
Abstract—Deoxyhypusine synthase catalyzes the first step in the posttranslational biosynthesis of the unusual amino acid hypusine [N^{ε}-(4-amino-2-hydroxybutyl)lysine] in eukaryotic translation initiation factor 5A (eIF-5A). eIF-5A and its single hypusine residue are essential for cell proliferation. Two series of 1,7-diaminoheptane derivatives were prepared and tested as inhibitors of human deoxyhypusine synthase. These include branched-chain saturated derivatives and both branched- and straight-chain unsaturated derivatives providing size and positional variation in branching and different torsional constraints. Of the branched-chain compounds, 7-amino-1-guanidinooctane (**39**) proved to be the most potent inhibitor in vitro (IC_{50}, 34 nM), while 1,7-diamino-*trans*-hept-3-ene (**20a**) displayed the greatest inhibition (IC_{50}, 0.7 μM) among the unsaturated compounds. Compound **39** also provided effective inhibition of hypusine production in Chinese hamster ovary cells in culture. Considerations of the in vitro inhibition data reported here, along with earlier findings, allowed some speculation concerning the conformation of the substrate spermidine during its productive interaction at the active site of deoxyhypusine synthase. Published by Elsevier Science Ltd.

Introduction

The amino acid hypusine (**I**) [N^{ε}-(4-amino-2-hydroxybutyl)lysine], which is unique to eukaryotic translation initiation factor 5A (eIF-5A), is formed post-translationally from the polyamine spermidine (**II**).[1,2] Transfer of the butylamine moiety of this polyamine to the ε-amino group of one particular lysine residue of the eIF-5A precursor (Lys^{50} in human eIF-5A precursor) to produce deoxyhypusine (**III**) [N^{ε}-(4-aminobutyl)lysine] is the first step in hypusine biosynthesis and is catalyzed by the enzyme deoxyhypusine synthase.[1,2] eIF-5A and the enzymes that catalyze its hypusine modification are abundant in all eukaryotic cells. Despite indication that eIF-5A, unlike other initiation factors, is not required for global protein synthesis,[3] there is mounting evidence that this protein and the hypusine modification, which marks its maturation, are vital for proliferation in all eukaryotic species.[2] Yeast cells are not viable if the two genes for eIF-5A are inactivated[4] and disruption of the deoxyhypusine synthase gene in these cells results in cessation of growth.[5] Effective inhibitors of deoxyhypusine synthase prevent the production of hypusine in eIF-5A in cells[6–8] and have been shown to arrest multiplication of mammalian cells, both normal and cancer, in culture.[7–9] Hence it was concluded that compounds targeted specifically to inhibit deoxyhypusine synthase could prove useful as novel antiproliferative agents. In consideration of recent reports that eIF-5A may act in HIV-1 replication as a Rev transactivator protein[10] and may be implicated as a cofactor for Rex function in HTLV replication,[11] compounds that inhibit deoxyhypusine synthase may also bear potential as anti-HIV-1 and anti-HTLV agents.



I

Key words: Deoxyhypusine synthase inhibitors; branched chain 1,7-diaminoheptanes; unsaturated derivatives of 1,7-diaminoheptane; diamines; diguanidines

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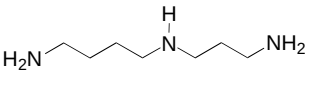
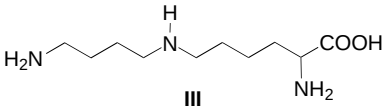


II



III

In our search for inhibitors of deoxyhypusine synthase, attention was focused toward compounds structurally related to the substrate spermidine.[6,7] The monoguanylated diamine, 1-amino-7-guanidinoheptane (GC_7), was found to display efficient inhibition in vitro through competition with spermidine for binding to enzyme.[6] In Chinese hamster ovary cells, GC_7 was a remarkable inhibitor causing complete inhibition of hypusine biosynthesis and curtailment of growth at concentrations as low as 1 μM in the medium.[8] Growth arrest without significant reduction in cellular spermidine or eIF-5A precursor underlines the critical role of hypusine in cell proliferation.[8] The efficient entry of GC_7 into cells by way of the polyamine transport system[8] is an additional advantage arising from its structural relationship to spermidine.

Besides their value as exogenous regulators of cellular function, deoxyhypusine synthase inhibitors that act as a consequence of their structural similarity to spermidine offer promise for further understanding of enzyme mechanism. Early findings with compounds similar in structure to spermidine led to speculation on features of the spermidine-binding site of the enzyme.[6] The crystal structure of a complex of deoxyhypusine synthase with bound NAD[12] recently determined by X-ray analysis has opened the way to a comprehensive computer-assisted study of this site and of the enzyme's catalytic mechanism. In conjunction with this study, as well as in a continuing effort to find more potent compounds to test as antiproliferative drugs, and because of the simple structure and potent inhibitory activity of GC_7, we were prompted to synthesize and test a number of modified 1,7-diaminoheptanes. Here we describe the work leading to the identification of 7-amino-1-guanidinooctane (**39**) as a new lead compound.

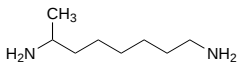
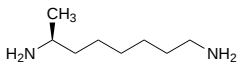
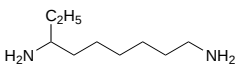
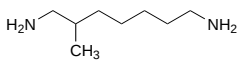
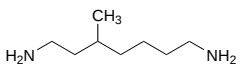
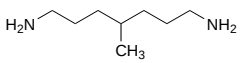
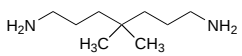
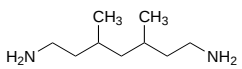
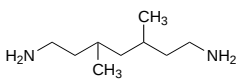
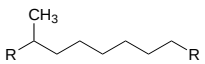
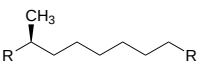
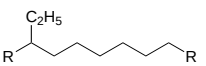
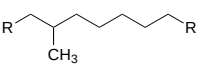
Chemistry

The saturated branched-chain diamines and their guanylated derivatives of Table 1, with the exception of those with a branch on the same carbon atom as one of the basic groups, were prepared as outlined in Scheme 1. Conversion of the known branched-chain pimelonitriles **1a**,[13] **1b**,[14] **1d**,[15] and **1e** (racemic and *meso*)[16] and **1c** (bp 115–118 °C/0.5 Torr, prepared in 80% yield from 3-methyl-1,5-dibromopentane[17] by treatment with NaCN in DMSO[18]) to the diamines **2a–e** was achieved by reduction with borane/THF. Reaction of **2c** and **2d** with equivalent amounts of *S*-methylisothiouronium sulfate yielded monoguanylated diamines **4c** and **4d**, respectively; treatment of **2a–e** with excess amounts of the reagent gave the diguanidines **3a–e**.

Scheme 2 outlines the preparation of the diamines and diguanidines of Table 2 with unsaturated bonds at the 2 position. 7-(2-Tetrahydropyranyloxy)-2-heptyn-1-ol (**5**) was prepared in 85% yield by reaction of the Grignard derivative of 1-(2-tetrahydropyranyloxy)-5-hexyne[19] with formaldehyde in a 1:1 mixture of THF and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU). A less satisfactory yield of **5** (50–60%) was obtained by Ferdinandi and Just[20] using a different procedure. Removal of the THP protecting group of **5** afforded diol **6**. Our overall yield of **6** (65%) is superior to that (38%) reported by Crombie et al.[21] for **6** prepared directly from 5-hexyn-1-ol. Reaction of **6** with mesyl chloride in methylene chloride provided **7**, which was converted to diazide **8** by addition of NaN_3 in DMSO. Selective reduction of the azido groups of **8** to amino groups without effect on the alkyne functionality was accomplished with triphenylphosphine in aqueous THF[22] to yield **9**. Transformation of **9** to *trans* diaminoalkene **10** was conducted by reduction with $LiAlH_4$ in THF and conversion of **9** to *cis* double-bonded amine **11** by hydrogenation using Lindlar catalyst (Pd-PbO-$CaCO_3$). Guanylation of **9**, **10**, and **11** with *O*-methylisourea hydrogen sulfate afforded the unsaturated guanidines **12**, **13**, and **14**, respectively.

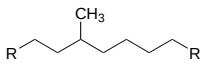
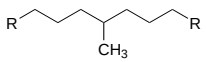
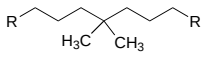
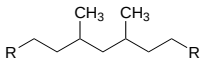
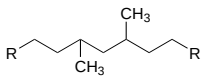
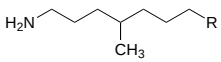
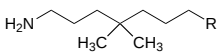
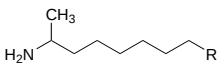
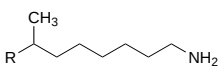
The acetylenic and olefinic diamines and diguanidines of Table 2, in which unsaturation is at the 3 or 4 position, were accessible through ring opening of oxiranes with the lithio derivative of 1-(2-tetrahydropyranyloxy)-4-pentyne[23] in mixtures of THF and a dipolar aprotic cosolvent. Addition of ethylene oxide to a 50% DMPU/THF solution of the pentynyllithium yielded the achiral 7-(2-tetrahydropyranyloxy)-3-heptyn-1-ol (**15a**) in 75% yield. A less satisfactory yield of **15a** (45%) was reported by Grob and Moesch[24] with the use of ethylene oxide and the Grignard derivative of 1-(2-tetrahydropyranyloxy)-4-pentyne. Using the racemic forms of propylene oxide and 1,2-epoxybutane in 13% hexamethylphosphoramide (HMPA)/THF solutions yields of 68% and 40% were realized for the racemic octynol and nonynol derivatives, **15b** and **15c**, respectively. In order to define the specificity of enzyme-inhibitor interaction, which will be discussed later, we employed optically pure (*S*)-(-)-propylene oxide to prepare the (*S*)-enantiomer of **15b**, (*S*)-**15b**, in 65% yield as a

Table 1. Physical and deoxyhypusine synthase inhibitory properties of branched-chain derivatives of 1,7-diaminoheptane

Compound	Structure[a]	Yield % (methods)[b]	mp °C	Recryst.[c] solvent	Formula	Anal.[d]	IC_{50}, μM
25b		90[e](O)	163–4	A	$C_8H_{20}N_2 \cdot 2HCl$	C,H,N,Cl	2.5 ± 0.2
(*S*)-**25b**		87[f](O)	197–8	A	$C_8H_{20}N_2 \cdot 2HCl$	C,H,N,Cl	13.2 ± 1.0
25c		74[g](O)	148	A	$C_9H_{22}N_2 \cdot 2HCl$	C,H,N,Cl	431 ± 137
2a		44(A)	150	A	$C_8H_{20}N_2 \cdot 2HCl$	C,H,N,Cl	69.8 ± 5.7
2b		60(A)	225	A	$C_8H_{20}N_2 \cdot 2HCl$	C,H,N,Cl	46.9 ± 9.6
2c		55(A)	248	A	$C_8H_{20}N_2 \cdot 2HCl$	C,H,N,Cl	>1000
2d		66(A)	236	A	$C_9H_{22}N_2 \cdot 2HCl$	C,H,N,Cl	>1000
2e		65(A)	233–4	B	$C_9H_{22}N_2 \cdot 2HCl$	C,H,N,Cl	>1000
meso-**2e**		68(A)	196	A	$C_9H_{22}N_2 \cdot 2HCl$	C,H,N,Cl	>1000
26b		40(K)	284–5	C	$C_{10}H_{24}N_6 \cdot H_2SO_4$	C,H,N,S	0.41 ± 0.08
(*S*)-**26b**		33(K)	glass		$C_{10}H_{24}N_6 \cdot H_2SO_4$	C,H,N,S	1.3 ± 0.1
26c		40(K)	240–4 dec	C	$C_{11}H_{26}N_6 \cdot H_2SO_4$	C,H,N,S	1.1 ± 0.2
3a		45(B)	295 dec	D	$C_{10}H_{24}N_6 \cdot H_2SO_4$	C,H,N,S	48.5 ± 3.1

(continued)

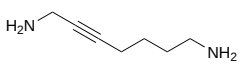
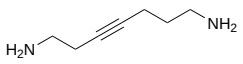
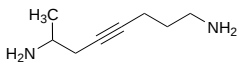
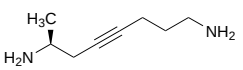
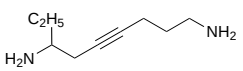
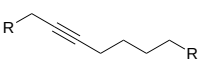
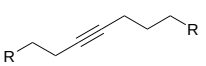
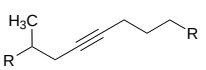
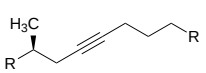
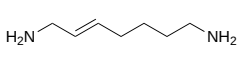
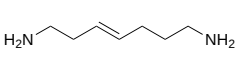
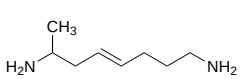
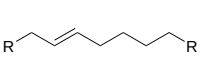
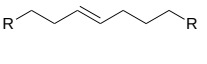
Table 1—*contd*

Compound	Structure[a]	Yield % (methods)[b]	mp °C	Recryst.[c] solvent	Formula	Anal.[d]	IC_{50}, μM
3b		50(B)	307–8 dec	C	$C_{10}H_{24}N_6 \cdot H_2SO_4$	C,H,N,S,	24.6 ± 1.9
3c		44(B)	294 dec	C	$C_{10}H_{24}N_6 \cdot H_2SO_4$	C,H,N,S	723 ± 160
3d		40(B)	305 dec	C	$C_{11}H_{26}N_6 \cdot H_2SO_4$	C,H,N,S	706 ± 126
3e		25(B)	295 dec	E	$C_{11}H_{26}N_6 \cdot H_2SO_4$	C,H,N,S	>1000
meso-**3e**		38(B)	272–4 dec	C	$C_{11}H_{26}N_6 \cdot H_2SO_4$	C,H,N,S	>1000
4c		43(C)	140		$C_9H_{22}N_4 \cdot 2HCl$[h]	C,H,N,Cl	17.2 ± 3.0
4d		27(C)	oil		$C_{10}H_{24}N_4 \cdot 2HCl$	C,H,N,Cl	17.8 ± 1.0
39		8[i]	269–70	C	$C_9H_{22}N_4 \cdot H_2SO_4$	C,H,N,S	0.034 ± 0.006
40		13[i]	glass		$C_9H_{22}N_4 \cdot 2HCl$[j]	C,H,N,Cl	5.8 ± 1.2

[a]R = -NHC(=NH)NH_2.
[b]The methods of preparation designated by the letters refer to general methods given in the Experimental.
[c]A, ethanol–ether; B, abs. Ethanol; C, water–ethanol; D, water-methanol; E, water.
[d]Analytical results were within 0.4% of the theoretical value.
[e]Yield from **19b** (Table 2).
[f]Yield from (*S*)-**19b** (Table 2).
[g]Yield from **19c** (Table 2).
[h]Monohydrate.
[i]Overall yield from **15b** (Experimental).

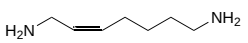
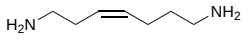
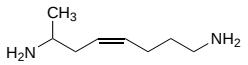
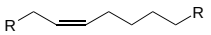
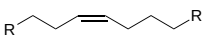
precursor of (*S*)-**19b**, (*S*)-**22b**, (*S*)-**25b**, and (*S*)-**26b**. (2*R*)-8-(2-Tetrahydropyranyloxy)-4-octyn-2-ol, the (*R*)-enantiomer of **15b**, was prepared by Hillis and Ronald[25] in 69% yield by this procedure. Our $[\alpha]_D$ of +9.9° for (*S*)-**15b** is consistent with that of −10° reported for the (*R*)-form of compound **15b**.[25] The partially blocked alkyne diols **15a–c** were converted to the diaminoalkynes **19a–c** by employing the same series of procedures described above in Scheme 2 for transformation of alcohol **5** to amine **9**. Refluxing of **19a** with $LiAlH_4$ in

Table 2. Physical and deoxyhypusine synthase inhibitory properties of unsaturated derivatives of 1,7-diaminoheptane

Compound	Structure[a]	Yield % (methods)[b]	mp °C	Recryst.[c] solvent	Formula	Anal.[d]	IC_{50}, μM
9		22[e] (D,E,F,G,H)	204–5	A	$C_7H_{14}N_2 \cdot 2HCl$	C,H,N,Cl	621 ± 14
19a		31[f](L,E,F,G,H)	192	A	$C_7H_{14}N_2 \cdot 2HCl$	C,H,N,Cl	1 ± 0.05
19b		23[f] (L,E,F,G,H)	126–7	A	$C_8H_{16}N_2 \cdot 2HCl$	C,H,N,Cl	21 ± 3
(*S*)-**19b**		22[f] (L,E,F,G,H)	156	A	$C_8H_{16}N_2 \cdot 2HCl$	C,H,N,Cl	12.3 ± 0.6
19c		9[f] (L,E,F,G,H)	150	A	$C_9H_{18}N_2 \cdot 2HCl$	C,H,N,Cl	200 ± 44
12		40 (K)	268 dec	B	$C_9H_{18}N_6 \cdot H_2SO_4$	C,H,N,S	248 ± 14
22a		41 (K)	280 dec	B	$C_9H_{18}N_6 \cdot H_2SO_4$	C,H,N,S	5.6 ± 0.4
22b		83 (N)	glass		$C_{10}H_{20}N_6 \cdot 2HCl$[g]	C,H,N,Cl	31 ± 1
(*S*)-**22b**		66 (N)	glass		$C_{10}H_{20}N_6 \cdot 2HCl$[g]	C,H,N,Cl	24 ± 2
10		30 (I)	243	A	$C_7H_{16}N_2 \cdot 2HCl$	C,H,N,Cl	41.9 ± 2.4
20a		22 (I)	238	A	$C_7H_{16}N_2 \cdot 2HCl$	C,H,N,Cl	0.73 ± 0.06
20b		24 (M)	oil		$C_8H_{18}N_2 \cdot 2HCl$	C,H,N,Cl	8 ± 0.5
13		54 (K)	325 dec	B	$C_9H_{20}N_6 \cdot H_2SO_4$	C,H,N,S	25 ± 1.6
23		77(K)	> 350	B	$C_9H_{20}N_6 \cdot H_2SO_4$	C,H,N,S	6.4 ± 0.4

(continued)

Table 2—*contd*

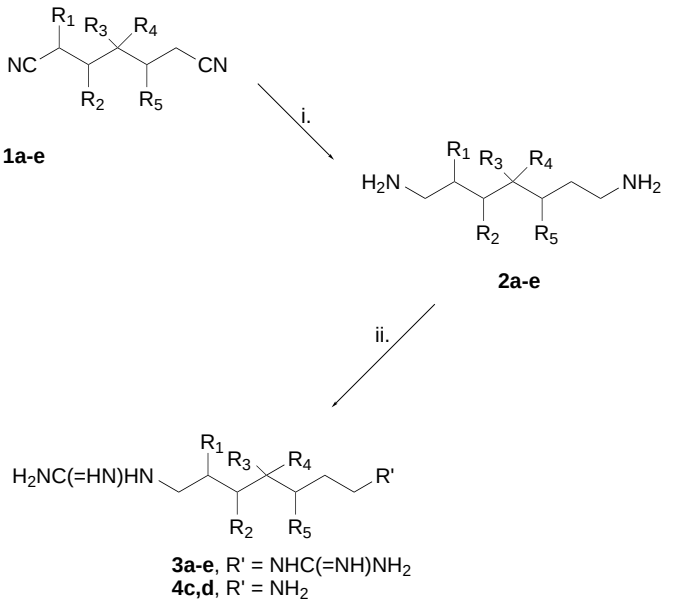
Compound	Structure[a]	Yield % (methods)[b]	mp °C	Recryst solvent[c]	Formula	Anal.[d]	IC_{50}, mM
11		72(J)	190–2	C	$C_7H_{16}N_2 \cdot 2HCl$	C,H,N,Cl	16.8 ± 2.5
21a		73 (J)	204–6	C	$C_7H_{16}N_2 \cdot 2HCl$	C,H,N,Cl	20.8 ± 3.0
21b		50(J)	128–32	A	$C_8H_{18}N_2 \cdot 2HCl$	C,H,N,Cl	107 ± 21
14		74(K)	315 dec	B	$C_9H_{20}N_6 \cdot H_2SO_4$	C,H,N,S	37 ± 3
24		82 (K)	315 dec	B	$C_9H_{20}N_6 \cdot H_2SO_4$	C,H,N,S	23 ± 3

[a]R = -NHC(=NH)NH_2.
[b]The methods of preparation designated by the letters refer to general methods given in Experimental.
[c]A, ethanol–ether; B, water–ethanol; C, methanol–ether.
[d]Analytical results were within 0.4% of the theoretical value.
[e]Overall yield from THP-hexyne.
[f]Overall yield from THP-pentyne.
[g]Hemihydrate.

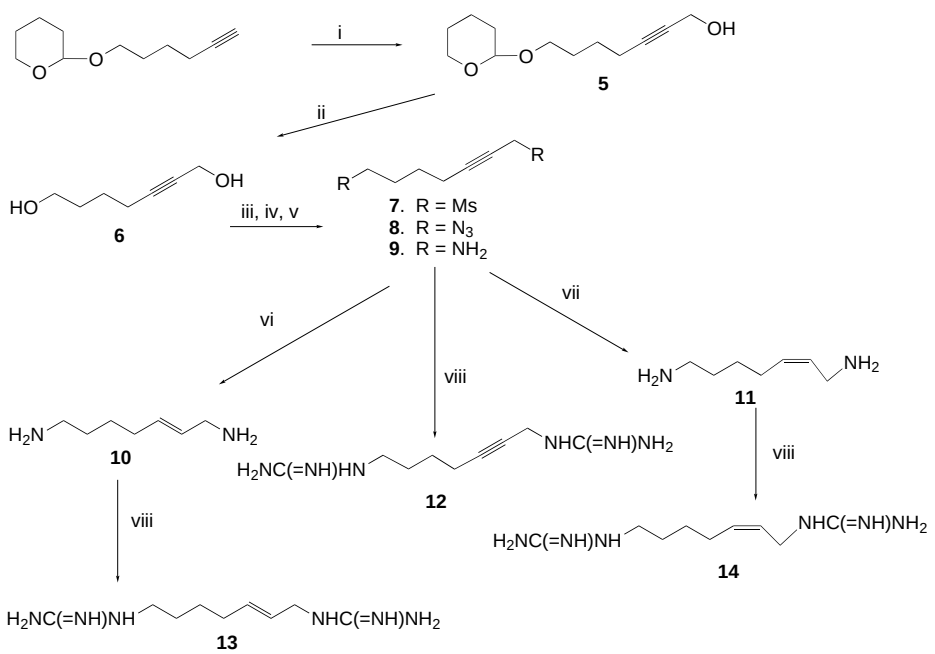
THF provided a moderate yield of *trans* diaminoalkene **20a**, whereas no **20b** was produced even upon prolonged treatment of **19b** under these conditions. Use of the reducing agent in diglyme at reflux temperature gave the target compound **20b** in acceptable yield. Hydrogenation of **19a** and **19b** using Lindlar catalyst yielded the *cis* double-bonded diamines **21a** and **21b**, respectively. Reaction of the unsaturated 1,7-diaminoheptanes **19a**, **20a**, and **21a** with *O*-methylisourea hydrogen sulfate afforded diguanidines **22a**, **23**, and **24**, whereas use of this reagent with 1,7-diaminooctynes **19b** and (*S*)-**19b** gave only low yields of impure products; their treatment with the more reactive guanylating reagent 1*H*-pyrazole-1-carboxamidine. HCl in DMF containing 2 equivalents of diisopropylethylamine afforded **22b** and (*S*)-**22b** in good yields (83 and 66%, respectively).

Scheme 4 describes the preparation of the 8- and 9-carbon saturated 1,7-diamines and diguanidines of Table 1. Catalytic hydrogenation of the diaminoalkynes **19b** and **19c** using Adams catalyst (PtO_2) afforded 1,7-diaminooctane (**25b**) and 1,7-diaminononane (**25c**), respectively, through complete reduction of the triple bonds. Guanylation of these diamines with *O*-methylisourea hydrogen sulfate provided the guanidine compounds **26b** and **26c**.

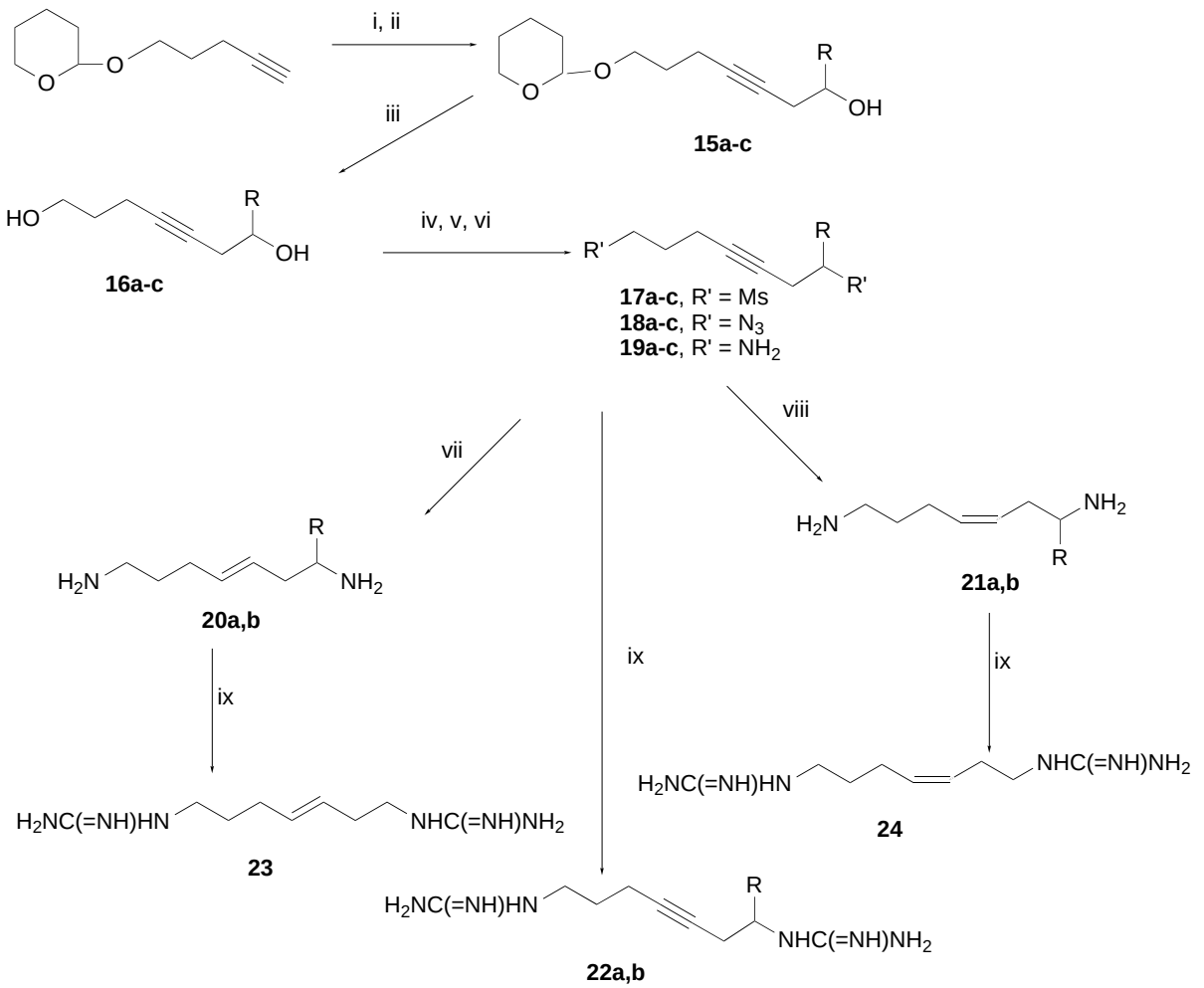
The racemic form of 7-amino-1-guanidinooctane (**39**) and that of 1-amino-7-guanidinooctane (**40**) (Table 1) were prepared as outlined in Scheme 5 starting with racemic 8-(2-tetrahydropyranyloxy)-4-octyn-2-ol (**15b**). The key intermediate, 1-amino-7-*tert*-butoxycarbonyl (*t*-Boc)aminooctane (**34**), was obtained through transformation of **15b** to 1-(2-tetrahydropyranyloxy)-7-aminooctane (**29**) by a series of reactions involving formation of mesylate **27**, its conversion to azide **28** and simultaneous reduction of the multiple bond and production of amine by catalytic hydrogenation. After protection of the amino group of **29** by reaction with di-*tert*-butyldicarbonate to form **30**, selective removal of the THP blocking group to produce **31** was followed by another series of reactions involving conversion of the alcohol to the mesylate **32**, its transformation to the azide **33** and finally catalytic hydrogenation to yield the mono blocked diamine **34**. Conversion of its free amino group to a guanidino group to form **35** by the use of *O*-methylisourea hydrogen sulfate was followed by removal of the *t*-Boc group with TFA yielding one of the target compounds **39**. Alternative amino group blocking allowed selective modification of the other amino group and hence synthesis of the second target compound. Introduction of the benzyloxycarbonyl group on the free amino group of **34** upon its treatment with



Scheme 1. (i) BH_3, THF, △; (ii) $[CH_3SC(=NH)NH_2]_2{\cdot}H_2SO_4$, H_2O, △.



Scheme 2. (i) C_2H_5MgBr, THF, △, DMPU, HCHO; (ii) PPTS, EtOH; (iii) CH_3SO_2Cl, TEA, CH_2Cl_2; (iv) NaN_3, DMSO; (v) Ph_3P, THF, H_2O; (vi) $LiAlH_4$, THF, △; (vii) H_2, Pd (Lindlar Catalyst), EtOH; (viii) $CH_3OC(=NH)NH_2{\cdot}H_2SO_4$, MeOH, TEA.

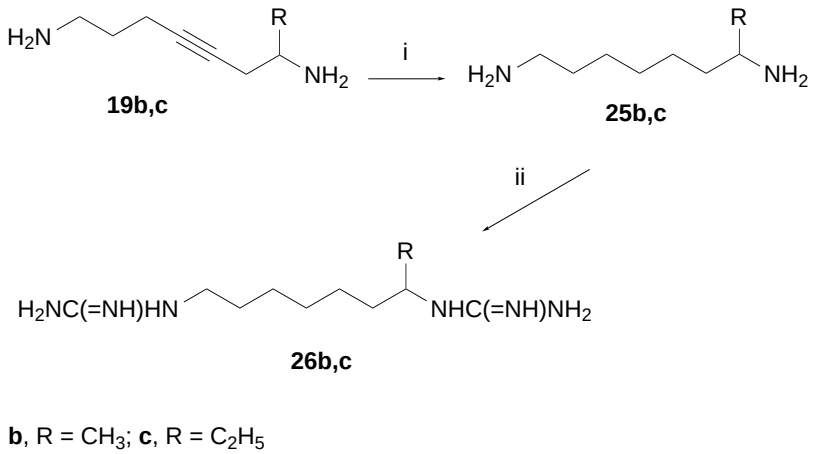


Scheme 3. (i) *n*BuLi, THF; (ii) (a) DMPU, ethylene oxide; (b) HMPA, propylene oxide, (c) HMPA, 1,2-epoxybutane; (iii) PPTS, EtOH; (iv) CH_3SO_2Cl, TEA, CH_2Cl_2; (v) NaN_3, DSMO; (vi) Ph_3P, THF, H_2O; (vii) (a) $LiAlH_4$, THF, (b) $LiAlH_4$, diglyme; (viii) H_2, Pd (Lindlar Catalyst), EtOH; (ix) (a) $CH_3OC(=NH)NH_2 \cdot H_2SO_4$, MeOH, TEA, (b) 1*H*-pyrazole-C(=NH)NH_2, DIEA, DMF.

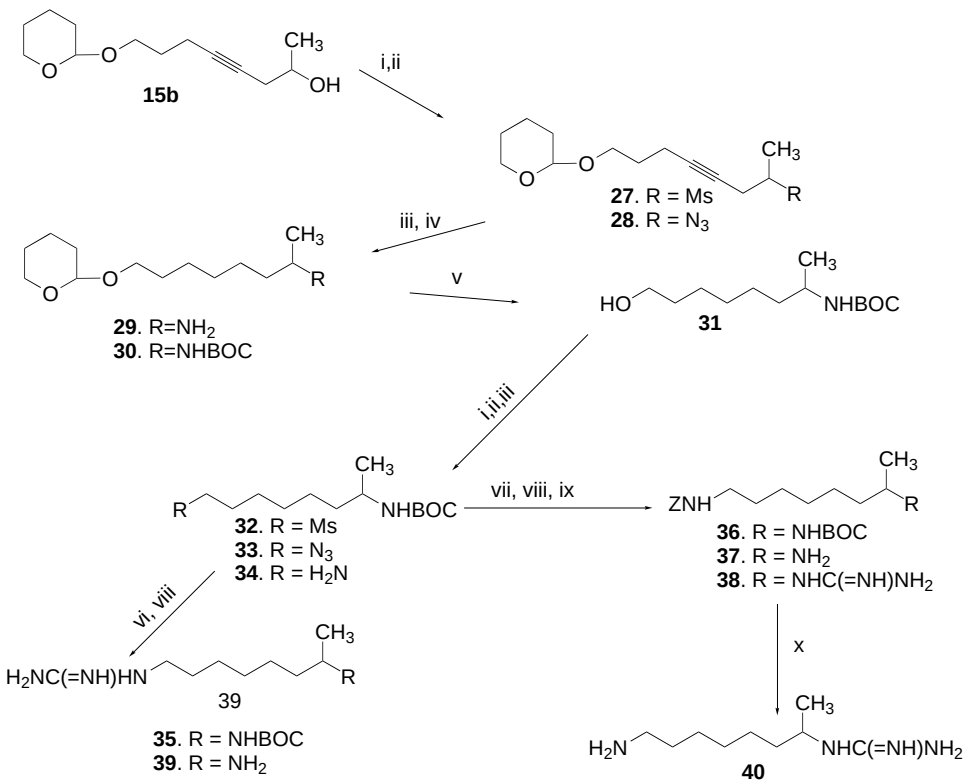
N-(benzyloxycarbonyloxy)succinimide to give **36** was followed by selective removal of the *t*-Boc blocking group with TFA affording **37**. Guanylation of this alternatively blocked diamine with 1*H*-pyrazole-1-carboxamidine to produce **38** and removal of the benzyloxycarbonyl group by catalytic hydrogenation provided **40**.

Results and Discussion

Table 1 lists the properties of the branched-chain saturated 1,7-diaminoheptane derivatives and Table 2 those of both straight- and branched-chain unsaturated 1,7-diaminoheptane derivatives prepared and evaluated as inhibitors of deoxyhypusine synthase. For comparison, Table 3 gives the inhibitor properties of 1,7-diaminoheptane (C_7) and its *mono* and *bis* guanylated derivatives, GC_7 and GC_7G, respectively, determined in parallel with those of the compounds of Tables 1 and 2. The competitive-type inhibition exerted by C_7, GC_7,and GC_7G against spermidine, together with their similarities in structure to this polyamine substrate, led us to the earlier conclusion that binding of each of these compounds occurs at the same site on the enzyme.[6,7] Because the present compounds also bear strong structural resemblance to spermidine, it is assumed that they too interact with deoxyhypusine synthase at the site normally occupied by this substrate.



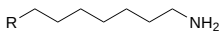
Scheme 4. (i) H_2, PtO_2 (Adams Catalyst), EtOH; (ii) $CH_3OC(=NH)NH_2 \cdot H_2SO_4$, MeOH, TEA.



Scheme 5. (i) CH_3SO_2Cl, CH_2Cl_2, TEA; (ii) NaN_3, DMSO; (iii) H_2, PtO_2 (Adams Catalyst), EtOH; (iv) $(BOC)_2O$, DMF; (v) PPTS, EtOH; (vi) $CH_3OC(=NH)NH_2 \cdot H_2SO_4$, MeOH, TEA; (vii) ZOSu, $CHCl_3$; (viii) TFA, 0 °C; (ix) 1*H*-pyrazole-C(=NH)NH_2, DIEA, DMF; (x) H_2, Pd black, MeOH.

Certain branched-chain and unsaturated derivatives of spermidine, specifically 1-methylspermidine [*N*-(3-aminobutyl)-1,4-diaminobutane],[26] *N*-(3-aminopropyl)-1,4-diamino-*cis*-2-butene,[27] and *N*-(3-aminopropyl)-1,4-diamino-*trans*-2-butene,[27] were reported to function as deoxyhypusine synthase substrates. Others, namely 1,1-dimethylspermidine[2] and *N*-(3-aminopropyl)-1,4-diamino-2-butyne,[27] do not act as substrates, but were shown to display some degree of inhibition. Hence, the compounds listed in Tables 1 and 2 were designed with

Table 3. Deoxyhypusine synthase inhibitory properties of 1,7-diaminoheptane and its guanylated derivatives

Compound	Structure[a]	IC_{50}, μM
C_7		4.5 ± 0.5
GC_7G		3.6 ± 0.2
GC_7		0.05 ± 0.0006

[a]R = $NHC(=NH)NH_2$.

the knowledge that the enzyme possesses tolerance at its spermidine binding site for a degree of carbon chain branching and unsaturation.

Comparison of the inhibitory properties of the compounds of Table 1 with those of C_7 and its guanyl derivatives (Table 3) reveals that diamines **2a**, **2b**, and **2c** with methyl branches at positions 2, 3, and 4, respectively, of the 1,7-diaminoheptane carbon chain, **2d** with 2 methyl groups at the 4 position and **2e** and *meso*-**2e** with one methyl group at position 3 and one at position 5 display either no significant degree of inhibition or much poorer inhibition than unbranched 1,7-diaminoheptane (C_7). The same relationship is seen with diguanidines **3a–e** versus 1,7-diguanidinoheptane (GC_7G), although in each case, except **3e** and *meso*-**3e**, the guanidines provide better inhibition than their amine counterparts. On the other hand, 1,7-diaminooctane (**25b**), the methyl group of which is on the same carbon as one of its amino groups, is a better inhibitor than the parent compound, C_7. The same relationship is seen with diguanidines **26b** and GC_7G. This, together with the fact that the racemic compound, **25b**, and the enantiomer, (*S*)-**25b**, differ significantly in inhibitory properties, as do **26b** and (*S*)-**26b**, is evidence of a specific interaction of the methyl branch at this position with the active site of the enzyme. The very restricted nature of this interaction is defined by the poor affinity of **25c**, which contains an ethyl group in place of the methyl group of **25b**. Thus, that region of the enzyme's active site that effectively accommodates the methyl branch of **25b** is of insufficient size to allow ready access of an ethyl group. This exclusion of the ethyl group is relaxed, however, when it is oriented at the same relative position in diguanidine **26c**; this compound provides excellent inhibition. The size of the guanidinium group is greater than that of the ammonium group and its charge is more delocalized.[28] For this reason the guanidinium group binds less strongly to anionic ligands. However, its ability to form pairs of zwitterionic hydrogen bonds provides it additional binding strength[28] so that its association with enzyme may, indeed, be as strong as, or stronger than, that of the ammonium group. Thus, the dramatic increase in the effectiveness of inhibition experienced with diguanidine **26c** over diamine **25c** may be explained by a change in carbon chain alignment caused by guanidinium group binding. The adjustment in chain position resulting from charge delocalization and/or size of the two basic centers could allow ready accommodation of the ethyl group of **26c** at that region of the enzyme that accepts the methyl group of diamine **25b** even though the ethyl group might be only partially positioned within this region. Basic center binding could, in a similar way, cause the increases in inhibition seen with the other branched chain diguanidines over their parent diamines (Table 1), although in cases other than **25b** versus **26b** it is unlikely that there are specific sites for interaction of inhibitor methyl branches with enzyme. The comparable degrees of inhibition given by the unbranched compounds C_7 and GC_7G are in accordance with this suggestion and indicate that the binding force exerted by guanidinium groups at both terminal positions in heptane derivatives approximates that exerted by ammonium groups at these positions.

In earlier studies with straight chain inhibitors of deoxyhypusine synthase it was found that with those in which separation of the two required terminal basic centers was most favorable for inhibition, that is, by an unbranched 7- or 8-carbon chain or by a straight 7-member chain in which the central carbon atom was replaced by O, S, or N, the combination of one guanidinium group and one ammonium group provided by far the most potent inhibition.[6,7] It was concluded on the basis of this finding that, even though both sites on the enzyme that normally bind the primary amino groups of spermidine can accommodate guanidino groups, one of these sites favors a guanidino groups over an amino group, while the other strongly prefers the amino group.[6] This preference is borne out here in

the case of the easily prepared monoguanylated diamines **4c** and **4d**, which are far superior as inhibitors to diamines **2c** and **2d** and diguanidines **3c** and **3d**. Thus, we were prompted to compare the inhibition given by the monoguanylated forms of 1,7-diaminooctane, **39** and **40**. Synthesis of these isomers, albeit somewhat involved, was undertaken with the assurance of revealing that site on the enzyme at which specific interaction with inhibitor methyl branch occurs. Because **25b** and **26b** are stronger inhibitors than are C_7 and GC_7G, respectively, we also hoped to obtain an inhibitor superior to GC_7. From the difference in IC_{50} values between **39** and **40** it is evident that methyl-branch interaction occurs in close vicinity to the site on the enzyme that prefers the amino group, as depicted in Figure 1. We speculated earlier that this is the site with which the amino group at the aminopropyl end of spermidine interacts during its productive binding.[6] Strong support for this binding mode comes from a recent report that 1-methylspermidine, the methyl branch of which is on the same carbon as the primary amino group at its aminopropyl end, is a substrate for deoxyhypusine synthase.[26]

Careful testing showed **39** to be a somewhat better inhibitor than GC_7. Although its increase in potency of inhibition over GC_7 as reflected in the IC_{50} values was not as pronounced as hoped for, **39** did give noticeably greater reductions in hypusine biosynthesis in cultured Chinese hamster ovary cells. Reductions of 20 and 60% were realized after 18 h at levels of 0.1 and 0.3 μM, respectively, in the medium as compared to 0 and 8% for GC_7 at the same respective levels; essentially complete inhibition of hypusine production was seen after 18 h at a 1 μM level of either inhibitor.

Examination of the IC_{50} values of Table 2 shows several striking features of the inhibition given by unsaturated forms of 1,7-diaminoheptane and its derivatives. Perhaps the most notable of these are the pronounced effects on inhibition of the position of the unsaturated bond in the carbon chain and of carbon chain methyl branching. The unsaturated bond in each of these compounds is either in the 2 or in the 3 position from a basic center and each compound is either a diamine or a diguanidine. Therefore, if these compounds, as believed, inhibit by binding in the position normally occupied by spermidine at the active center of deoxyhypusine synthase, it seems obvious that they can be oriented in either of two directions (i.e., either with their unsaturated bond within the active site region in which the aminopropyl portion of spermidine binds or within that region in which the aminobutyl part binds). Spermidine derivatives with double or triple bonds at the C-6-7 position act as substrates or inhibitors for deoxyhypusine synthase.[27] Since the unsaturated bond in each

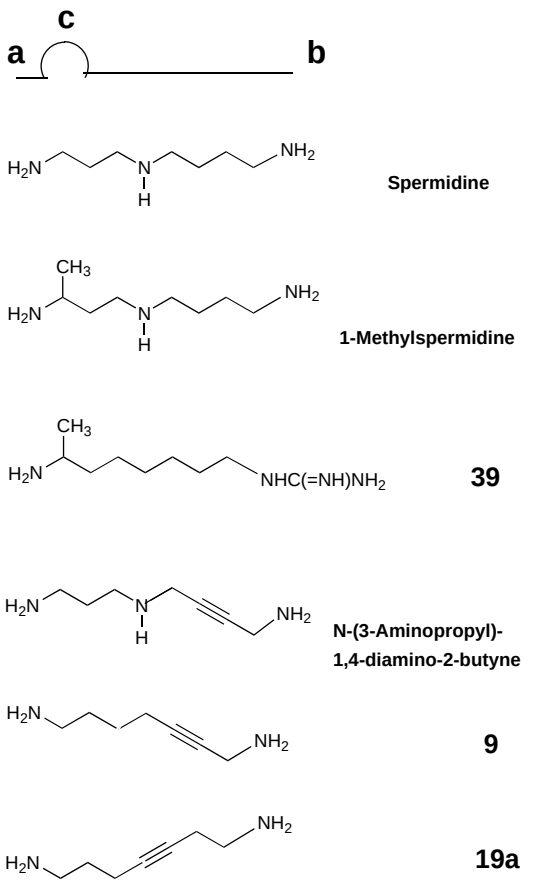


Figure 1. Proposed orientation of representative compounds at the spermidine binding site of deoxyhypusine synthase. **a** and **b** represent those portions of the site (depicted above the structures of the compounds) that bind the primary amino groups of spermidine; the amino group at the aminopropyl end of spermidine interacts with **a**, which prefers an amino group, whereas the aminobutyl moiety of spermidine is directed toward **b**, which favors a guanidino group. **c** represents that portion of the binding site that interacts specifically with a methyl group on the same carbon of a compound as (one of) its primary amino group(s).

of these spermidine derivatives is located at position 2 from the aminobutyl end, one might presume that each of those compounds of Table 2 with unsaturation at position 2 (i.e., compounds **9**, **12**, **10**, **13**, **11**, and **14**) can bind to enzyme with its unsaturated bond in the aminobutyl region of the spermidine binding site, as depicted for compound **9** in Figure 1. Indeed, the IC_{50} values for these inhibitors are not inconsistent with the approximate relative inhibitory properties reported for the unsaturated spermidine derivatives. The poorest of the inhibitors with unsaturation at position 2 are those with the triple bonds, (i.e., **9** and **12**) while the poorest inhibition by the unsaturated spermidine derivatives is

that given by the derivative with the triple bond at the C-6-7 position.[27]

It is more difficult to speculate as to the mode of binding of those inhibitors that contain unsaturated bonds at the 3 position. A methyl branch on the terminal carbon atom nearest the unsaturated bond in each case causes a pronounced loss in inhibitory potency, for example in **19b** versus unbranched **19a** and in **20b** versus unbranched **20a**. This is in contrast to the enhancement of inhibition caused by a methyl branch at a terminal carbon position in the saturated inhibitors (Table 1) and may signify opposite modes of binding for the saturated and unsaturated inhibitors. The evidence cited above for specific interaction of the methyl groups at the terminal carbon atoms of the saturated inhibitors with the aminopropyl end of the enzyme's spermidine binding site, as indicated by the orientation of compound **39** in Figure 1, suggests that, like inhibitors with unsaturated bonds in the 2 position (i.e., **9** in Figure 1) those with unsaturation at the 3 position may bind to enzyme with their unsaturated areas within the aminobutyl region of the spermidine binding site, as shown for **19a** in Figure 1.

Interestingly, of the unsaturated 1,7-diaminoheptane derivatives (Table 2), the 3-alkynes **19a** and **22a** and the 3-*trans* alkenes **20a** and **23** are the most efficient inhibitors. Those derivatives with *cis* double bonds in the 3 position, **21a** and **24**, are significantly less effective. The restricted rotations resulting from unsaturation between carbon atoms confers on these compounds certain spatial characteristics. Thus the configurations of the 3-*cis* alkenes, **21a** and **24**, are such that the distances between carbon atoms adjoining their doubly bonded carbons are considerably less than those between the carbons adjoining the triply bonded carbons of **19a** and **22a** and the *trans* doubly bonded carbons of **20a** and **23**. Since the latter distances, those in the more effective inhibitors, approximate that between terminal carbons of a saturated 4-carbon chain in its fully extended conformation, it seems reasonable to suggest that during productive binding a sizable portion of the spermidine molecule is oriented in its binding site in a fully, or almost fully, extended conformation and that this conformation extends to, or includes its central nitrogen atom.

Experimental

Melting points were determined on a Thomas-Hoover apparatus in open capillary tubes and are uncorrected. TLC was performed on Merck silica gel 60 F_{254} analytical plates visualized with UV, tolidine-Cl_2, ninhydrin, Sakaguchi and/or aqueous $KMnO_4$. Flash chromatography was conducted on Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded on a Varian Gemini 300 spectrometer. Chemical shifts are expressed relative to internal standard TMS or HOD at 4.8 ppm (D_2O). Mass spectra were obtained on a JEOL SX 102 spectrometer (FAB). The optical rotations were recorded at 20° using a Perkin-Elmer 341 polarimeter with a 1 dm cuvette. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, Georgia, and were within 0.4% of the theoretical values.

2-Methyl-1,7-diaminoheptane dihydrochloride (2a). Method A. A solution of pimelonitrile **1a** (2.04 g, 15 mmol) in 20 mL of THF was added slowly to BH_3-THF (1 M, 75 mL, 75 mmol) at room temperature under argon. The reaction was heated at reflux for 2 h and then cooled and carefully hydrolyzed with 6 N HCl (50 mL). Most of the THF was distilled away and the solution was cooled, basified (solid NaOH), and extracted with $CHCl_3$ (5×20 mL). The combined extracts were dried over Na_2SO_4, concentrated and distilled to afford 0.95 g (44%) of free amine (bp 60 °C/0.9 Torr). A solution of this amine (2 mmol) in 5 mL of ethanol was treated with 2 N HCl in ethanol (2.2 mL) and the solvent removed under vacuum. The remaining solid was recrystallized from ethanol-ether to give **2a**: mp 150 °C, ^{1}H NMR (D_2O) δ 0.98–1.00 (d, 3H), 1.38–1.50 (m, 6H), 1.67–1.75 (m, 2H), 1.80–1.87 (m, 1H), 2.96–3.03 (m, 4H); MS (FAB, *t*-glyc) 145 (M + H). Similarly prepared were **2b** (bp of free amine, 58–60 °C/0.6 Torr): ^{1}H NMR (D_2O) δ 0.91–0.93 (d, 3H), 1.37–1.45 (m, 4H), 1.50–1.56 (m, 1H), 1.62–1.69 (m, 4H), 2.98–3.06 (m, 4H); MS (FAB, *t*-glyc) 145 (M + H); **2c** (bp of free amine, 59–60 °C/0.6 Torr): ^{1}H NMR (D_2O) δ 0.90–0.92 (d, 3H), 1.21–1.29 (m, 2H), 1.36–1.45 (m, 2H), 1.49–1.53 (m, 1H), 1.65–1.72 (m, 4H), 2.99 (t, 4H); MS (FAB, *t*-glyc) 145 (M + H); **2d** (bp of free amine, 66–68 °C/0.7 Torr): ^{1}H NMR (D_2O) δ 0.90 (s, 6H), 1.25–1.30 (m, 4H), 1.62–1.66 (m, 4H), 2.98 (t, 4H); MS (FAB, *t*-glyc) 159 (M + H); **2e** (bp of free amine, 65 °C/0.4 Torr): ^{1}H NMR (D_2O) δ 0.89–0.91 (d, 6H), 1.18 (t, 2H), 1.50–1.55 (m, 2H), 1.60–1.69 (m, 4H), 3.01–3.07 (m, 4H); MS (FAB, *t*-glyc) 159 (M + H); *meso*-**2e** (bp of free amine, 67–72 °C/0.5 Torr): ^{1}H NMR (D_2O) δ 0.91–0.93 (d, 6H), 1.12–1.20 (m, 1H), 1.23–1.32 (m, 1H), 1.41–1.48 (m, 2H), 1.65–1.73 (m, 4H), 2.97–3.10 (m, 4H); MS (FAB, *t*-glyc) 159 (M + H).

2-Methyl-1,7-diguanidinoheptane sulfate (3a). Method B. A mixture of diamine **2a** (free base, 0.217 g, 1.5 mmol) and *S*-methylisothiuronium sulfate (0.46 g, 3.3 mmol) in 2.5 mL of water was heated at reflux for 1 h. After cooling, 20 mL of absolute ethanol was added. The oil that formed crystallized upon trituration with ethanol. Recrystallization from aqueous methanol gave 0.22 g (45%) of **3a**: mp 295 °C dec.; ^{1}H NMR (D_2O) δ 0.92–0.94 (d, 3H), 1.33–1.45 (m, 6H), 1.54–1.62 (m, 2H), 1.74–1.80 (m, 1H), 3.02–3.08 (m, 2H), 3.16–3.21 (m,

2H); MS (FAB, *t*-glyc) 229 (M + H). Similarly prepared were: **3b**: ^{1}H NMR (D_2O) δ2 0.89–0.92 (d, 3H), 1.34–1.47 (m, 5H), 1.56–1.60 (m, 4H), 3.16–3.25 (m, 4H); MS (FAB, *t*-glyc) 229 (M + H); **3c**: ^{1}H NMR (D_2O) δ 0.88–0.90 (d, 3H), 1.18–1.26 (m, 2H), 1.32–1.42 (m, 2H), 1.45–1.51 (m, 1H), 1.54–1.66 (m, 4H), 3.17 (t, 3H); MS (FAB, *t*-glyc) 229 (M + H); **3d**: ^{1}H NMR (D_2O) δ 0.87 (s, 6H), 1.21–1.27 (m, 4H), 1.51–1.57 (m, 4H), 3.16 (t, 4H); MS (FAB, *t*-glyc) 243 (M + H); **3e**: ^{1}H NMR (D_2O) δ 0.88–0.90 (d, 6H), 1.15 (t, 2H), 1.40–1.48 (m, 2H), 1.55–1.67 (m, 4H), 3.17–3.30 (m, 4H); MS (FAB, *t*-glyc) 243 (M + H); *meso*-**3e**: ^{1}H NMR (D_2O) δ 0.89–0.91 (d, 6H), 1.06–1.13 (m, 1H), 1.23–1.30 (m, 1H), 1.33–1.42 (m, 2H), 1.61–1.64 (m, 4H), 3.18–3.25 (m, 4H); MS (FAB, *t*-glyc) 243 (M + H).

4-Methyl-1-amino-7-guanidinoheptane dihydrochloride (4c). Method C. A mixture of diamine **2c** (free base, 0.288 g, 2 mmol) and *S*-methylisothiuronium sulfate (0.278 g, 2 mmol) in 3 mL of water was heated at reflux for 1 h. Purification of **4c** was carried out by ion exchange chromatography on a 1×12.5 cm column of Dowex 50 X 2 (H^+, 200–400 mesh) using a logarithmic gradient generated from water in a 100 mL constant volume chamber by displacement with 2.5 N HCl. **4c** emerged from the column between 75 and 140 mL well separated from unreacted **2c** and the fully guanylated compound **3c**. The oil that remained after removal of solvent crystallized after long standing at room temperature to afford 0.224 g (43%) of product: mp 140 °C; ^{1}H NMR (D_2O) δ 0.89–0.91 (d, 3H), 1.20–1.28 (m, 2H), 1.33–1.44 (m, 2H), 1.47–1.54 (m, 1H), 1.57–1.74 (m, 4H), 2.99 (t, 2H), 3.18 (t, 2H); MS (FAB, *t*-glyc) 187 (M + H). Similarly prepared was **4d**: ^{1}H NMR (D_2O) δ 0.89 (s, 6H), 1.24–1.31 (m, 4H), 1.54–1.65 (m, 4H), 2.98 (t, 2H), 3.17 (t, 2H); MS (FAB, *t*-glyc) 201 (M + H).

7-(2-Tetrahydropyranyloxy)-2-heptyn-1-ol (5). Method D. To a solution of ethyl magnesium bromide (1 M in THF, 103 mL, 0.103 mol) under argon at room temperature was added dropwise with stirring 1-(2-tetrahydropyranyloxy)-5-hexyne (16.5 g, 90.6 mmol). The mixture was then heated at reflux for 15 min and cooled to room temperature. After addition of DMPU (103 mL), formaldehyde produced in an outside vessel by heating of paraformaldehyde (6.2 g, 206 mmol) at 165–170 °C was forced into the reaction mixture over a period of 30–45 min by a very slow stream of argon. The temperature of the reaction was allowed to rise during this period. After an additional 30 min, the mixture was poured into a solution of ammonium chloride (12 g) in 500 mL of water and the crude product was extracted with ether (3×500 mL). The combined extracts were washed with brine, dried over Na_2SO_4 and evaporated. The residue was flash chromatographed (3/7, ethyl acetate/hexane) to afford 16.3 g (85%) of **5** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.52–1.75 (m, 10H), 2.24–2.29 (m, 2H), 3.38–3.54 (m, 2H), 3.66–3.89 (m, 2H), 4.25 (m, 2H), 4.59 (t, 1H).

Hept-2-yn-1,7-diol (6). Method E. A mixture of **5** (15.9 g, 75 mmol) and pyridinium-*p*-toluene sulfonate (1.89 g, 7.5 mmol) in 600 mL of 95% ethanol was stirred at 53–58 °C for 5 h. Following evaporation of solvent, the residue was flash chromatographed (1/1, acetone/hexane) to give 7.3 g (76%) of **6** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.55–1.71 (m, 4H), 2.26–2.30 (m, 2H), 3.69 (t, 2H), 4.25 (s, 2H).

Hept-2-yn-1,7-dimesylate (7). Method F. A solution of **6** (6.4 g, 50 mmol) and triethylamine (15.2 g, 150 mmol) in 500 mL of methylene chloride was cooled in an ice–salt bath and mesyl chloride (12.6 g, 110 mmol) was added over a period of 10 min with vigorous stirring. After an additional 15 min, the reaction mixture was washed successively with cold 10% HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated to afford 11.5 g (81%) of **7** as a colorless oil, which moved as a single component on TLC (2/3, ethyl acetate/hexane, R_f 0.26): ^{1}H NMR ($CDCl_3$) δ 1.64–1.71 (m, 2H), 1.84–1.91 (m, 2H), 2.31–2.36 (m, 2H), 3.02 (s, 3H), 3.10 (s, 3H), 4.26 (t, 2H), 4.84 (s, 2H).

Hept-2-yn-1,7-diazide (8). Method G. To a solution of **7** (8.5 g, 30 mmol) in 60 mL of DMSO was added powdered NaN_3 (5.85 g, 90 mmol) portionwise with stirring. Stirring was continued overnight. The reaction was diluted with 120 mL of water and extracted with ether (3×100 mL). The combined extracts were washed with water and dried over Na_2SO_4. The residue was flash chromatographed (1/9, ethyl acetate/hexane) to afford 2.8 g (52%) of **8** as a slightly yellow oil.

1,7-Diaminohept-2-yne dihydrochloride (9). Method H. A mixture of **8** (2.7 g, 15 mmol) and triphenylphosphine (7.8 g, 30 mmol) in 60 mL of THF containing water (0.81 g, 45 mmol) and a few boiling stones was allowed to react overnight at room temperature. After removal of solvent, the solid residue was transfered to a mortar with the aid of approximately 20 mL of water and pulverized. Sufficient HCl solution (approximately 6 mL of 5 N) was added with stirring to render the mixture strongly acidic (congo red test paper). The supernatant was collected by suction filtration and the residue washed with water. The combined extracts and washings were reduced in volume to about 10 mL, cooled, basified (solid NaOH) and extracted with methylene chloride (5×20 mL). The combined extracts were dried over Na_2SO_4. The oil obtained was flash chromatographed (7/2/1, methylene chloride/methanol/NH_4OH, lower layer used as eluant) to give 1.53 g (81%) of free amine. A portion of this amine in ethanol was treated

with 2 N HCl in ethanol. The solid obtained upon evaporation was recrystallized from ethanol-ether to give **9**: mp 204–205 °C; ^{1}H NMR (D_2O) δ 1.59–1.66 (m, 2H), 1.73–1.81 (m, 2H), 2.31–2.35 (m, 2H), 3.03 (t, 2H), 3.80 (s, 2H); MS (FAB, *t*-glyc) 127 (M + H).

1,7-Diamino-*trans*-hept-2-ene dihydrochloride (10). Method I. A solution of **9** (free base, 0.63 g, 5 mmol) in 15 mL of THF was added slowly to $LiAlH_4$ (1 M, 15 mL, 15 mmol) in THF at room temperature. The mixture was heated to reflux for 18 h and cooled. To this was added carefully in succession, water (0.6 mL), NaOH (15%, 0.6 mL) and water (1.8 mL). The mixture was filtered by suction and the precipitate washed well with water and methylene chloride. The filtrate and washings were combined, reduced in volume to about 10 mL, cooled, made strongly basic (solid NaOH) and extracted with methylene chloride (5×20 mL). The purified amine obtained upon flash chromatographed (7/2/1, methylene chloride/ methanol/ NH_4OH, lower layer used as eluant) was dissolved in ethanol and treated with 2 N HCl in ethanol to afford 0.302 g (30%) of **10**: ^{1}H NMR (D_2O) δ 1.45–1.54 (m, 2H), 1.63–1.73 (m, 2H), 2.12–2.19 (m, 2H), 2.98–3.03 (t, 2H), 3.56–3.58 (d, 2H), 5.57–5.66 (m,1H), 5.90–5.99 (m, 1H); MS (FAB, *t*-glyc) 129 (M + H).

1,7-Diamino-*cis*-hept-2-ene dihydrochloride (11). Method J. A solution of **9** (free base, 0.63 g, 5 mmol) in 100 mL of ethanol was hydrogenated over activated Lindlar catalyst (100 mg) at atmospheric pressure and room temperature. When the hydrogen uptake reached the level of one molar equivalent (112 mL), the catalyst was removed by filtration and the solvent was evaporated. Isolation of the product by flash chromatography (7/2/1, methylene chloride/methanol/NH_4OH, lower layer used as eluant) and treatment with alcoholic HCl in the usual manner afforded 0.72 g (72%) of **11**: ^{1}H NMR (D_2O) δ 1.49–1.54 (m, 2H), 1.67–1.71 (m, 2H), 2.15–2.22 (m, 2H), 2.99–3.04 (t, 2H), 3.67–3.70 (d, 2H), 5.50–5.58 (m, 1H), 5.81–5.97 (m, 1H); MS (FAB, *t*-glyc) 129 (M + H).

1,7-Diguanidinohept-2-yne sulfate (12). Method K. To a solution of **9** (0.2 g, 1 mmol) in 2 mL of methanol was added a solution of *O*-methylisourea sulfate (0.52 g, 3 mmol) and 2 mL of triethylamine in 2 mL of water. After standing overnight, ethanol was added to complete crystallization. Recrystallization from water–ethanol afforded 0.124 g (40%) of **12**: ^{1}H NMR (D_2O) δ 1.58–1.61 (m, 2H), 1.65–1.72 (m, 2H), 2.29–2.30 (m, 2H), 3.21 (t, 2H), 4.00 (s, 2H); MS (FAB, *t*-glyc) 211 (M + H), 309 (M + H + H_2SO_4).

1,7-Diguanidino-*trans*-hept-2-ene sulfate (13). Prepared from **10** using **Method K**: ^{1}H NMR (D_2O) δ 1.45–1.50 (m, 2H), 1.57–1.62 (m, 2H), 2.10–2.14(m, 2H), 3.18 (t, 2H), 3.77–3.79 (d, 2H), 5.48–5.57 (m, 1H), 5.71–5.80 (m, 1H); MS (FAB, *t*-glyc) 213 (M + H), 311 (M + H + H_2SO_4).

1,7-Diguanidino-*cis*-hept-2-ene sulfate (14). Prepared from **11** using **Method K**: ^{1}H NMR (D_2O) δ 1.43–1.50 (m, 2H), 1.58–1.63 (m, 2H), 2.12–2.18 (m, 2H), 3.19 (t, 2H), 3.85–3.87 (d, 2H), 5.48–5.57 (m, 1H), 5.69–5.78 (m, 1H); MS (FAB, *t*-glyc) 213 (M + H), 311 (M + H + H_2SO_4).

7-(2-Tetrahydropyranyloxy)-3-heptyn-1-ol (15a). Method L. A solution of 1-(2-tetrahydropyranyloxy)-4-pentyne (12.6 g, 75 mmol) in 70 mL of THF was cooled in an ice–salt bath and n-butyllithium (2.5 M in hexane, 33 mL, 82.5 mmol) was added slowly under argon with stirring. The ice-salt bath was replaced by an ice bath and the reaction mixture was allowed to stir for 2 h, cooled to −50 °C and 70 mL of DMPU was added. This was followed by the rapid addition of ethylene oxide (10 g, 225 mmol) from an inverted cylinder. The mixture was allowed to come to room temperature over a period of 6 h and stirring was continued for 18 h. The mixture was poured into 750 mL of ice water and extracted with ether (3×100 mL). The combined extracts were washed with water, and with brine, dried over $MgSO_4$, and evaporated. Flash chromatography (2/3, ethyl acetate/hexane) of the residue afforded 11.9 g (75%) of **15a** as a light-pink oil: ^{1}H NMR ($CDCl_3$) δ 1.51–1.83 (m, 8H), 2.23–2.32 (m, 2H), 2.39–2.47 (m, 2H), 3.44–3.55 (m, 2H), 3.66–3.77 (m, 2H), 3.78–3.90 (m, 3H), 4.59–4.64 (m, 1H).

8-(2-Tetrahydropyranyloxy)-4-octyn-2-ol (15b and (*S*)-15b). Obtained as colorless oils using **Method L** with the exceptions that DMPU was replaced by HMPA (10.5 mL) and ethylene oxide by propylene oxide: yields; racemic **15b**, 68%; (*S*)-**15b**, 65%; for (*S*)-**15b**, $[\alpha]_D$ + 9.9° (c 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.22–1.24 (d, 3H), 1.52–1.82 (m, 8H), 2.27–2.36 (m, 4H), 3.44–3.52 (m,2H), 3.79–3.87 (m, 1H), 3.88–3.92 (m, 1H), 4.59 (t, 1H) (spectra identical for **15b** and (*S*)-**15b**).

1-(2-Tetrahydropyranyloxy)-4-nonyn-7-ol (15c). Prepared by **Method L**: in 40% yield, using HMPA and 1,2-epoxybutane, respectively, in place of DMPU and ethylene oxide: ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H), 1.49–1.85 (m, 10H), 2.09–2.42 (m, 4H), 3.48–3.49 (m, 2H), 3.59–3.62 (m, 1H), 3.73–3.89 (m, 2H), 4.59 (s, 1H).

Hept-3-yn-1,7-diol (16a). Prepared from **15a** in 86% yield using **Method E**: ^{1}H NMR ($CDCl_3$) δ 1.70–1.79 (m, 2H), 2.27–2.33 (m, 2H), 2.39–2.45 (m, 2H), 3.66–3.70 (t, 2H), 3.74–3.77 (t, 2H).

Oct-3-yn-1,7-diol (16b and (*S*)-16b). Prepared in 85% and 80% yields, respectively, from **15b** and (*S*)-**15b**

using **Method E**: for (*S*)-**16b**, $[\alpha]_D + 15.4°$ (c 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.22–1.24 (d, 3H), 1.72–1.79 (m, 2H), 2.28–2.36 (m, 4H), 3.75 (t, 2H), 3.88–3.94 (m, 1H). (Spectra identical for **16b** and (*S*)-**16b**).

Non-4-yn-1,7-diol (16c). Prepared from **15c** in 77% yield using **Method E** and flash chromatographed in 1/1, acetone/hexane for purification: ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H), 1.50–1.60 (m, 2H), 1.73–1.80 (m, 2H), 2.28–2.34 (m, 2H), 2.38–2.44 (m, 2H), 3.61–3.65 (m, 1H), 3.76 (t, 2H).

Hept-3-yn-1,7-dimesylate (17a). Obtained as an oil (97%) from **16a** using **Method F**: ^1H NMR ($CDCl_3$) δ 1.90–1.95 (m, 2H), 2.32–2.36 (m, 2H), 2.61–2.67 (m, 2H), 3.04 (s, 3H), 3.06 (s, 3H), 4.27 (t, 2H), 4.35 (t, 2H).

Oct-3-yn-1,7-dimesylate (17b and (*S*)-17b). By **Method F** from **16b** and (*S*)-**16b** (98% and 100%, respectively): ^1H NMR ($CDCl_3$) δ 1.46–1.49 (d, 3H), 1.88–1.96 (m, 2H), 2.34 (t, 2H), 2.54–2.56 (m, 2H), 3.03 (s, 3H), 3.04 (s, 3H), 4.34 (t, 2H), 4.81–4.86 (m, 1H). (Spectra identical for **17b** and (*S*)-**17b**).

Non-3-yn-1,7-dimesylate (17c). By **Method F** from **16c** (99%): ^1H NMR ($CDCl_3$) δ 1.01 (t, 3H), 1.77–1.85 (m, 2H), 1.89–1.97 (m, 2H), 2.32–2.34 (m, 2H), 2.59–2.62 (m, 2H), 3.03 (s, 3H), 3.06 (s, 3H), 4.35 (t, 2H), 4.65–4.69 (m, 1H).

Hept-3-yn-1,7-diazide (18a). From **17a** using **Method G** (70%): ^1H NMR ($CDCl_3$) δ 1.71–1.81 (m, 2H), 2.26–2.33 (m, 2H), 2.43–2.50 (m, 2H), 3.34–3.44 (t, 4H).

Oct-3-yn-1,7-diazide (18b and (*S*)-18b). By **Method G** from **17b** and (*S*)-**17b** (65% and 70%, respectively): ^1H NMR ($CDCl_3$) δ 1.31–1.33 (d, 3H), 1.72–1.81 (m, 2H), 2.31–2.33 (m, 2H), 2.36–2.39 (m, 2H), 3.41 (t, 2H), 3.57-3.63 (m, 1H). (Spectra identical for **18b** and (*S*)-**18b**).

Non-3-yn-1,7-diazide (18c). By **Method G** from **17c** (52%): ^1H NMR ($CDCl_3$) δ 1.00 (t, 3H), 1.56–1.71 (m, 2H), 1.73–1.81 (m, 2H), 2.28–2.33 (m, 2H), 2.40–2.43 (m, 2H), 3.34–3.38 (m, 1H), 3.42 (t, 2H).

1,7-Diaminohept-3-yne dihydrochloride (19a). From **18a** using **Method H**: ^1H NMR (D_2O) δ 1.85–1.90 (m, 2H), 2.35 (t, 2H), 2.61 (t, 2H), 3.09–3.17 (m, 4H); MS (FAB, *t*-glyc) 127 (M + H).

1,7-Diaminooct-3-yne dihydrochloride (19b and (*S*)-19b). From **18b** and (*S*)-**18b** by **Method H**: ^1H NMR (D_2O) δ 1.36–1.38 (d, 3H), 1.84–1.93 (m, 2H), 2.35–2.40 (m, 2H), 2.50–2.67 (m, 2H), 3.12 (t, 2H), 3.50–3.57 (m, 1H); MS (FAB, *t*-glyc) 141 (M + H). (Spectra identical for **19b** and (*S*)-**19b**).

1,7-Diaminonon-3-yne dihydrochloride (19c). From **18c** by **Method H**: ^1H NMR (D_2O) δ 0.99 (t, 3H), 1.71–1.81 (m, 2H), 1.83–1.93 (m, 2H), 2.36 (t, 2H), 2.53–2.70 (m, 2H), 3.11 (t, 2H), 3.32–3.38 (m, 1H); MS (FAB, *t*-glyc) 155 (M + H).

1,7-Diamino-*trans*-hept-3-ene dihydrochloride (20a). From **19a** (free base) by **Method I**: ^1H NMR (D_2O) δ 1.71–1.81 (m, 2H), 2.12–2.19 (m, 2H), 2.36–2.43 (m, 2H), 2.98–3.08 (m, 4H), 5.49–5.56 (m, 1H), 5.64–5.71 (m, 1H); MS (FAB, *t*-glyc) 129 (M + H).

1,7-Diamino-*trans*-oct-3-ene dihydrochloride (20b). Method M. To a solution of racemic **19b** (free base, 0.28 g, 2 mmol) in 8 mL of diglyme was added slowly with stirring under argon $LiAlH_4$ (0.5 M in diglyme, 13 mL, 6.5 mmol). The mixture was heated at reflux for 18 h, cooled and hydrolyzed carefully with water using external cooling. After filtration with the use of celite, the solvent was removed under high vacuum. The residue was dissolved in 10 mL of water, basified (solid NaOH) and extracted with methylene chloride (5×20 mL). Satisfactorily purified product could not be obtained by flash chromatography. Therefore the material was chromatographed by the ion exchange procedure described in **Method C**. Compound **20b** emerged from the column between 50 and 75 mL and was obtained as a colorless oil (0.085 g, 24%) upon removal of solvent: ^1H NMR (D_2O) δ 1.28–1.31(d, 3H), 1.71–1.81 (m, 2H), 2.13–2.20 (m, 2H), 2.33–2.38 (m, 2H), 3.00 (t, 2H), 3.37–3.44 (m, 1H), 5.48–5.57 (m, 1H), 5.64–5.74 (m, 1H); MS (FAB, *t*-glyc) 143 (M + H).

1,7-Diamino-*cis*-hept-3-ene dihydrochloride (21a). From **19a** free base by **Method J**: ^1H NMR (D_2O) δ 1.71–1.81 (m, 2H), 2.16–2.23 (m, 2H), 2.43–2.50 (m, 2H), 2.99–3.08 (m, 4H), 5.46–5.51 (m, 1H), 5.63–5.71 (m, 1H); MS (FAB, *t*-glyc) 129 (M + H).

1,7-Diamino-*cis*-oct-3-ene dihydrochloride (21b). From racemic **19b** free base by **Method J**: ^1H NMR (D_2O) δ 1.30–1.32 (d, 3H), 1.71–1.81 (m, 2H), 2.16–2.23 (m, 2H), 2.39–2.45 (m, 2H), 3.01 (t, 2H), 3.39–3.46 (m, 1H), 5.45–5.54 (m, 1H), 5.65–5.73 (m, 1H); MS (FAB, *t*-glyc) 143 (M + H).

1,7-Diguanidinohept-3-yne sulfate (22a). From **19a** by **Method K**: ^1H NMR (D_2O) δ 1.75–1.82 (m, 2H), 2.29 (t, 2H), 2.48 (t, 2H), 3.27–3.36 (m, 4H); MS (FAB, *t*-glyc) 211 (M + H), 309 (M + H + H_2SO_4).

1,7-Diguanidinooct-3-yne dihydrochloride (22b and (*S*)-22b). Method N. To solutions of **19b** and (*S*)-**19b** (free bases, 0.14 g, 1 mmol) in 1 mL of DMF were added 1*H*-pyrazole-1-carboxamidine.HCl (0.31 g, 2.1 mmol) and diisopropylethylamine (0.383 mL, 2.2 mmol) under

argon. After standing overnight, the mixtures were diluted with 10 mL portions of ether. The oils that formed were washed well with ether, dissolved in water and chromatographed by the ion exchange procedure described in **Method C**. Products, which eluted between 150–250 mL, were obtained as colorless somewhat hygroscopic foams (0.248 g, 83% for **22b** and 0.196 g, 66% for (*S*)-**22b**) upon removal of solvent and drying under high vacuum: ^{1}H NMR (D_2O) δ 1.27–1.29 (d, 3H), 1.74–1.82 (m, 2H), 2.28–2.34 (m, 2H), 2.41–2.58 (m, 2H), 3.30 (t, 2H), 3.73–3.79 (m, 1H); MS (FAB, *t*-glyc) 225 (M + H), 261 (M + H + HCl). (Spectra identical for **22b** and (*S*)-**22b**).

1,7-Diguanidino-*trans*-hept-3-ene sulfate (23). From **20a** by **Method K**: ^{1}H NMR (D_2O) δ 1.62–1.72 (m, 2H), 2.07–2.14 (m, 2H), 2.26–2.32 (m, 2H), 3.15–3.25 (m, 4H), 5.47–5.54 (m, 1H), 5.56–5.64 (m, 1H); MS (FAB, *t*-glyc) 213 (M + H), 311 (M + H + H_2SO_4).

1,7-Diguanidino-*cis*-hept-3-ene sulfate (24). From **21a** by **Method K**: ^{1}H NMR (D_2O) δ 1.62–1.71 (m, 2H), 2.11–2.18 (m, 2H), 2.31–2.38 (m, 2H), 3.17–3.27 (m, 4H), 5.44–5.52 (m, 1H), 5.57–5.65 (m, 1H); MS (FAB, *t*-glyc) 213 (M + H) 311 (M + H + H_2SO_4).

1,7-Diaminooctane dihydrochloride (25b and (*S*)-25b). Method O. Solutions of **19b** and (*S*)-**19b** (free bases, 0.28 g, 2 mmol) in 100 mL portions of ethanol were hydrogenated over activated Adams catalyst (25 mg) at atmospheric pressure and room temperature. When the hydrogen uptake reached the theoretical level (2 mol equiv, 90 mL), catalyst was removed by filtration and the solvent evaporated. Conversion of the free amines to the dihydrochlorides was conducted in the usual manner to afford **25b** and (*S*)-**25b**: ^{1}H NMR (D_2O) δ 1.28–1.30 (d, 3H), 1.33–1.61 (m, 6H), 1.59–1.75 (m, 4H), 3.00 (t, 2H), 3.33–3.40 (m, 1H); MS (FAB, *t*-glyc) 145 (M + H). (Spectra identical for **25b** and (*S*)-**25b**).

1,7-Diaminononane dihydrochloride (25c). From **19c** free base by **Method O**: ^{1}H NMR (D_2O) δ 0.97 (t, 3H), 1.40 (m, 6H), 1.59–1.76 (m, 6H), 3.00 (t, 2H), 3.18–3.26 (m, 1H); MS (FAB, *t*-glyc) 159 (M + H).

1,7-Diguanidinooctane sulfate (26b and (*S*)-26b). From **25b** and (*S*)-**25b**, respectively, by **Method K**: ^{1}H NMR (D_2O) δ 1.17–1.20 (d, 3H), 1.30–1.40 (m, 6H), 1.55–1.65 (m, 6H), 3.18 (t, 2H), 3.50–3.57 (m, 1H); MS (FAB, *t*-glyc) 229 (M + H), 327 (M + H + H_2SO_4). (Spectra identical for **26b** and (*S*)-**26b**).

1,7-Diguanidinononane sulfate (26c). From **25c** by **Method K**: ^{1}H NMR (D_2O) δ 0.97 (t, 3H), 1.38 (m, 6H), 1.55–1.73 (m, 6H), 3.16–3.23 (m, 3H); MS (FAB, *t*-glyc) 243 (M + H), 341 (M + H + H_2SO_4).

8-(2-Tetrahydropyranyloxy)-4-octyn-2-mesylate (27). Prepared from racemic **15b** (4.2 g, 18.6 mmol) by **Method F** using 1.5 equiv of triethylamine and 1.1 equiv of mesylchloride. Cold 5% citric acid replaced cold 10% HCl in washing the reaction mixture. The product (5.2 g, 92%) was obtained as a colorless oil, which moved as a single component on TLC (2/3, ethyl acetate/hexane, R_f 0.57): ^{1}H NMR ($CDCl_3$) δ 1.47–1.49 (d, 3H), 1.53–1.81 (m, 8H), 2.24–2.29 (m, 2H), 2.52–2.59 (m, 2H), 3.04 (s, 3H), 3.44–3.52 (m, 2H), 3.79–3.89 (m, 2H), 4.58 (t, 1H), 4.79–4.85 (m, 1H).

8-(2-Tetrahydropyranyloxy)-4-octyn-2-azide (28). The application of **Method G** to **27** (5.1 g, 16.8 mmol) afforded 2.81 g (67%) of **28** as a slightly yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.31–1.33 (d, 3H), 1.53–1.84 (m, 8H), 2.26–2.32 (m, 2H), 2.35–2.39 (m, 2H), 3.44–3.53 (m, 2H), 3.56–3.60 (m, 1H), 3.76–3.88 (m, 2H), 4.60 (t, 1H).

1-(2-Tetrahydroxypyranyloxy)-7-aminooctane (29). Method P. A solution of **28** (2.76 g, 11 mmol) in 25 mL of ethanol was hydrogenated over Adams catalyst (25 mg) at 40 lbs/in^2 for 6 h. Removal of catalyst by filtration and evaporation of solvent afforded 2.2 g (96%) of the saturated amine **29** as a colorless oil that chromatographed as a single material on TLC (17/2/1, methylene chloride/methanol/NH_4OH, lower layer used for solvent, R_f 0.43); ^{1}H NMR ($CDCl_3$) δ 1.07–1.09 (d, 3H), 1.22–1.58 (m, 14H), 1.67–1.82 (m, 2H), 2.49 (s, 2H), 2.90–2.92 (m, 1H), 3.33–3.52 (m, 2H), 3.67–3.88 (m, 2H), 4.56 (t, 1H).

1-(2-Tetrahydropyranyloxy)-7-*tert*-butyloxycarbonylaminooctane (30). To a solution of amine **29** (2.1 g, 9.16 mmol) in 2.5 mL of DMF was added in portions with stirring a solution of di-*tert*-butyl dicarbonate (2 g, 9.2 mmol) in 2.5 mL of DMF. After stirring for an additional 30 min the solvent was removed under high vacuum. The oil that remained was dissolved in ether, washed with cold 5% citric acid, saturated $NaHCO_3$, water and brine, and dried over $MgSO_4$. Removal of ether afforded 2.85 g (94%) of **30** as a colorless oil: TLC (2/3, ethyl acetate/hexane, R_f 0.74); ^{1}H NMR ($CDCl_3$) δ 1.09–1.11 (d, 2H), 1.33–1.58 (m, 14H), 1.44 (s, 9H), 1.71–1.84 (m, 2H), 3.34–3.54 (m, 2H), 3.61–3.66 (m, 1H), 3.69–3.90 (m, 2H), 4.29–4.33 (m, 1H), 4.58 (t, 1H).

7-*tert*-Butyloxycarbonylaminooctane-1-ol (31). Obtained from **30** (2.8 g, 8.5 mmol) with the use of **Method E**. Purification by flash chromatography (2/3, ethyl acetate/hexane) provided **31** (1.8 g, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.09–1.12 (d, 3H), 1.34 (m, 8H), 1.44 (s, 9H), 1.54–1.58 (m, 2H), 3.61 (m, 1H), 3.64 (t, 1H).

7-*tert*-Butyloxycarbonylaminooctane-1-mesylate (32). Application of *Method F* (as used in preparation of **27**) to **31** (1.7 g, 7 mmol) afforded **32** (1.96 g, 87%) as a white solid: mp 54 °C after recrystallization from hexane: TLC (2/3, ethyl acetate/hexane, R_f 0.47); ^{1}H NMR ($CDCl_3$) δ 1.10–1.12 (d, 3H), 1.36–1.39 (m, 8H), 1.45 (s, 9H), 1.73–1.78 (m, 2H), 3.01 (s, 3H), 3.61 (m, 1H), 4.23 (t, 2H); Anal. ($C_{14}H_{29}NO_5S$) C,H,N,S.

7-*tert*-Butyloxycarbonylaminooctane-1-azide (33). From **32** (1.93 g, 6 mmol) by **Method G** as a colorless oil (1.45 g, 89%): ^{1}H NMR ($CDCl_3$) δ 1.09–1.11 (d, 3H), 1.34 (m, 8H), 1.44 (s, 9H), 1.54–1.62 (m, 2H), 3.25 (t, 2H), 3.62 (m, 1H), 4.28 (br, 1H).

1-Amino-7-*tert*-butyloxycarbonylaminooctane (34). The application of **Method P** to azide **33** (1.36 g, 5 mmol) afforded the *t*-Boc diamine **34** (1.2 g, 98%) as an oil: TLC (17/2/1, methylene chloride/methanol/NH_4OH, lower layer used for solvent, R_f 0.40); ^{1}H NMR ($CDCl_3$) δ 1.07–1.10 (d, 3H), 1.31 (m, 8H), 1.42 (s, 9H), 1.47–1.49 (m, 2H), 2.72 (t, 2H), 2.90 (s, 2H), 3.60 (m, 1H), 4.34–4.37 (d, 1H).

1-Guanidino-7-*tert*-butyloxycarbonylaminooctane hemisulfate(35). Guanylation of amine **34** (0.37 g, 1.5 mmol) using **Method K** followed by flash chromatography (2/2/1, methylene chloride/methanol/NH_4OH, R_f 0.31) afforded **35** (0.24 g, 48%) as a white powder after drying under high vacuum : mp 184 °C dec.; ^{1}H NMR ($CDCl_3$) δ 1.09–1.11 (d, 3H), 1.32 (m, 8H), 1.43 (s, 9H), 1.59 (m, 2H), 3.21 (m, 2H), 3.55 (m, 1H); Anal. ($C_{14}H_{30}N_4O_2 \cdot 0.5\ H_2SO_4$) C, H, N, S.

1-Benzyloxycarbonylamino-7-*tert*-butyloxycarbonylaminooctane (36). To a solution of amine **34** (0.73 g, 3.25 mmol) in 5 mL of $CHCl_3$ at ice bath temperature under argon was added dropwise with stirring a solution of *N*-(benzyloxycarbonyloxy)succinimide (0.81 g, 3.25 mmol) in 5 mL of $CHCl_3$. The mixture was allowed to warm to room temperature and kept overnight. Upon extraction successively with cold 5% citric acid, saturated $NaHCO_3$ and brine and removal of solvent, a solid residue was obtained and recrystallized from ethyl acetate-pentane to afford the differentially blocked diamine **36** (1.05 g, 85%): mp 52–53 °C; TLC (2/3, ethyl acetate/hexane, R_f 0.66); ^{1}H NMR ($CDCl_3$) δ 1.09–1.11 (d, 3H), 1.31–1.49 (m, 10H), 1.44 (s, 9H), 3.18 (m, 2H), 3.61 (m, 1H), 4.29 (s, 1H), 4.69 (s, 1H), 5.10 (s, 2H), 7.36 (s, 5H); Anal. ($C_{21}H_{34}N_2O_4$) C, H, N.

7-Amino-1-benzyloxycarbonylaminooctane (37). Method Q. To 4 mL of trifluoroacetic acid at 0 °C was added **36** (0.95 g, 2.5 mmol). After 1 h at this temperature, the acid was removed by rotary evaporation without external heating. The residue was washed several times by trituration with ether and flash chromatographed (7/2/1, methylene chloride/methanol/NH_4OH, lower layer used for elution) to afford **37** (0.63 g, 91%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.14–1.16 (d, 3H), 1.32–1.50 (m, 10H), 2.97–3.02 (m,1H), 3.15–3.22 (m, 2H), 5.10 (s, 2H), 7.36 (s, 5H).

7-Guanidino-1-benzyloxycarbonylaminooctane (38). Guanylation of amine **37** (0.42 g, 1.5 mmol) was conducted according to **Method N** using 1 equiv each of reagent and diisopropylethylamine. Flash chromatography (7/2/1, methylene chloride/methanol/NH_4OH, lower layer used for elution) afforded **38** (0.3 g, 62%) as a strongly basic oil: ^{1}H NMR ($CDCl_3$) δ 1.05–1.46 (m, 13H), 2.99–3.14 (m, 2H), 3.52–3.61 (m, 1H), 5.06 (s, 2H), 7.32 (s, 5H).

7-Amino-1-guanidinooctane sulfate (39). Removal of the *t*-Boc group from **35** (0.2 g, 0.6 mmol) was carried out by **Method Q**. The residual oil obtained upon removal of acid was crystallized under ether and recrystallized from water–ethanol to give **39** (0.07 g, 46%): ^{1}H NMR (D_2O) δ 1.28–1.30 (d, 3H), 1.38 (m, 6H), 1.61 (m, 4H), 3.18 (t, 2H), 3.32–3.39 (m, 1H).

1-Amino-7-guanidinooctane dihydrochloride (40). A solution of **38** (0.25 g, 7.8 mmol) in a mixture of 5 mL of methanol and 2 mL of N HCl was hydrogenated over Pd black (50 mg) at 40 lbs/in^2 for 2 h. The catalyst was removed by filtration and the residue that remained upon evaporation of solvents was dissolved in water and chromatographed on an ion exchange column using the conditions outlined under **Method C**. The target compound **40** emerged from the column between 80 and 150 mL and was obtained as a hygroscopic glass (0.16 g, 76%) upon removal of solvent and drying under high vacuum: ^{1}H NMR (D_2O) δ 1.19–1.21 (d, 3H), 1.36 (m, 6H), 1.54 (m, 2H), 1.67 (m, 2H), 3.00 (t, 2H), 3.51–3.57 (m, 1H).

Enzyme assay and determination of cellular hypusine biosynthesis

Human recombinant deoxyhypusine synthase was prepared as outlined[29]. The enzyme was assayed by a published procedure[30]. The assay mixtures included, in a total of 0.02 mL, 0.2 M glycine–NaOH buffer, pH 9.5, 10 μM eIF-5A precursor protein (prepared by overexpression of a human eIF-5A cDNA in *Escherichia coli*), 1 mM dithiothreitol, 0.5 mM NAD^+, 5.4 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 deoxyhypusine was measured after 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, $IC_{50} = I[(100\text{-}x)/x]$, where I = inhibitor concentration and x = the percentage inhibition[6], using the KaleidaGraph program. Methods for cultures of Chinese hamster ovary cells and for the determination of hypusine in these cells are given elsewhere[8]. Values for hypusine formed at 18 h after addition of ^{3}H-labeled spermidine are given as percentage reduction in the corresponding value in untreated cells.

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