CHEMICAL REVIEWS

REVIEW

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Guanylated Diamines, Triamines, and Polyamines: Chemistry and Biological Properties

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CONTENTS

1. INTRODUCTION

Naturally occurring polyamines (PAs) play a pivotal role in the life of all organisms from bacteria to man. The human PAs putrescine, spermidine, and spermine are fundamental for cell viability, having a multitude of in vivo functions, and their study is an expanding field of research. The polycationic nature of these polyamines is important for their biological activities and for their in vivo interaction with macromolecules such as enzymes or the polyanionic nucleic acids. Several reviews have been reported so far on the chemistry, synthesis, and biological properties of polyamines.[1]

However, a particular class of polyamines, namely, the guanylated polyamines, attracted the attention of many chemists and biologists in the last decades. A guanylated polyamine is a polyamine with one or more of its amino moieties as part of a guanidine function. These compounds can be sometimes referred to as polyaminoalkylguanidines. Replacement of an amino group in a biologically active polyamine compound by the strongly basic guanidinium results often in a significant increase

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of its potency and/or selectivity. In fact, the guanidine group is a common structural key element in a variety of natural and synthetic compounds, which show interesting biological properties or chemical behavior and have therefore found important applications in medicinal, bioorganic, supramolecular chemistry, and, most recently, asymmetric synthesis. A guanidinium group is commonly used by proteins and enzymes to recognize and bind anions through ion pairing and hydrogen bonding. The specific patterns of hydrogen bonding recognition together with the high basicity ($pK_a \approx 13.5$) makes the guanidium group able to play several key roles in recognition, electrophilic catalysis, and biological activity in many enzymes. As a consequence, guanylated polyamines may possess biochemical and biophysical properties amplified with respect to their parent polyamines. Moreover, being strictly related to natural bioamines and amino acids, the guanylated polyamines often have high solubility in water and bioavailability, which makes them excellent drugs or lead compounds. Guanylated polyamines (in particular guanylated diamines and triamines) play important roles in biological processes and might be produced directly by plants, animals, and humans. However, despite their simple chemical structures, these compounds showed difficulties from a synthetic point of view, mostly due to the nature of guanidine moiety, which makes these compounds highly polar and hard to handle and to purify. In addition, the presence of two or more nitrogens or guanidine moieties represents an additional issue: for instance, the regioselctive guanylation of polyamines still represents a big challenge for chemists. Different synthetic methodologies have been reported to overcome these issues such as the shrewd use of a protecting group strategy or the use of solid-phase synthesis. Moreover, possessing these compounds different reactive moieties, side reactions sometimes constitute a problem.

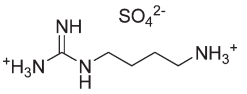
This review will cover systematically the preparation methodologies and chemical properties of guanylated diamines, triamines, and, more generally, polyamines. Finally, a description of their most important biological properties will be reported.

2. GUANYLATED DIAMINES

Guanylated diamines are a quite large class of compounds that could be divided into monoguanylated diamines or biguanylated diamines, depending on the number of guanidine functions present on the molecule. Some guanylated diamines, such as agmatine, are natural compounds and can be found in plants, bacteria, and animals, where they explain several biological functions. On the other hand, the greatest number of guanylated diamines have been synthesized so far by chemists as derivatives of the natural compounds and have proved to possess different pharmacological activities. Guanylated diamines can be synthesized generally through simple guanylation of diamines by the use of different guanylating agents.[2] However, sometimes, syntheses could be problematic because of the peculiar structures of some of these compounds.

2.1. Agmatine

Among monoguanylated diamines, the natural compound agmatine (AG) **1** (Figure 1) represents the most attracting one, due to its physiological properties in animals, in particular as neurotransmitter[3] and regulator of polyamines concentration.[4] The term "agmatine" was coined in 1910 by Albrecht Kossel, who first identified the substance in herring sperm.[8] After Kossel's discovery, agmatine was found in many plants, bacteria, and invertebrates in following years.[5] Recently, AG was shown to occur also in mammals,[9] by identifying its presence in bovine brain, recognizing it in the clonidine-displacing compound previously studied by other groups.[6a,10] AG is known as an intermediate in the polyamine metabolism of various bacteria, fungi, parasites, and marine fauna,[5] as well as in the metabolism of mammals where polyamines have been attributed an important function in cellular growth.[6] AG is an amine and organic cation. Structurally it is a monoguanylated diaminobutane, also referred to as [1-(4-aminobutyl)guanidine]. It is a biogenic amine mainly present in the diprotonated form at physiological pH. AG is chemically characterized as follows: its molecular mass is 130 Da, the UV absorption maximum of 200 nm suggests an aliphatic structure, and a ninhydrin positive reaction confirms the existence of an amino group.[7]



Agmatine 1

Figure 1. Agmatine sulfate.

2.1.1. Biosynthesis and Biological Role of Agmatine. A short summary of general biogenic amines metabolism is described because of its strict correlation with agmatine metabolism. Biogenic amines are a class of compounds synthesized during normal metabolic processes in all organisms. Biogenic amines are biological regulators, including catecholamines, polyamines, and agmatine. In eukaryotic cells, the polycationic polyamines, putrescine, spermidine, and spermine, are essential factors for embryonal development, differentiation, and cell proliferation.[11] The total intracellular concentration is very accurately regulated by metabolic modulation or uptake in cells and increases rapidly in proliferating or differentiating cells.[12]

2.1.1.1. Polyamine Metabolism. Polyamine Biosynthesis. The precursor for polyamine biosynthesis is ornithine, a nonproteic amino acid, an intermediate of the urea cycle, and derived from arginine by the action of arginase. Ornithine decarboxylase (ODC) is required for the first step in polyamine synthesis, in which ornithine is decarboxylated to produce putrescine. ODC is a pyridoxalphosphate-dependent enzyme and is the first rate-limiting step in polyamines biosynthesis. This enzyme is active as a homodimer with a very short half-life and is degraded by the 26S proteasome, without ubiquitination. The degradation of ODC is regulated by the antizyme, a small protein induced by polyamine that regulates also the polyamine transporter.[13] Another rate-limiting step in polyamine biosynthesis is the decarboxylation of *S*-adenosylmethionine (SAM) by *S*-adenosylmethionine decarboxylase (SAMDC) that yields decarboxylated SAM, which, in turn, donates its propyl amine moiety to form spermidine and spermine by two specific aminopropyl transferases, spermidine synthase and spermine synthase. The SAMDC is a pyruvoyl-containing decarboxylase, and its degradation is regulated by ubiquitination. On the contrary, spermidine and spermine synthase are constitutively expressed and are primarily regulated by the availability of their substrates.

Polyamine Catabolism. Spermidine/spermine *N*1-acetyltransferase (SSAT) is a propylamine acetyltransferase that monoacetylates spermidine and may form either mono- or diacetylates spermine. This inducible enzyme transfers an acetyl group from

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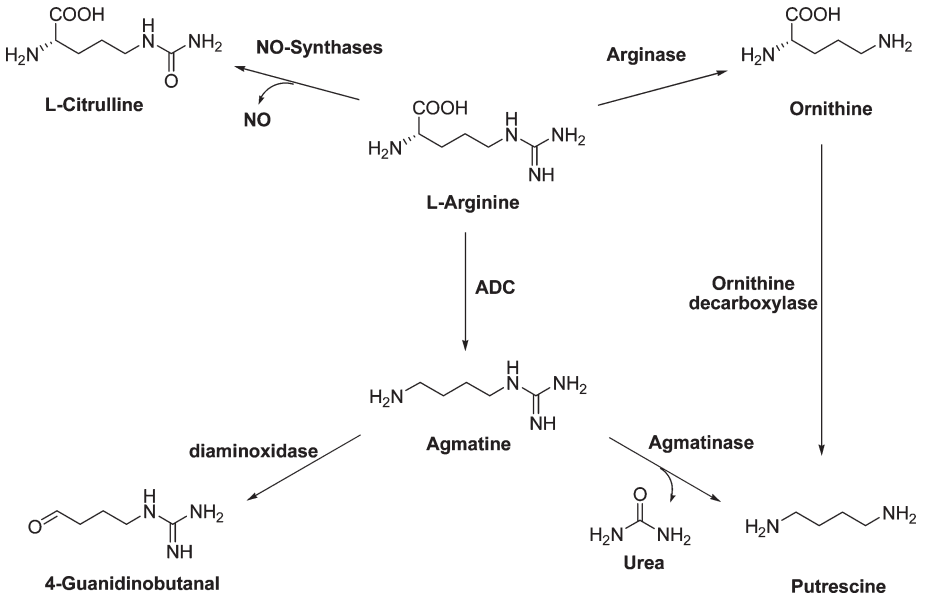

Figure 2. Agmatine metabolism.

acetyl-coenzyme A to the N1 position of spermidine or spermine. These acetylated polyamines have two potential fates. First, diamines and acetylated polyamines could be exported by the putative diamine transporter and eliminated in urine. Second, acetylated spermidine is also substrate for a flavin-dependent polyamine oxidase (PAO), which catalyzes its conversion back to putrescine. The reaction produces spermidine or putrescine, depending on the starting substrate, 3-acetoaminopropanal and H_2O_2. Recently, a new flavin-dependent enzyme has been characterized, a spermine oxidase (SMO) that can oxidize nonacetylated spermine to produce spermidine, 3-aminopropanal, and H_2O_2.[14]

2.1.1.2. Agmatine Metabolism. Biosynthesis. In bacteria and plants and fungi, AG arises enzymatically from the activity of arginine decarboxylase (ADC) on arginine (Figure 2). The presence of this enzyme in mammals has been debated for a long time. However, Li et al. in 1994 succeeded in identifying and characterizing mammalian agmatine by ion and molecular weight exclusion chromatography, high-pressure liquid chromatography and mass chromatography.[6a] In *E. coli*, ADC exist in constitutive (biosynthetic) and inducible (biodegradative) isoforms that are both cytosolic, and their activities are dependent on pyridoxal-phosphate and Mg^{2+}. In mammals, AG is synthesized by a mammalian form of ADC[15,16,6b] whose cDNA sequence has been recently identified.[16b] AG can be degraded by agmatinase in the brain[17] and by diamine oxidase in peripheral tissues.[18] The physiological role of agmatine in normal brain function is still unknown, in part because of the absence of adequate pharmacological tools to manipulate its synthesis and degradation. The mammalian ADC is probably associated with mitochondrial membranes and is able to decarboxylate both arginine and ornithine. ADC activity is inhibited by Ca^{2+}, Co^{2+}, and polyamines. The ADC activity is constitutive in mammalian cells and high in confluent cells, so it may serve as a constitutive source of polyamines.[6] In contrast to bacterial ADC, mammalian ADC uses ornithine in addition to arginine, whereby it is not a typical ornithine decarboxylase, because it is neither cytosolic nor inhibited by diflouromethylornithine, a universal and irreversible inhibitor of all isoforms of ornithine decarboxylase (ODC).[19] The optimum temperature of mammalian ADC is 30 °C. At the optimum bacterial temperature of 37 °C, the enzyme activity of the mammalian ADC is only one-third as active as it is at 30 °C. Only the optimum pH (8.25) is similar between mammalian and bacterial ADC.[6,20]

Catabolism. AG is degraded by two distinct ways depending on the tissue where it is contained:

- By diamine oxidase (DAO) in peripheral tissues (kidney), which catalyzes the degradation to guanidinobutyraldehyde (guanidinobutanal), which is finally excreted from the body. The heterogeneous location of DAO suggests that certain tissues or organs may have the capacity to regulate local agmatine levels.[16a]
- By agmatinase activity (agmatine ureohydrolase), which catalyzes the formation of urea and putrescine, or by agmatine deaminase. Agmatinase is the only enzyme specific for agmatine catabolism. The enzyme requires Mn^{2+} as cofactor in the active site and possesses a mitochondrial target sequence in the N-terminal of the protein.[20] Gilad et al. obtained the first evidence of a breakdown of agmatine to putrescine in rat brains.[21]

2.1.1.3. Organ Distribution of Agmatine. AG has been demonstrated in almost all organs of the rat, whereby the highest concentrations were found in the stomach. In lower concentrations it was found in the lungs, vas deferens, adrenals, kidneys, heart, liver, skeletal muscles, and brain.[20] At the subcellular level, AG was found to be localized mainly in large dense core vesicles in the cytoplasm and in the immediate vicinity of the endoplasmatic reticulum and the mithocondria.[22]

2.1.1.4. Agmatine Action in Polyamine Metabolism. AG regulates polyamine metabolism by acting on different enzymes involved in the polyamine pathway. It competitively inhibits nitric oxide synthase (NOS), which catalyzes the following reaction:

$$\text{L-arginine} + \text{NADPH} + H^+ + O_2 \rightleftarrows \text{citrulline} + \text{NO} + \text{NADP}^+$$

This evidences an important role in modulating NO production as an endogenous regulator.[23] Particularly, AG irreversibly

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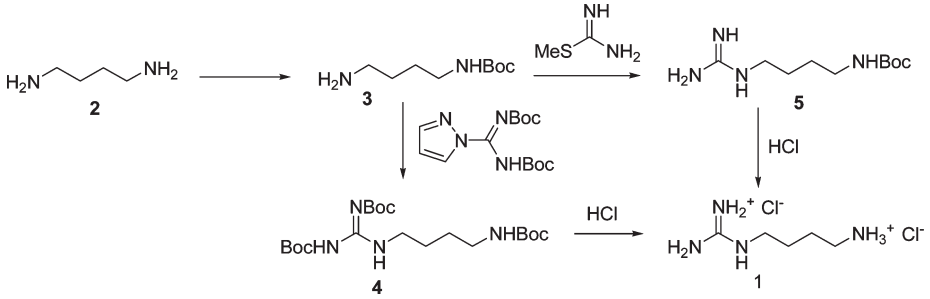
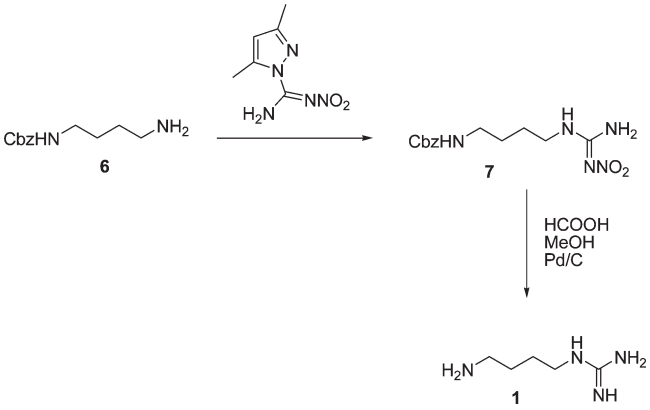
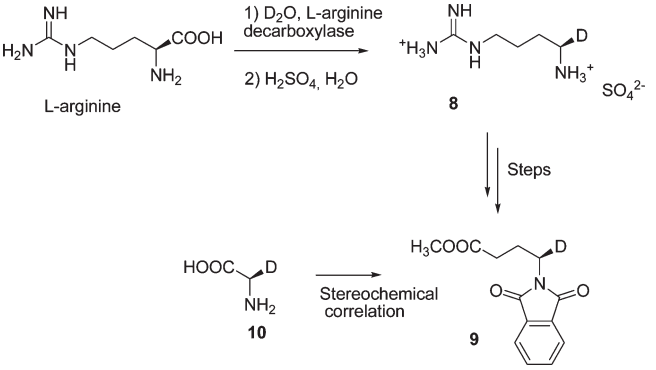
Scheme 1. Synthesis of Agmatine Hydrochloride



Scheme 2. Synthesis of Agmatine from Nitroagmatine



Scheme 3. Synthesis of Agmatine via Enzymatic Process



inhibits the neuronal NOS (nNOS) and downregulates the inducible form (iNOS), exhibiting a neuroprotective role.[24] AG induces SSAT and antizyme of ODC, which, as mentioned previously, inhibits both enzyme activity and polyamine transporter. Finally, it is metabolized by agmatinase to form urea and putrescine; hence, it is considered a polyamine precursor.

2.1.1.5. Biological Functions of Agmatine. Central Nervous System. AG binds to *N*-methyl *D*-aspartate (NMDA) receptors and acts as an antagonist at NMDA receptor channels. NMDA antagonists are known to block opioid withdrawal symptoms.[25,26] AG also binds to α2-adrenergic receptors.[6a,20] However, several studies reported that AG is not an agonist at this site. AG is considered as one of the endogenous ligands for imidazoline receptors and seems to have a role in depression because various findings indicate a potential pathophysiological role of the imidazoline binding site in the development of depression. Halaris et al.[24] found raised agmatine concentration in the plasma of depressed patients.

Cardiovascular Properties of Agmatine: Interaction with Neurohumoral System (NO, Sympathetic Nervous System). AG is a weak competitive inhibitor of various NO synthase (NOS) isoenzymes. The clearest effect is inhibition of iNOS, while less inhibition of endothelial NO synthase (eNOS) was observed.[23] This effect seems to contribute to vasodilatation. The stimulation of presynaptic α2-receptors leads also to vasodilatation. Hence, AG is able to reduce blood pressure in rats.[27,28]

Cellular Growth Processes. Because polyamines participate in DNA replication and cellular proliferation, it was a plausible hypothesis that AG might also be involved in cellular growth processes.[29] It has been speculated that, due to AG rapid metabolism to putrescine, the antiproliferative effects of AG could be attributed to other catabolism polyamines. However, it seems that influence of AG in the cell growth might be due to two distinct pathways. First, AG acts to stimulate an antizyme that induces the reduction of intracellular putrescine, a well-known proliferation promoter. Second, AG stimulates the spermidine/spermine acetyltransferase (SSAT), the key rate-limiting enzyme for polyamine intraconversion, and simultaneously inhibits *S*-adenosylmethionine decarboxylase (SAMDC), an enzyme that also has a modulating effect on intracellular polyamine content.

Metabolic Effects. It has long been known that AG increases insulin release from rat pancreatic islets of Langerhans cells, glucose uptake into the isolated rat respiratory diaphragm, glucose oxidation in isolated fat cells, and the glycogen content in the respiratory diaphragm. These activities seem to be correlated to the interaction of AG to specific imidazoline binding sites of the pancreas. AG has also proved to induce a moderate increase in insulin secretion.[30] However, it seems that AG might enhance insulin secretion via its metabolites, such as the amines putrescine and spermidine, which are necessary for proinsulin biosynthesis.

2.1.2. Natural Sources of Agmatine. Being that AG is one of the products of the metabolism of L-arginine, it is not surprising to find it in variable amounts in plants and foods. In addition to Kossel's early discovery, in 1910 AG was also found by Engeland and Kutscher in ergot. A few years later, in 1924, Kiesel reported the presence of this aminoalkylguanidine in ears of rye that were free from ergot.[31,32] Later, AG was isolated from

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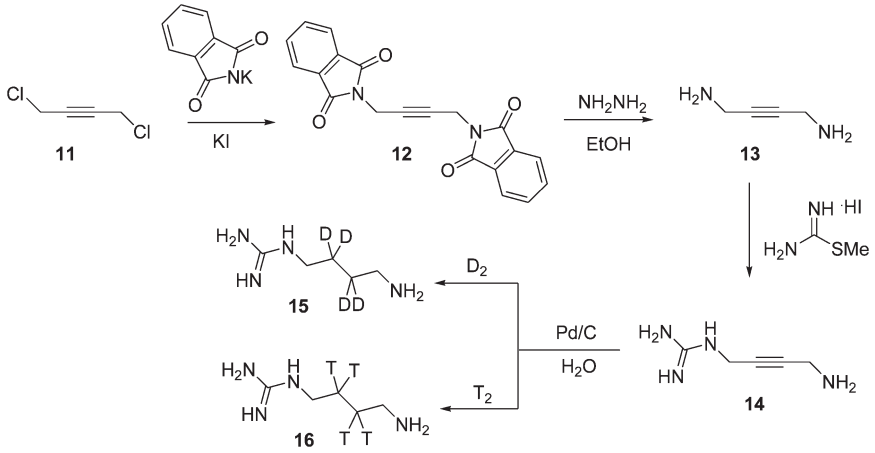
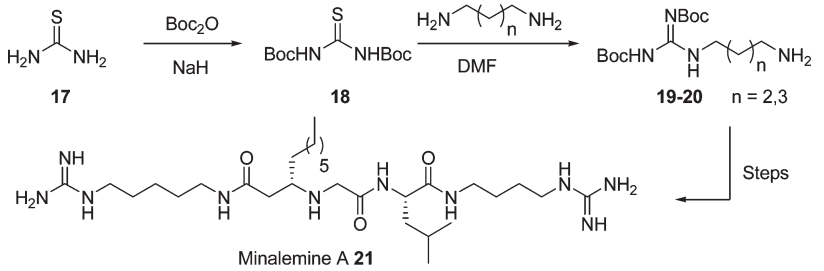
Scheme 4. Synthesis of Labelled Agmatine



Scheme 5. Synthesis of Minalemine A21



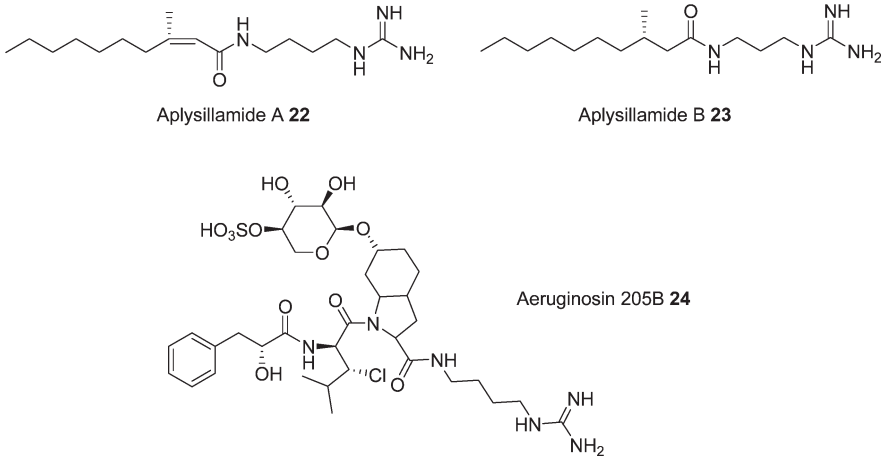


Figure 3. Natural products containing agmatine motif.

a few invertebrate sources such as *Hymeniacidon caruncula*, *Halichondria panacea*,[33] and, notably, the sponge *Geodia gigas*,[34] and from several cephalopods.[35] A study by Kawabata et al. in 1978 revealed that relatively high concentrations of AG could be detected in fresh abalone and top-shell muscles ranging from 40 to 200 mg/kg. Both shellfishes belong to gastropods, which contain large amounts of arginine instead of creatine as phosphagen. Maybe the AG could be formed by arginine decarboxylase present in the muscles of shellfishes.[36] More recently, AG was detected in meat products in low levels, ranging from 0.9 to 7.9 mg/kg,[37] and its production was observed also during the storage of fresh anchovies.[38] Finally, AG, together with other biogenic amines, was found in beer in relatively low levels (<2 mg/L).[39]

2.1.3. Synthesis of Agmatine. Agmatine sulfate is commercially available (Sigma). However, because of its mode of

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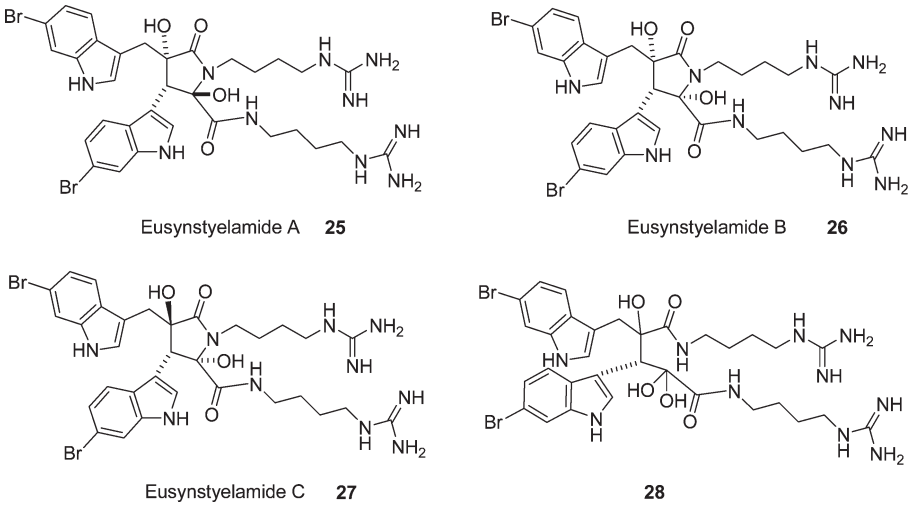
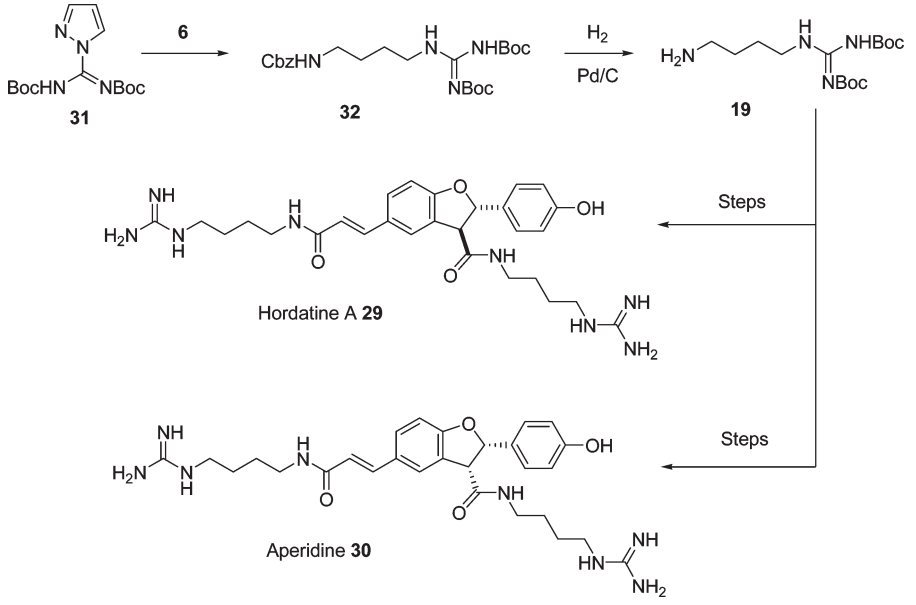


Figure 4. Structures of Eusynstyelamides A−C.

Scheme 6. Synthesis of Hordatine A and Aperidine



preparation, AG might contain small amounts of L-arginine (L-Arg).[40] Mansuy and co-workers reported a synthetic procedure that allowed one to obtain agmatine without any trace of L-Arg.[41] The 1,4-diaminobutane **2** was selectively protected with *tert*-butoxycarbonyl (Boc) protecting group, affording amine **3**, which was in turn monoguanylated using *N*,*N*′-bis(*tert*butyloxycarbonyl)pyrazole-1-carboxamidine. The protected agmatine **4** was then converted into the hydrochloridic salt of **1** by removal of Boc groups in methanolic HCl. (Scheme 1).

A similar approach was reported by Beria et al.[42] This synthetic procedure differs from the previous one for the use of *S*-methylisothiourea hydroiodide as guanylating agent, which led to the formation of the intermediate **5** (Scheme 1). Another approach to synthesize AG was reported by Golding et al. through the use of 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide (DMNPC). (Scheme 2). The benzyloxycarbonyl (Cbz)-diaminobutane **6** was guanylated to afford the guanidine derivative **7**. The nitro group was removed through catalytic transfer hydrogenolysis with HCOOH. The use of the nitroguanidine group as a masked guanidine offers the advantage of low basicity and easy chromatographic purification. This favors a multistep synthesis since nitroguanidines can be easily carried through several stages of a synthesis before their final reduction to guanidines.[43]

Enzymatic approaches to the synthesis of AG have been described. Richards and Spenser reported in 1982 an in depth study of the mechanism of decarboxylation of L-arginine by L-arginine decarboxylase from *E. coli*. The authors demonstrated in their study that the catalyzed reaction, which involves the conversion

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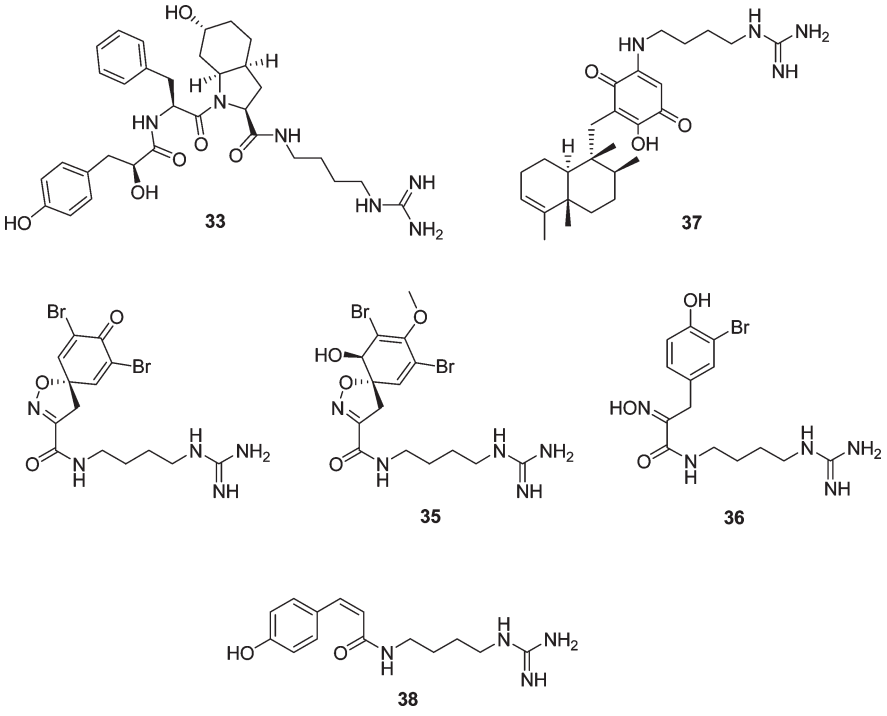

Figure 5. Further natural products containing agmatine motif.

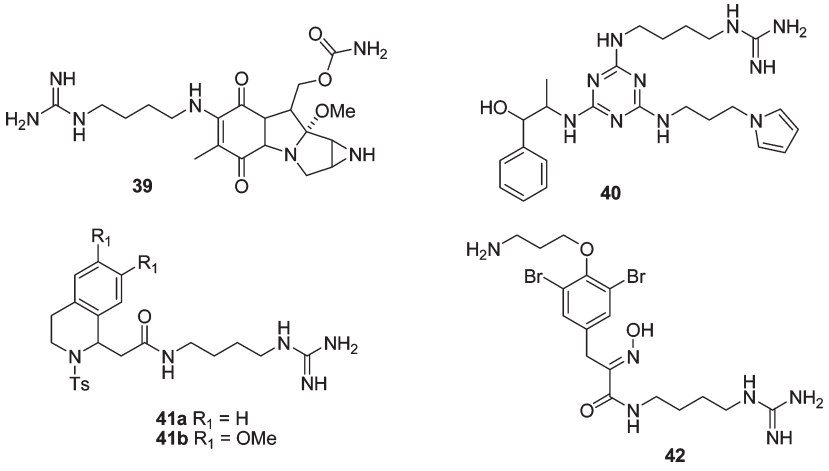

Figure 6. Synthetic compounds containing agmatine motif.

of a chiral center within the L-α-amino acid $RCH(NH_2)COOH$ into a prochiral stereocenter with the corresponding amine RCH_2NH_2, occurs through a stereochemically defined course. The conversion of L-arginine into AG takes place with net retention of configuration. L-arginine was converted into *R*-(−)-(1-^2H)agmatine sulfate **8** by reaction with L-arginine decarboxylase in deuterium oxide. Agmatine **8** was converted in 3 steps into (−)-methyl-*R*-4-phthalimido-(4-^2H)butyrate **9**, whose configuration was determined by correlation with an authentic specimen of **9**. The absolute configuration of **9** rests on the stereochemical correlation with that of *R*-(2-^2H)glycine **10**. The authors demonstrated through this strategy that the catalyzed decarboxylation of L-arginine to give AG proceeds with net retention of configuration[44] (Scheme 3).

Laboratory biosyntheses of AG using arginine decarboxylases from different fonts have been also described. Panagiotidis et al. described the formation of AG from arginine decarboxylase from *E. coli* strain K-12,[45] while Prasad and Adiga used for the same purpose arginine decarboxylase purified from cucumber (*Cucumis sativus*) seedlings.[46] Finally, an interesting synthesis of tritium and deuterium labeled AG has been recently described. Radiolabeled AG could represent a unique tool to identify molecular targets and dissect metabolic pathways. Moreover, it could be useful in the evaluation of pharmacological mechanisms and for pharmacokinetic studies.[47] The synthesis starts from 1,4-dichloro-2-butyne **11**, which was coupled with potassium phthalimide to yield derivative **12**. Hydrazinolysis of **11** with hydrazine in EtOH led to diamine **13**, which was in turn monoguanylated with *S*-Me-isothiourea sulfate in water at room temperature, leading to **14** in 70% yield. Finally, treatment of **14** with deuterium gas or tritium gas

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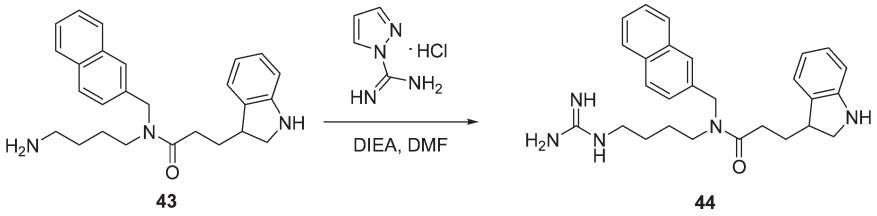
Scheme 7. Guanylation of Primary Amine with 1*H*-Pyrazole-1-carboxamidine



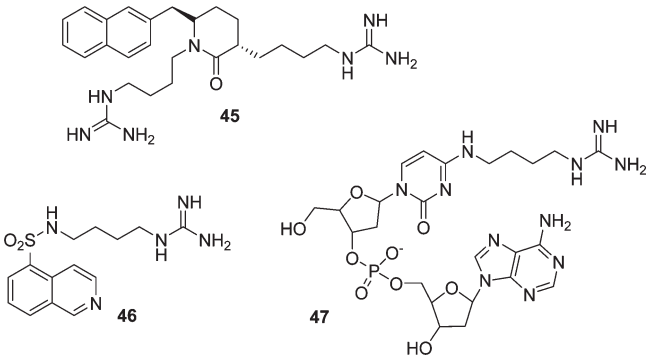
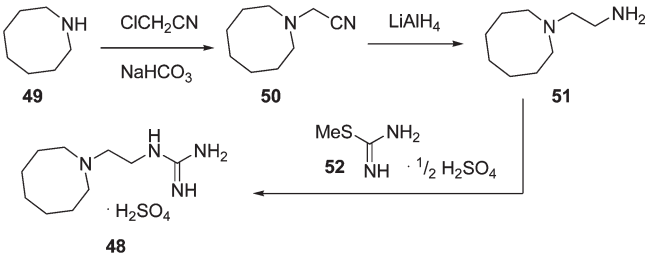


Figure 7. Drugs containing agmatine motif.

Scheme 8. Synthesis of Guanethidine



in the presence of Pd/C led to the desired labeled compounds **15** and **16**, respectively. (Scheme 4).

2.1.4. Agmatine Motif in Natural Compounds. Being that agmatine is a direct biosynthetic derivative of arginine, it is easy to find it as a part of the structure of some natural compounds and in particular of natural peptides. Commercial or synthetic AG is used in the total synthesis of many natural products. Minalemine A **21** is a small natural peptide containing AG motif. Moreover, a homoagmatine motif is also present in the molecule. The total synthesis of Minalemine A was recently described (Scheme 5). The di-Boc-agmatine **19** and the di-Boc-homoagmatine **20** were used as synthons in the total synthesis and obtained through reaction of a large excess of diaminobutane (putrescine) or diaminopentane (cadaverine) and di-Boc-thiourea **18**. This latter guanylation reaction proceeded under mild conditions, affording **19** and **20** in 91−92% yield. The following coupling reaction led to natural compound **21**.[48]

Other examples of natural products containing the AG motif are represented by Aplysillamides A and B (**22**−**23**), new antimicrobial guanidine alkaloids isolated from the Okinawan marine sponge *Psammplysilla pure*,[49] and glycopeptides Aeruginosins 205A and 205B, endowed with high serine protease inhibitory activity and isolated from the cyanobacterium *Oscillatoria agardhii* (NIES-205)[50] (Figure 3). Total synthesis of Aeruginosin 205B **24** was recently reported by Hanessian and co-workers, who established the structure and the absolute configuration of **24**.[51] Recently, two novel biologically active short peptides, aeruginosins KY642 and KY608, and the known aeruginosin 98A containing AG motif were isolated from the cyanobacterium *Microcystis sp.* strain.[52]

Eusynstyelamides A, B, and C **25**−**27** isolated from ascidian *Eusynstyela latericius*[53] constitute an interesting class of AG containing natural products. Eusynstyelamide **28**, an optically active modified tryptophan−arginine dipeptide dimer, was reported, without specified configuration, by Ireland and co-workers from *E. misakiensis* in 1994[54] (Figure 4). Eusynstyelamides A−C exhibited inhibitory activity against neuronal nitric oxide synthase (nNOS). Compounds **25** and **26** displayed mild inhibitory activity toward *Staphylococcus aureus* (IC_{50} 5.6 and 6.5 mM, respectively) and mild inhibitory activity toward the C4-plant regulatory enzyme pyruvate phosphate dikinase (PPDK) (IC_{50} values of 19 and 20 mM, respectively). Total synthesis of Eusynstyelamide A (±) **25** was recently reported, and agmatine-di-Boc **19** was used to construct the guanidine portion.[55]

Hordatine A **29** was initially discovered in 1996 from barley as an antifungal compound, which was predominantly distributed in the shoots of seedlings.[56] Later, Hordatine A **29** and Aperidine **30** were isolated from beer as active ingredients, able to bind to muscarinic M_3 receptor.[57] In addition, these compounds have exhibited antagonist activity against the α_{1A}-adrenoceptor. The absolute stereochemistry of natural Hordatine A **29**, together with that of Aperidine **30**, was determined by the total synthesis of each enantiomer of **29** and **30**.[58] The Cbz-diaminobutane **6** was guanylated by the use of guanylating agent **31**. Selective deprotection of **32** by hydrogenolysis gave di-Boc-agmatine **19**, which was in turn used for the total synthesis of **29** and **30** (Scheme 6).

Other examples of AG containing natural compounds are represented by the aquatic peptide Microcin SF608 **33**,[59] the extracts of the sponge *Suberea clavata* compounds **34**−**36**,[60] and the Nakijiquinone H **37**, isolated from Okinawan marine sponges of the family *Spongiidae*.[61] The cis-*p*-Coumaroylagmatine **38** was isolated from *Albizzia julibrissin* Durazz as a bioactive substance for nyctinasty of trees. The cis-*p*-Coumaroylagmatine was synthesized from AG and cis-*p*-coumaric acid, which was prepared by means of photoisomerization of the commercially available trans-isomer. It was derivatized to the hydroxysuccinimide ester and then coupled with AG using dicyclohexylcarbodiimide (DCC)[62] (Figure 5).

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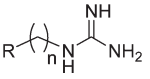
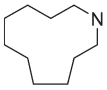
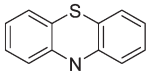
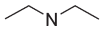
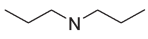
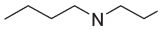
Table 1. Guanethidine Derivatives



Cmpd	R	n	Yield %	Cmpd	R	n	Yield %
48		2	74	**48g**		2	70
48a		2	53	**48h**		2	63
48b		2	83	**48i**		2	41
48c		2	80	**48j**		3	52
48d		3	82	**48k**		2	66
48e		4	66	**48l**		2	63
48f		2	70	**48m**		2	73

2.1.5. Agmatine in the Synthesis of Biological Active Compounds. Agmatine has been used by medicinal chemists in the synthesis of biological active compounds. The Mytomycin A derivative **39** containing AG[63] was described and assayed as antitumoral agent, proving, however, to not be active. Triazine derivative **40** was synthesized through the reaction of appropriate chlorotriazine and agmatine and evaluated for its antimalarial activity.[64] Compounds **41a**−**b** were evaluated as bradykinin-1 antagonists,[65] while compound **42** was evaluated as an inhibitor of mycothiol-*S*-conjugate amidase[66] (Figure 6).

AG derivative **44** was synthesized through the guanylation of amine **43** with 1*H*-pyrazole-1-carboxamidine hydrochloride **31** and was evaluated as mimetic of the melacortins' active core[67] (Scheme 7).

Other agmatine containing compounds are the chemokine receptor CXCR4 antagonist **45**,[68] the derivative **46** evaluated for its vasodilatory activity[69] and the dinucleotide **47** synthesized as anti-HIV-1 integrase inhibitor[70] (Figure 7).

Finally, a series of derivatives with different structures and biological activities, but all including AG backbone, have been described. These compounds were evaluated as inhibitors of furin and furin-like proprotein convertases,[71] inhibitors of the neuropilin-1 vascular endothelial growth factor A (VEGF-A) interaction,[72] vasopressin antagonists,[73] antimicrobial agents,[74] inhibitors of myeloid differentiation factor 88,[75] cysteine proteases inhibitors,[76] thrombin inhibitors,[77] hypoglycemic agents,[78] and finally Aeruginosin derivatives.[79]

2.2. Monoguanylated Diamines

Monoguanylated diamines are an attractive class of guanylated compounds of which agmatine is a member. These molecules are structurally quite simple, and due to their strict correlation with

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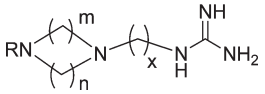
Table 2. Analogues of Guanethidine



compound	R	*m*	*n*	*x*	yield %
53a	H	2	2	2	60
53b	CH_3	2	2	2	51
53c	CH_3	2	2	3	80
53d	CH_2CH_3	2	2	2	90
53e	$HOCH_2CH_3$	2	2	0	66
53f	Ph	2	2	2	78
53g	*p*-Cl-Ph	2	2	2	70
53h	*o*-MeO-Ph	2	2	2	84
53i	Bn	2	2	2	81
53j	$PhCH_2CH_2$	2	2	2	68
53k	CH_3	2	3	2	75
53l	Ph	2	3	2	51
53m	CH_3	3	3	2	54
53n	Ph	3	3	2	56

natural polyamines, asparagines and agmatine possess several and different biological properties. From a synthetic point of view, the main challenge in the synthesis of monoguanylated diamines is represented by the selective introduction of only one guanidine moiety into a diamine backbone. Several strategies have been reported for the synthesis of monoguanylated derivatives, most of them based on a selective guanylation reaction on a diamine. In the case of monoguanylated diamines, the choice of an appropriate guanylating agent could represent sometimes a key factor, allowing the introduction of particular substituents and favoring the purification/isolation procedures. The earliest reports that described the synthesis of monoguanylated diamines appeared during the 1960s. These compounds were generally synthesized by medicinal chemists because of their biological properties, in particular cardiovascular and antimicrobial properties, starting from commercial appropriate diamines. The guanylating agents of choice at that time were the *S*-alkylisothioureas and the *O*-alkylisoureas, namely, the reactive forms of thioureas and ureas. Nucleophilic addition of an amine on *S*- or *O*-alkyliso-(thio)ureas leads to the formation of a guanidine with elimination of *S*-alkyl or *O*-alkyl leaving groups. These reactions are generally catalyzed by a base. However, in some cases, the use of thioureas activated by metals (generally Hg salts) as guanylating agent was preferred, mostly depending on the substrate to be synthesized. In a few other cases, the use of different guanylating agents in the synthesis of monoguanylated diamines was reported, mainly related to the particular structural properties or reactivity of the substrates to be guanylated. On the other hand, the regioselective monoguanylation of diamines can be achieved through the shrewd use of a protecting group strategy or by the use of solid-phase synthesis. Herein, monoguanylated diamines will be classified on the basis of the methodologies used for their synthesis.

2.2.1. Synthesis of Monguanylated Diamines with *S*-Alkylisothioureas or *O*-Alkylisoureas. The first example of synthesis of a monoguanylated diamine was reported by Mull et al. in 1960 regarding the antihypertensive agent Guanethidine **48**.[80] Guanethidine is a monoguanylated ethylendiamine whose amine moiety is constituted by an octahydro-1-azocyne. The synthesis is reported in Scheme 8. Octahydro-1-azocyne **49** was first reacted with chloroacetonitrile to give the cyano derivative **50**. Subsequent $LiAlH_4$ reduction led to compound **51**, which has a diamine skeleton. The primary amine **51** was then guanylated with *S*-Me-isothiourea sulfate **52** in water at reflux, affording Guanethidine **48**. Accordingly, derivatives **48a**−**m** of guanethidine containing different amine moieties and having different length alkylic chains were synthesized (Table 1).

Following the same synthetic approach, the same authors reported a couple of years later the synthesis of a new series of guanethidine analogues **53a**−**n**, which were assayed as antihypertensive agents.[81] These compounds are reported in Table 2.

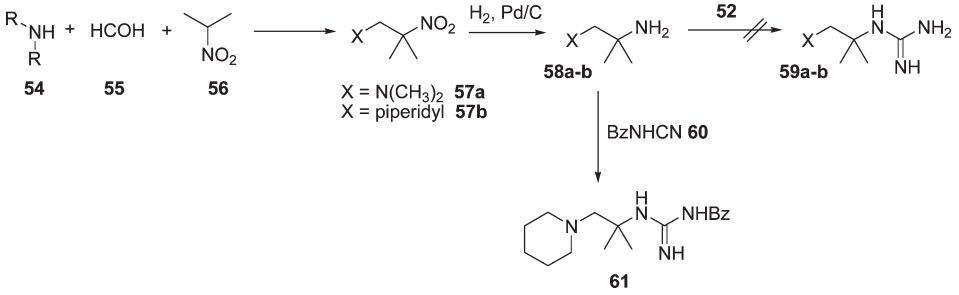
Attempts to synthesize similar monoguanylated diamines to those described above were described by Tsuji and Ueda in 1964.[82] The synthetic approach for the synthesis of compounds **59a**−**b** also provided the use of *S*-Me-isothiourea **52** as guanylating agent. Diamines **58a**−**b** were obtained in two steps via Mannich reaction followed by hydrogenation of the nitro group. However, in this case, the guanylation step did not afford the desired compounds **59a**−**b**, possibly due to the steric hindrance of diamines. To overcome this issue, a different strategy was applied based on the guanylation of **58b** with benzoylcyanamide **60**, affording compounds **61**. However, attempts to hydrolyze **61** to the desired **59b** were unsuccessful, and the starting amine **58b** was recovered from the hydrolysis step (Scheme 9).

The steric hindrance of diamines might represent a problem for guanylation or, on the contrary, favor monoguanylation over biguanylation of diamines. An in depth investigation on guanylation of steric hindered diamines has been reported.[83] When the diamine **62** was reacted with *S*-Me-isothiourea **52**, the monoguanylated diamine **64** was obtained and selective guanylation was observed. On the other hand, diamine **63** was not guanylated, and the authors recovered only the sulfate salt of diamine **65** from the reaction mixture. The authors hypothesized that the *N*1-amino group might catalyze the guanylation of the *N*2-amino group, and that the lack of this catalysis together with steric reasons would prevent the guanylation step. In the case of amine **63**, the *N*2-amino group is sterically hindered and does not react with *S*-Me-isothiourea. The *N*1-amino group, a tertiary amine, is not guanylated but reacts with *S*-Me-isothiourea sulfate, affording ammonium intermediate **63a**. The newly formed $RR'NH^+$ group is not able to catalyze the guanylation step on *N*2-amine, since the catalysis of the amino group is due to the lone pair of nitrogen atoms. Hence, the absence of *N*1-amine catalysis together with steric hindrance explains these results. On the other hand, in the case of amine **62**, the *N*1-amino group is a primary amine not sterically hindered. When **62** was reacted with *S*-Me-isothiourea, guanylated intermediate **62a** was obtained. However, at this stage, the *N*1-nitrogen is guanylated and protonated and cannot catalyze the guanylation of *N*2-aminogroup. Hence, in this case, steric hindrance favored a selective monoguanylation on *N*1-amine (Scheme 10).

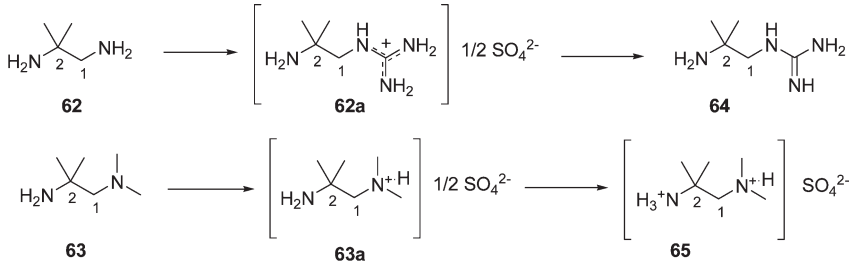
The use of monoacylated diamines was also described in the same paper to overcome biguanylation side reactions. Acyl diamines **66** and **67** were selectively guanylated with *S*-Me-isothiourea sulfate **52**. Hydrolysis of **68a**−**b** led to monoguanylated diamine **69**[83] (Scheme 11).

Monoguanylation of a diamine can be influenced not only by steric but also by electrostatic factors. An interesting paper in this

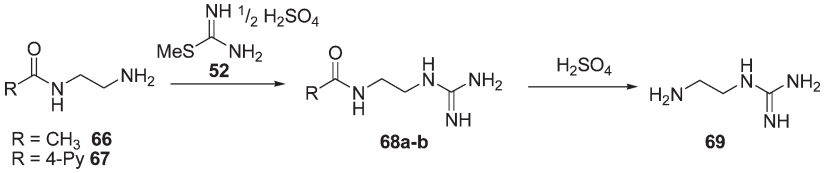
Scheme 9. Synthesis of Sterically Hindered Guanylated Diamines



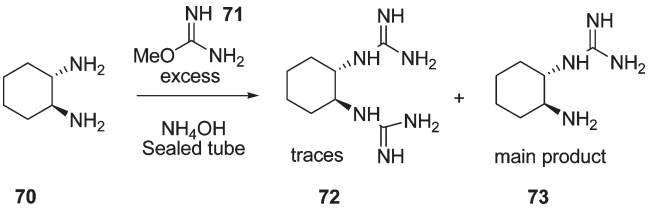
Scheme 10. Selective Guanylation of Hindered Diamines



Scheme 11. Monoguanylated Ethylendiamine



Scheme 12. Synthesis of Monoguanylated Cyclohexyldiamine



connection was reported in 1979 by Dietrich et al., who described the selective monoguanylation of cyclohexyldiamine **70**.[84] The authors reacted cyclohexyldiamine **70** with a large excess of *O*-Me-isourea **71** in the presence of aqueous ammonia as base with the aim to obtain the biguanylated diamine **72**. However, also by forcing the reaction conditions and performing the reaction in a sealed tube, they were able only to recover monoguanylated diamine **73** as the major product. Traces of biguanylated product **72** were detected (Scheme 12). The monoguanylation seems to be due primarily to electrostatic and not to steric factors. In fact, the same reaction carried out using 2-methyl-1-nitroisourea led to the full conversion of **70** into the biguanylated derivative (see the section on biguanylated diamines). Monoguanylated diamine **73** was obtained as the major product also when a large excess of *S*-Me-isothiourea **52** was used as guanylating agent under the same reaction conditions.

Hence, in principle, the synthesis of structurally simple monoguanylated diamines through direct guanylation of diamines with *S*-Me- or *O*-Me-iso(thio)ureas is a fast and easy approach. Monoguanylated diamines **74** and **1**, having different length alkylic chains, were synthesized in one step starting from commercially available alkyldiamines and *O*-Me-isourea sulfate in refluxing water. To overcome the formation of biguanylated side products, a half equivalent of *O*-Me-isourea was generally used. In certain cases, the use of Cbz-monoprotected diamines such as **75a**−**b** was preferred to overcome a biguanylation side reaction. Protecting group benzyloxycarbonyl (Cbz) was then removed by hydrogenolysis, leading to the desired monoguanylated derivatives **77a**−**b**[85] (Scheme 13).

However, examples of monoguanylation reaction by the use of equimolar amounts of diamine and guanylating agents also have

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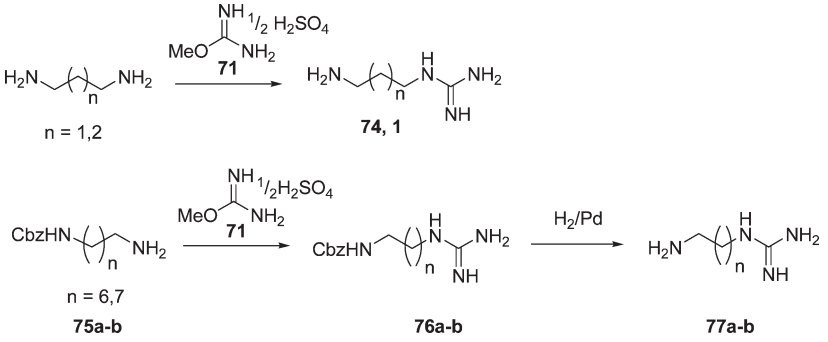
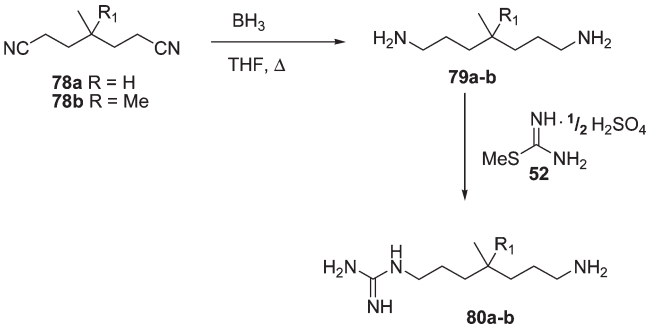
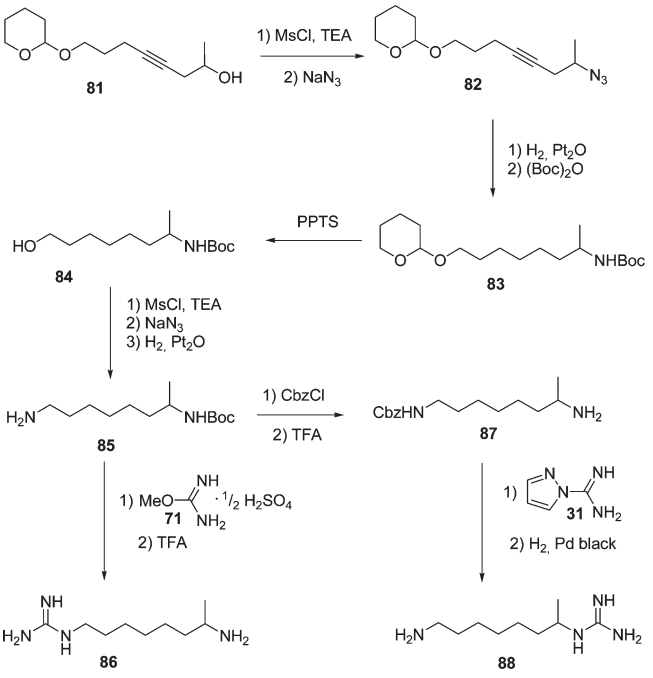
Scheme 13. Synthesis of Monoguanylated Diamines with *O*-Me-isourea



Scheme 14. Synthesis of Monoguanylated Diamines Using *S*-Me-Isothiourea



Scheme 15. Synthesis of Monoguanylated Unsymmetric Diamines



been described. Lee and Folk reported in 1998 the synthesis of a series of 1,7-diaminoheptane derivatives as inhibitors of the deoxyhypusine synthase, some of them presenting a monoguanylated diamine structure.[86] The synthesis of some of these derivatives is outlined in Scheme 14. The pimelonitriles **78a**−**b**, prepared from the appropriate bromides by reaction with NaCN, were converted into diamines **79a**−**b**, which were in turn monoguanylated with an equivalent amount of *S*-Me-isothiourea sulfate **52**. The yields of this latter reaction are low, 43% (**80a**) and 27% (**80b**), respectively, due to the formation also of corresponding biguanylated side products. Monoguanylated diamines **80a**−**b**, their biguanylated derivatives, and unreacted diamines were purified by ion-exchange chromatography.

The same authors also demonstrated how it is possible to carry out the monoguanylation of an unsymmetric diamine through an appropriate protecting group strategy. *N*-Boc-diamine **85** bearing a methyl group at C1 of the alkylic chain was obtained in an 8-step sequence from alkyne **81**. At this stage, guanylation on the primary amine with Me-*O*-isourea **71** sulfate followed by Boc-deprotection lead to monoguanylated diamine **86**. Protection of primary amine **85** with CbzCl followed by selective deprotection of Boc-protecting group lead to amine **87**. This latter compound was finally guanylated with pyrazole **31** and deprotected by hydrogenolysis, affording **88**, the isomer of **86**[86] (Scheme 15).

On the contrary, the synthesis of monoguanylated diamines containing a secondary amino group does not present difficulties concerning biguanylation side reactions. An example is described in Scheme 16. Diamines **91** and **94**, synthesized from nitrile **90** or diamine **93**, respectively, contain both primary and secondary amino groups. Guanylation using 1 equiv of *O*-Me-isourea **71** occurred selectively on the less sterically hindered primary amine, leading to products **92** and **95**.[87]

A similar case is the synthesis of *N*-(4-diethylamino-1-methylbutyl)guanidine **97** (Scheme 17) through the reaction of 2-amino-5-diethylamino pentane **96** with *S*-methylisothiourea sulfate **52** in refluxing water.[88] This monoguanylated diamine was used to synthesize a small library of antimalarial and antitubercular agents **98a**−**e** with pyrimidine structure (Table 3). Reaction of guanidine **97** with appropriate solid-phase supported α,β-unsaturated carbonyl compounds followed by acidic cleavage led to the formation of pyrimidines **98a**−**e** in two steps.

S-Me-isothiourea was also employed as guanylating agent in the synthesis of analogues of natural compounds. The synthesis of natural amino acid arginine derivatives was recently described. These compounds were planned with the aim to

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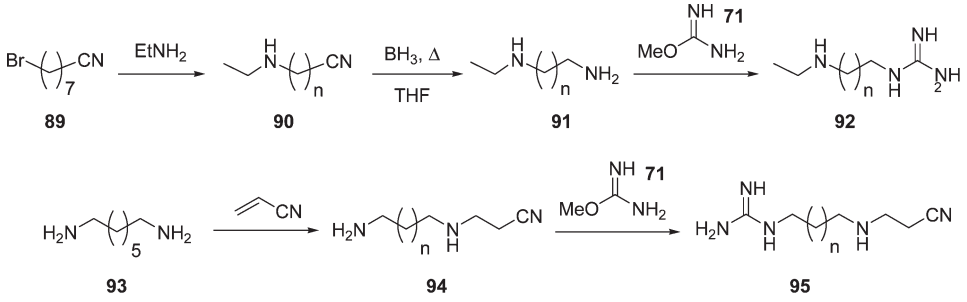
Scheme 16. Synthesis of Monoguanylated Diamines Containing a Secondary Amine



Scheme 17. Synthesis of Monoguanylated Diamines Containing Tertiary Amine

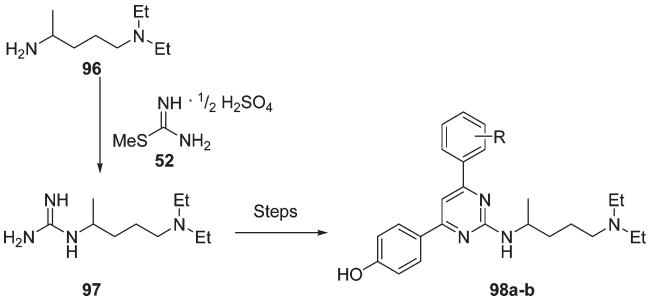


Table 3. Pyrimidines Derived from Monoguanylated Diamine 97

compound	R	compound	R
98a	H	**98d**	3,4-diOMe
98b	4-Me	**98e**	2,5-diOMe
98c	4-OMe		

be incorporated into pharmaceutically interesting polypeptides. Arginine derivatives **100a**−**d** were obtained from *N*-Me-lysine **99** through guanylation with *S*-Me-isothioureas **101a**−**d** as reported in Scheme 18. Cyclic *S*-Me-isothioureas **103a**−**b** were also used, affording derivatives **102a**−**b**. Guanylation reactions were carried out in 2N NaOH for 9 days, and the desired monoguanylated derivatives were obtained in 40−90% yields.[89] Purification of highly polar products was accomplished by chromatography on 50 × 8 Dowex ion-exchange resin with NH_4OH elution.

The synthesis of monoguanylated diamines as building blocks for the synthesis of natural product Distamycin A analogues was described by Beria et al. Particularly, the authors reported the synthesis of analogues of the anticancer agent Brostallicin **104** (Figure 8), an analogue of Distamycin A containing a monoguanylated ethylendiamine motif.[42]

Commercially available diamines were first protected with Boc-anhydride, and the resulting compounds **105a**−**c** were guanylated with the appropriate *S*-Me-isothiourea hydroiodide in refluxing ethanol. Deprotection of Boc derivatives **106a**−**c** with methanolic HCl led to the desired monoguanylated diamines **107a**−**c**, which were in turn used for the synthesis of Brostallicin analogues (Scheme 19). Deprotection of **106c** with $SnCl_4$ constitutes an alternative approach.[90]

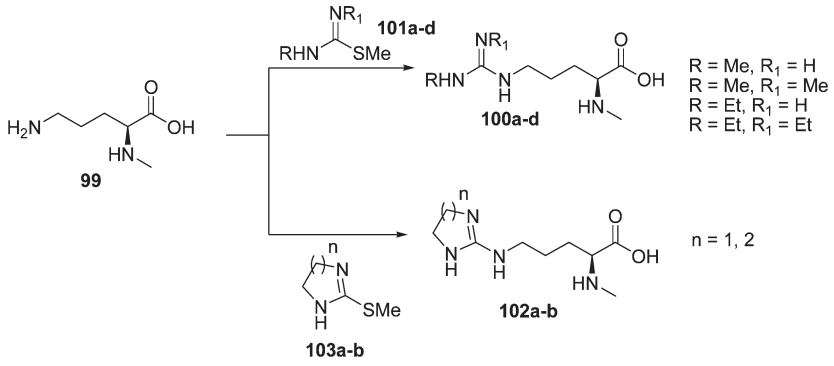
From the early 1990s, many chemists started to use the di-Boc-*S*-Me-isothiourea as guanylating agent in the synthesis of guanidines.[91] The use of this reagent offers advantages especially with regard to purification procedures. Guanidines and in particular molecules containing both a guanidine and an amine moiety are highly polar compounds, hard to handle and to purify, being sometimes also soluble in water. The use of di-Boc-isothioureas in multistep syntheses allows the introduction of the guanidine moiety into molecules in the early stages of the synthetic pathway without purification problems. The Boc protecting group can then be removed in the final step of the synthesis. The use of di-Boc-*S*-Me-isothiourea as guanylating agent in the synthesis of monoguanylated diamines was widely documented in several papers by Botta and co-workers in the last two decades. Starting from 1992, the research groups of Delle Monache and Botta were involved in the isolation and characterization of novel monoguanylated diamines from the extracts of the Venezuelan plant *Verbesina caracasana* fries.[92,93] The compounds obtained from the extracts of the plant were named **G1**−**G7** according to the chromatographic elution order and were characterized through NMR spectroscopy and electron ionization mass spectroscopy (EI-MS).[94] The structures of the compounds are reported in Figure 9.

To confirm the assigned structures, the authors reported the syntheses of **G1**−**G7** compounds. All the derivatives (with the exception of **G7**) have a monoguanylated diamine core with a 4-carbon alkyl chain. In the case of **G1**, **G2**, and **G5**, the amino moiety is protected as an amide, and a prenyl moiety is bound to the guanidine group. On the contrary, **G3** showed a free primary amine. The key step for the syntheses of **G1**−**G7** derivatives was represented by the guanylation of 1,4-diaminobutane (or protected 1,4-diaminobutane) with di-Boc-*S*-Me-isothioureas. The synthesis of **G3** (prenylagmatine) is reported in Scheme 20.[95]

Commercially available *N*,*N*′-di-Boc-*S*-Me-isothiourea was first alkylated affording the prenyl derivative **108**, which was in turn reacted with 1,4-diaminobutane to give the desired monoguanylated diamine **109** in 70% yield. The use of an excess of diamine (2.6 equiv) was fundamental to overcome the formation of the diguanylated product. The guanylation reaction was carried out at 50 °C in aqueous tetrahydrofuran (THF). Finally, Boc deprotection with CH_3SO_3H afforded **G3** as mesyl salt, which was purified by Dowex 1 × 2−200 ion-exchange resin, first followed by a further purification by a column of Sephadex LH-20 preswelled in MeOH. This purification procedure was

dx.doi.org/10.1021/cr100423x |*Chem. Rev.* 2011, 111, 5247–5300

Scheme 18. Synthesis of Arginine Derivatives through Guanylation of Diamine 99



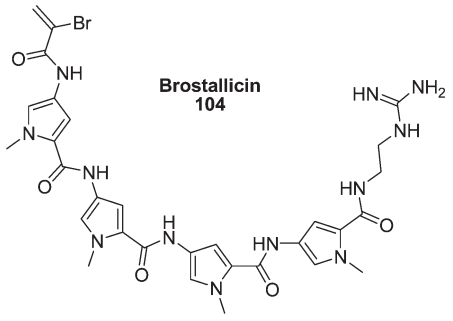


Figure 8. Structure of Brostallicin.

required by the high polarity of the compound, which cannot be purified by simple silica gel column chromatography. Prenylagmatine **G3** was also obtained from hydrolysis of **G2** (caracasandiamide) in KOH as reported in Scheme 21.[96,97] Component **G2** was named caracasandiamide because of the presence of two prenylagmatine motifs bound to a cyclobutane core through amide bonds. The determination of the structure of **G2** was made by ^{1}H NMR and ^{13}C NMR studies, which revealed spectra similar to that of **G1**. Moreover, a fast atom bombardment mass spectrometry (FABMS) analysis revealed a molecular peak at *m*/*z* 777, which led to the hypothesis of a cyclobutane structure. Hydrolytic and synthetic studies were employed to determine the structure of **G2**. A second hydrolysis of cyclobutane **110** led to the formation of a second prenylagmatine molecule and the cyclobutane derivative **111**. The structure of this latter compound was finally confirmed by synthesis from cinnamoyl derivative **112**.[96]

The synthesis of **G1** and **G5** was similar to that reported for **G3**.[98,99] *S*-Me-isothioureas-di-Boc **108** and **113** were reacted with 1,4-diaminobutane, affording desired guanylated diamines **109** and **114**. Then, the primary amines were reacted with the 3,4-dimethoxycinnamic acid to afford amides **115** and **116**, which were converted into **G1** and **G5**, repectively, after acidic Boc deprotection[95] (Scheme 22).

Because of the good activity of **G1** and **G5** as hypotensive agents, the same authors reported the synthesis of a series of analogues of these latter compounds. The **G1** derivatives **120a**−**f** have a prenyl moiety bound to the guanidine group, whereas the amine portion is protected as amide. The guanylated diamine portion was synthesized as reported in Scheme 22. Primary amine **109** was coupled with appropriate acyl chlorides to afford derivatives **119a**−**f**. The Boc deprotection was accomplished with CH_3SO_3H (Scheme 23). Compounds **120g**−**h** have a longer alkylic chain than **G1** and were obtained following the same synthetic procedure starting from precursors **117**−**118**. These latter compounds were obtained through the coupling of prenylated *S*-Me-isothiourea with 1,5-diaminopentane and 1,6-diaminoheptane, respectively.[100]

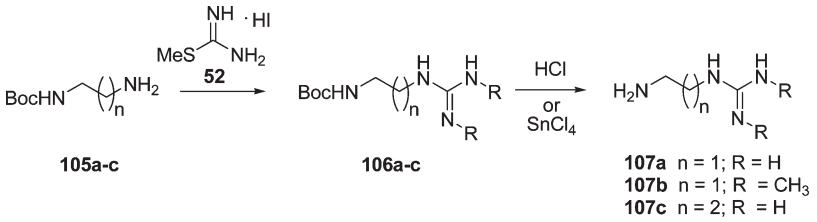
The synthesis of a series of **G5** analogues, which differ from the lead compound for the length of the alkylic chain, was accomplished as reported in Scheme 24. The Boc-protected monoguanylated diamines **122a**−**e** were obtained through the reaction of the appropriate diamines with commercially available di-Boc-*S*-Me-isothiourea **121** at 50 °C. Then, cinnamoylation and Boc deprotection led to **G5** derivatives **124a**−**e**.[95]

The synthesis of **G3** analogues was also reported. These compounds were planned to be assayed as polyaminooxidase inhibitors (PAO) and differ from **G3** for the alkyl substituent on the guanidine moiety in place of the prenyl group and in some cases for the length of the alkylic chain. For the synthesis of **127**, a series of alkylated *S*-Me-isothiureas **125** were first synthesized and then reacted with 1,4-diaminobutane or appropriate diamines. Acidic deprotection of the Boc group with methansulfonic acid led to desired compounds (Scheme 25). The derivative **127l** shows a phenylmethyl chain instead of the classic alkyl chain.[101] This compound was obtained by the reaction of *S*-Me-isothiourea with (4-(Boc-amino)methyl)aniline. Structures of compounds **127** are reported in Table 4.

Intermediates **126a**−**b** (propargyl and allyl) were further manipulated and used as precursors for the synthesis of a new series of nitric oxide synthase (NOS) inhibitors. Monoguanylated diamines **126a**−**b** were reacted with carbonate **128**, affording the NOS inhibitor−NO donating drugs (NINOD) **129a**−**b**. Removal of the Boc group was carried out with anhydrous trifluoroacetyl (TFA), giving **130a**−**b**[102] (Scheme 26).

As a further development of previous works, Botta and coworkers reported the synthesis of monoguanylated diamines endowed with hypoglycemic properties. These monoguanylated diamines differ for the substituents bound to the amine or the guanidine portion and for the length of the alkyl chain. Different approaches were used for the synthesis of these compounds.[103] In some cases, the authors obtain desired compounds using *S*-Me-isothiourea-di-Boc as guanylating agent. A first series of compounds (**132**, **134**, **136**, and **138**, Table 5) having different substituents on the guanidine moiety and a primary amino group

Scheme 19. Use of $SnCl_4$ in the Synthesis of Monoguanylated Diamines



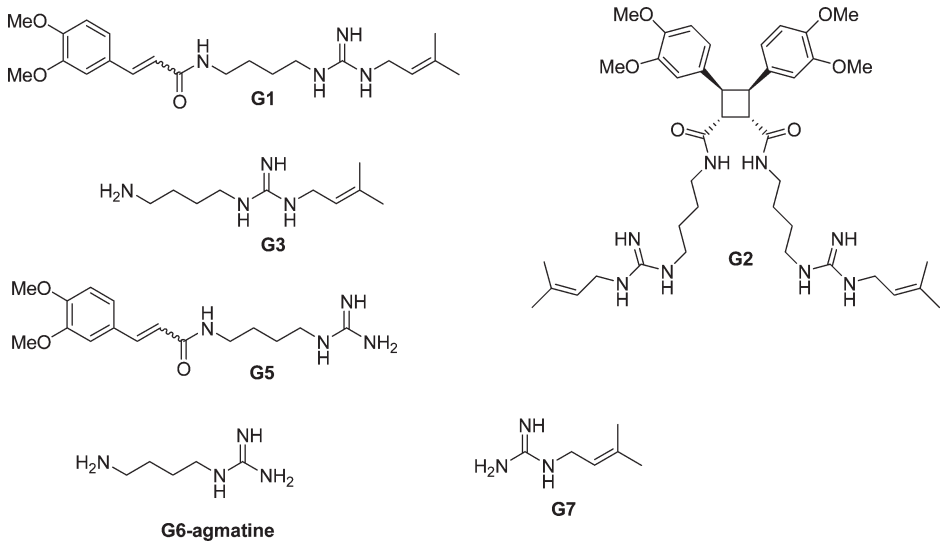
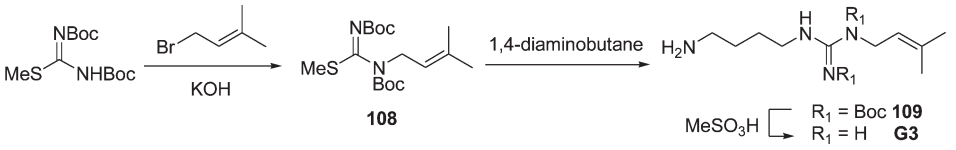


Figure 9. Structures of monoguanylated diamines from the extracts of *Verbesina caracasana* fries.

Scheme 20. Synthesis of G3



is illustrated in Scheme 27. Some of these compounds are particularly interesting from a structural point of view, since the classic alkyl chain was replaced by cycloalkyl or benzyl chains. Monoguanylation of different commercially available diamines was accomplished with substituted di-Boc-*S*-Me-isothioureas in THF at 50 °C.

The *S*-methylisothiourea-di-Boc was also used by Linney et al. for the synthesis of a series of monoguanylated diamines **142** and **149**, evaluated as histamine H_3-receptor antagonists.[104] These compounds show a general structure A (Table 6) where benzyl or alkyl groups are bound to the guanidine moiety and the amine portion is a cyclic tertiary amine (pyrrolidine, piperidine, azepane, or octahydro-1-azocine). The synthetic routes (*pathways a*−*c*) are reported in Scheme 28. Compounds **142** were synthesized through reaction of benzyl isothioureas **139** with (3-aminopropyl)pyrrolidine in refluxing THF, followed by deprotection of Boc with 4 M HCl/dioxane solution. Compound **144** bearing a *p*-chlorophenyl on the guanidine portion was obtained from *S*-Me-isothiourea **143** and (3-aminopropyl)pyrrolidine in refluxing ethanol.

On the other hand, the monoguanylated diamines **149** were obtained through a longer synthetic pathway. The guanylating agent **121** was alkylated with the appropriate amine (3-buten-1-amine, 4-penten-1-amine, or 5-hexen-1-amine) to give thioureas **145**. Then, reaction of these latter compounds with appropriate amines in refluxing THF led to the formation of the guanidine moiety (compounds **146**). Ozonolysis of the terminal double bond followed by reductive amination with secondary amines led to the formation of **148**, which were converted into the desired monoguanylated diamines **149** by HCl mediated Boc cleavage.

2.2.2. Synthesis of Monoguanylated Diamines with Activated Thioureas and Carbodiimides. Thioureas are common reagents for the synthesis of guanidines. However,

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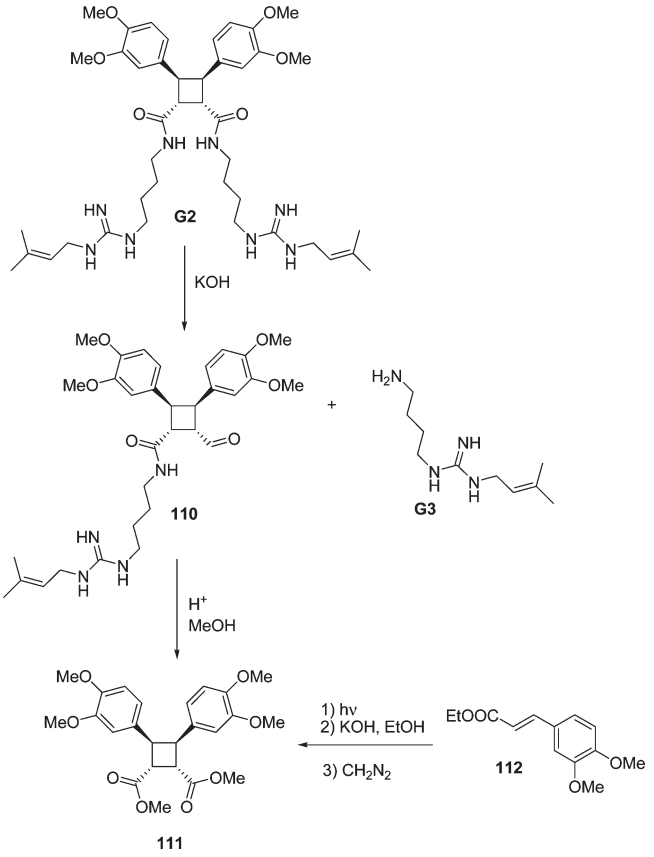
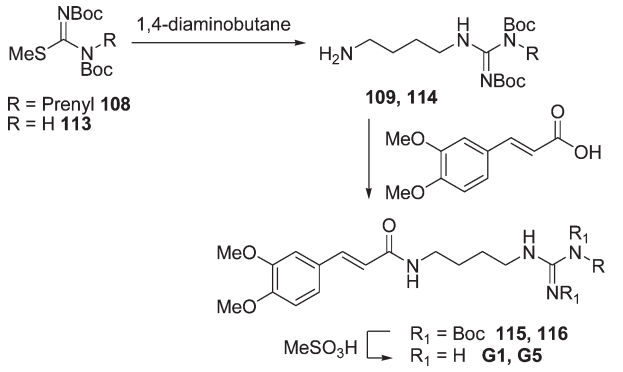
Scheme 21. Determination of the Structure of G2



Scheme 22. Synthesis of G1 and G5



thioureas are unable to react directly with amines and produce guanidines. Usually, the conversion of a thiourea into a guanidine requires an initial activation, generally achieved with Cu(II) or Hg(II) salts or with the Mukaiyama reagent (2-chloro-*N*-methylpyridinium iodide). Activation of thiourea led to the formation of the corresponding carbodiimide, which in turn reacts with an amine, leading to guanidine. Alternatively, the synthesis of guanidine can be accomplished directly through the reaction of an amine with an appropriate commercially available carbodiimide.

The use of carbodiimides generated from thioureas is generally required for the synthesis of sterically hindered monoguanylated diamines. As an example, in Scheme 29 the synthesis of a series of monoguanylated diamines **154a**−**e** by the use of thiourea in the presence of $HgCl_2$ is outlined. Compounds **154** bear a benzyl moiety on the internal nitrogen N3 of the guanidine group. Benzyl amine **150** was alkylated with the appropriate dibromoalkane. Then the bromine derivatives **151** were alkylated with pyrrolidine to form intermediates **152a**−**e**. Removal of Boc protecting group led to secondary amines **153a**−**e**, which were in turn guanylated with thiourea in the presence of $HgCl_2$.[104] Secondary amines do not react or react slowly with guanylating agents such as alkyl-*S*-isothioureas or alkyl-*O*-isoureas. On the contrary, guanylation of secondary amines with thioureas/Hg(II) system proved to be very efficient, and desired compounds were obtained in good yields (>65%).

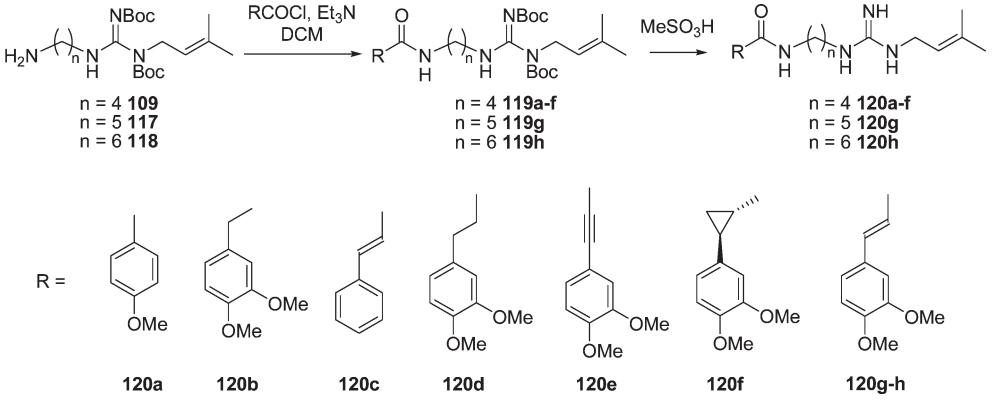
An interesting synthesis of a series of monoguanylated diamines, where one of the guanidine nitrogens is part of a piperidine or a piperazine ring, was recently described by Botta and co-workers.[103] Also in this case, the use of carbodiimide as guanylating agent was chosen because of the peculiarity of the target compounds, whose synthesis proved to be not possible with alkylated isoureas or isothioureas reagents. The synthesis is outlined in Scheme 30. The Boc-diaminohexane **155** was first reacted with *p*-*F*-phenylisothiocyanate affording thiourea **156**, which was converted into the stable carbodiimide **157** using the Mukaiyama reagent. Reaction of **157** with piperidine or *N*-Me-piperazine led to desired compounds **158a**−**b**, which were in turn deprotected with TFA and isolated as triflic salts **159a**−**b**. Similarly, monoguanylated diamines **162a**−**b** were synthesized as reported in Scheme 30. The *S*-Me-isothiourea-di-Boc was activated with $HgCl_2$ and then reacted with piperidine or *N*-Me-piperazine, leading to di-Boc-guanidine **160a**−**b**. The reaction likely proceeded through a carbodiimide intermediate. Mitsunobu reaction of **160a**−**b** with appropriate *N*-Boc-amino alcohol led to guanylated diamines **161a**−**b**, which were then converted into desired compounds **162a**−**b** by acidic removal of Boc.

However, the use of activated thiourea as guanylating agent is popular also in the synthesis of structurally simple monoguanylated diamines such as the monoguanylated diamine cationic amphiphiles **165a**−**c**, which is shown in Scheme 31. Diamines **163a**−**c** were reacted with di-Boc-thiourea in the presence of $HgCl_2$ and Et_3N, leading to **164a**−**c**. This latter compound was first methylated with MeI, deprotected with TFA, and finally purified using chloride ion-exchange chromatography over Amberlist A-26 chloride ion-exchange resin affording monoguanylated ethylendiamines **165a**−**c**, which bear a lipid portion.[105]

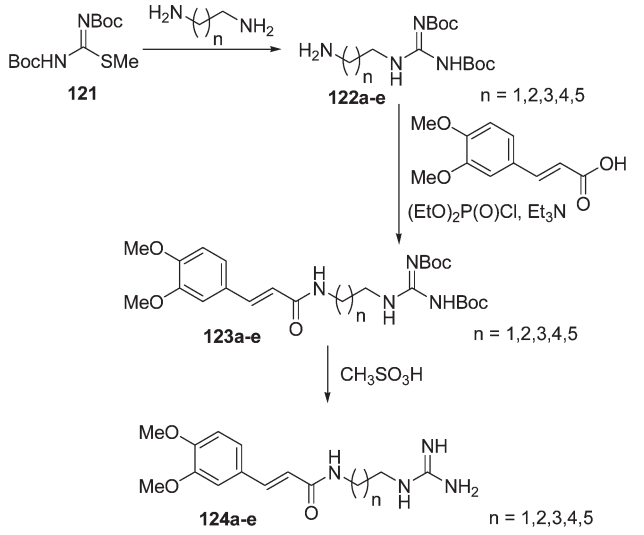
Similarly, the superbasic monoguanylated diamines **169** and **173** were obtained via carbodiimide as reported in Scheme 32. Appropriate thioureas **166** and **170** were converted into corresponding carbodiimides **167** and **171** through reaction with yellow HgO followed by vacuum distillation. Then reaction of carbodiimides with the primary amines **168** and **172** led to desired compounds **169** and **173a**−**b**, which were purified by distillation under vacuum. The pK_a values of these compounds range between 24.7 and 27.2, proving that these guanidines have a basicity similar or higher than the superbase N^1,N^1,N^3,N^3-tetramethylguanidine (TMG).[106]

Finally, noteworthy is the synthesis of compounds **176** and **179**, which can be considered as monoguanylated diamines having a phenyl group in place of the alkylic chain. In this case, the use of a stronger guanylating agent such as carbodiimide is

Scheme 23. Synthesis of G1 Derivatives



Scheme 24. Synthesis of G5 Derivatives



necessary due to poor nucleophilicity of aniline nitrogens. The di-Boc-thiourea **174** or the cyclic thiourea **177** were activated in the presence of $HgCl_2$. Final products were purified with Amberlyte resin[107] (Scheme 33).

2.2.3. Synthesis of Monoguanylated Diamines via Alkylation of tri-Boc-Guanidines (or tri-Cbz-Guanidines). The synthesis of monoguanylated diamines through alkylation of protected guanidines with alkyl alcohols or alkyl halides sometimes represents a valid option, especially in those cases where the lack of commercially available diamines makes impossible the use of previously described methodologies. The previously mentioned paper by Botta and co-workers on hypoglycemic agents describes the synthesis of some monoguanylated diamines **183** through the reaction of tri-Boc-guanidine with *N*-Boc-amino alcohols via Mitsunobu reaction[103] (Scheme 34, Table 7). The use of tri-Boc-guanidine **180** instead of *S*-Me-thioureas was preferred in this case because of the higher yields or the availability of substrates.

Similarly, the synthesis of compound **187** bearing a phenylpropyl moiety on guanidine through the use of tri-Cbz-guanidine **184** was reported (Scheme 35). Mitsunobu reaction of tri-Cbz-guanidine with cinnamic alcohol led to guanidine **185**, which was in turn reacted with 4-(*N*-Boc)amino butanol through a second Mitsunobu alkylation. Deprotection of **186** by hydrogenolysis led to the desired compound **187**.[103]

2.2.4. Synthesis of Monoguanylated Diamines with Other Guanylating Agents. The guanylating agent 1*H*-pyrazole-1-carboxamidine, already seen for the synthesis of agmatine (Scheme 1), has been recently used for the synthesis of monoguanylated diamine lipids **188a**−**c** (Scheme 36). The reaction proceeds under very mild conditions, affording desired compounds in only one step. The absence of toxic mercuric salts makes the use of 1*H*-pyrazole-1-carboxamidine highly preferable in comparison with thioureas.[105]

An intriguing synthetic approach to monoguanylated diamines was that used in the synthesis of the natural compound Smirnovine **191**, a prenyl guanidine isolated from *Smirnovia turkestana* Bge.[108] and later from *Galega orientalis* Lam. cv. Gale. Prenyl diamine **189** was prepared according to literature.[109] However, attempts to guanylate **189** with *S*-Me-isothiourea failed. Hence, compound **189** was first reacted with phenyl cyanate, affording intermediate **190**, which upon ammonolysis followed by treatment with picric acid afforded Smirnovine picrate **191**[110] (Scheme 37).

Trifyl guanidines, first reported in 1998, are becoming popular reagents for the preparation of guanidines.[111] Di-Boc-trifylguanidine **192** is efficiently converted by various primary amines into guanidines. The use of triflic guanidine **192** for the synthesis of a series of di-Boc-monoguanylated diamines **193a**−**h** was reported by the authors of this review. The use of triflic guanidine was preferred due to the mild reaction conditions (low temperature), which allowed one to obtain desired compounds in higher yields with respect to the use of di-Boc-*S*-Me-isothiourea[112] (Scheme 38).

All the compounds were obtained in ≥90% yields. However, noteworthy is the behavior of 1,3-diaminopropane in the reaction with triflic guanidine **192**. Compound **193b** was never isolated because it spontaneously cyclized into the stable 6-membered guanidine **194**.

2.2.5. Macrocyclization Reaction of di-Boc-Monoguanylated Diamines. The previously mentioned synthesis of Boc compounds **193a**−**h** was part of a work aiming to investigate the behavior of these substrates under heating conditions and to study their conversion into macrocyclic amidinoureas.[112] As reported previously, many authors synthesized

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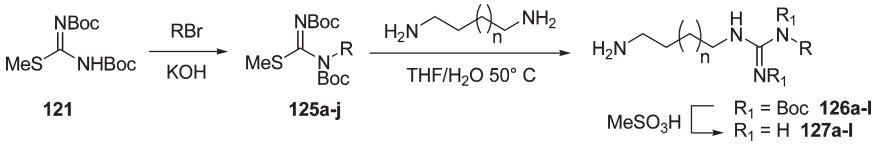
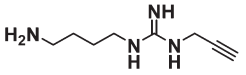
Scheme 25. Derivatives of G3 as NOS Inhibitors



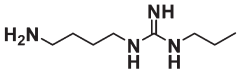
121 125a-j

Table 4. G3 Derivatives

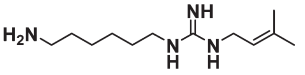
127a



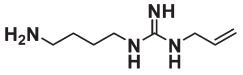
127e



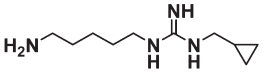
127i



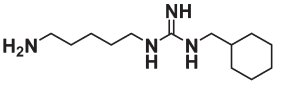
127b



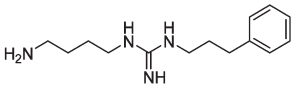
127f



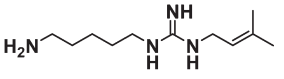
127j



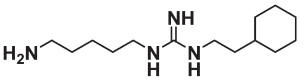
127c



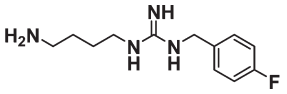
127g



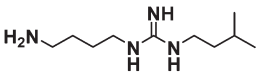
127k



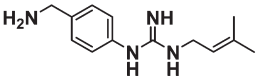
127d



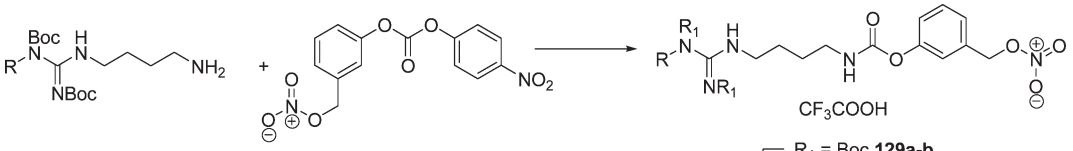
127h



127l



Scheme 26. Synthesis of NOS Inhibitor−NO Donating Drugs (NI-NOD)



126a-b 128

monoguanylated diamines by the use of *S*-Me-isothiourea-di-Boc **121**. Guanylation reactions were accomplished generally by heating the appropriate amine and the di-Boc-*S*-Me-isothiourea, leading to desired compounds in ~70% yields. However, it was observed that, by carrying out this guanylation reaction at ≥70 °C, an intramolecular cyclization of **193** occurred due to the nucleophilic attack of the primary amine on the carbonyl moiety of the Boc protecting group, leading to macrocycles **195** (Scheme 39).

The formation of the macrocycles **195** is strictly dependent on the length of the alkylic chain of monoguanylated diamines.

Table 5. Compounds 132, 134, 136, 138

compound	R	*n*	compound	R
132a	cyclopropylmethyl	5	**134**	γ,γ-dimethylallyl
132b	γ,γ-dimethylallyl	6	**136**	cyclopropylmethyl
132c	isopentyl	5	**138**	γ,γ-dimethylallyl

Compounds **193c−d** with 4−5 carbon chain length led to formation of dimers **196c−d**, whereas compounds **193e−l** with 6−9 carbon side chains led to a mixture of macrocycles and

dx.doi.org/10.1021/cr100423x |*Chem. Rev.* 2011, 111, 5247–5300

Scheme 27. Monoguanylated Diamines with Cyclic Alkyl Chain

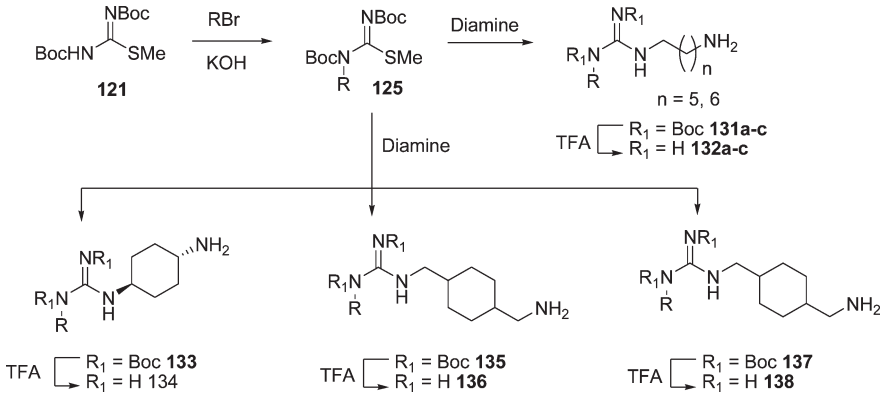
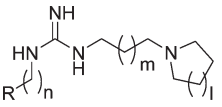


Table 6. Structures of Compounds 142 and 149



General Structure A

compound	R	*l*	*m*	*n*	compound	R	*l*	*m*	*n*
142a	Ph	1	1	1	**149c**	4-Cl-Ph	1	3	1
142b	4-Br-Ph	1	1	1	**149d**	4-Cl-Ph	1	1	2
142c	3-Br-Ph	1	1	1	**149e**	4-Cl-Ph	1	2	2
142d	2-Br-Ph	1	1	1	**149f**	4-Cl-Ph	1	3	2
142e	4-I-Ph	1	1	1	**149g**	cyclohexyl	1	3	1
142f	4-CF_3-Ph	1	1	1	**149h**	cyclohexyl	1	3	1
142g	4-MeO-Ph	1	1	1	**149i**	1-adamantyl	1	3	1
142h	4-Cl-Ph	1	1	1	**149j**	1-adamantyl	2	3	1
149a	4-Cl-Ph	1	1	0	**149k**	1-adamantyl	3	3	1
149b	4-Cl-Ph	1	2	1	**149l**	1-adamantyl	4	3	1

dimers. It is interesting also the behavior of the ethylendiamine derivatives **193a**. This compound when heated at 70 °C was directly converted into the stable 5-membered cyclic guanidine **199**.

2.2.6. Solid-Phase Synthesis of Monoguanylated Diamines. Solid-phase synthesis is an attractive alternative in the synthesis of polyamines and guanidines. Examples of monoguanylated diamines solid-phase synthesis also have been reported in the literature. The advantage of this approach is represented by the absence of chromatographic purifications, which in some cases proved to be tedious and troublesome due to the high polarity of guanylated diamines.

The earliest example of solid-phase synthesis of the Boc-monoguanylated diamine **203** was reported by Dodd and Wallace in 1998 by the use of the Merrifield resin as solid support. Reaction of Merrifield resin with thiourea led to the supported thiourea **200**, which was in turn converted into Boc-protected **201**. Alkylation of **201** with *N*-Boc-aminopropanol via Mitsunobu reaction led to thiourea **202**. Then, the cleavage from the resin with methanolic ammonia led to desired compound **203**[113] (Scheme 40).

A similar approach was reported by Mioskowski and co-workers in 2000. The synthetic approach is described in Scheme 41. Merrifield resin was first reacted with CS_2 and primary amines, affording dithiocarbamates **204**. These latter compounds were converted into chlorothioformamidines **205**, which were stable intermediates, easy to be prepared and handled. Reaction with an amine led to thiourea **206**. It is noteworthy that no double amine addition occurred at this stage. Finally, addition of a second amine (or diamine) led to desired monoguanylated derivatives **207a**−**c**.[114]

A solid-phase approach with the Rink amide resin for the synthesis of the monoguanylated diamines **210a**−**b** is reported in Scheme 42. The supported thioureas **208a**−**b** were reacted with the appropriate diamine, leading to monoguanylated diamines **209a**−**b**. Cleavage from the resin was accomplished with TFA.[115]

An interesting solid-phase approach to three monoguanylated propandiamines has been reported in 2005 by Corelli and co-workers. The authors focused on the use of dimethylpyrrole as the protecting group of the primary amine, which could be formed by the reaction of the appropriate diketone with amine. Moreover, this protecting group, masking completely the amine moiety, prevents biguanylation side reactions. The TantaGel S-NH_2 (TG), a standard type of resin used for peptide synthesis, solid-phase organic synthesis, and combinatorial chemistry, was chosen as the solid support because it swells well even in aqueous solvent.[116] (Scheme 43). The TG **211** was transformed in 5 steps into the supported diketone **212**, which was reacted under microwaves irradiation with aminopropanol to give the desired dimethylpyrrole **213**. Mitsunobu reaction of **213** with *S*-Me-isothiourea-di-Boc **121** led to **214**, which was in turn reacted with an appropriate amine, affording the supported compounds **215a**−**c**. Treatment of **215a**−**c** with NH_2OH led to the cleavage of the pyrrole and to the formation of desired monoguanylated diamines **216a**−**c**. Similarly, compound **218**, a monoguanylated diamine having a phenyl in place of the alkylic chain, was synthesized. The diketonic resin was regenerated at the end of the process.

2.3. Biguanylated Diamines

Biguanylated diamines are compounds possessing two guanidine moieties spaced by an alkyl or aryl chain. Several examples of biguanylated diamines are reported in the literature. Most of these compounds are symmetrical, possessing two identical guanidine moieties spaced by a simple alkyl chain. However, examples of unsymmetrical biguanylated diamines are known,

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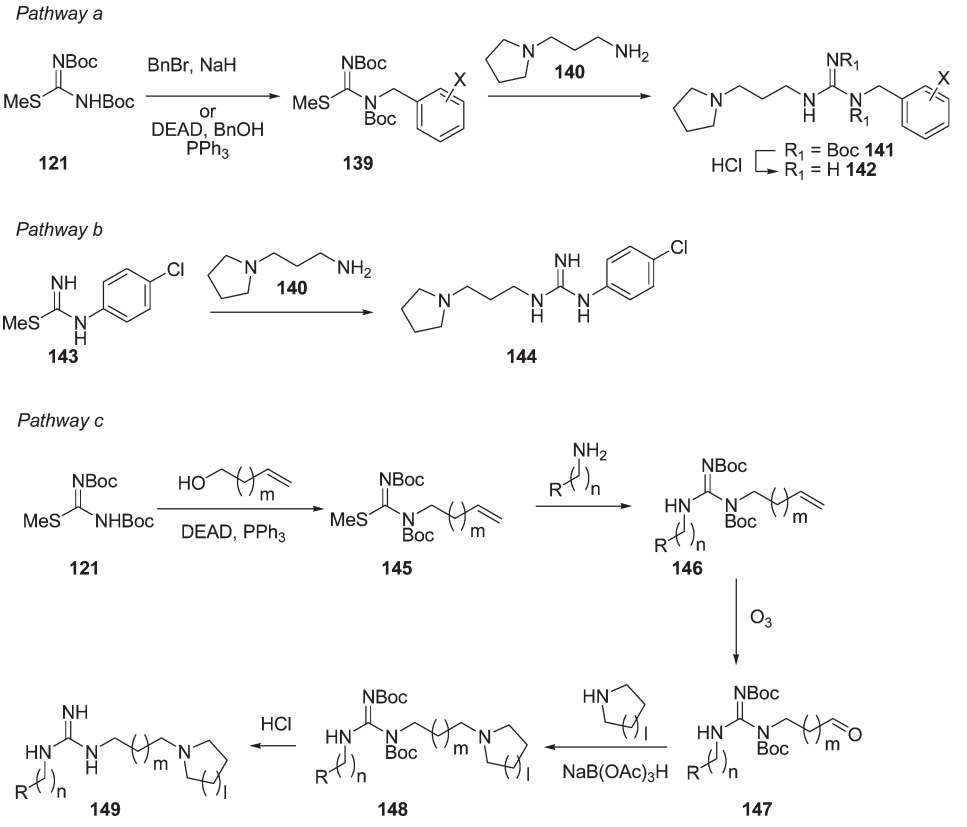
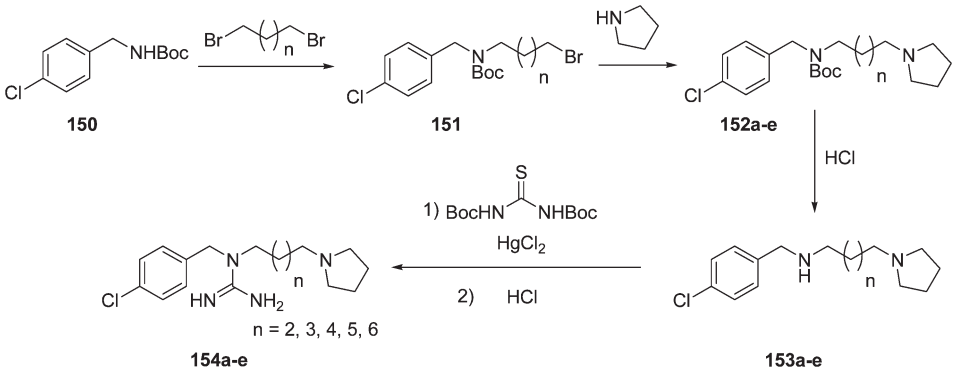
Scheme 28. Different Strategies for the Synthesis of Monoguanylated Diamines Containing a Pyrrolidine Moiety



Scheme 29. Synthesis of Monoguanylated Diamines with di-Boc-Thiourea and $HgCl_2$



often representing an attracting synthetic challenge. From a synthetic point of view, these compounds could be obtained using the same procedures used for monoguanylated diamines, namely, guanylation reactions of diamines with ureas, thioureas, or carbodiimides.

2.3.1. Symmetric Biguanylated Diamines. One of the first examples of biguanylated diamines is represented by the natural compounds Synthalin A and B, which were isolated in 1926 from *Galega officinalis*. Synthalin was an oral antidiabetic drug, marketed in Europe by Schering AG of Berlin as a synthetic drug with insulin-like properties and orally administered. However, it was toxic to the liver and kidney and was withdrawn from the market in the early 1940s. The first synthesis was reported in 1929 by Slotta and Tschesche through guanylation of diamines with *S*-Et-isothiourea dibromide.[117,118] Recently, the synthesis of Synthalin A was also reported by Mourer et al. during their studies on *p*-guanidinoethyl calixarenes evaluated as antibacterial agents. However, from these studies emerged that synthalin A possesses low antibacterial activity unlike guanidinocalixarenes containing only one guanidine moiety[119] (Scheme 44).

After the pioneering work of Slotta, Rose, and Swain reported in 1956, one of the first examples of biguanylated diamines synthesis was identified. The synthesis starts from hexamethylene diisothiocyanate **219**, which was reacted with *p*-Cl-aniline in

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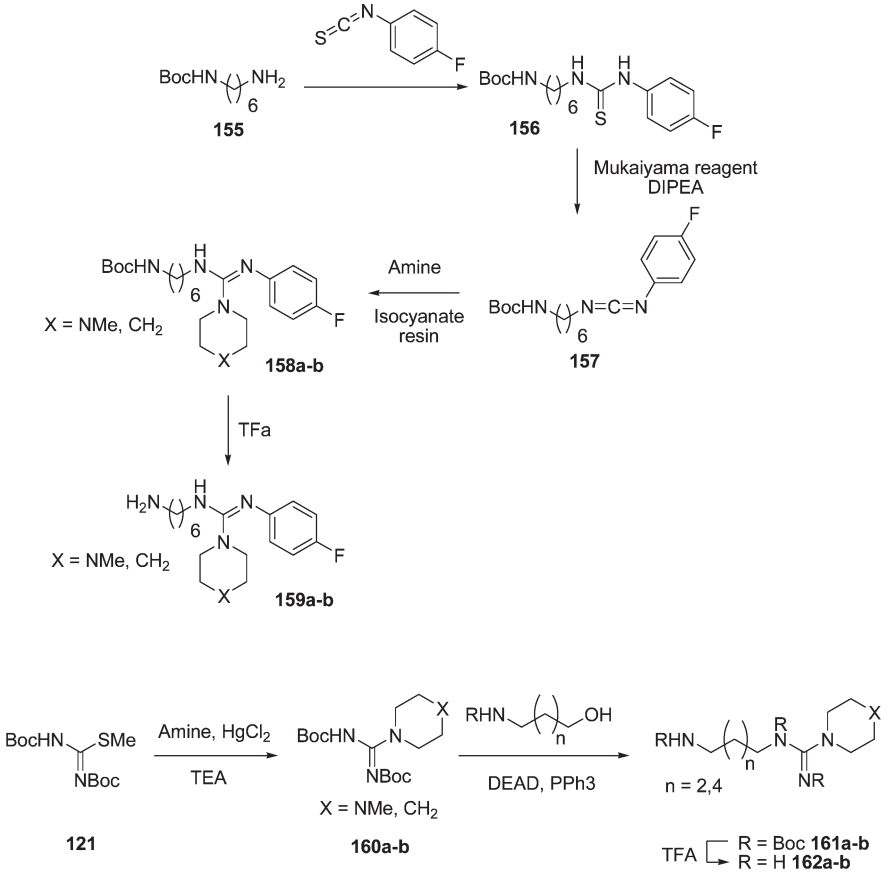
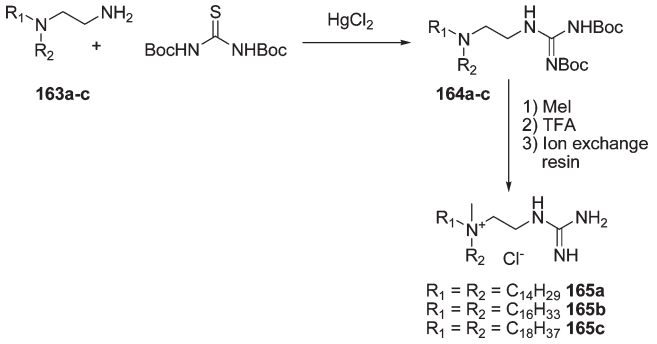
Scheme 30. Monoguanylated Diamines Modified at Guanidine Portion



Scheme 31. Monoguanylated Diamine Cationic Amphiphiles



boiling ethanol to give **220**. Following amination in alcoholic ammonia in the presence of HgO led then to desired compounds **221**[120] (Scheme 45).

Another early example of biguanylated diamines is the synthesis of **72** having a cyclohexyl chain.[84] This compound was synthesized to be evaluated as anion complexone of phosphate and carboxylated anions. The synthesis is reported in Scheme 46. Reaction of **70** with the reactive nitroguandine **222** led to biguanylated product **223** together with the cyclic side derivative **224** (10%). Attempts to reduce the formation of **224** were unsatisfactory. Removal of the nitro group through hydrogenolysis in acidic solution led to desired compound **72** in 30% overall yield. The low overall yield could be overcome in principle by the use of *S*-Me-isothiourea or *O*-Me-isourea as guanylating agent. In fact, the reaction of ethylendiamine and 1,3-diaminopropane with *S*-Me-isothiourea or *O*-Me-isourea in a sealed tube at 70 °C led to **225** and **226** in 85% and 58% yields, respectively. However, in the specific case of **70**, it has been observed that reaction of diamine **70** with isothiourea or isourea led to the formation of monoguanylated compound **73** as the major product and only to 5% of **72**, possibly due to electrostatic factors (see Scheme 12). During a biguanylation or polyguanylation reaction, a primary amine intermediate is supposed to be formed. The reactivity of this intermediate diminishes rapidly with the number of positively charged guanidinium groups introduced. As a consequence, the rate of the reaction for the introduction of the final guanidium group becomes slow compared to the side reaction of the reagents (decomposition) and generally mixtures of monoguanylated−biguanylated diamines that are difficult to separate are obtained. Ion-exchange chromatography was used for purification of compounds **72**.

As shown above, symmetric biguanylated diamines are obtained in principle through a biguanylation reaction on an appropriate diamine substrate using an excess of the guanylating agent. The synthesis of a series of biguanylated diamines **227a**−**d** through a guanylation reaction with Et-*S*-isothiourea in water was described in 1984 by Menichi and Hubert-Habart.

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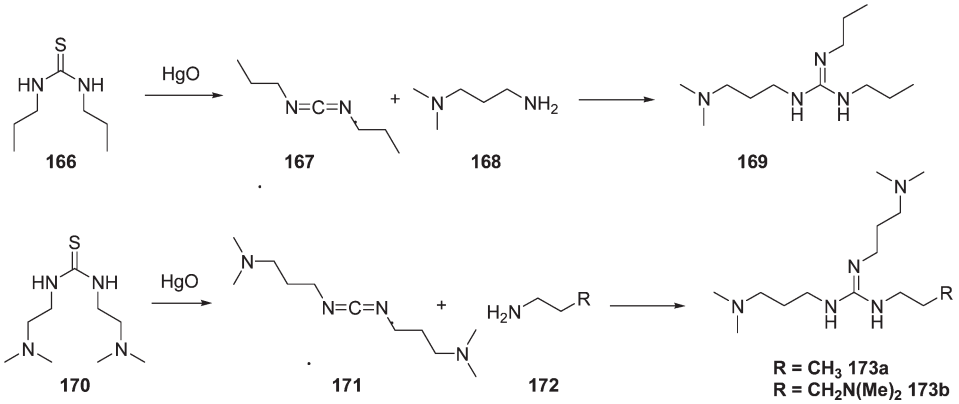
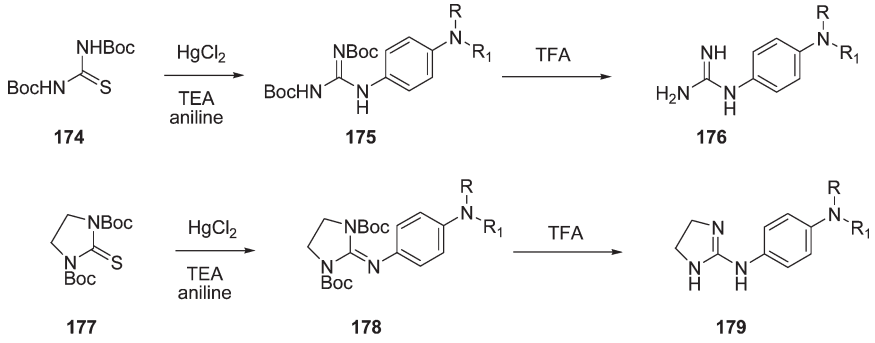
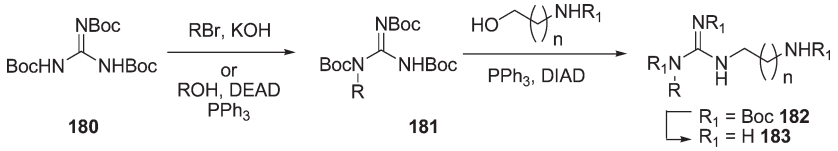
Scheme 32. Superbasic Monoguanylated Diamines



Scheme 33. Monoguanylated Dianilines



Scheme 34. Mitsunobu Reaction in the Synthesis of Monoguanylated Diamines



Compounds **227a**−**d** having different length alkylic chains were in turn converted into bisaminopyrimidine derivatives **228**. Indeed, reaction of the guanidine portion with an appropriate β-dicarbonyl compound led to the formation of a pyrimidine core[121] (Scheme 47).

Similar approaches with *O*-Me-isourea or *S*-Me-isothiourea as guanylating agents were described, for instance, in the synthesis of new inhibitors of deoxyhypusine synthase,[85] or in the synthesis of Arcaine **229**, a known weight-reducing compound. Arcaine is also a well-known inhibitor of NO synthase and a NMDA antagonist acting as a competitive inhibitor at the polyamine site.[122] Its properties are strictly related to its analogy with agmatine.[123] Arcaine also has been isolated in *Lathyrus sativus*, and mediation of its synthesis in vitro from agmatine by the transamidinase has been demonstrated.[124] A series of Arcaine analogues was described by Hamilton and co-workers with inhibitory effects on the binding of the channel-blocking drug [^{3}H]MK-801 to the NMDA receptor complex.[125] This series of compounds is characterized by a 1,3-dimethylphenyl chain between the two guanidine portions, which makes the synthetic route more complex because of the lack of commercially appropriate diamine building blocks.[126] Guanylated diamines such as Arcaine act to decrease [3H]MK-801 binding at low concentrations and therefore are classified

Table 7. Structures of Compounds 183a−f

compound	R	*n*	compound	R	*n*
183a	H	6	**183d**	3-methyl-1-butyl	5
183b	γ,γ-dimethylallyl	1	**183e**	cyclopropylmethyl	5
183c	H	5	**183f**	γ,γ -dimethylallyl	2

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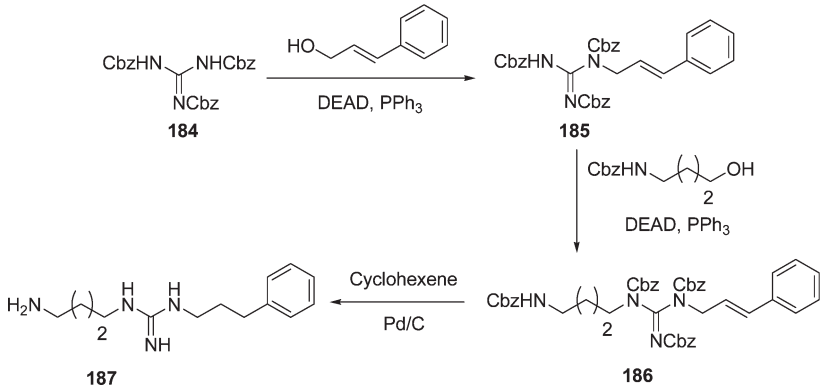
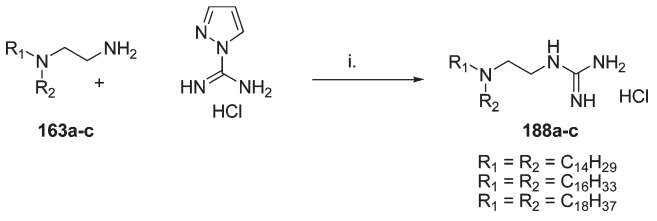
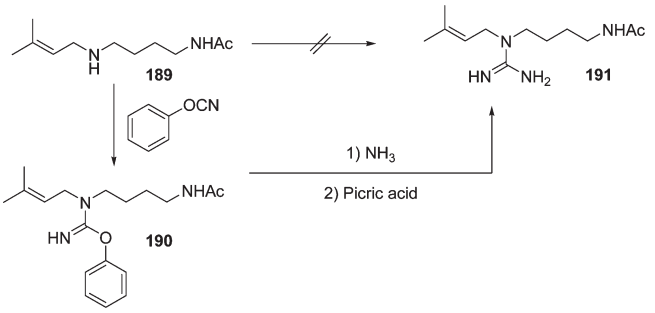
Scheme 35. Synthesis of Monoguanylated Diamine 187 from tri-Cbz-Guanidine 184



Scheme 36. Use of 1*H*-Pyrazole-1-carboxamidine in the Synthesis of 188a−c



Scheme 37. Synthesis of Smirnovine



as antagonists. The synthesis of the monosubstituted bisguanidine derivatives is shown in Scheme 48. The 5-bromo-*m*-xylene **230** was oxidized to 5-bromoisophthalic acid with $KMnO_4$, followed by esterification with ethanol and sulfuric acid. The resulting 5-bromoethyl isophthalate was used in Suzuki coupling. The product biphenyl diester **231** was reduced to the diol with $LiAlH_4$, which was converted to the bisazide by a Mitsunobu-type reaction with diphenylphosphorylazide (DPPA) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU). The bisazide was reduced to the bisamine **232** with H_2/Pd−C in methanol followed by guanylation, which was accomplished with bis(Boc)thiourea in the presence of mercuric chloride to give the bis(Boc)-protected guanidine **233**. Deprotection of the Boc groups to give **234** was achieved with HCl gas in ethyl acetate. A second route was developed for disubstituted guanidiniums. 5-Hydroxyisophthalic acid **235** was esterified and then alkylated with methyl iodide to give bismethyl 5-methoxyisophthalate **236**. This was hydrolyzed with LiOH to the diacid followed by acid chloride formation with oxalyl chloride and reaction with *n*-propylamine to give **237**. Reduction of the resulting amide was achieved with borane−THF, followed by acidic workup to give the hydrochloride salt. The diamine was then biguanylated with bis(Boc)thiourea in the presence of mercuric chloride and then converted into desired compounds **239** by acidic removal of the Boc protecting group. Several analogues **240** were then synthesized and are reported in Table 8. The most potent compound synthesized proved to be the *N*,*N*′-bis(propyl)guanidine **240h**.

Aminoimidazolines can be considered as the cyclic analogues of the guanidine group. This aspect results clearly from the two sequent examples where a series of biguanidines **227a**−**b** and **241a**−**d** and Synthalin A were synthesized together with the corresponding biimidazolines **242a**−**g**.[127] Hence, imidazolines **242** can be considered as a particular kind of biguanylated diamines and were obtained by reaction of different diamines with 2-methylmercapto-4,5-dihydroimidazole iodide. Treatment of hydroiodidric salts **242** with picric acid and purification with a basic anion exchange resin led to corresponding hydrochloridric salts (Scheme 49).

Dardonville et al. also reported the synthesis of a series of biguanylated anilines **245** and **247** endowed with antitripanosomal and antiplasmodial activity[128] and DNA affinty.[129] These compounds have a chain between the two guanidine functions constituted by biphenyl methane, biphenyl ether, or biphenyl thioether. The authors substituted this arylalkyl chain with a tricyclic moiety to obtain compounds **248**−**249**. The synthesis of **245** and **247** is reported in Scheme 50. Appropriate anilines **243** were guanylated with thiourea or 2-thio-4,5-dihydroimidazole in the presence of $HgCl_2$ as activating agent. The use of activated thiourea as guanylated agent has to be ascribed to the poor nucleophilicity of anilines in the guanylation reaction (Table 9).

Guanylation reactions of diamines are often long, and biguanylation of the appropriate substrate could require several hours. In the past decade, microwave-assisted synthesis allowed for the drastic reduction of times of many reactions. Microwave synthesis has been also applied to the synthesis of symmetric biguanylated diamines **227b** and **251**. 1,6-Diaminohexane and diamine **250** were reacted with *S*-Me-isothiourea sulfate in the presence of K_2CO_3 in a microwave oven at 450 W, leading to

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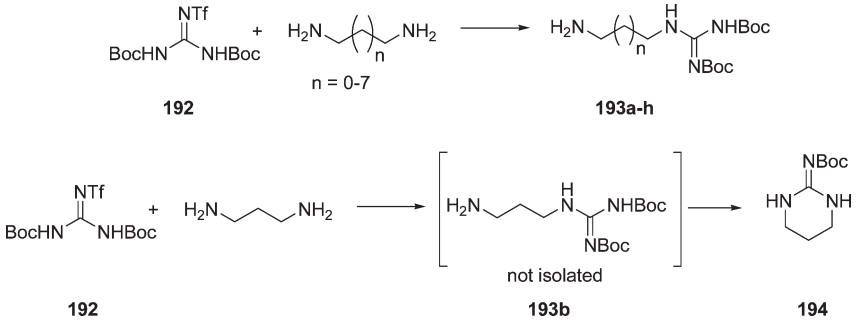
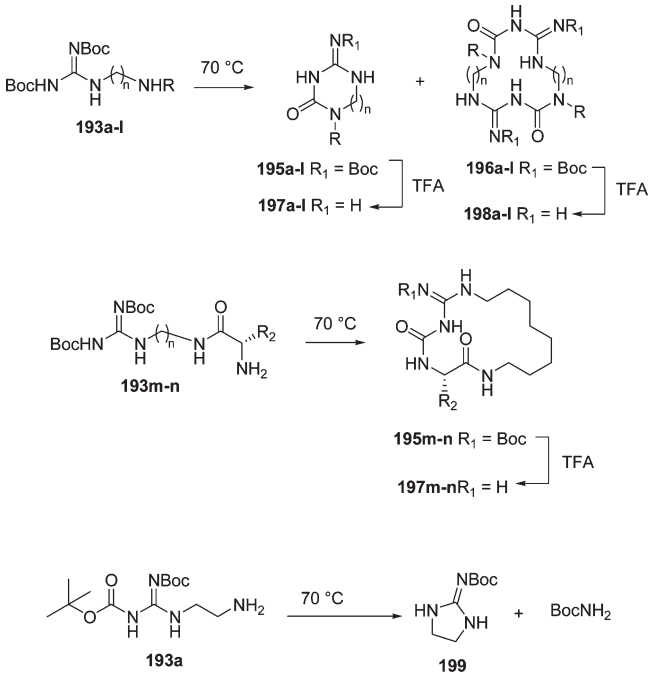
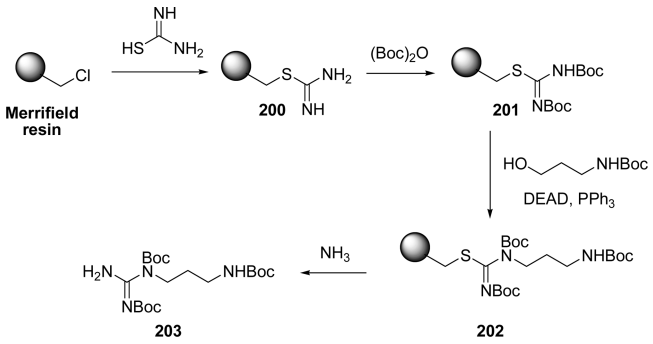
Scheme 38. Use of Triflic Guanidine 192 in the Synthesis of 193a−h



Scheme 39. Cyclization of di-Boc-Monoguanylated Diamines

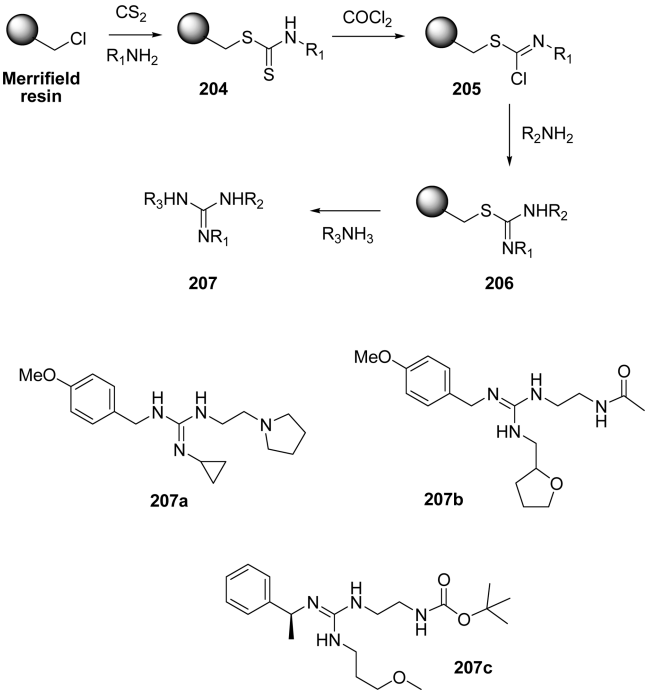


Scheme 40. Solid-Phase Synthesis of Monoguanylated Diamine 203



guanidines **227b** and **251** in only 18−25 min (Scheme 51). These final compounds were then washed with $CHCl_3$ and

Scheme 41. Use of Merrifield Resin in the Synthesis of Guanylated Diamines



directly converted into 2-substituted aminopyrimidines through reaction with appropriate dicarbonyl compounds with the possibility to use final compounds in supramolecular or metal-coordinating chemistry.[130]

A synthetic approach to biguanylated diamines as antimalarial agents different from those previously described was reported into an interesting paper by Calas, Vial, and co-workers. In this work, the authors reported the synthesis of analogues of Synthalin B using *S*-Me- or *S*-Et-isothioureas. Nearby, a different strategy was also described. The 1,12-diaminododecane was converted into a guanylating agent **254** in a 3-step sequence. Reaction of diamine with CS_2 gave the isothiocyanate **252**, which was converted into the corresponding thiourea **253** by reaction with dimethylamine. Then, methylation with CH_3I led to bisguanylating agent *S*-Me-isothiourea **254**. Hence, in this case, the diamine is the guanylating agents, and different primary or secondary amines could be introduced. Reaction of **254** with appropriate amine led to desired

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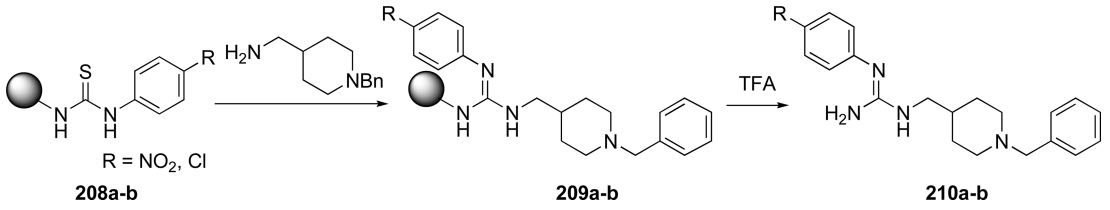
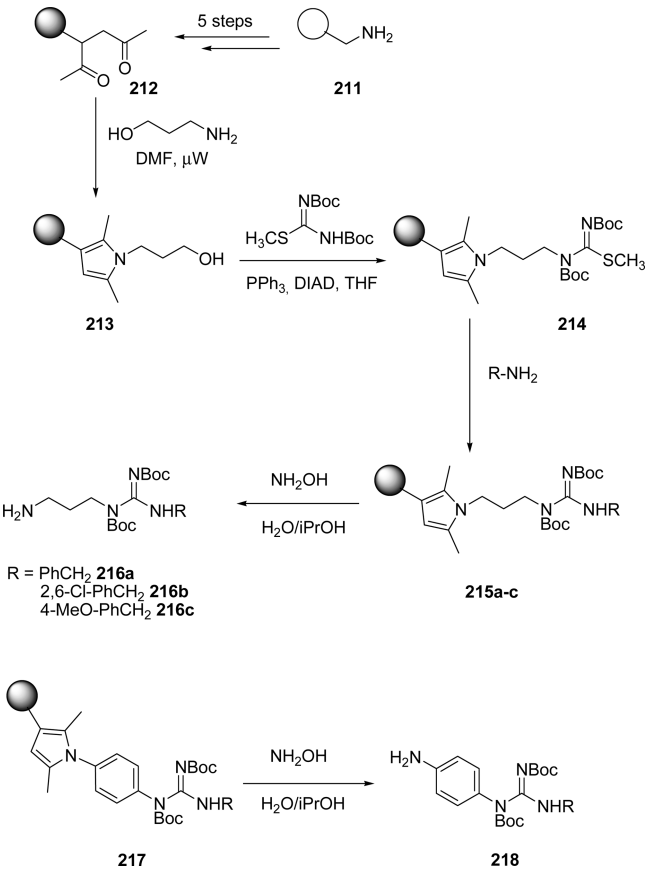
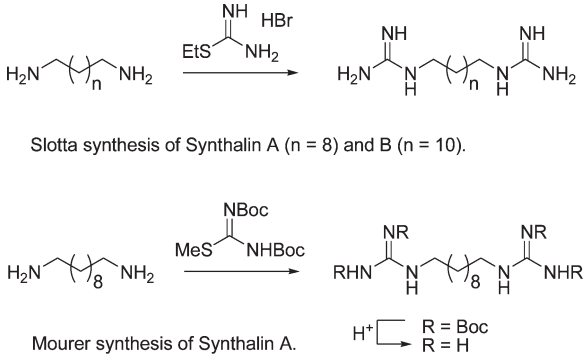
Scheme 42. Synthesis of 210a−b Using the Rink Amide Resin



Scheme 43. Dimethylpyrrole As Protecting Group in the Synthesis of Solid-Phase Synthesis of Monoguanylated Diamines



Scheme 44. Syntheses of Synthalin A and Synthalin B



biguanylated diamines **255**−**256** (Scheme 52). The great advantage of this synthetic approach is represented by its versatility, due to the possibility of synthesizing a library of compounds **257**−**258a**−**c** in two steps starting from the common building block **252** (Table 10). On the other hand, the yields for this reaction are sometimes low (i.e., 39% for **255**). However in the case of **255**, the low yield could be due to the lower nucleophilicity of $NCNH_2$ with respect to NH_3.[131]

Finally, a synthetic approach to symmetric biguanylated diamines by the use of carbodiimide was reported in 2003 by Richeson et al., who described the synthesis of biguanylated ethylendiamine **260** to be used as a potential guanidate ligand. Guanidate anions could be in fact sterically and electronically flexible ligands for the design of transition metal and main group metal complexes. Compound **260** was obtained through the reaction of ethylendiamine with diisopropylcarbodiimide **259** in toluene at 100 °C in 60% yield. This synthetic approach as suggested by the authors could lead to the synthesis of a larger library of potential ligands by the use of different commercial diamines. The NH groups of guanidines could favor the introduction of ligand **260** into appropriate metal complexes. As reported, compound **260** reacts smoothly with tetrakis(benzyl) complex of Zr and Ti to yield complexes **261** and **262**[132] (Scheme 53).

2.3.2. Unsymmetric Biguanylated Diamines. Unsymmetric biguanylated diamines represent a synthetically interesting challenge, since alternative strategies have to be planned to introduce two different guanidine moieties into a diamine backbone. In principle, the easiest approach is to guanylate a monoguanylated diamine, prepared selectively as reported before. A clear example is represented by the synthesis of **G3** derivative **264**, which was obtained through a double guanylation sequence with the appropriate *S*-Me-isothioureas.[133]

In the previous case, the unsymmetry element was represented by the two guanidine portions. Slightly more complicated are the biguanylation of unsymmetric diamines. Earliest reports described the synthesis of **267a**−**c** through guanylation of diamines **265a**−**c** with *S*-Me-isothiourea sulfate[82] (Scheme 55). Speculations on the guanylation of sterically hindered amino groups were made by the authors. The first guanylation reaction seems to occur at a secondary amine, while the primary amine did not react for steric reasons. After the first guanylation occurred, the intermediates **266a**−**c** were able to catalyze the guanylation of the primary amine. The lone pairs of guanidine nitrogens should catalyze the guanylation at the sterically hindered amine, leading to compounds **267a**−**c**.[83]

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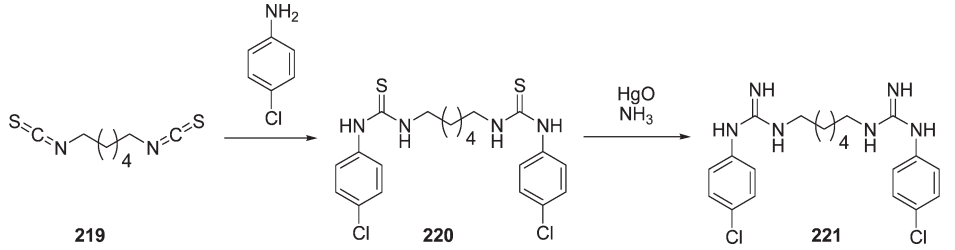
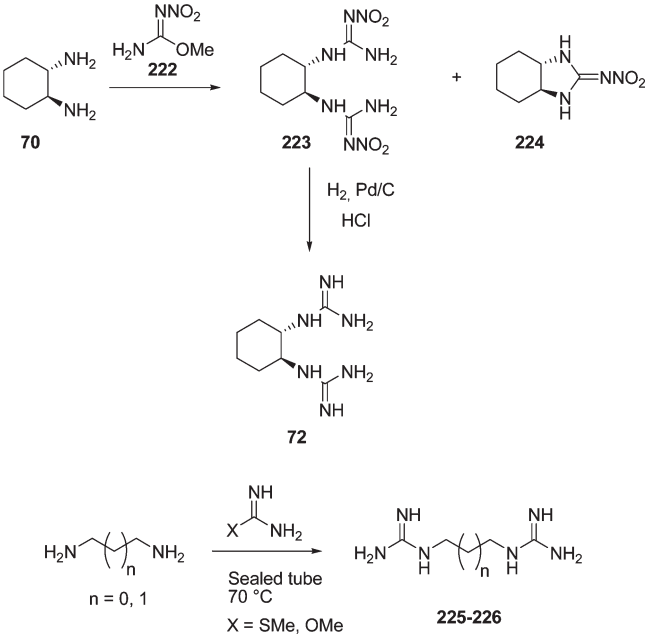
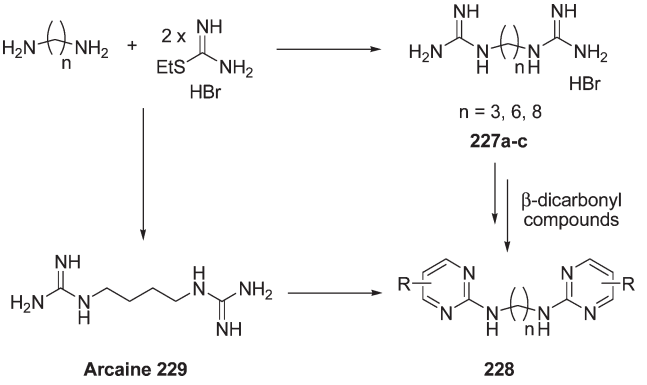
Scheme 45. Use of HgO in the Synthesis of Biguanylated Diamine 221



Scheme 46. Use of Nitro Guanylating Agent 222



Scheme 47. Synthesis of Arcaine and Its Derivatives



In some cases, the most difficult aspect in the synthesis of unsymmetric biguanylated diamines is represented by the synthesis of the diamine itself. Lee and Folk reported the synthesis of a series of unsymmetric biguanylated diamines, whose unsymmetry element was represented by the alkylic chain. The authors synthesized these compounds as inhibitors of deoxyhypusine synthase. A first series of derivatives **270** was synthesized as reported in Scheme 56. The branched chain pimelonitriles **268**, prepared from corresponding dibromides by reaction with NaCN,[134] were reduced to appropriate diamines **269** with BH_3-THF, which were in turn converted into biguanylated products **270** after reaction with *S*-Me-isothiourea sulfate. Compounds **270a**−**e** differ from each other in the presence of one or two methyl groups on the alkylic chain (Table 11).

A second series of biguanylated diamines **275**−**277** having an unsaturated alkylic chain was also described. In this case, the unsaturated bond (double or triple) is the element for the unsymmetry. The synthesis is outlined in Scheme 57 and takes place from 7-(2-tetrahydropyranyloxy)-2-heptyn-1-ol **272**, prepared from **271** by Grignard addition on formaldehyde. Deprotection of the tetrahydropyranyl (THP) group in acidic medium led to diol **273**, which was converted into diamine **274** in a 3-step sequence. Finally, biguanylation of diamine occurred with *O*-Me-isourea sulfate **71** in MeOH, leading to **275**. Alkyne intermediate **274** was also converted into corresponding *E* and *Z* alkenes, which were then guanylated under the same reaction conditions, affording biguanylated diamines **276** and **277**, respectively.

Finally, a third series of nonsymmetric biguanylated diamines, having an unsaturated and branched alkyl chain, was also described (Scheme 58). Diols **279a**−**c** were obtained in two steps from alkyne **278**. Then **279a**−**c** were converted into diamines **280a**−**c** following the same procedure described above. Finally, biguanylated products **282a**−**b** were obtained using *O*-Me-isourea **71** as guanylating agent. Reduction of triple bond of diamines **280** followed by guanylation led to alkenes **281a** and **283a** and to saturated diamines **284b**−**c**.[86]

Other interesting examples of unsymmetric biguanylated diamines can be found in the synthesis of natural compounds. The bisguanidinium toxin (+)-saxitoxin (STX) can be considered a biguanylated diamine, because it possesses two guanidine moieties, incorporated into 5- and 6-membered rings and spaced by an alkylic chain. The (+)-saxitoxin, a paralytic shellfish poison, is among the most lethal nonproteinaceous substances known, its acute toxicity resulting from its ability to disable ionic conductance through the voltage-gated sodium channel. The authors planned a synthetic strategy (Scheme 59) where the two guanidine functions were incorporated into 9-membered ring **I**, which could be in turn obtained from alkene **II**. Compounds **I** and **II** are examples of cyclic biguanylated diamines. In the first synthesis of STX, described in 2006, alkene **II** was synthesized in a 2-step sequence from *S*-Me-isothiourea **III** through an intramolecular cyclization. It is noteworthy that the intramolecular

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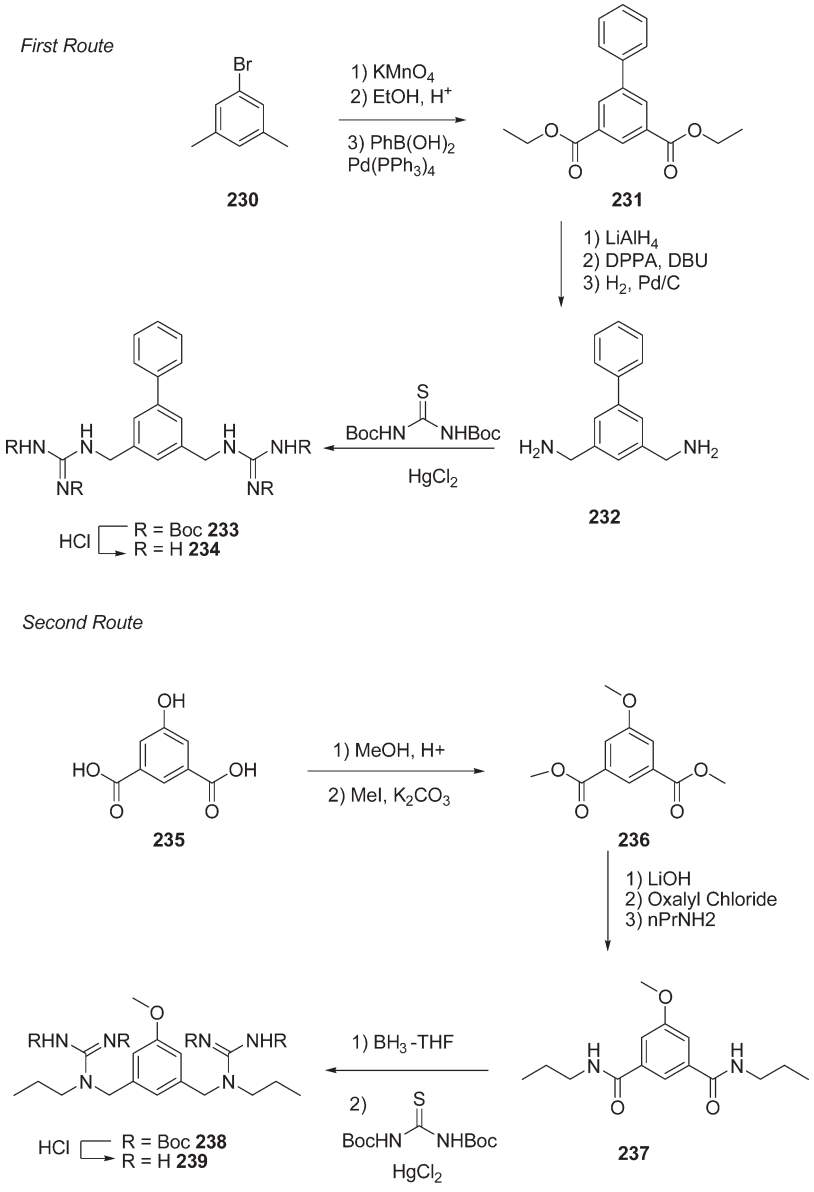
Scheme 48. Different Routes for the Synthesis of Some Arcaine Analogues



guanylation reaction was performed in the presence $AgNO_3$/Et_3N. The latter conditions presumably trigger formation of a reactive *N*-sulfonylcarbodiimide, which in turn is intercepted by the pendant amine.[135]

In 2007, Du Bois and co-workers reported their attempts to synthesize intermediate **II** through a ring-closing metathesis (RCM) reaction starting from the nonsymmetric biguanylated diamine intermediate **IV**. The synthesis of biguanyl compound **290** is reported in Scheme 60 and starts from *N*,*O*-acetal **285**, which was converted into **286** by the stereoselective addition of divinyl zinc. Alcohol **286** was then converted into the azide **287**. The introduction of the first guanidine moiety was carried out at this stage using an imidoyl chloride reagent (MbsN)=CCl_2. Further transformations led to amine **289**, which was thus guanylated with the appropriate *S*-Me-isothiourea, leading to biguanylated diamine **290**. Unfortunately, the RCM strategy did not work, and the authors were unable to obtain cycle **291** using different types of Ru catalysts.[136,137]

Stellettadine A is a natural product isolated from marine sponge *Stelletta sp.* in Japan.[138] This structurally unique alkaloid consists of norsesquiterpene and Arcaine (1,4-diguanidinobutane) units, and it is able to induce larval metamorphosis in ascidians. The total synthesis of (*S*)-Stellettadine A was described by Mori and co-workers (Scheme 61), and it is based on a convergent approach where intermediates **292** and **5** are coupled to give compound **293**. Intermediate **292** was obtained from (*S*)-citronellal, while intermediate **5** was obtained through Boc-protection of the primary amine of agmatine sulfate. The second guanidine moiety was introduced using a different guanylating agent. Removal of the Boc protecting group of **293** to give **294** was followed by guanylation by Mosher's procedure,[139] with aminoiminomethanesulfonic acid NH_2C-(=NH)SO_3H and Et_3N in MeOH, to give (*S*)-**295** in good yield. Treatment of (*S*)-**295** with methanolic KOH effected selective removal of one of the acyl groups to give the target compound (*S*)-Stellettadine A in 67% yield.[140,141]

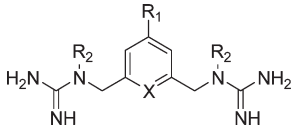
dx.doi.org/10.1021/cr100423x |*Chem. Rev.* 2011, 111, 5247–5300

Recently, the total synthesis of the related natural compound (±)-bistellettadine also has been described through a similar synthetic procedure.[142]

2.3.3. Bisbiguanylated Diamines. A special case of biguanylated diamines is represented by the (bis)biguanylated diamines. These compounds differ from common biguanylated diamines by the fact that the guanidine moieties are replaced by bisguanidine groups. From a synthetic point of view, it is clear that common guanylating agents shown above cannot be used and different strategies had to be planned. The general approach for the synthesis of bisbiguanylated diamines is the use of an appropriate *N*-cyanoguanidine. Addition of an appropriate amine on the cyano group led to desired bisguanidine moiety.

The first example of bisbiguanylated diamines was described by Rose and Swain in 1956. The authors reported the synthesis of two series of bisbiguanylated diamines **297** and **299** as potential antibacterial agents according to previously developed methodologies for the synthesis of monodiguanides.[143,144] Two

Table 8. Structures of Some Arcaine Derivatives



compound	R_1	R_2	X	compound	R_1	R_2	X
240a	H	H	CH	**240i**	H	allyl	CH
240b	OCH_3	H	CH	**240j**	H	*n*But	CH
240c	OiPr	H	CH	**240k**	H	*i*But	CH
240d	*O*-CyHex	H	CH	**240l**	H	Bn	CH
240e	Ph	H	CH	**240m**	OCH_3	*n*Pr	CH
240f	NHBz	H	CH	**240n**	OH	*n*Pr	CH
240g	H	Et	CH	**240o**	H	H	N
240h	H	*n*Pr	CH	**240p**	H	*n*Pr	N

Scheme 49. Diimidazolines 242a−g As Analogues of Biguanylated Diamines 241a−d

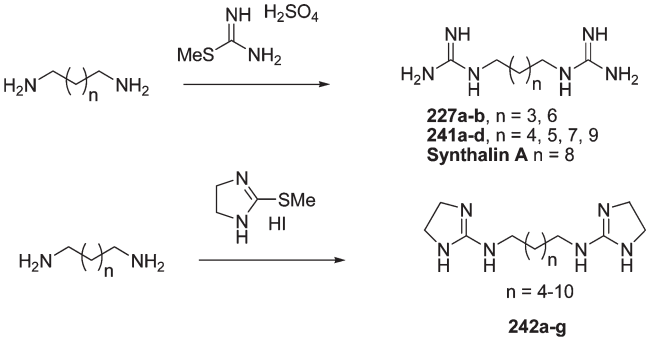
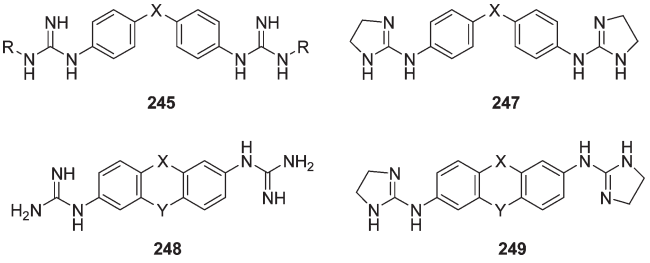
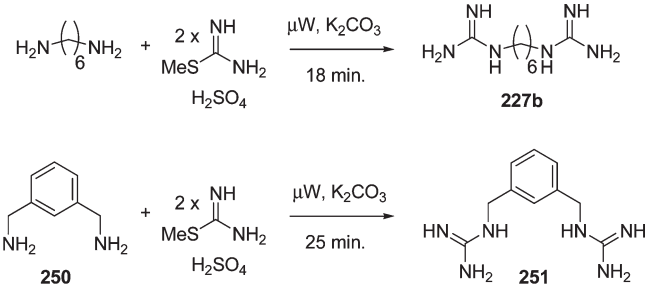


Table 9. Structures of Biguanylated Anilines

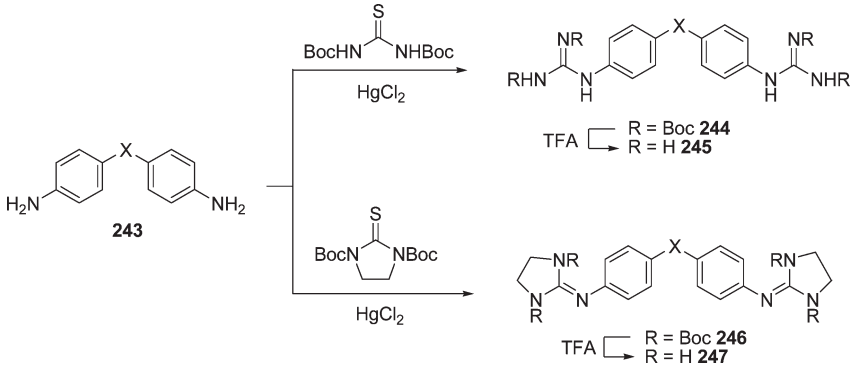


compound	X	Y	R
245a	CH_2		
245b	CH_2		$(EtO)_2ChCH_2$
247a	CH_2		
245c	O		
247b	S		
245f	S		
248	CH_2	CH_2	
249	CH_2	CH_2	

Scheme 51. Microwave-Assisted Synthesis of Biguanylated Diamines



Scheme 50. Biguanylated Anilines



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Scheme 52. Alternative Approach for the Synthesis of Biguanylated Diamines

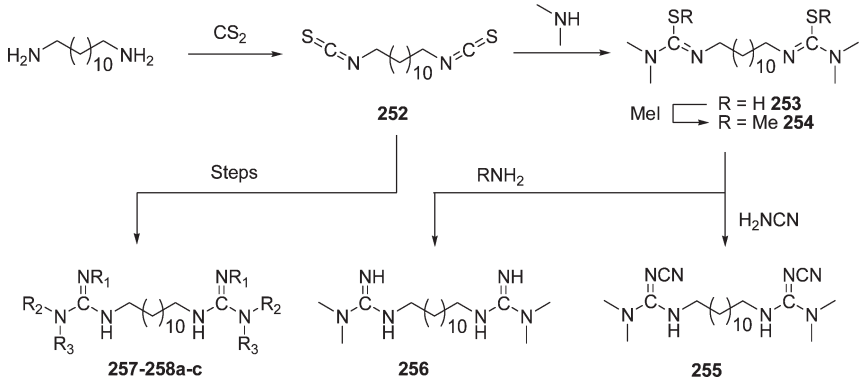
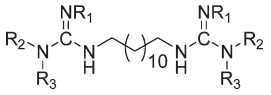
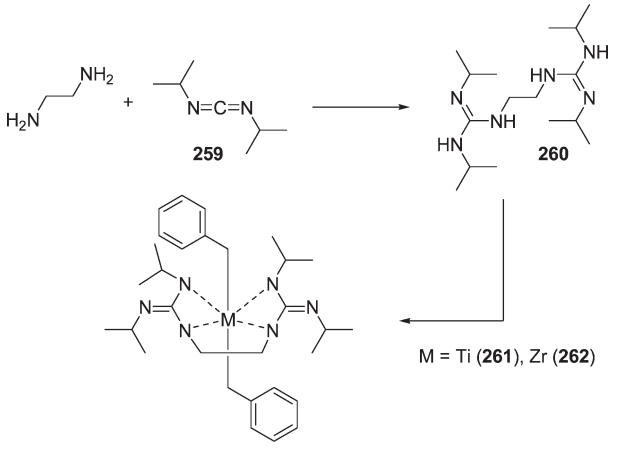


Table 10. Structures of Biguanylated Diamines 255, 256, 257, and 258a−c

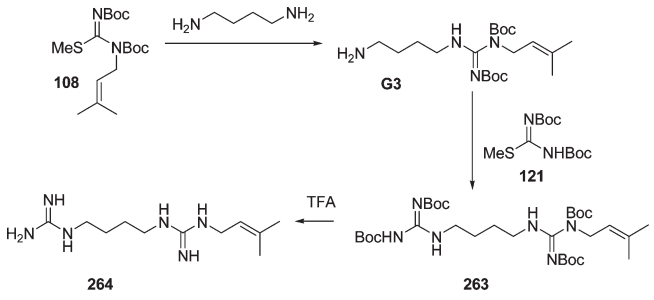


compound	R_1	R_2	R_3
255	CN	CH_3	CH_3
256	H	CH_3	CH_3
257	C_6H_{11}	C_6H_{11}	H
258a	$-(CH_2)_2-$		H
258b	$-(CH_2)_3-$		H
258c	$-CH_2-C(CH_3)_2-CH_2-$		

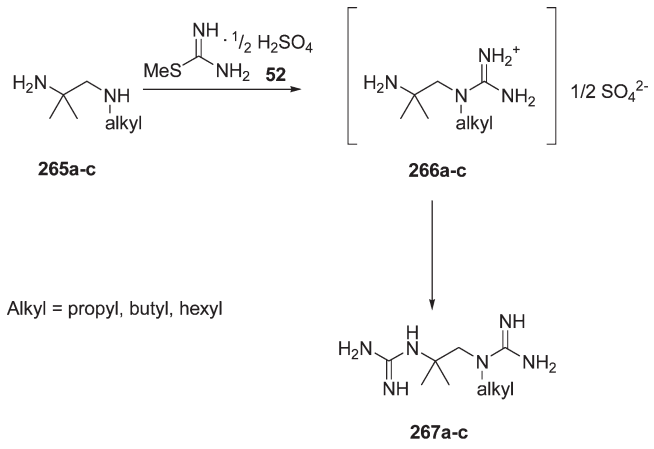
Scheme 53. Biguanylated Diamines As Metal Ligands



Scheme 54. Synthesis of the Unsymmetric Guanylated Diamine 264



Scheme 55. Sterically Hindered Unsymmetric Biguanylated Diamines



alternative synthetic approaches were described on the basis of structural characteristic of desired final compounds. The first approach requires the reaction of a *N*-phenylcyanoguanidine **296** with each of the amino group of an appropriate diamine. The second approach, more generally used, is the complementary process in which the biscyano guanidine **298**, readily prepared from the appropriate diamine and two molecular portions of sodium dicyanimide, interacts with 2 equiv of a general amine NHRR′. (Scheme 62).

Reactions were performed in nitrobenzene or 2-ethoxyethanol depending on the substrate. However, for the synthesis of compounds **299**, when R and/or R′ were alkyl groups, solvent was not employed, and usually, biscyanoguanidines **298** were directly fused with alkylamines at 150−160 °C.[145] Compound **297** having n = 4 is the bactericidal and bacteriostatic agent

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Chlorhexidine, a well-known drug used today for skin infections, wounds, and burns, as well as in obstetrics and bladder irrigation. Finally, Chlorhexidine is frequently used in dental hygiene, because it maintains a significant antibacterial activity even at high dilution.[146,147]

Synthesis of Chlorhexidine derivatives **301** was accomplished through a synthetic approach similar to that described by Rose and Swain (Scheme 63, Table 12). Compounds **301** are bisbiguanylated diamines whose diamino portion is represented by a heterocycle, namely, a substituted piperazine. Compounds were obtained from cyanides **300** through reaction with appropriate amines.[148]

Scheme 56. Biguanylated Diamines Widely Substituted on the Alkyl Chain

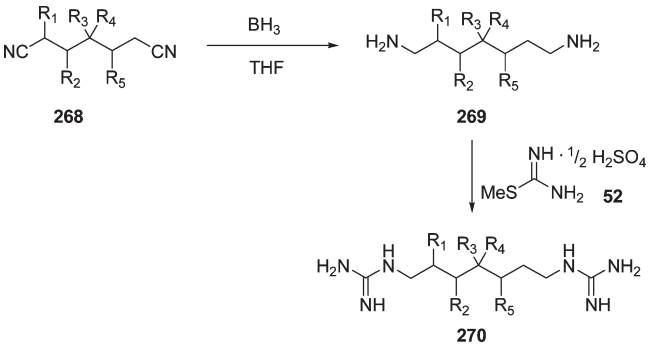


Table 11. Structures of Compounds 270a−e

compound	R_1	R_2	R_3	R_4	R_5
270a	CH_3	H	H	H	H
270b	H	CH_3	H	H	H
270c	H	H	CH_3	H	H
270d	H	H	CH_3	CH_3	H
270e	H	CH_3	H	H	CH_3

Chlorhexidine is generally sold as Chlorhexidine digluconate (CHG) salt, and studies on the identification and isolation of impurities in stressed CHG solutions have been described.[149] Among the 11 impurities identified, some of them proved to have a bisbiguanylated diamine backbone, and their structures has been confirmed through total synthesis (Scheme 64). The unsymmetrical chlorexidine impurities **303** and **305** have been synthesized according to general procedure, with specific attention paid to using half of the prescribed stoichiometric equivalent of a specific reagent.[150]

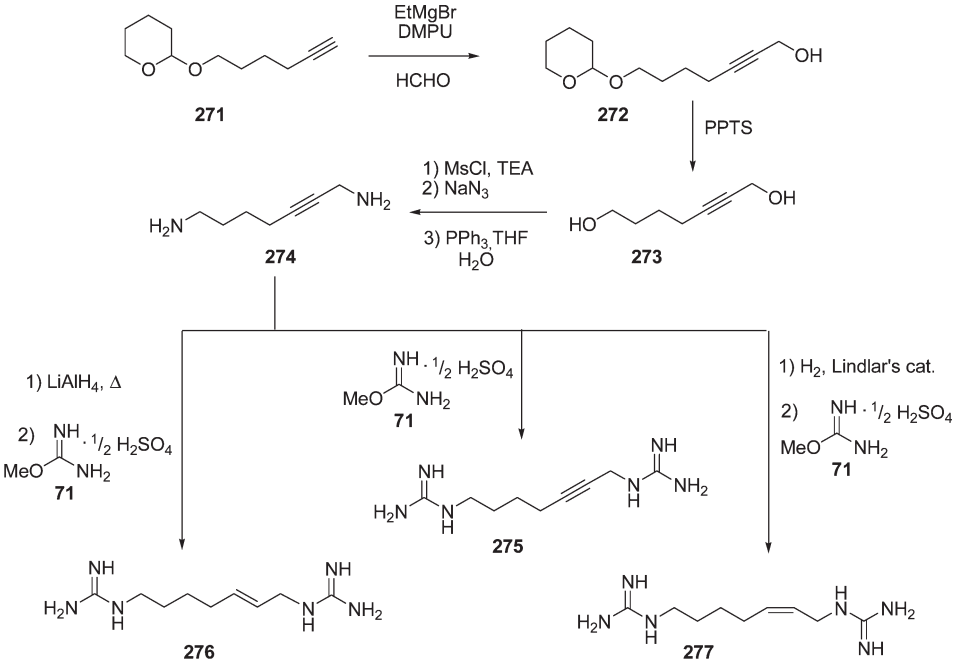
The synthesis of detuterium-labeled[151] and carbon-14-labeled[152] Chlorhexidine has been also described. The synthesis of deuterium-labeled Chlorhexidine was reported by Hudlicky and co-workers and starts from the commercially available diol **306**. Deuteration of triple bonds, added to an adequate protecting group strategy, led to deuterium-labeled diol **307**. This latter compound was first converted into the corresponding dibromide and then into labeled diamine **308**. Finally, conversion of diamine **308** into deuterated Chlorhexidine **310** was accomplished following the Rose−Swain approach (Scheme 65).

Using the standard methodology, syntheses of bisbiguanylated diamines related to Chlorhexidine but having different length alkylic chains have been described.[144,153,154]

3. GUAZATINE

Until the early 1990s, the term guazatine was the common name for bis(8-guanidinooctil)amine as proposed by IUPAC. Later, the chemical definition of guazatine was reconsidered by BSI and it was proposed as "A mixture from the reaction products from polyamines, comprising mainly the octamethylenediamine, iminodioctamethylenediamine, and octamethylene bis(iminooctamethylene) diamine, and carbamonitrile".

Scheme 57. Synthesis of Unsymmetric Biguanylated Diamines Containing Unsaturated Bonds



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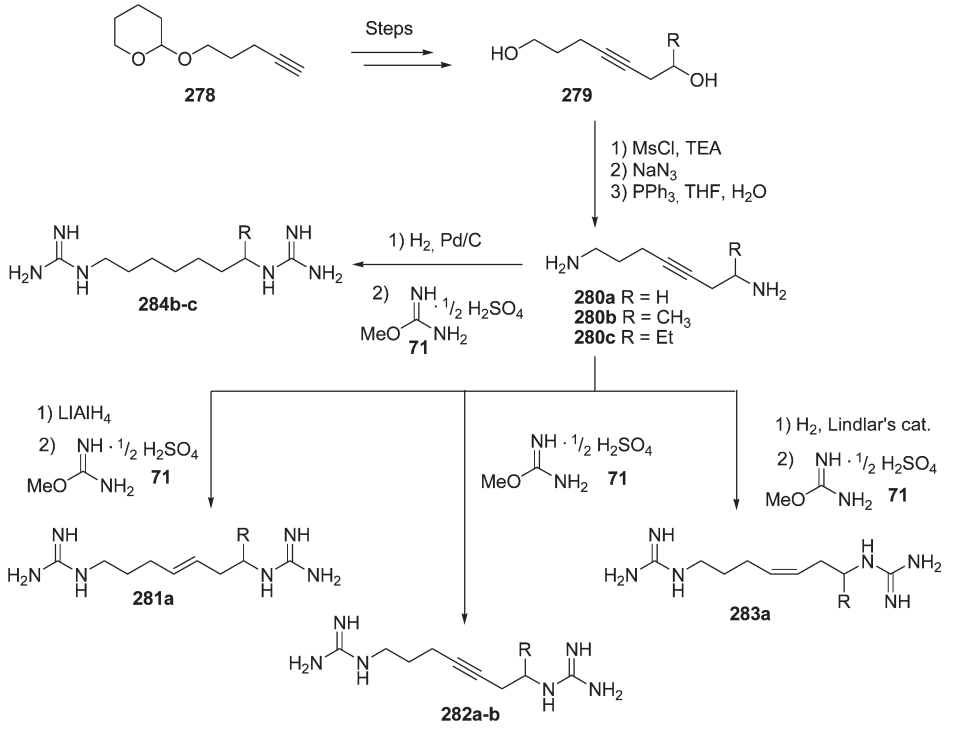
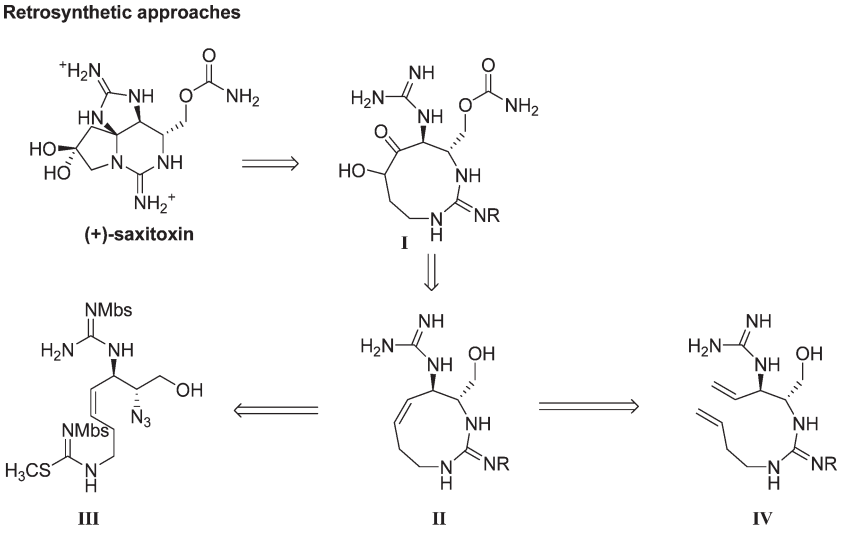
Scheme 58. Different Strategies for the Synthesis of Unsymmetric Biguanylated Diamines Containing Unsaturated Bonds



Scheme 59. Retrosynthetic Approach to (+)-Saxitoxin



Hence, from a chemical point of view, guazatine is a mixture of mono-, bi-, and triguanylated diamines, triamines, and tetramines, even if the term guazatine is still commonly used to refer to the biological active ingredients. Single components of guazatine have been recently separated and fully characterized. A coding system, defined by the Codex Alimentarius Commission, is used for the compounds that make up guazatine. In this system, "N" represents any amino group. Thus NN stands for $H_2N-(CH_2)_8-NH_2$, NNN stands for $H_2N-(CH_2)_8-NH-(CH_2)_8-NH_2$, and so on. "G" stands for any amino group (NH or NH_2) of the above that is guanidated. For example, GG stands for $H_2N-C(NH)NH-(CH_2)_8-NH-C(NH)-NH_2$.[155] Guazatine can be considered as an ideal bridge between guanylated diamines and triamines.

3.1. Analysis of Guazatine and Separation of Its Components

Analytical protocols for the separation and identification of the main components of guazatine mixture have been reported in the literature. The first approach was described in the early 1990s

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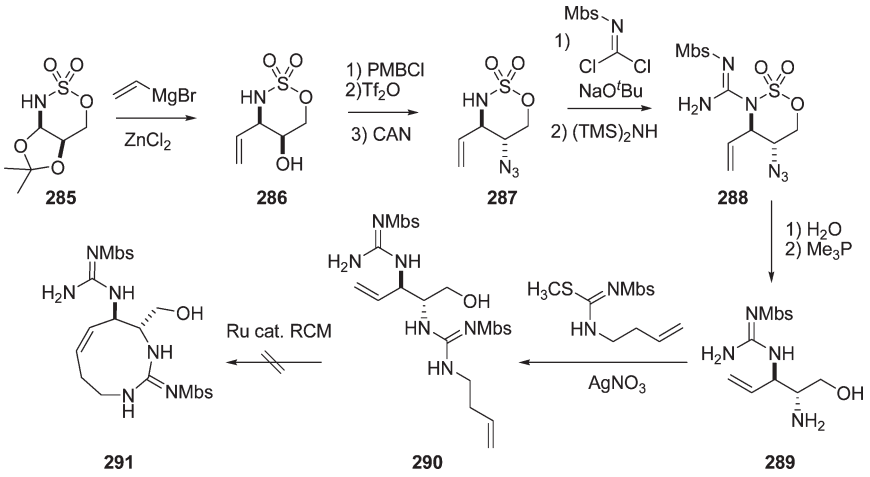
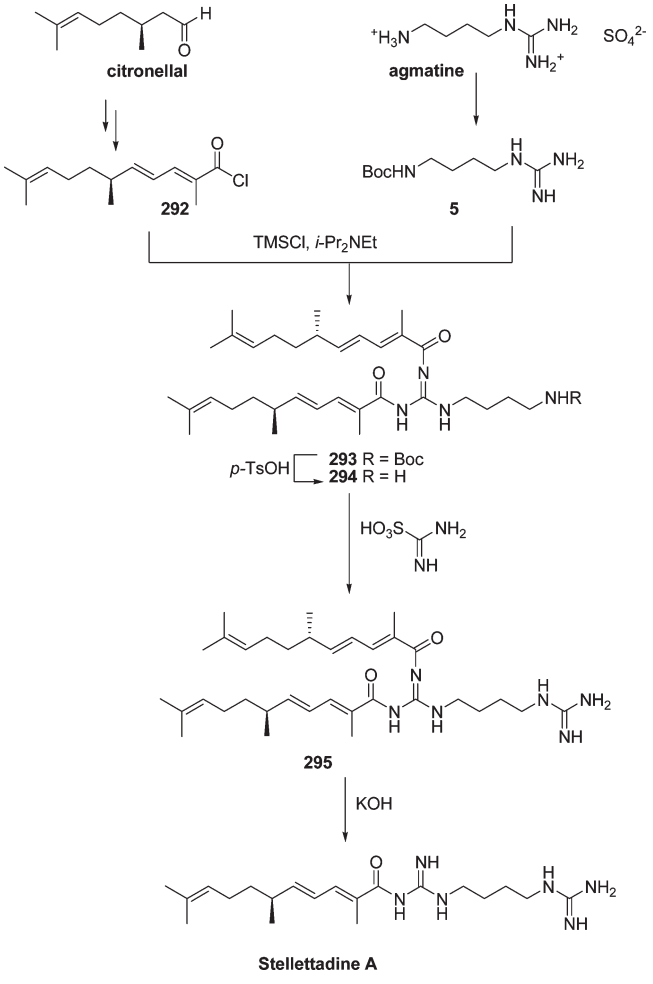
Scheme 60. Biguanylated Diamine Intermediate in the Total Synthesis of (+)-Saxitoxin



Scheme 61. Synthesis of Stellettadine A



using gas chromatography−mass spectrometry (GC-MS) and FABMS analysis.[156] Standard guazatine purity was determined by titration of the guanidinic groups. Separation and identification of the main components of guazatine was obtained by GC after derivatization with hexafluoroacetylacetone. The guanylated diamines and triamines proved to be the most abundant components of guazatine. A typical composition of free guazatine is shown in Table 13. Structures of main components are shown in Figure 10. It can be seen that diamine derivatives account for ~40% of the constituents of guazatine, triamines for ~46%, tetramines for ~11%, and other amine derivatives for ~3%. The most abundant individual components are the fully guanidated triamine (GGG, 30.6%) and the fully guanidated diamine (GG, 29.5%), followed by the monoguanidated diamine (GN, 9.8%) and by a diguanidated triamine (GGN, 8.1%).

In a more recent liquid chromatography−mass spectrometry (LC-MS) protocol of analysis and characterization without derivatization, each compound has been separated and identified with fragmentation studies using the spectra recorded with various fragmentor energies by Dreassi et al.[157] This method allowed one to obtain through chromatographic analysis the main diamines, triamines, and tetramine (GN, GG, GNG, GGN, GGG, and GGGG) that cover >87% of the total contents of the guazatine mixture. Mass spectrum obtained by direct injection of a sample of standard guazatine and its chromatographic profile obtained by LC-MS are shown in Figures 11 and 12.

The same components were also isolated in a parallel LC−electrospray ionization−MS (LC-ESI-MS) method for the quantitative detection of guazatine residues in maize and hard wheat.[158] The main compounds of guazatine mixture are illustrated in Figure 12. The amount of single components in a standard guazatine mixture turned out identical to that reported in Table 13.

It is noteworthy that the component GNG, whose structure is sometimes erroneously associated with the term guazatine, as previously seen, constitutes only 4.5% of the guazatine mixture. Possibly this ambiguity could be due to the antifungal activity of guazatine, which is mainly derived from the GNG component, as will be shown later.

3.2. Synthesis of Carbon-14 Labeled Guazatine

Syntheses of single components of guazatine are reported in the appropriate chapters of this review. However, an interesting method for small-scale laboratory simulation of the industrial

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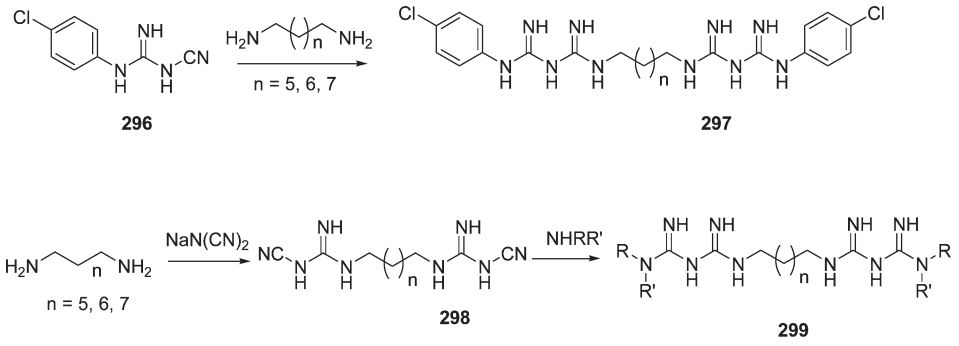
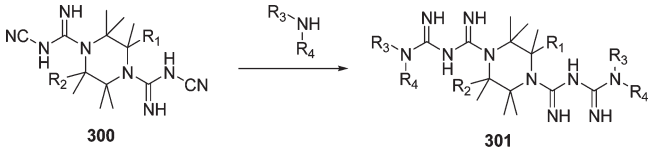
Scheme 62. Synthesis of Chlorhexidine and Bisbiguanylated Diamines



Scheme 63. Synthesis of Chlorhexidine Analogues



process for the manufacture of guazatine, with carbon-14 labeling of the terminal positions of the octamethylene chains, has been reported in the literature, and it is shown in Scheme 66.[159] Dibromide **311** was converted into the carbon-14 labeled dicyanide **312** through reaction with carbon-14 labeled sodium cyanide. Reduction of **312** led to diamine **313**, which was in turn converted into triamine and other oligomers with Ni catalyzed elimination of ammonia. Industrially, this stage is carried out under hydrogen pressure to control the rate of reaction and also to ensure the complete saturation of imine intermediates that may be formed. On the gram scale in the laboratory, however, it has been found more convenient to carry out the reforming reaction under nitrogen at atmospheric pressure and to rely on hydrogen adsorbed on the surface of the nickel catalyst for saturation of imine intermediates. Finally, the guanylation step was carried out with cyanamide in aqueous acetic acid, leading to compounds **315**.

3.3. Biological Properties of Guazatine

Guazatine is a nonsystemic contact fungicide that disturbs the membrane function of fungi and is used in agricultural seed treatment for control of certain seedborne diseases of wheat, barley, and oats. Guazatine belongs to the class of long-chain guanidine fungicides, as well as the dodine (guanidine-*n*-dodecane). It has been assumed that guazatine might act as a nonspecific toxicant, affecting several cellular functions, such as membrane permeability, uptake of nutrients, and respiration. World Health Organization (WHO) has classified guazatine as moderately hazardous with an oral LD_{50} value in rats of 280 mg/kg bw. In a number of short-term studies in rats, the overall NOAEL (no observed adverse effect level) of guazatine was 200 ppm, equivalent to 10 mg/kg bw per day. In a 1-year study in dogs, the NOAEL was 25 ppm, equal to 0.8 mg/kg bw per day. Several studies in rats concluded that guazatine is not genotoxic and carcinogenic, while in a study of developmental toxicity in rabbits, there were no signs of fetotoxicity or teratogenicity.[160] On citrus fruit, guazatine is used as a bulk

Table 12. Structures of Compounds 301a−s

compound	R_1	R_2	R_3	R_4
301a	H	H	CH_3	*p*-ClPh
301b	H	H	H	*p*-ClPh
301c	H	H	CH_3	*p*-ClPh
301d	CH_3	H	H	*p*-ClPh
301e	CH_3	CH_3	H	*p*-ClPh
301f	H	H	H	*m*-ClPh
301g	H	H	H	3,4-Cl_2-Ph
301h	H	H	H	*p*-BrPh
301i	H	H	H	*p*-FPh
301j	H	H	H	*p*-MeOPh
301k	H	H	H	*p*-MePh
301l	H	H	H	H
301m	CH_3	H	H	H
301n	CH_3	CH_3	H	H
301o	H	H	H	$CH(CH_3)_2$
301p	CH_3	CH_3	H	$CH(CH_3)_2$
301q	H	H	CH_3	CH_3
301r	CH_3	H	CH_3	CH_3
301s	CH_3	CH_3	CH_3	CH_3

dip after harvest and in the packing line as a spray.[161] Having only low mammalian toxicity, it is a useful replacement for the highly toxic organomercurial seed dressings.[162]

Botta and co-workers reported the antifungal activity of single components of guazatine against *Candida* strains.[157] The data compared with commercial mixture and fluconazole are summarized in Table 14. The guazatine mixture showed potent antifungal activity on all but one *Candida* strains, with MIC_{50} values ranging between 10 and 80 μM. The activity of isolated compounds was highly variable. GN, GG, and, to a lesser extent, GGGG and GGN showed modest activity within the concentration range tested. Conversely, GNG and GGG showed an overall good activity and MIC_{50} values that were in most cases lower than those of guazatine and fluconazole, taken as the reference drugs. GNG and GGG were particularly effective against fluconazole-resistant clinical isolates of *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis*. A guazatine mixture was also found to inhibit selectively *Zea mays* L. PAO with K_i values of 7.5×10^{-9} M at pH 6.5 and 25.0 °C.[163] At a structural level, guazatine extensively interacts with aromatic residues at the active site of PAO.[164] A remarkable feature revealed by the

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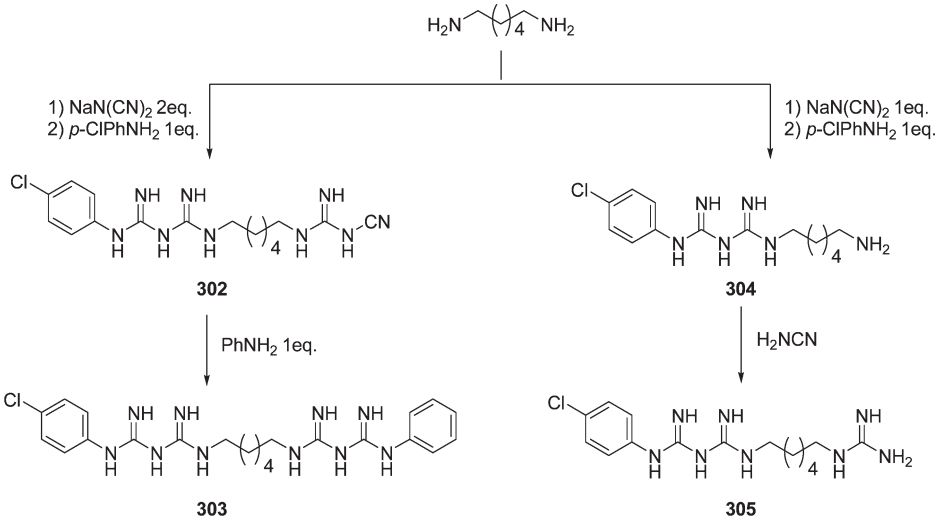
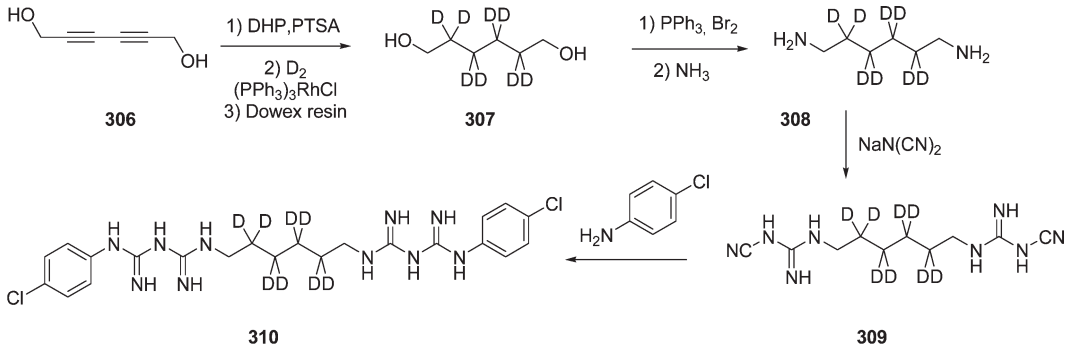
Scheme 64. Impurities in Stressed CHG Solutions



Scheme 65. Labelled Chlorhexidine



structure of the PAO−guazatine complex is the fact that the long inhibitor chain completely fills the catalytic tunnel.[165] Recently, a guazatine mixture was found to be able to inhibit the growth of *Penicillium digitatum* strains at the minimal concentration of 3.1 ppm.[166] Moreover, guazatine inhibited isolated strains of *Geotrichum citri-aurantii* (3.1−75 ppm), a fungus that causes a postharvest disease in mature and injured citrus fruits.[167]

Finally, guazatine component GNG 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 Δ1-pyrroline.[168] For this reason, guazatine was tested as, and found to be, an inhibitor of deoxyhypusine synthase,[169] which catalyzes cleavage of spermidine at the same site. However, the well-known toxicity of this compound, commonly used as a fungicide in agriculture, makes it unsuitable for physiological studies.

Table 13. Typical Composition of Free Guazatine Reported on International Portal on Food Safety, Animal and Plant Health

component	%	component	%
NN	0.8	GGG	30.6
GN	9.8	GNNG	1.4
GG	29.5	GGGN	1.4
NNN	<0.1	GGGG	5.1
NGN	0.8	tetramines	3.1
GNN	1.7	GGGGG	1.1
GGN	8.1	pentamines	1.4
GNG	4.5	hexamines and above	0.6

4. GUANYLATED TRIAMINES

Guanylated triamines are compounds with a triamine backbone whose one, two, or three nitrogens could be part of a guanidino moiety. The number of examples of guanylated triamines in the literature is relatively small when compared with guanylated diamines. However, in the last 20 years, a number of papers describing this class of compounds has appeared in the literature. Monoguanylated triamines can be classified in GNN or NGN compounds according to the nomenclature used for guazatine. Biguanylated triamines can be divided into GNG and GGN compounds. Moreover, these compounds can be still further classified in symmetric and unsymmetric compounds

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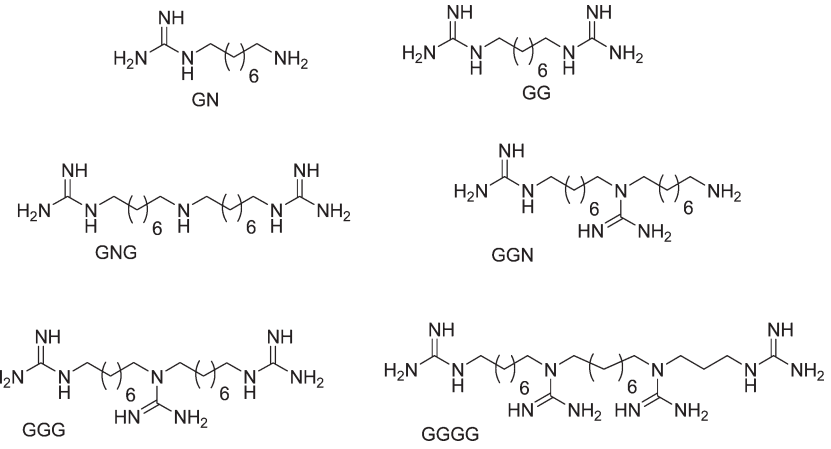

Figure 10. Components of the guazatine mixture.

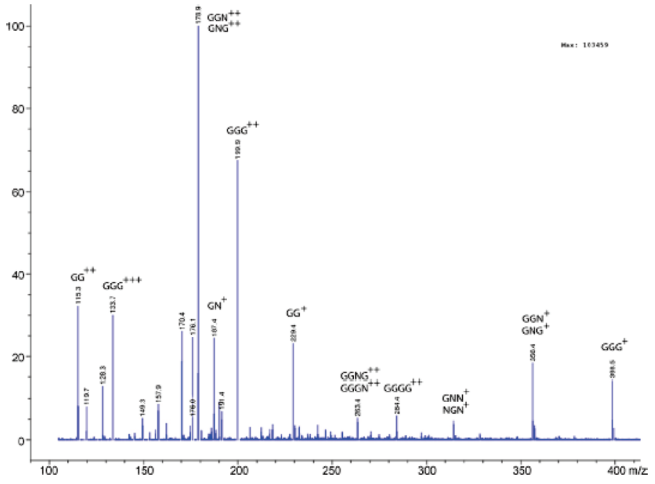

Figure 11. Mass spectrum obtained by direct injection of a sample of standard guazatine.

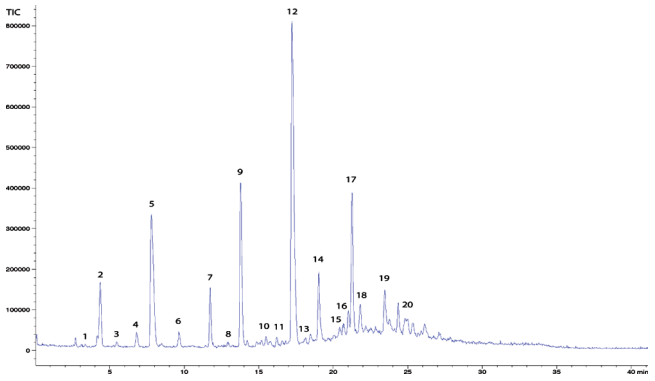

Figure 12. Chromatographic profile obtained for a sample of commercial guazatine (350 μg/mL) by LC−MS. (1) NN; (2) GN; (3) NNN; (4) GNN; (5) GG; (6) NGN;(7) GNG; (8) GGNN; (9) GGN; (10) GNGN; (11) GNNG; (12) GGG; (13) GGNG; (14) GGGN; (15) GGNGG; (16) GGGNG; (17) GGGG; (18) GGGGN; (19) GGGGG; (20) GGGGGG.

depending on the substituents on the guanidine moiety or on the length of the alkylic chain. Finally, triguanylated triamines will be

Scheme 66. Synthesis of Labeled Guazatine

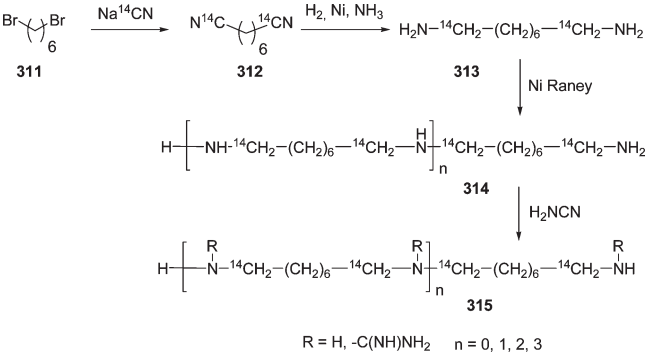

Table 14. Antifungal Activity of Guazatine Components

	MIC$_{50}$ (μM)						
Candida strains	GN	GG	GNG	GGG	GGGG	GM[a]	F[b]
C. albicans	>80	>80	40	5	80	80	0.8
C. krusei	>80	>80	20	10	40	20	>80
C. parapsilosis	>80	>80	20	>80	40	20	3.2
C. glabrata	>80	80	40	>80	>80	40	52
C. tropicalis		40	1.25	1.25	10	10	

[a] Guazatine mixture. [b] Fluconazole.

referred to as GGG, and also in this case they could be divided into symmetric and unsymmetric.

A triamine compound generally contains one secondary amino function. In principle, the main problem in the synthesis of guanylated triamines starting from triamines could be represented by the selective guanylation of a primary over a secondary amine or vice versa. Despite secondary amines being generally more nucleophilic than primary amines, these latter compounds react faster with electrophiles such as acylating or guanylating agents.[170,171] The lower reactivity of a secondary over a primary amine toward electrophiles has been widely documented and ascribed to steric factors.[172] Hence, guanylation of a triamine will occur faster at its primary amine functions. Guanylation of a

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triamine at its secondary function still represents an interesting synthetic challenge.

4.1. Monoguanylated Triamines

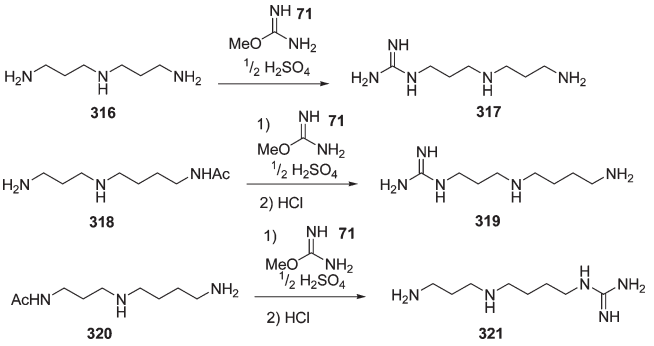
Few examples have been reported so far on the synthesis of monoguanylated triamines, and their preparations are strictly correlated with those of monoguanylated diamines. Monoguanylated triamines of GNN type can be synthesized through selective guanylation at the primary amine function. On the other hand, examples of triamines monoguanylated at their secondary amino group are rare and their synthesis is a bit more tedious.

Syntheses of the monoguanylated spermidine and caldine have been described in the 1990s through the guanylation of these natural triamines (Scheme 67). Caldine (norspermidine), a natural triamine found in plants, bacteria, and algae[173] and endowed with antitumoral activity, was selectively monoguanylated with 1/2 equiv of *O*-Me-isourea sulfate **71** to give compound **317**. On the contrary, guanylation of spermidine was more tricky because of its unsymmetric structure. However, starting from commercial acetylspermidines **318** and **320**, selective guanylation with OMe-isourea sulfate **71** was accomplished leading to, after deprotection, derivatives **319** and **321**.[85]

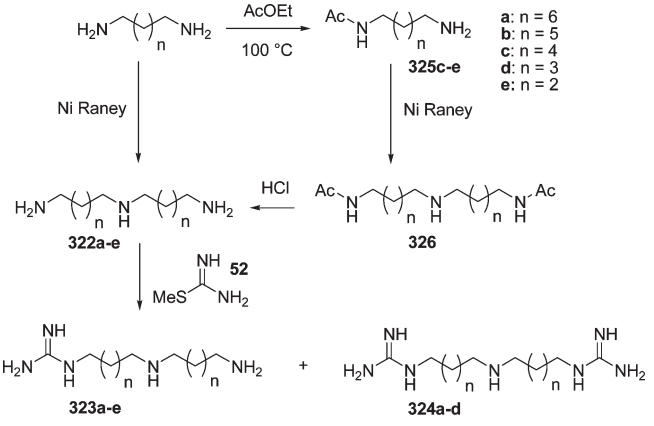
A similar approach using *S*-Me-isothiourea as guanylating agent was reported by the same authors two years later during their studies on the synthesis of deoxyhypusine synthase inhibitors.[87] In this paper, an intriguing synthesis of several triamine building blocks was also described. (Scheme 68). Triamines **322a**−**e** were prepared through conversion of primary to secondary amines with Ni-Raney. The 7- and 8-carbon chain diamines reacted with Ni catalyst in refluxing benzene affording **322a**−**b**. The 4-, 5-, and 6-carbon chain diamines reacted rapidly with Ni-Raney but did not produce triamines **322c**−**d**. Hence, these latter compounds were prepared in a different way from monoacetyl derivatives **325c**−**e**. These latter compounds reacted fast with Ni-Raney, affording desired symmetric triamines **326c**−**e**, which were in turn hydrolyzed to **322c**−**e**. Finally, reaction of **322a**−**e** with an equivalent amount of *S*-Me-isothiourea sulfate **52** led to monoguanylated triamines **323a**−**e**. The use of a double equivalent amount of guanylating agent led to biguanylated triamines **324a**−**d**.

A more versatile approach to monoguanylated triamines, and in the specific case spermidine, was also described in the same

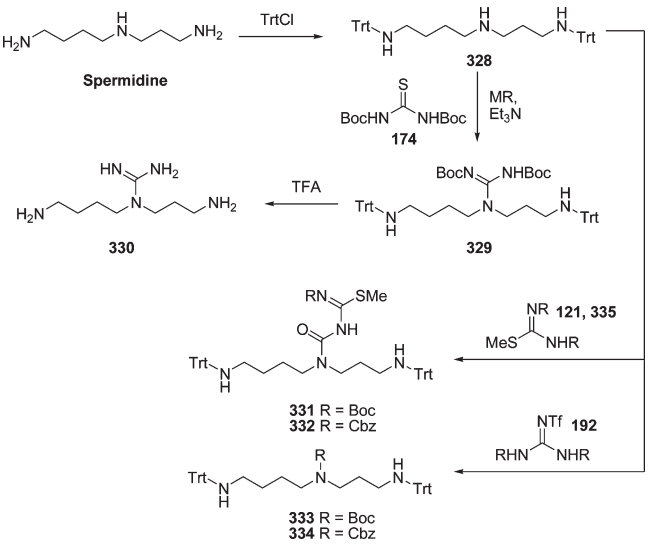
Scheme 67. Synthesis of Monoguanylated Triamines



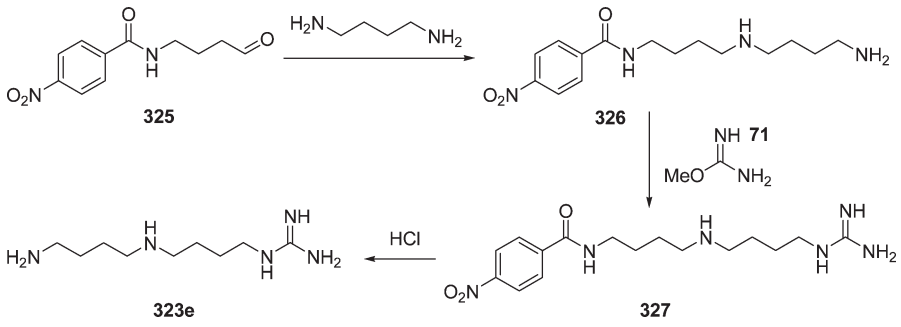
Scheme 68. Synthesis of Mono- and Biguanylated Triamines



Scheme 70. Guanylation of Spermidine at Its Secondary Amine Function



Scheme 69. Synthesis of Monoguanylated Triamine 323e through Reductive Amination Reaction



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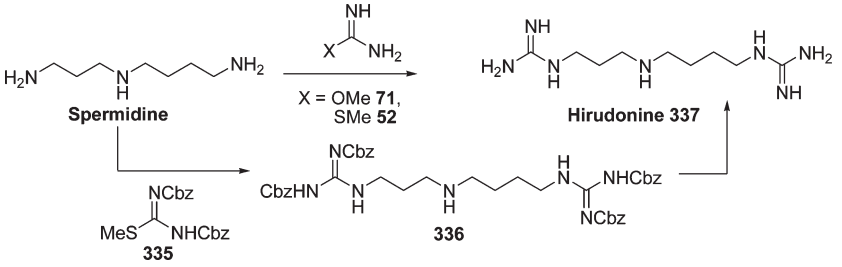
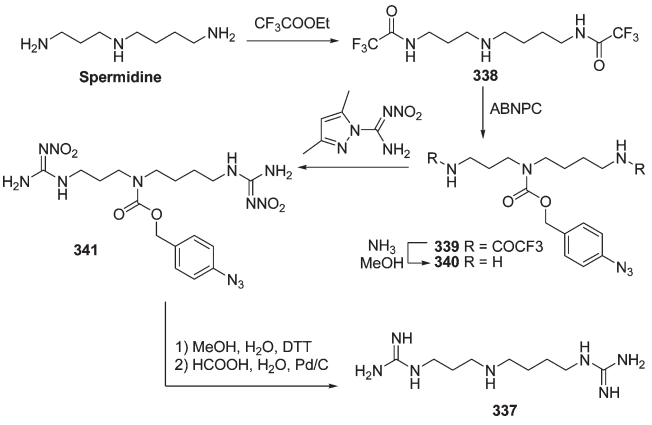
Scheme 71. Synthesis of Hirudonine



Scheme 72. Alternative Synthesis of Hirudonine



Scheme 73. Synthesis of GNG-type Biguanylated Triamines

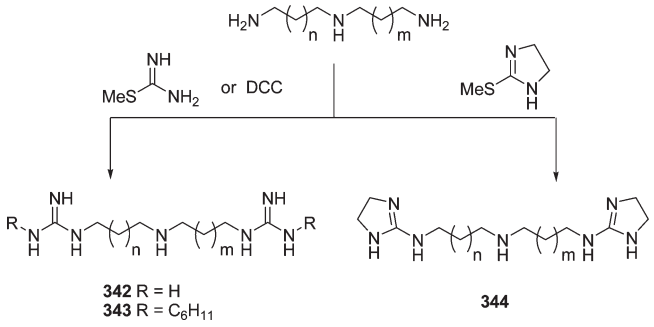
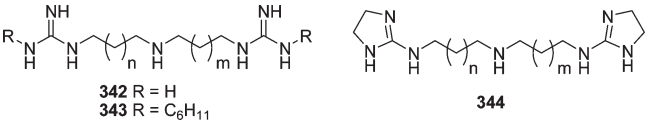


Table 15. Structures of Compounds 342, 343, and 344



compound	*n*	*m*	compound	*n*	*m*
342a	0	0	**344a**	0	0
342b	0	1	**344b**	0	1
342c	1	1	**344c**	1	1
343	0	1			

paper. The triamine **326** was synthesized through reductive amination between the protected aminoaldehyde **325** and the 1,4-butandiamine in the presence of $NaBH_3CN$ as imine reducing agent. Then, selective guanylation with *O*-Me-isourea sulfate **71** and hydrolysis of benzoyl group led to compound **323e**[87] (Scheme 69). This synthetic approach could represent a versatile method that could allow the synthesis of a plethora of monoguanylated triamines, symmetric and unsymmetric, starting from readily available building blocks such as aldehydes and diamines, overcoming the availability of commercial triamines.

As mentioned above, the synthesis of compounds of the NGN type is a bit more difficult because sterically demanding secondary amines react less smoothly and efficiently than primary amines. In this connection, an interesting study on the guanylation of spermidine at secondary amine function was reported by Athanassopoulos et al.[174] After protection of primary amines of spermidine with trityl protecting groups, the authors explored the use of different guanylating agents. The use of di-Boc-*S*-Me-isothiourea **121** led to the formation of urea **331** arising from the attack of secondary amine on the *tert*-butoxycarbamate of the guanylating agent. When di-Cbz-*S*-Me-isothiourea **335** was used, compound **334** was observed in 34% yield together with small amounts of **332**. The reaction of **328** with powerful guanylating agent triflic guanidines **192** produced side products **333** and **334**. Finally, the authors explored the use of di-Boc-thiourea **174** in the presence of the Mukayama's reagent (MR) or with DCC. In the first case, the reaction went to completion in a few minutes, affording **329** in 96% yield, whereas in the second case, guanylation was not completed even under stressed conditions. (Scheme 70).

4.2. Biguanylated Triamines

Biguanylated triamines can be obtained as previously described through simple guanylation of the appropriate triamine with 2 equiv of guanylating agents.[87] Guanylation of primary amine occurs faster than that on secondary amine function. A well-known biguanylated triamine is the natural compound Hirudonine **337**, namely, the N^1-N^8-biguanylated spermidine, a rare guanylated triamine located in the central nervous system of the leech *Hirudo medicinalis* L.[175] and whose crystal structure has been described in 1987.[176] Syntheses of Hirudonine have been accomplished through double guanylation of spermidine with *O*-Me-isourea sulfate **71**,[85] with *S*-Me-isothiourea sulfate **52**[177] or with di-Cbz-*S*-Me-isothiourea **335** followed by hydrogenolysis of the protecting group[178] (Scheme 71).

A more articulated synthesis of Hyrudonine was also described by the use of nitroguanidine group as a masked guanidine.[43,179]

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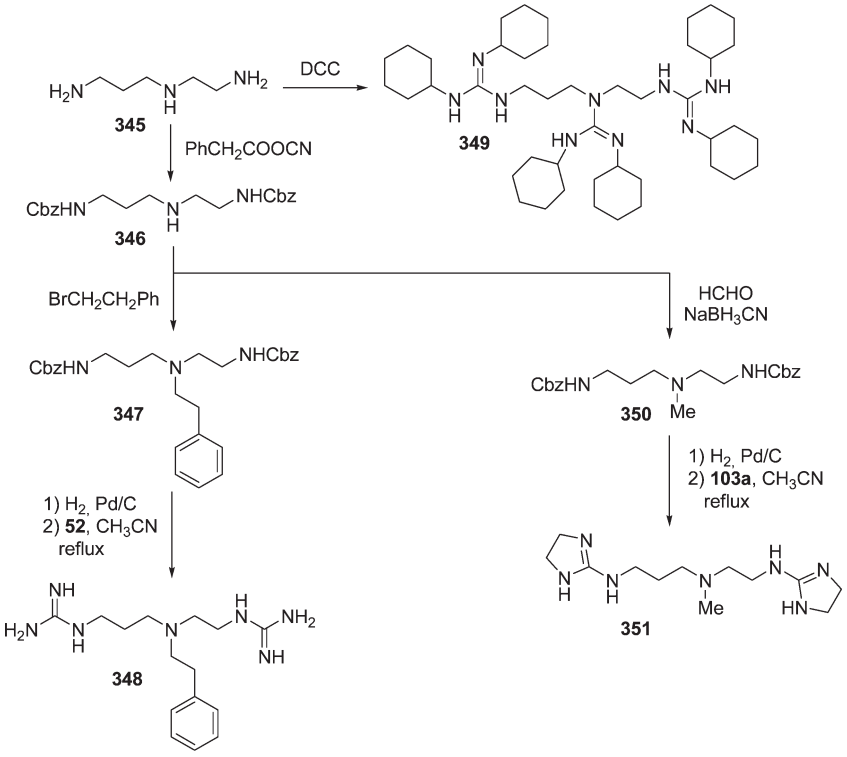
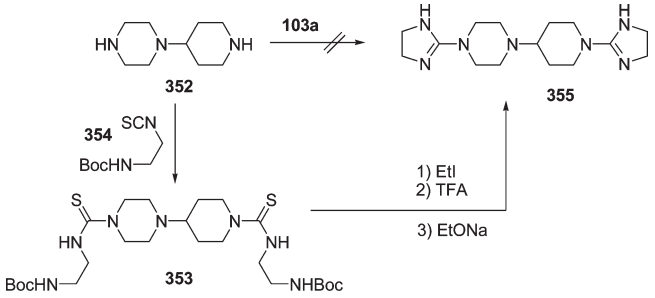
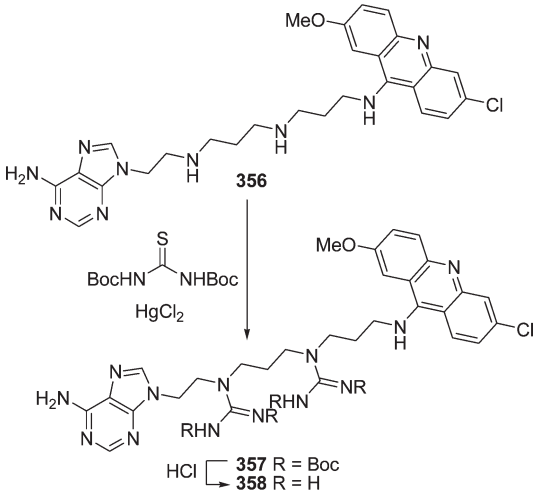
Scheme 74. Syntheses of Bi- And Triguanylated Triamines



Scheme 75. Synthesis of the Conformationally Restricted Compound 355



Scheme 76. Synthesis of GGN-type Biguanylated Triamine 358



Nitroguanidine, due to its relative low basicity, reduced the purification problems, making synthetic transformations easier. Treatment of spermidine with CF_3COOEt gave product **338** as trifluoracetate salt, which was then converted into the fully protected intermediate **339** using 4-azidobenzyl-4-nitrophenyl carbonate (ABNPC). Deprotection of terminal amino groups led to **340**, which was then biguanylated using 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide (DMNPC). Deprotection of *N*4-amino group was accomplished with excess of dithiothreitol (DTT) followed by hydrogenolysis, which afforded Hyrudonine **337** (Scheme 72).

The synthesis of a series of GNG type biguanylated triamines **342**−**343** was also reported by Dardonville et al. through simple guanylation of appropriate triamines with *S*-Me-isothiourea sulfate **52** or dicyclohexylcarbodiimide (DCC).[127b] A series of imidazolium derivatives **344** was also reported, with the aminoimidazolinium moiety being the cycloalkylated version of the guanidine group (Table 15, Scheme 73).

The synthesis of guanylated triamines **348**, **349**, and **351** whose secondary nitrogens were differently alkylated proved to be more articulated.[127b] Triamine **345** constituted the common starting scaffold, which was protected at its primary amino groups. Alkylation of **346** with bromoethylbenzene led to **347** that was then deprotected and guanylated with *S*-Me-isothiourea sulfate **52**. Reductive amination between **346** and formaldehyde

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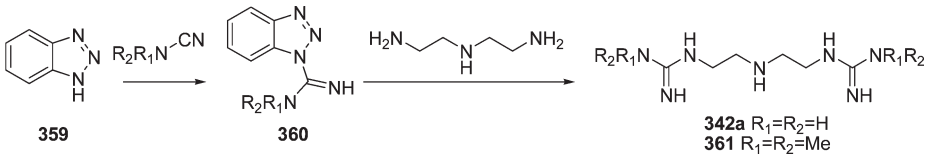
Scheme 77. Use of Benzotriazole 360 in the Synthesis of Biguanylated Triamines



Scheme 78. Synthesis of Iminoctadine Derivatives

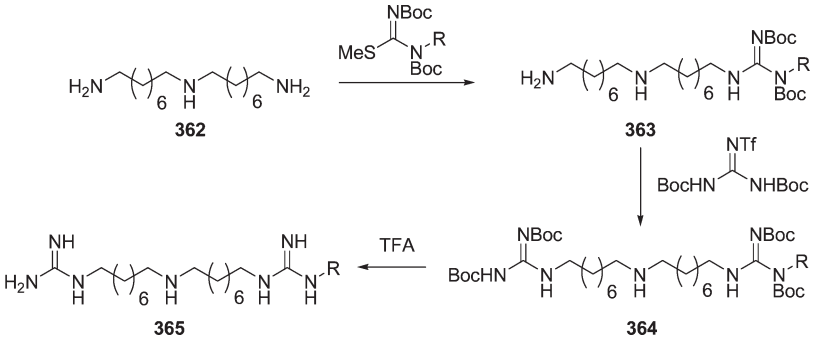


Table 16. Structures of Iminoctadine Derivatives

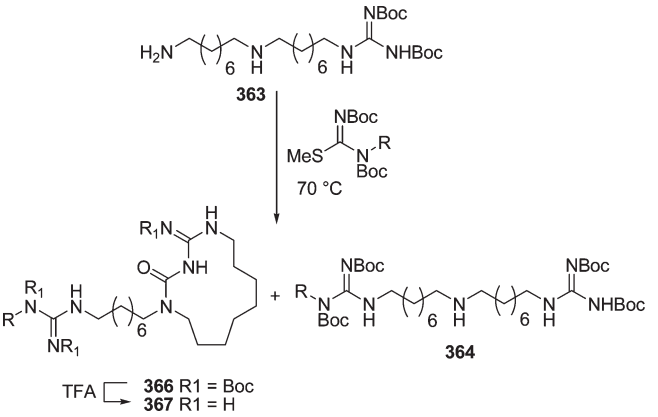
compound	R	compound	R
365a	H	**365e**	isobut-2-enyl
365b	prenyl	**365f**	benzyl
365c	cyclopropylmethyl	**365g**	propargyl
365d	crotyl		

led to **350**, which was converted in 2 steps into imidazolinium derivative **351**. Finally, the triguanylated triamine **349** was obtained from **345** through reaction with DCC in refluxing CH_3CN (Scheme 74).

Noteworthy is the synthesis of conformationally restricted compound **355**, which can be considered as the tetracyclic version of a classical biguanylated triamine. The triamine skeleton **352** is constituted by 1-(4-piperidyl)piperazine. Attempts to transform **352** into **355** with 2-*S*-Me-4,5-dihydroimidazole iodide **103a** proved to be unsuccessful, possibly for sterical reasons. Hence, an elegant way was described to generate imidazolinium moiety, based on a methodology reported by Ariga and Anslyn.[180] Compound **352** was reacted with isothiocyanate **354**, yielding thiourea **353**. This latter compound was converted into **355** in a one-pot, three-step sequence. Sulfur of **353** was alkylated with EtI to form ethyl isothiourea intermediate. Removal of Boc of this intermediate with TFA generated the intramolecular nucleophile that cyclized in the presence of EtONa to give desired compound **355**[181] (Scheme 75).

Guanylation of secondary amines proved to be difficult as previously seen. In this context, an interesting example is the synthesis of the GGN type biguanylated triamine **358** as potential anticancer alkylating agent.[182] Triamine **356** was rationally designed and contains an aminoacridine intercalator for DNA binding, an adenine moiety for site recognition, and a triaminoalkyl linker. One of the amino moieties is part of the aminoacridine

Scheme 79. Macrocyclization of di-Boc-Monoguanylated Triamine 363

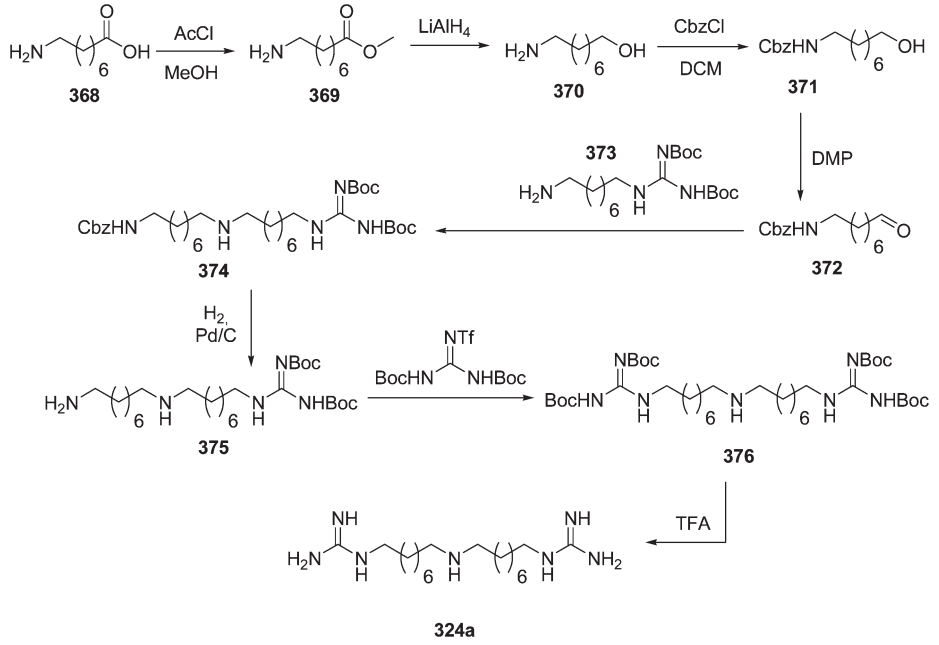


group, while the other two amino moieties are secondary amines. Guanylation of **356** proceeded using the strong guanylating agent di-Boc-thiourea, which was activated to carbodiimide with $HgCl_2$. Then, deprotection in acidic medium led to desired compound **358** (Scheme 76).

Just because of the poor tendency of certain amines to be guanylated, several new guanylating agents have been developed so far. Among these, the benzotriazole **360** appeared as a highly reactive guanylating agent in guanylation of sterically hindered or poorly nucleophilic secondary amines, superior than the corresponding pyrazol- or triflyl compounds. Guanylating properties of benzotriazole **360** were shown in the synthesis of biguanylated triamines **342a** and **361**. Compounds were obtained through a μW assisted one-pot parallel synthetic sequence. Guanylation occurred at 60 °C in 6 h and afforded biguanylated triamines

dx.doi.org/10.1021/cr100423x |*Chem. Rev.* 2011, 111, 5247–5300

Scheme 80. Synthesis of Iminoctadine



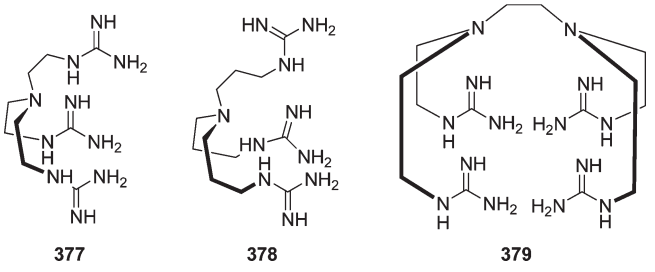


Figure 13. Guanylated polyamines as metal ligands.

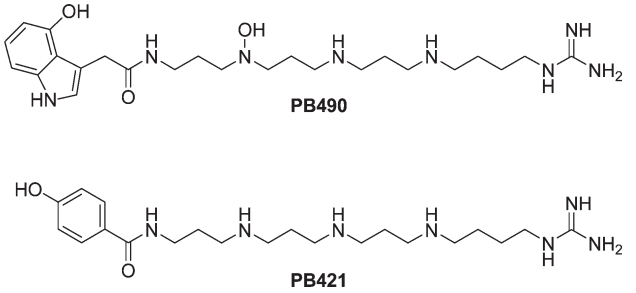


Figure 14. Natural guanylated polyamines found in the venom of the spider *Paracoelotes birulai*.

342a and **361** in 99% yields.[183] Traces of corresponding mono- and triguanylated triamines were detected via ESI-MS analysis (Scheme 77).

An in depth study on biguanylated triamines related to GNG and GGG components of guazatine mixture was recently reported by Botta and co-workers. The GNG component of guazatine, namely, the iminoctadine, proved to be an excellent inhibitor of PAO from *Zea mays* (ZmPAO) with K_i = 7.5 nM. Hence, a series of guanidines **365** was synthesized and assayed as inhibitors of ZmPAO.[133] Compounds **365** are unsymmetric due to different substituents on the guanidine moieties, and for this reason, two distinct guanylation steps were required. The synthesis of these derivatives started from commercial 1,17-diamino-9-azaheptadecane **362**, which was first monoguanylated at 50 °C with different di-Boc-*S*-Me-isothioureas. These latter compounds were synthesized apart through alkylation reaction with appropriate bromides or via Mitsunobu reaction with appropriate alcohols. Compounds **363** were then further guanylated with trifylguanidine at room temperature, affording **364**, which were in turn deprotected to give desired biguanylated triamines **365a**−**g** as triflic salts (Scheme 78, Table 16).

Because of the antifungal properties of guazatine, some of these compounds were later assayed as antifungal agents. Just in this context, authors discovered an interesting side reaction during the synthesis of biguanylated triamines. When monoguanylated triamine **363** was reacted with di-Boc-*S*-Me-isothioureas **121** at $T \geq 70$ °C, the formation of macrocyclic amidinoureas **366** was observed[184] (Scheme 79). This side reaction was due to an intramolecular nucleophilic addition of secondary amino moiety on the carbonyl of Boc-protecting group.[185,114] Macrocyclization is self-catalyzed, and it starts just at $T \geq 70$ °C, while at lower temperatures, simple guanylation was observed. It is not still clear if amines **363** were first cyclized and then guanylated or vice versa. However, the authors reported that in some cases macrocycles **366** were not formed and only the linear biguanylated triamines were isolated. These data leave one to think that the guanylation step might occur first and then be followed by the macrocyclization. Macrocyclic amidinoureas were obtained in ∼40% yields and, after deprotection, were assayed as antifungal agents, revealing in some cases a biological

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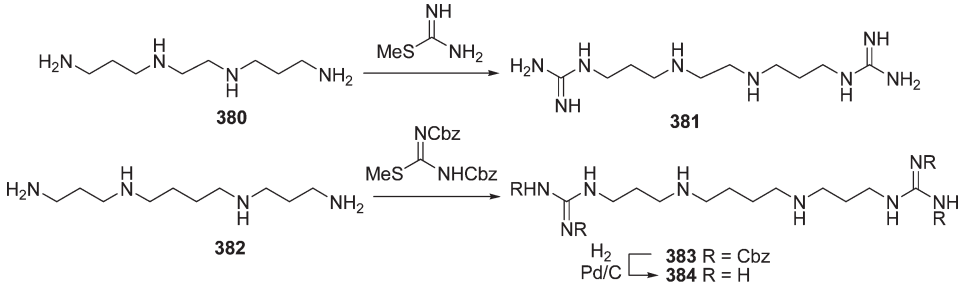
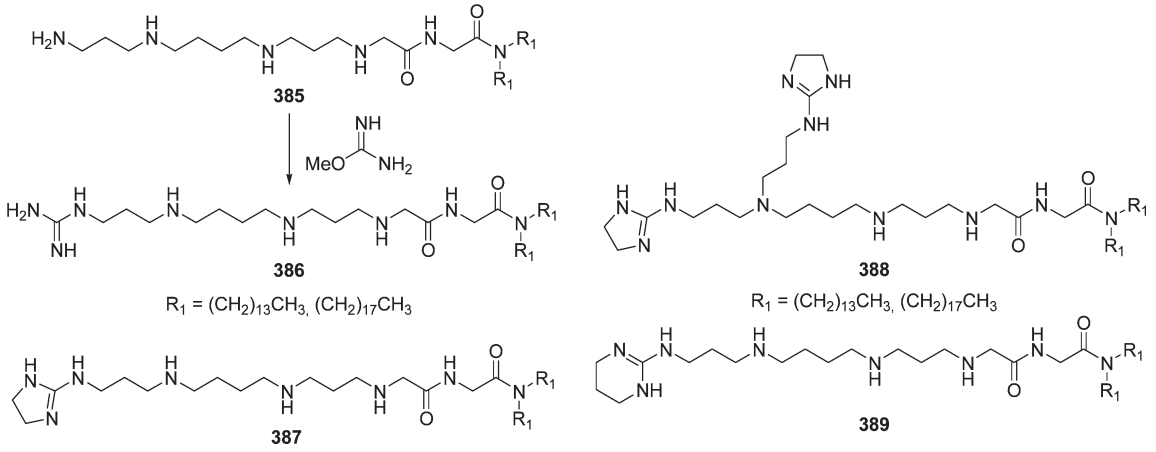
Scheme 81. Synthesis of Biguanylated Polyamines



Scheme 82. Synthesis of Monoguanylated Polyamine 386 and Its Derivatives



profile better than that of common antifungal drugs.[186] This behavior of Boc-protected guanylated polyamines could represent a problem, which in any case, being side reaction temperature dependent, could be overcome by the use of strong guanylating agents able to operate at lower temperature, such as the trifylguanidine.

The key commercial synthon for the synthesis of guazatine derivatives, namely, the 1,17-diamino-9-azaheptadecane **362**, was recently retired from the market. For this reason, Botta and co-workers were forced to elaborate a new synthetic strategy to obtain biguanylated triamines. In particular, the synthesis of the guazatine component iminoctadine through a convergent approach has been described[187] (Scheme 80). In a parallel work, the authors synthesized the building blocks, namely, the aldehyde **372** and the monoguanylated diamine **373**. Aldehyde **372** was obtained starting from aminocaprilic acid **368**, which was first esterified and then reduced at alcohol **370**. This latter compound was protected at primary amine as Cbz-derivative **371** and then oxidized with Dess-Martin periodinane (DMP), affording aldehyde **372**. Reductive amination between aldehyde **372** and amine **373**, prepared through guanylation of 1,8-diaminooctane with di-Boc-*S*-Me-isothiourea, led to monoguanylated triamine **374**. Then, hydrogenolysis followed by guanylation of primary amine with trifylguanidine led to biguanylated triamine **376**. Finally, Boc-cleavage with TFA led to iminoctadine **324a** (the GNG component of guazatine mixture) as triflic salt.

5. GUANYLATED POLYAMINES

In addition to the previously described guanylated diamines and triamines, in this chapter, the attention will be focused on the guanylated polyamines, namely, those compounds possessing a polyamine backbone with 4 or more amino moieties, where at least one is included into a guanidine function. Hence, the term "polyamine" will be used in this chapter to indicate those compounds containing ≥4 amino functions. Polyamines, such as the natural spermine, are natural products widely found in microorganisms, plants, and animals and are involved in several important biological functions. Some natural alkaloids especially from marine microorganisms and plants also possess a polyamine structure. Finally, polyamines are sometimes used in the synthesis of complexes with metals. Even if examples of polyamines guanylated at one or more of their amino moieties have been described so far in the literature, the amount of these compounds still remains slight when compared to guanylated diamines or triamines. Despite the fact that di-, tri-, and polyamines are widespread in nature, natural guanylated polyamines are quite rare.[188] The synthesis of guanylated polyamines is generally the consequence of a chemical modification on polyamines, made for different purposes, to alter their biological activities, solubility, or electronic profiles by the introduction of one or more guanidinum groups.

An interesting early example of polyguanylated polyamines was reported in 1979 in the field of anion coordination chemistry.

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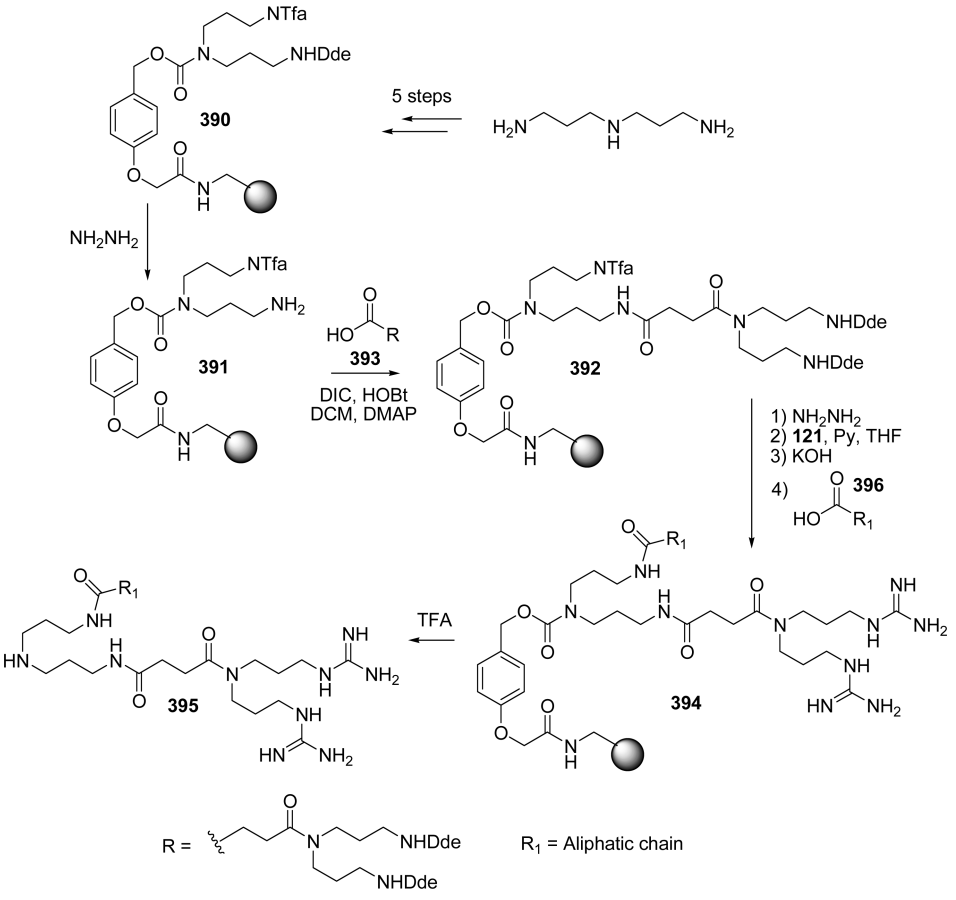
Scheme 83. Solid-Phase Synthesis of Biguanylated Polyamine 395



A series of polyguanidinium slats was synthesized via polynitrile intermediates and used as anion complexes for different metals.[84] The synthesis of these compounds (Figure 13) was accomplished starting from the appropriate polyamines through the reaction with *N*-nitro-OMe-isourea as guanylating agent as previously described in Scheme 46 for the synthesis of biguanylated diamine **72**.

Recently, two natural guanylated polyamines have been found and fully characterized in the lyophilized venom of the spider *Paracoelotes birulai* (Araneidae: Amaurobiidae). The structure of these compounds, named **PB490** and **PB421**, reported in Figure 14, was elucidated through high-performance liquid chromatography-MS (HPLC-MS) analysis. Both of these compounds present a monoguanylated pentamine structure.[189]

Synthetic approaches to guanylated polyamines closely follow those already seen for guanylated di- or triamines. Guanylation at primary amino groups occurs faster than at secondary ones and can be carried out using di-Cbz-*S*-Me-isothiourea or simple *S*-Me-isothiourea sulfate, as for the synthesis of compounds **381** and **384**[178−190] (Scheme 81).

Similarly, more complex guanylated polyamines **386**−**389** at primary amino functions were obtained with *O*-Me-isourea sulfate or with the corresponding cyclic 2-methylmercapto derivatives.[191]

A solid-phase synthesis of guanylated polyamines as cationic lipids and able to bind DNA was reported by Bradley and co-workers.[192] These compounds contain the general features of a cationic lipid, namely, one or two guanidinium polar head groups attached to one or two hydrophobic tails, such as single- or double-hydrocarbon chain of 12−18 carbons in length. Biguanylated polyamine **395** was synthesized as reported in Scheme 83 starting from the resin **390**, which was obtained in 5 steps from bis-(3-aminopropyl)amine. An aminomethyl polystyrene resin was used as a solid support. Resin **390** was treated with hydrazine to give amine **391**, which was in turn converted in scaffold **392** by treatment with acid **393**. The two Dde groups were then selectively removed, and the free amino groups were guanylated with di-Boc-*S*-Me-isothiourea to give **394**. Trifluoroacetyl protecting group was then removed, and free amino group was coupled with a range of acids **396**. Finally, cleavage from the solid support afforded biguanylated polyamines **395**. Similarly, monoguanylated polyamines **400** were synthesized in 6 steps as reported in Scheme 84. Also in this case, guanylation was accomplished with di-Boc-*S*-Me-isothiourea.

An interesting synthesis of polyamines guanylated only at their secondary amine functions was recently described. Selective guanylation was accomplished through an appropriate manipulation of protecting groups and by the use of di-Boc-thiourea activated by the Mukayama's reagent (MR)[174] (Scheme 85).

Noteworthy is the work done by Woster and co-workers in recent years regarding the synthesis of antitrypanosomal compounds with a (bis)biguanylated polyamine structure. These compounds were synthesized as guanylated derivatives

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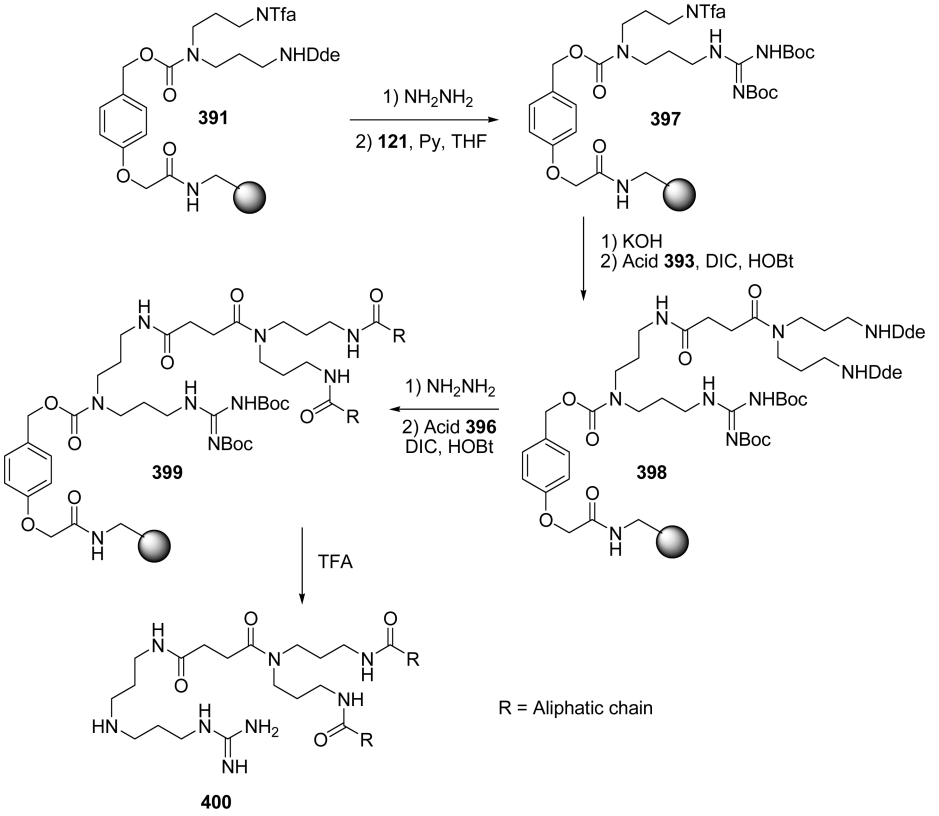
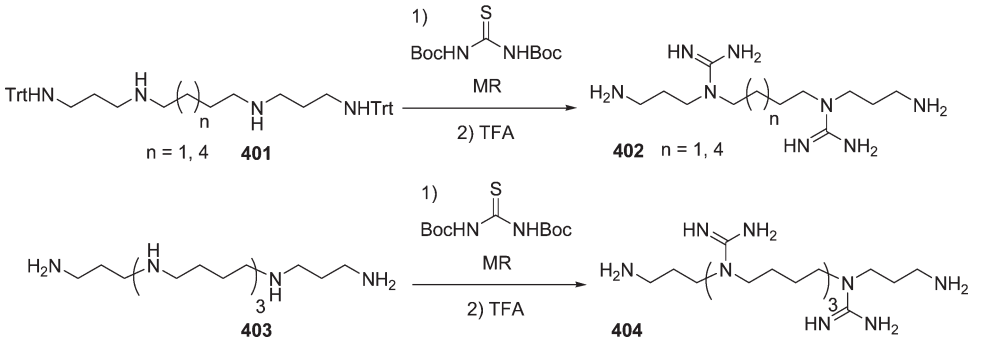
Scheme 84. Solid-Phase Synthesis of Monoguanylated Polyamine 400



Scheme 85. Polyamines Guanylated at Their Secondary Amine Functions



of natural polyamines, which are known to act as inhibitors of trypanosomes interfering with their polyamine metabolism. Moreover, the introduction of a bisguanidine function was planned in connection with the antimicrobic agent Chlorexidine **297**, which proved to act also as an inhibitor of trypanothione reductase from *Trypanosoma cruzi*.[193] Two series of compounds were synthesized as reported in Scheme 86. Biguanylated polyamines **409** were obtained starting from commercial amines **405**, which were converted into *N*-cyano intermediates **406**. These latter compounds were then coupled with diamine **408**, synthesized according to reported procedures.[194] Finally, cleavage of mesityl protecting groups led to guanylated polyamines **409**. On the contrary, bisbiguanylated compounds **410** were synthesized from the reaction of cyanoguanidines **407** with diamine **408** followed by mesityl deprotection (Scheme 86). Compounds synthesized[195−197] were reported in Table 17.

Another interesting approach to guanylated polyamines endowed with NOS inhibitory activity was recently described by Silverman and co-workers. A series of enantiopure guanylated derivates bearing a nitro group on the guanidine moiety were synthesized starting from Boc-L-Arginine-(NO_2)−OH **411** as described in Scheme 87. Arginine derivative **411** was converted into amide **412**, which was in turn reduced with BH_3−THF complex at −10 °C to give polyamine **413**. Low reaction temperature was required to overcome the cleavage of the Boc

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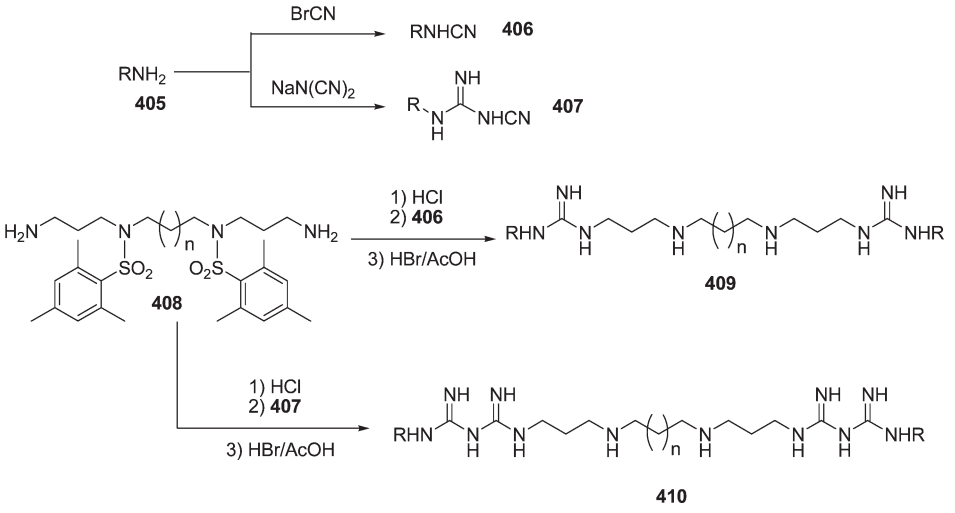
Scheme 86. Synthesis of Bisbiguanylated Polyamines



protecting group. Further manipulation of this latter compound led to derivatives **415** and **418**, which were assayed as NOS inhibitors[198] (Scheme 87).

X-ray crystal structure of nNOS with inhibitor **418** bound was also reported, revealing that the hydroxyl group provided a direct interaction with the heme proprionate moiety of the enzyme.[199] Further nitroarginine derivatives **421**, **423**, and **425** endowed with NOS inhibitorial activity were synthesized in a few steps from aldehyde **419** through reductive amination with the appropriate amine (Scheme 88).[200]

A clear example of natural guanylated polyamine is represented by the Martinelline, an alkaloid isolated from an organic extract of Amazonian plant *Martinella iquitosensis* roots. Martinelline **442** can be considered a nonpeptidic natural tetramine with a tricyclic pyrroloquinoline core. Three of its nitrogens are part of a guanidine moiety. Martinelline was first isolated by Witherup, Varga, and co-workers together with its derivative Martinellic acid, and it proved to be an effective inhibitor for several G-protein coupled receptor systems including bradykinin B1 and B2 as well as histaminergic, α1-adrenergic, and muscarinic receptors.[201] Total synthesis of Martinelline has been reported at the same time by the groups of Batley and Ma. In both cases, the tricyclic core **429** has been synthesized through a three-component imino-Diels–Alder reaction. Further manipulation of intermediate **429** led to triamine **430**. Ma et al. described a single-step biguanylation of **430** using *S*-Me-isothiourea **432** in the presence of $AgNO_3$,[202] leading to **434**. On the other hand, Batley et al. converted **429** into Troc-derivative **431**, which was in turn manipulated to biguanylated compound **435** in a 3-step sequence. First, guanylation was performed with the appropriate *S*-Me-isothiourea **432** in the presence of $HgCl_2$, leading to **433**. Removal of the protecting group of **433** with Zn dust followed by guanylation with the protected *S*-Me-isothiourea **125g** in refluxing CH_3CN afforded **435**.

Martinelline side chain **439** was synthesized from crotylamine **437**, in turn obtained in 6 steps from alcohol **436**. Amine **437** was guanylated with trifylguanidine **192** or di-Boc-*S*-Me-isothiourea **121**, leading to intermediate **438**, which after tetrabutyl ammonium fluoride (TBAF) mediated deprotection was converted into desired product **439**. Coupling of acid **440**, obtained by basic hydrolysis of **435**, with alcohol **437** followed by Boc deprotection with TFA, led to Martinelline **442**.[203,204] Further approaches to the synthesis of Martinelline heterocyclic core have been described[205,206] as well as the first asymmetric total synthesis of (−)-Martinelline.[207] In all cases, guanylation steps have been performed by the use of Boc-*S*-Me-isothioureas in the presence of $AgNO_3$.

Table 17. Structures of Compounds 409 and 410

compound	*n*	R	compound	*n*	R
409a	1	3,3-diphenylpropyl	**409h**	5	2,2-diphenylethyl
409b	1	2,2-diphenylethyl	**410a**	1	3,3-diphenylpropyl
409c	2	3,3-diphenylpropyl	**410b**	1	2,2-diphenylethyl
409d	2	3,3-diphenylethyl	**410c**	2	3,3-diphenylpropyl
409e	5	cycloheptyl	**410d**	2	2,2-diphenylethyl
409f	5	benzyl	**410e**	5	3,3-diphenylpropyl
409g	5	3,3-diphenylpropyl	**410f**	5	2,2-diphenylethyl

6. BIOLOGICAL PROPERTIES OF GUANYLATED DIAMINES, TRIAMINES, AND POLYAMINES

Guanylated diamines and polyamines, as partially shown during the description of synthetic methodologies, possess several and different biological properties, often not strictly related to their chemical structures. It is difficult to make a classification of biological properties of guanylated polyamines on the basis of their structures because a similar biological activity might be possessed by a monoguanylated diamine as well as a triamine or a bi- or polyguanylated polyamine. Moreover, because of the strict structural relations between guanylated polyamines and biogenic polyamines, a certain compound can show activity toward different targets even not correlated each another. Herein, guanylated di-, tri-, and polyamines will be classified on the basis of their chief biological activities.

6.1. Antimicrobial Activity

Guanylated polyamines were found to possess activity toward different microbial agents, particularly against bacteria, fungi, and protozoa.

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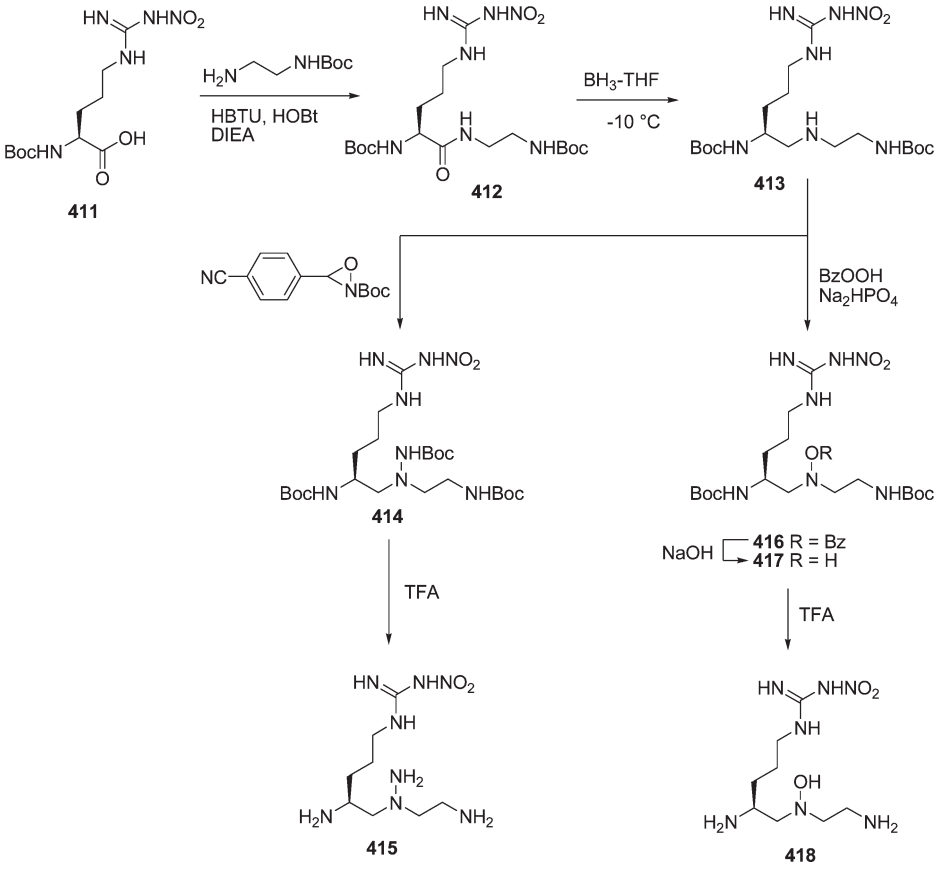
Scheme 87. Synthesis of Nitroguanylated Polyamines 415 and 418



Antibacterial Agents. Chlorhexidine is probably the oldest known drug with guanylated diamine structure possessing antibacterial activity. Chlorhexidine showed activity against several bacteria such as *S. aureus* (MIC = 3 μg mL^{-1}), *E. coli* (MIC = 3 μg mL^{-1}), and *S. thypi* (MIC = 1.5 μg mL^{-1}).[148,153,208] Studies to evaluate the structure−activity relationships (SARs) on Chlorhexidine **297** and its derivatives were carried out, in particular to establish the optimum distance that should exist between the two guanidine groups and the most effective types of substituents on these ending groups (Figure 15). The highest antibacterial activity was shown by derivatives with bisbiguanylated diamine structure, and in particular from those compounds where n = *5*, *6*, or 7. The introduction of hydroxyl or carbonyl substituents into the terminal aryl groups was detrimental for activity, as was the replacement of bisguanidine by guanidine. Replacement of aryl groups with alkyl substituents led to less active compounds.[209] Heterocyclic derivatives **301** proved to be active as Chlorhexidine in particular against *S. aureus*.[148] The importance of bisbiguanylated moieties was demonstrated by the fact that the biguanylated diamine Synthalin A proved to have antibacterial activity lower than Chlorexidine against *S. aureus* with MIC = 64 μg mL^{-1}.[119]

Clinical studies on Chlorexidine proved that it is an efficient inhibitor of dental plaque.[210,211] Yi et al. reported that analogues of Chlorexidine with *n* = 9 and 10 are quite active against *S. aureus* but less active than lead compound against other *Staphylococcus* strains.[154]

Antiprotozoal Agents. Protozoans have little in common with each other, and so agents effective against one pathogen may not be effective against another. However, some guanylated polyamines proved to be very active against Malaria and Trypanosoma species. Compounds **98** synthesized from agmatine derivatives **97** showed activity against *P. falciparum* (MIC = 2−10 μg mL^{-1}) comparable to that of antimalarial drug pyrimethamine.[88] Biguanylated diamines **256**−**257** showed potent antimalarial activity with IC_{50} of 0.35, 0.5, and 0.6 nM, respectively, against *P. falciparum*. A SAR study revealed that the optimal length of the alkyl chain between two guanidines is 12 methylene groups. Moreover, the basicity of guanidine moieties is strictly correlated with the antimalarial activity of these compounds as well as their ability to form a hydrogen bond with the target(s).[131] Also biguanylated diamines **227a**−**c**, **241a**−**d**, and **242a**−**g** synthesized by Dardonville et al. showed good antimalarial activity.[127a,212] On the other hand, biguanylated and bisbiguanylated polyamines **409** and **410** synthesized by Woster and co-workers proved to be excellent antitrypanosomal agents with IC_{50} values against *Trypanosoma brucei brucei* between 0.09 and 3.35 μM. All these compounds proved to inhibit selectively the enzyme trypanothione reductase without affecting the human enzyme glutathione reductase.[195] Derivatives **245** and **247** synthesized by Dardonville et al.[128a] showed nanomolar activity against *Trypanosoma brucei rhodesiense* and *P. falciparum*. Also in this case, the

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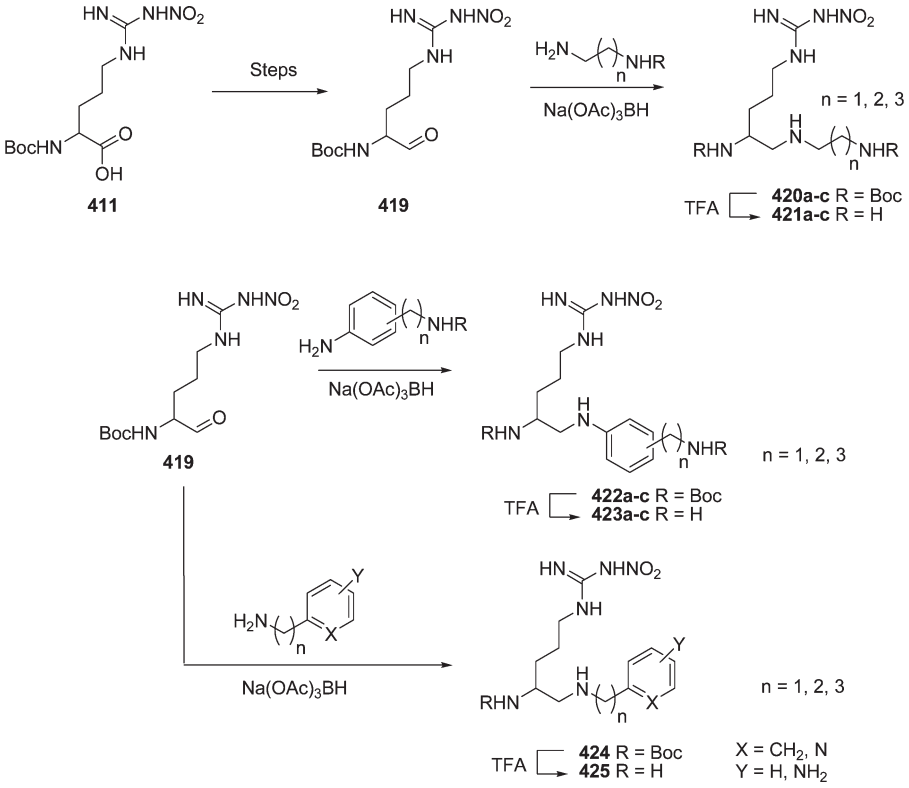
Scheme 88. Alternative Syntheses of Nitroguanylated Polyamines



presence of a biguanide proved to be essential for antiprotozoal activity. A mechanism of antitrypanosomal action due in part to the formation of a DNA complex has been hypothesized.

Antifungal Agents. Guazatine is a well-known antifungal agent, and its guanylated components proved to have good activity against *Candida* species. The biguanylated triamines **365**, analogues of guazatine components, also showed an excellent antifungal profile with MIC values better than those of antifungal drug Fluconazole against different *Candida* strains.[133] It is noteworthy that macrocyclic amidinuoureas **367**, derived from cyclization of guanylated triamines **363**, showed very good antifungal activity not only against *Candida* strains (MIC = 1.25−5 μM) but also against filamentous fungi from *Aspergillus* species (MIC = 4 μM).[184]

6.2. Antihypertensive Agents

The antihypertensive activity of guanylated diamines is known since the 1960s when guanethidine sulfate **48** (also named Ismelin) proved to be able to lower the arterial pressure of unanesthetized renal and neurogenic dogs and blocked symphatetic efferent transmission at the nerve terminal.[80] Studies on Guanethidine derivatives showed that the essential features for antihypertensive activity were represented by the eight-membered ring and by the ethylendiamine backbone.[81] Natural guanidines from *Verbesina caracasana* isolated by Botta and co-workers proved to be good antihypertensive agents. Compound **G1** decreases blood pressure dose dependently and increases cardiac inotropism, respiratory frequency, and tidal volume. Cardiovascular effects of **G1** were dependent on arterial vasodilatation, and interaction with the cardiac β_1-adrenoreceptors and **G1** might be considered a hypotensive drug as potent as papaverine and reserpine and more active than guanethidine.[99] Cardiovascular and respiratory responses to compounds **G1**−**G6** obtained from *Verbesina caracasana* and administered by i.v. route in anesthetized rats were also explored and were shown in Table 18. From these studies emerged that **G2** was the most active drug in lowering blood pressure and increasing cardiac inotropism, while **G1** was the most active compound in stimulating breathing.[94,97]

A similar biological behavior was also shown by derivatives **120a**−**h**, which were tested for cardiovascular and respiratory effects in comparison with **G1** (Table 19). All the derivatives showed hypotensive activity, and SAR studies were reported. Compound **120c** appeared to be the most interesting compound, being a hypotensive agent of low-mild potency, devoid of significant tachycardic effects, and with stimulating respiratory effects.[100]

6.3. Nitric Oxide Synthase (NOS) Inhibitors

Nitric oxide synthases (NOSs) are a family of eukaryotic enzymes that catalyze the production of nitric oxide (NO) from L-arginine (Scheme 91). NO is an important cellular signaling molecule, having a vital role in many biological processes. Low levels of NO, generated by endothelial NO synthase (eNOS), exert homeostatic functions and counteract inflammation, whereas high amounts of NO, generated by the inducible isoform of NOS (iNOS), cause tissue destruction and cellular death. Because L-arginine

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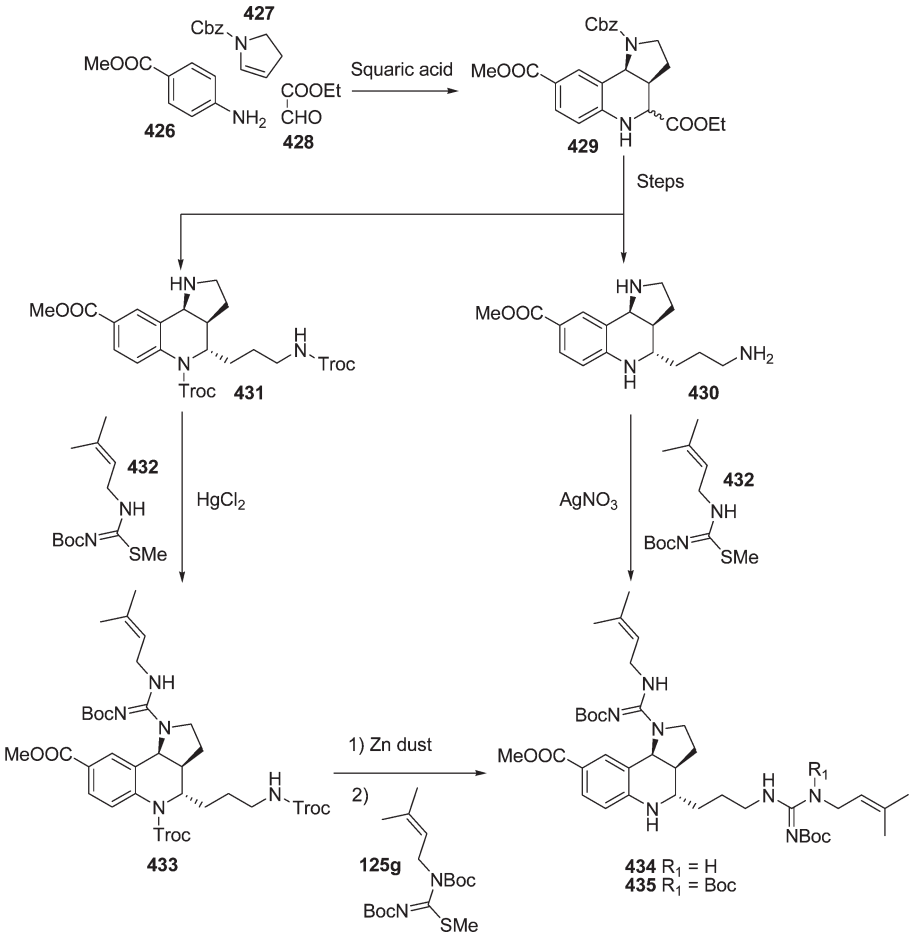
Scheme 89. Synthesis of the Biguanylated Core of Martinelline



is the natural substrate of NOSs, it is clear that synthetic guanylated diamines and polyamines could interact with these enzymes.

Guanylated compounds **127** showed NOS inhibitory activity toward nNOS (neuronal) and iNOS (inducible). Data are shown in Table 20.[101]

Nitric oxide synthase (NOS) inhibitor NO-donating drugs (NI-NODs) **129**−**130** were obtained starting from guanylated diamines. These compounds inhibit IL-1β production at 100 μM, modulate PGE_2 production, and protect against apoptosis. In addition, in a rodent model of colitis, NI-NOD1 and NI-NOD2 proved to potently decrease inflammation. Finally, in human umbilical endothelial cells (HUVECs) assay, **129**−**130** (100 μM) cause a significant decrease in L-citrulline production, demonstrating that all these compounds act as NOS inhibitors.[102] Nitroguanylated polyamines **415**, **418**, **421**, **423**, and **425** also proved to be very efficient nNOS inhibitors (K_i = 0.54 for **415**, 0.12 for **418**, and 0.21−2.20 for **421**, **423**, and **425**).[198] Most of the compounds also showed a certain activity against iNOS and eNOS superior to that of N^{ω}-nitro-L-arginine (L-NNA), an extensively studied inhibitor of NOS. The major limitation of L-NNA as a therapeutic agent is its poor isoform selectivity.[213,214] Compounds **415**−**418** in particular were rationally designed on the basis of the L-NNA structure with the aim to improve their selectivity.[215]

6.4. Guanylated Diamines As Hypoglycemic Agents

Galegine, a natural guanidine first isolated from *Galega officinalis*, has been used for a certain time as a hypoglycemic agent until the "formin" class of agents was discovered in 1950s. Among the "formins", the most famous compound is the metformin, a biguanide, still used in the treatment of diabetes. Before, diguanidine Synthalin also proved to possess hypoglycemic properties.[216,217]

Hypoglycemic activity of these compounds seems to be related to the presence of the guanidine group, and for this reason, several guanidines have been tested so far as potential hypoglycemic agents.[218] In this context, some guanylated di- and polyamines have been described to possess hypoglycemic properties.[123] In particular, monoguanylated diamine **136** showed to be a good antihyperglycemic and food intake modulating compound[103] (Table 21). Compound **136** showed good antihyperglycemic activity, −29% in blood glucose levels, and a 40% reduction in food intake. A stronger reduction in glycemia with respect to controls (−43%) 60 min after insulin suppression test (IST) was observed, and water consumption (−59%) reflected the observed efficacy.

All compounds were administered subcutaneously twice a day at doses equivalent to 25 mg/kg **127i**. Values are expressed as % variation with respect to control.

6.5. Inhibitors of Cell Growth

Deoxyhypusine Synthase Inhibitors. Deoxyhypusine (N'-(4-aminobutyl)lysine) is an intermediate in the post-translational

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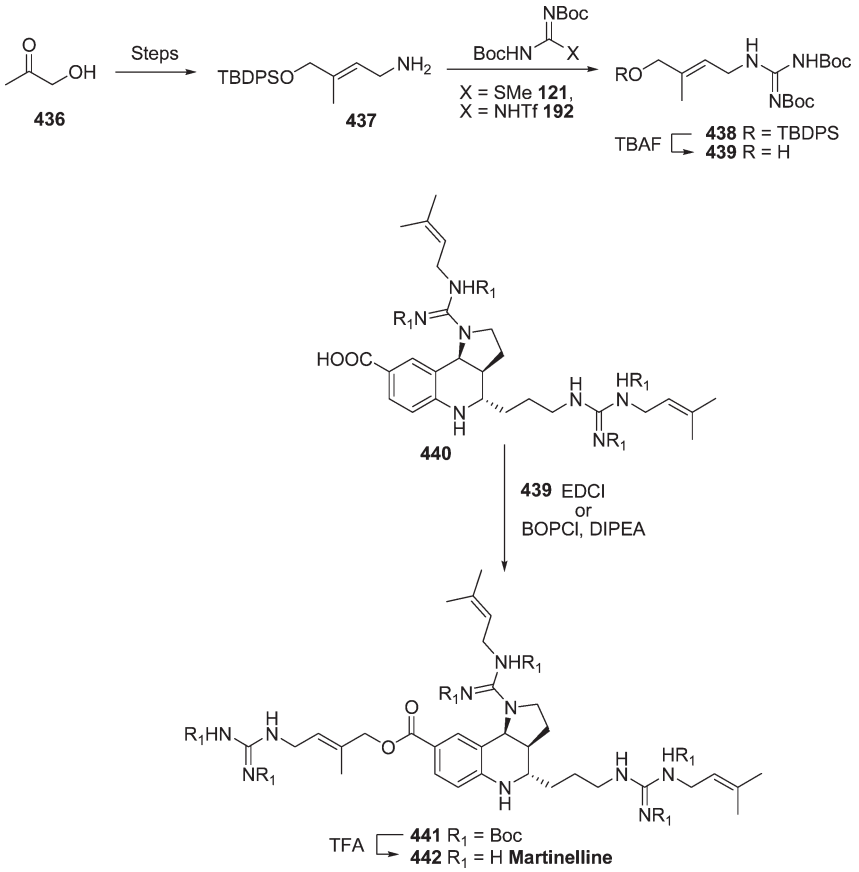
Scheme 90. Total Synthesis of Martinelline



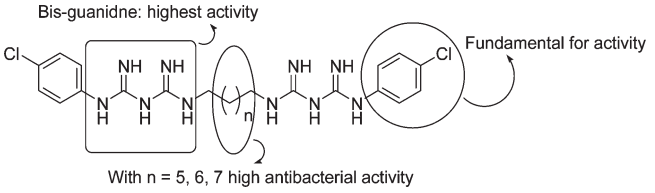


Figure 15. Structure−activity relationships of Chlorhexidine.

biosynthesis of the unique amino acid hypusine (*N*′-(4-amino-2-hydroxybutyl)lysine). Hypusine occurs at a single position in one cellular protein, the eukaryotic translation initiation factor 5A (e-IF-5A). There is evidence that e-IF-5A, which is abundant in many eukaryotic cells, and its hypusine modifications are essential for cell proliferations. Deoxyhypusine synthase (DHS) catalyzes the first two steps in the post-translational modification of a single lysine residue of the precursor e-IF-5A to the hypusine. DHS converts lysine to deoxyhypusine by a NAD-dependent transfer of the butylamine moiety of spermidine.[219] In the second step of the modification, deoxyhypusine hydroxylase catalyzes the hydroxylation of deoxyhypusine, yielding hypusine. Specific inhibitors of DHS or deoxyhypusine hydroxylase result in cellular growth arrest and alter tumor cell proliferation and differentiation (Scheme 92).

Jakus, Folk, and co-workers described the synthesis of several guanylated polyamines as potential DHS inhibitors. The authors reported that inhibitory activity was shown by the compounds possessing two charged primary amino or guanidine groups or one of each. The efficacy of the inhibition seems to be related to the maximum possible distance between the amino groups and is adversely affected by substitutions on secondary amino groups or in the carbon chains. Monoguanylated diamines are much more effective inhibitors than the parent diamines or their biguanylated counterparts. Compound 77**a**, namely, the monoguanylated diaminoheptane, proved to be a very efficient DHS inhibitor with a K_i = 10 nM.[85,87] Biological data of some guanylated inhibitors of DHS are reported in Table 22.

PAO Inhibitors. Polyamine oxidases (PAOs) are flavin adenine dinucleotide (FAD) dependent enzymes involved in the catabolism of ubiquitous polyamines via oxidative deamination of spermidine, spermine, and/or their acetylated derivatives at the secondary amino group. PAOs represent a heterogeneous family of enzymes whose properties vary depending on the biological source. The great interest devoted to PAOs results from the physiological relevance of their substrates, which are essential growth factors, as well as from the cytotoxic properties of their reaction products. Hence, the search of inhibitors of PAOs can contribute to the study of polyamine homeostasis and to design new antitumor drugs. *Zea mays* PAO (ZmPAO), the first PAO whose tertiary structure was

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Table 18. Antihypertensive Activity of G1−G7 Compounds

	blood pressure				
compound (μM/kg)	systolic (Δ%)	diastolic (Δ%)	heart rate (Δ%)	dP/dt max (Δ%)	respiratory frequency (Δ%)
G1 (4.12)	−26 ± 5	−29 ± 5	+5 ± 1	+57 ± 6	+21 ± 5
G2 (4.12)	−46 ± 5	−68 ± 4	−11 ± 3	+77 ± 6	−70 ± 3
G3 (4.12)	−11 ± 1	−17 ± 4	−4 ± 1	+23 ± 3	+4 ± 1
G5 (4.12)	−8 ± 3	−8 ± 2	0	+11 ± 4	+4 ± 1
G6 (4.12)	−8 ± 2	−10 ± 2	0	+16 ± 3	+12 ± 4
G7 (4.12)	−27 ± 4	−33 ± 4	−27 ± 6	+32 ± 5	+70 ± 8
Clonidine (0.1)	−16 ± 2	−17 ± 1	−15 ± 2	−21 ± 4	
Papaverine (5)	−18 ± 3	−16 ± 2	−5 ± 1	−19 ± 2	

Table 19. Antihypertensive Activity of Compounds 120a−h

	blood pressure, mmHg				
compound	systolic	diastolic	dP/dt_{max}, mmHg/s	heart rate, beats/min	respiratory frequency, beats/min
120a	−26 ± 3	−35 ± 5	+3249 ± 212	−16 ± 2	+17 ± 3
120b	−22 ± 4	−31 ± 1	+3088 ± 250	−24 ± 4	+16 ± 4
120c	−47 ± 4	−59 ± 6	+6070 ± 474	+19 ± 5	+12 ± 2
120d	−15 ± 3	−14 ± 3	+2252 ± 173	−12 ± 3	+6 ± 4
120e	−14 ± 4	−24 ± 5	+3424 ± 321	+14 ± 5	+14 ± 6
120f	−30 ± 1	−26 ± 2	+2781 ± 283	−19 ± 4	+19 ± 1
120g	−34 ± 2	−40 ± 4	+5028 ± 116	+8 ± 3	−10 ± 2
120h	−9 ± 2	−8 ± 1	+648 ± 33	+4 ± 1	+4 ± 2
G1	−21 ± 3	−18 ± 2	+2996 ± 104	+22 ± 3	+23 ± 3

Scheme 91. Mechanism of Action of NOS

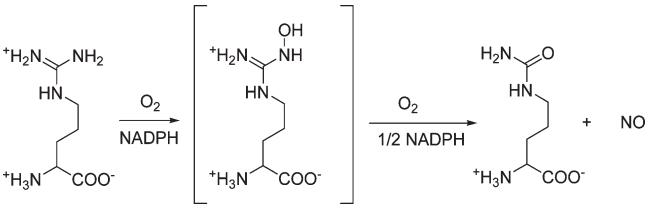

Table 20. NOS Inhibitory Activity of Compounds 127a−i

	K_i, μM			K_i, μM	
compound	nNOS	iNOS	compound	nNOS	iNOS
127a	50	15	**127e**	>500	>500
127b	40	20	**127g**	500	Nd
127c	100	200	**127h**	>500	>500
127d	50	30	**127i**	200	Nd

determined, was chosen as a model protein in designing new inhibitors suitable for every animal or plant PAO. Biguanylated triamines **365** described by Botta and co-workers showed high inhibitory activity toward ZmPAO.[133] Data are reported in Table 23. The optimum length for alkylic chain of biguanylated triamines is C = 8, while the most active compound proved to be **365c** bearing a cyclopropylmethyl moiety bound to one of the guanidine portion.[220] Monoguanylated diamines **127** were also tested showing ZmPAO inhibitory activity in the mM range.

LSD1 inhibitors. Recently, Woster and co-workers described the synthesis of a series of bisbiguanylated polyamines able to inhibit the enzyme lysine-specific demethylase 1 (LSD1), which plays an important role in the epigenetic control of gene expression. Enzyme LSD1 is emerging as an important new target for the development of new antitumor drugs and inhibitors of LSD1 proved to be able to induce the re-expression of aberrantly silenced tumor suppressed genes in tumor cells in vitro. Compound **410e** showed the best pharmacological profile, causing 17.2% LSD1 activity remaining in vitro following treatment at 10 μM.[195,196]

Table 21. Reductions of Glycemia Levels at Insulin Suppression Test and of Cumulative Water and Food Intake, After 5 Days of Treatment in Male db/db Mice

compound	glucose time 0/60 min	water intake %	food intake %
127i	−31/−13	−53	−19
G3	−13/−11	−53	−37
132a	−42/−18	−45	−19
136	−29/−43	−59	−40
138	−8/−29	−29	−21
134	−14/−22	−44	−27

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Scheme 92. Mechanism of Action of Deoxyhypusine Synthase

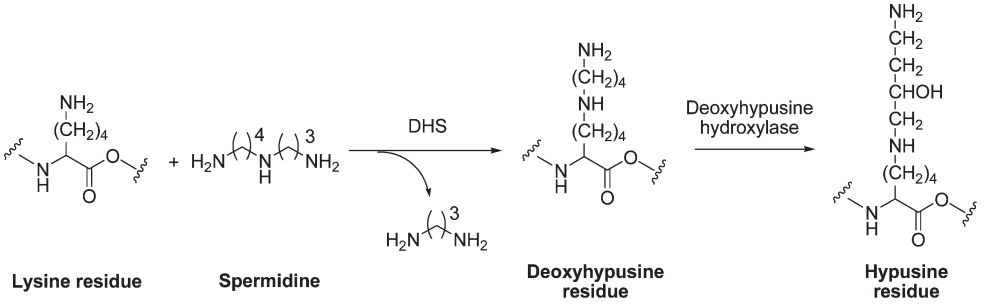

Table 22. DHS Inhibitory Activity of Some Guanylated Polyamines

compound	IC_{50}, mM	K_i, mM	compound	IC_{50}, mM	K_i, mM
guazatine	17.2	12.1 ± 1.6	**77a**	0.017	0.0097 ± 0.0005
241a	48.3	35.2 ± 4.8	**77b**	0.37	0.24 ± 0.02
241b	3.0	1.7 ± 0.08	**317**	1.2	0.74 ± 0.03
227b	8.5	5.65 ± 0.51	**319**	0.57	0.33 ± 0.05
342c	204	154 ± 20	**321**	0.24	0.15 ± 0.04
agmatine **1**	71		hirudonine **337**	7.45	4.89 ± 1.32

Table 23. ZmPAO Inhibitory Activity of Compounds 365a−g

compound	K_i (nM)	compound	K_i (nM)
365a	7.5	**365e**	0.5
365b	3.0	**365f**	1.0
365c	0.08	**365g**	0.7
365d	1.1		

7. CONCLUSIONS

Guanylated di-, tri-, and polyamines represent an attractive class of compounds both from a synthetic as well as a biological point of view and have attracted the attention of many chemists, pharmacologists, and biologists in the last decades. The presence of a guanidino and an amino groups on the same molecule makes these compounds unique and able to have multiple functions. In organic synthesis, guanylated polyamines can be used as organic superbases or metal ligands. At the same time, the strict correlation of guanylated polyamines with natural aminoacids and biogenic amines makes these compounds able to interact with multiple biological substrates and consequently to possess different biological activities. Guanylated polyamines show good biochemical properties, being organic compounds endowed with high solubility in water. This property, due to the presence of basic and protonable moieties, makes the guanylated polyamines excellent drug candidates or templates in the synthesis of novel bioactive compounds. However, from a synthetic point of view, the synthesis of these compounds sometimes remain a trick because of the poor reactivity of certain building blocks, the poor yields, and the occurrence of side reactions. The development of novel efficient and selective guanylation methodologies will represent a challenge for the next future.

This review intended to make a systematic classification of guanylated di-, tri-, and polyamines on the basis of their structure first and then on the basis of their most important biological activities. Because of the wide field of application of guanylated polyamines in medicinal as well as organic chemistry, the interest on these compounds will be destined to increase. The purpose of the review is to offer to a large number of scientists a systematic and complete overview on such fascinating molecules.

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Dr. Daniele Castagnolo obtained a degree cum laude in medicinal chemistry at the University of Siena (Italy) in 2003 working on the synthesis of enantiomerically pure amines. In 2006, he received a Ph.D. in medicinal chemistry from the University of Siena working on the synthesis of antimycobacterial compounds and on the development of enyne metathesis methodologies for the synthesis of antifungal agents. During his Ph.D. studies, he joined the group of Prof. Joahnn Mulzer at the Univesrity of Vienna as visiting student working on the synthesis of the antibiotic branimycin. Dr. Castagnolo did his postdoctoral studies at the Helsinki University of Technology working with Prof. Petri Pihko on the synthesis of nonanomeric [6.5]-spiroketals. Since December 2007, he has been working as Research Associate at the University of Siena in collaboration with Prof. Maurizio Botta. Dr. Castagnolo's research interests are in the drug design and synthesis of guanylated polyamines

endowed with antimicrobial activity as well as the development of novel metal-catalyzed reactions.

Prof. Silvia Schenone obtained a degree in medicinal chemistry in 1987 and in Pharmacy in 1988, both with laude at the University of Genoa. In 1992, she received a Ph.D. in Medicinal Chemistry and became a researcher in 1992. From 2001, she has been an associate Professor of Medicinal Chemistry at the same University. At present, she is involved in the synthesis of heterocyclic compounds as tyrosine kinase inhibitors. Moreover, she is interested in the preparation of adenosine receptor antagonists and anti-inflammatory agents. Prof. Schenone's team is also involved in structural studies on biological macromolecules (enzymes, receptors, growth factors) and molecular modeling applications in medicinal subjects. She is the author of more than 100 publications.

Prof. Maurizio Botta obtained a degree in chemistry at the University of Rome in 1974. After working as a temporary assistant in organic chemistry at the University of Rome, he got a fellowship from the University of New Brunswick (Canada),where he earned his Ph.D. in chemistry in December 1979 under the direction of Prof. K. Wiesner. He was invited researcher in the laboratory of Prof. S. Hannessian at the University of Montreal, and in 1987, he became an associate professor of medicinal chemistry at the Faculty of Pharmacy of the University of Siena, where he has been a full professor since 2000. Since 2008, he has been an adjunct professor at the Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology (SHRO, Temple University, Philadelphia, U.S.A.). Botta's research interests are in the drug design and synthesis of antiviral, antitumor, and antifungal agents.

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