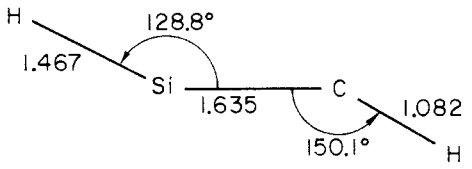

Figure 3. Theoretical equilibrium geometry for *trans*-silaacetylene predicted at the DZ + d CI level of theory.

subsequently discovered that the linear HSiCH is not even a minimum on the potential surface. However, all is not lost in light of Gordon and Pople's finding[48] that silaacetylene favors a bent equilibrium geometry. The only (as best we can determine) predicted equilibrium geometry for silacetylene is that of Hoffmann,[49] seen in Figure 3, which reveals a trans bent structure. The silicon–carbon bond distance (1.64 Å) is significantly less than that in silaethylene (1.71 Å), but notably longer than would be anticipated for a hypothetical Si≡C triple bond. A cis bent equilibrium geometry is also conceivable, and work in progress is designed to pursue this and other points.[49] It may also be hoped that a suitable choice of substituents might actually place the silaacetylene energetically below the silylidene isomer. Experiments probing the latter point would be particularly welcome.

Particular thanks are due to Dr. John D. Goddard for many stimulating and informative discussions and for his contributions to the research reviewed here. I am also grateful to Diane Hood, Yasunori Yoshioka, Jozef Bicerano, and Mark Hoffmann, who carried out much of the work described in this Account. This research was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy, under Contract DE-AC03-76SF00098, the National Science Foundation under Grant CHE-8009320, the Robert A. Welch Foundation, and the University of Texas. The hospitality and intellectual stimulation provided by Professor David P. Craig and Dr. Leo Radom at the Australian National University, where this Account was composed, are sincerely appreciated.

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New Chemistry of Naturally Occurring Polyamines

BRUCE GANEM

Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853

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In 1677, when Anton von Leeuwenhoek first observed living spermatozoa with his primitive microscope, he also discovered a crystalline substance from human seminal fluid. It was described in his famous letter of that year to the Royal Society of London:[1]

> "*Et cum praedicta materia paucillum temporis steterat, in ea observabantur trilaterales figurae ab utraque parte in aculeum desinentes quibusdam longitudo minutissimae arenae, aliquae aliquantulum majores, ut fig. A. Praeterea, adeo nitidae ac pellucidae, ac si crystallinae fuissent.*"

We now recognize that it was spermine phosphate, a salt of the first known naturally occurring polyamine, that had so easily and spontaneously precipitated under van Leeuwenhoek's lens. However it took a long succession of distinguished naturalists and medical investigators more than 250 years to unravel the identity of this substance.[2] In a brilliant series of papers after the First World War, Rosenheim[3,4] and Wrede[5] used organic synthesis to established conclusively the correct composition of spermine and a related base, spermidine.

Together with the simpler diamines putrescine and cadaverine, which were discovered in decomposing animal carcasses, these four aliphatic bases constitute the principal members of an ubiquitous family of natural products. Pathways for the biosynthesis of polyamines have been uncovered in so many animals, plants, and microorganisms that it is safe to say at least some representatives are present in all eukaryotic and prokaryotic cells. Thus, it seems surprising that compounds so widely distributed throughout nature and whose discovery predates that of DNA by some 200 years have seldom merited more than passing mention in scientific textbooks. More often than not, polyamines were viewed as odd curiosities of physiology and metabolism.

$NH_2(CH_2)_4NH_2$	putrescine, **1**
$NH_2(CH_2)_5NH_2$	cadaverine, **2**
$N^1H_2(CH_2)_3N^4H(CH_2)_4N^8H_2$	spermidine, **3**
$N^1H_2(CH_2)_3N^4H(CH_2)_4N^8H(CH_2)_3N^{12}H_2$	spermine, **4**

That this is no longer the case can best be gauged by the explosive proliferation of scientific literature on the polyamines since the early 1970s. Only highlights of some major developments will be presented here; research on polyamines has been the subject of numerous monographs[6–8] and reviews.[9–14]

Bruce Ganem is Professor of Chemistry at Cornell. He was born in Boston, MA, and received his Bachelor of Arts degree from Harvard College in 1969. After completing Ph.D. studies with G. Stork at Columbia University, he was a National Institutes of Health Postdoctoral Fellow with W. S. Johnson at Stanford before joining the Cornell Faculty in 1974. He has been a Visiting Professor at the University of Chicago and a J. S. Guggenheim Foundation Fellow.

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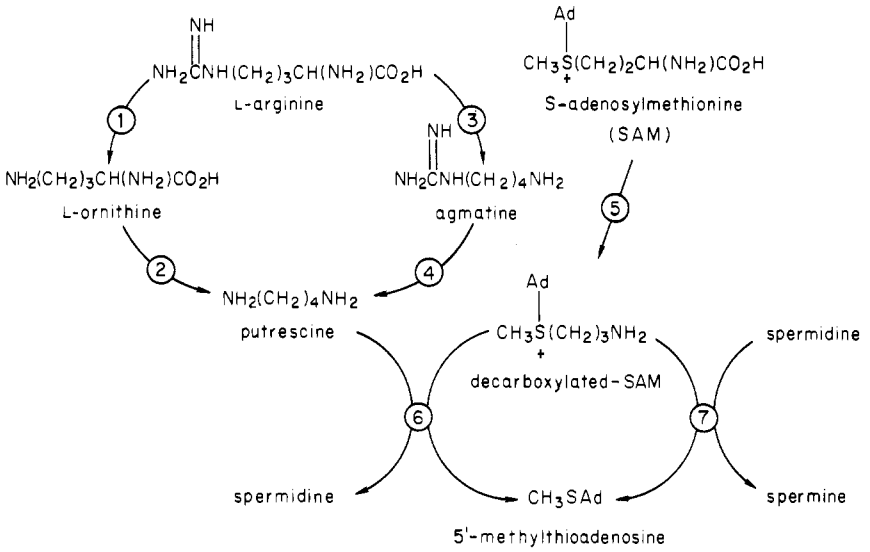
Scheme I
Polyamine Biosynthesis[a]



[a] Steps: (1) arginase; (2) ornithine decarboxylase; (3) arginine decarboxylase; (4) agmatinase; (5) SAM-decarboxylase; (6) spermidine synthase; (7) spermine synthase.

Function of Native Polyamines

Pioneering studies by the Tabors and Rosenthal[15,16] demonstrated that all prokaryotic and eukaryotic cells synthesize putrescine and spermidine. Prokaryotes usually contain more 1 and 3 and generally lack spermine, which seems to be confined to nucleated eukaryotic cells. At physiological pH, these bases are very largely protonated and exhibit net charges close to 2+, 3+, and 4+, respectively. Conformationally mobile polyamine cations can associate with adjacent phosphate groups of polynucleotides to stabilize them against denaturation and shearing. The same kinds of binding exert a profound influence on the secondary and tertiary structure of nucleic acids, although the physiological significance of such effects in vivo remains to be established. These interactions may account for the direct in vitro effect of polyamines on DNA and RNA polymerases, methylases, and hydrolases as well as on the assembly of mRNA, tRNA, and rRNA into the protein synthesizing apparatus.[17] As we shall see, pK_a's of the basic sites in polyamines are proportional to internitrogen distances, which can range from two to five carbon atoms. Consequently the binding of polyamines to macromolecules is a very complex phenomenon: the pairwise formation of electrostatic interactions requires the correct spacing between nitrogens in the polyamine chain, which in turn influences both base strength and conformational flexibility. In these and other respects, charged polyamine structures are distinct from metal ions, whose sequestration from the circulation depends upon diffusion or transport across membranes. By contrast, the endogenous synthesis of polyamines permits their levels to be regulated precisely according to the needs of the cell.

The major pathway of polyamine biosynthesis utilizes the amino acids L-ornithine and *S*-adenosylmethionine (SAM) in a stepwise transfer of γ-aminopropyl groups to putrescine as shown in Scheme I.[13] The cascade of enzymes mediating this process comprises an extraordinarily complex control mechanism.[18,19]

Early studies suggest that polyamines serve as growth promoters for a variety of microorganisms.[20,21] However in view of their widespread occurrence at substantial (up to millimolar) concentrations and their relatively slow catabolism, it is unlikely that they act as inducible metabolic regulators akin to cyclic AMP. It now appears that as free bases putrescine, spermidine, and spermine play important roles in cellular differentiation and proliferation.[13] A strong case can be made that normal levels of 1, 3, and 4 are required for the uninterrupted growth of most cells and that polyamines enhance the efficiency and fidelity of certain metabolic processes, thereby streamlining and accelerating the cell cycle progression.[13,22-25]

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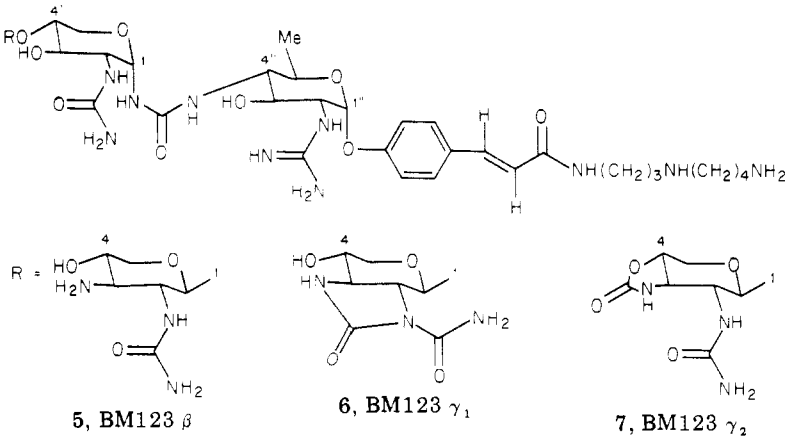


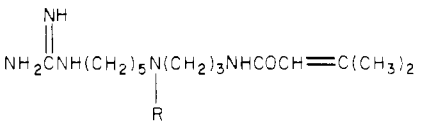
5, BM123 β 6, BM123 γ_1 7, BM123 γ_2

Polyamines have also been implicated as useful diagnostic markers in a number of diseases, such as cystic fibrosis,[26,27] and in human malignancies.[23] For instance, Russell has reported a dramatic elevation (up to 50-fold) of urinary polyamines in human subjects with various solid tumors or leukemia. Following surgical tumor excision, polyamine levels returned to near normal.[28,29] Such dramatic clinical observations should be interpreted cautiously since they are very likely epiphenomena obscuring a more complex metabolic situation. Nevertheless, Bachrach has pointed out that a simple polyamine urinalysis may eventually constitute a routine diagnostic test for malignant tumors and for chemotherapy evaluation.[7]

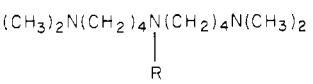
Polyamine Conjugates

Besides their presence in native form as free aliphatic bases, the common polyamines often occur conjugated[30] with sugars,[31] steroids,[32] phospholipids,[33] and peptides[34] and also as substructural units within numerous families of plant alkaloids.[35] For years these latter substances have been regarded by organic chemists as secondary metabolic natural products, offering little in the way of interesting pharmacological activity.[36,37] Quite recently, however, many unique polyamine derivatives have been found that possess remarkably diverse biochemical profiles. Among the most fascinating are the glycocinnamoylspermidines LL-BM123 β, γ_1, and γ_2 (**5–7**) which are broad-spectrum antibiotics isolated from an unidentified species of *Nocardia*.[31] The γ_1 and γ_2 components are of special interest in view of their potent activity against Gram-negative organisms and their protective effects against infection.

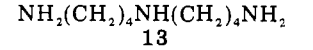
From the red–orange sponge *Acarnus erithacus*, Carter and Rinehart have isolated the antiviral and antimicrobial acarnidines **8–10**, a family of trifunctional



8, R = $CO(CH_2)_{10}CH_3$
9, R = *cis*-$CO(CH_2)_3CH{=}CH(CH_2)_5CH_3$
10, R = $COC_{13}H_{21}$



11, R = $CO(CH_2)_{14}CH_3$
12, R = *trans*-$COCH{=}CH(CH_2)_{12}CH_3$



13

homospermidines active against *Herpes simplex*, *Bacillus subtilis*, and *Pseudomonas atrovenetum*.[38] Solapalmitine (**11**) and solapalmitenine (**12**), are two tetramethylated acyl derivatives of bis(4-aminobutyl)-amine (**13**) that were shown by Kupchan to possess significant tumor-inhibitory activity.[39]

Like **13**, many of the more specialized polyamines with unusual carbon–nitrogen frameworks often surface as conjugates. the polyamino acid hypusine (**14**), found in various animal tissues[40] and recently in human lymphocytes,[41] would seem to be the product of coupling L-lysine with the known diamine hydroxyputrescine.[42] However labeling studies reveal a more roundabout biogenesis in which spermidine provides hypusine's aminobutyl carbons.[41] Two relatively new polyamines, thermospermine (**15**) and thermine (**16**), were discovered in the extreme thermophile *Thermus thermophilus*, a bacterium which grows normally at 75 °C.[43,44]

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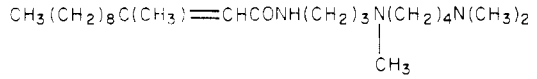
$NH_2(CH_2)_2CH(OH)CH_2NH(CH_2)_4CH(NH_2)CO_2H$
14

$NH_2(CH_2)_4NH(CH_2)_3NH(CH_2)_3NH_2$
15

$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
16

Two unnamed cytotoxic agents, **17** and its dihydro derivatives, were extracted from the coral reef coelenterate *Sinularia brongersmai* by Schmitz et al. in 1979[45]



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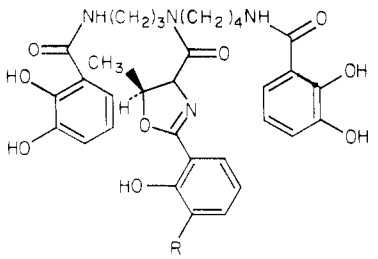
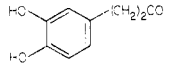


18, R = OH
19, R = H

and are apparently the first spermidine metabolites to be discovered in marine organisms. Other acylated spermidines include many different classes of siderophores (or siderochromes)—iron-chelating substances that utilize hydroxamate or catecholate groups to transport or sequester iron(III). A few such structures show promise in the treatment of Cooley's anemia.[46] Agrobactin (**18**), and parabactin (**19**) are representative catechol-type siderophores.[47]

Kukoamine A (**20**) is a spermine derivative with some of the characteristic features of a siderophore, yet its

$RNH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NHR$

20, R = 

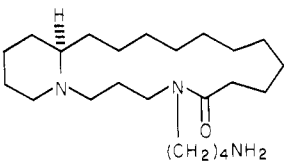
principal physiological effect is as a powerful antihypertensive agent.[48] This polyamine is one of the active components of "jikoppi", a crude herbal extract of the plant *Lycium chinense* used for centuries by medical practicioners in Asia and the Orient. Besides being clinically effective against high blood pressure, this extract exhibits hypoglycemic, fever-lowering, and antistress–antiulcer activity. The biogenetic origin of **20** raises interesting questions since spermine is not customarily found in prokaryotes.

By the mid-1970s, polyamines and their conjugates were beginning to emerge as wide-ranging biological effector molecules with bright prospects for pharmaceutical development.

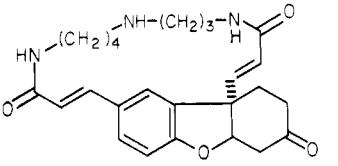
Previous Synthetic Efforts

The first reported syntheses of spermidine and spermine by Rosenheim[3,4] relied, in principle, on the stepwise reaction of $PhO(CH_2)_3Br$ with putrescine to attach one, then two (symmetric) γ-aminopropyl groups. This very simple approach predictably suffered from problems of regioselectivity and polyalkylation; nevertheless it was an exemplary total synthesis in its time. More than 50 years have since elapsed, yet the state of the art in synthetic polyamine chemistry has advanced surprisingly little, and few genuinely site-specific reactions have been developed. The biosynthetic processes drawn in Scheme I pay tribute to Nature's own discriminating touch in differentiating one amine group from another.

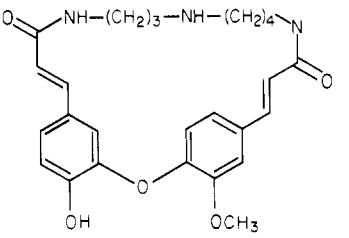
Classical efforts at partial or total synthesis of polyamine conjugates have focused on the macrocyclic lactams oncinotine (**21**),[49] lunaridine (**22**),[50–52] and co-



21, oncinotine



22, lunaridine



23, codonocarpine

donocarpine (**23**)[53–56] and their derivatives. The strategy in most cases was to elaborate the substituted polyamine backbone from a simple amine and then close the macrocycle late in the synthesis.

Along conceptually different lines, three groups have resorted to prior derivatization of a polyamine building block. Swiss workers first synthesized threefold protected spermidine **24** in seven steps[57] and then converted it to the dihydropalustrines (**25**). Quick et al. prepared bis(*tert*-butoxycarbonyl)spermidine (**26**),[58] which proved instrumental in the first total synthesis of codonocarpine.[55] Finally Bergeron et al.[59] have described monobenzylspermidine (**27**) as a useful precursor for manmade siderophores and other structures in which the different primary amine groups of **27** need not be distinguished.

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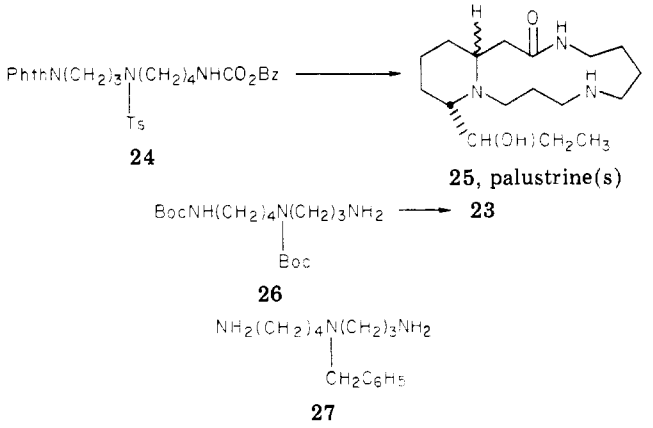


The Chemical Problem—Selectivity Considerations

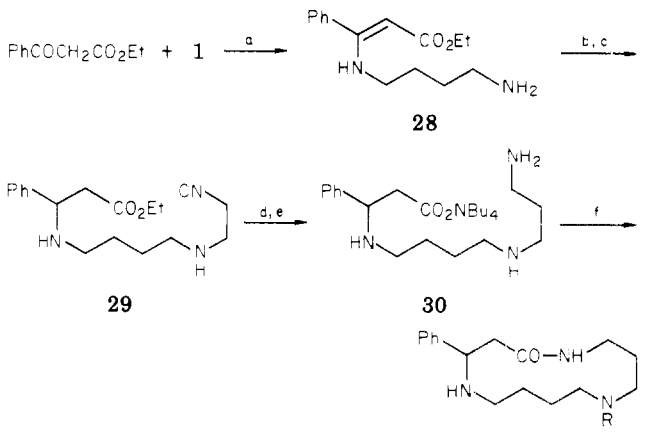
Our own interest in polyamine chemistry originated with the macrocyclic spermidine alkaloids[60] celacinnine (**32**), celabenzine (**33**), and celafurine (**34**), whose synthesis appeared to pose a critical test for new lactamization methods.[61] A straightforward, unambiguous preparation of acyclic triamino carboxylate salt **30** was accomplished as shown in Scheme II.

As we had hoped, cyclization of **30** using catecholborane in pyridine produced diaminolactam **31** in nearly 70% yield, which then underwent selective cinnamoylation, benzoylation, or furoylation to furnish the natural products **32–34**.[62,63] Since that time, both Wasserman[64] and Yamamoto[65] have reported syntheses of celacinnine by rather different approaches.

Although the overall yields in Scheme II were quite acceptable, we became intrigued with the possibility of assembling the core ring structure **31** directly from spermidine and cinnamate (or its biochemical equivalent), thereby mimicking Nature. For this, a protocol was required to distinguish the three different nitrogens of spermidine which appear in both 1,3 and 1,4 positions on the 4-azaoctane chain.

Secondary amines are usually more nucleophilic than primary; however, the nitrogens in spermidine do not behave independently. Powell and co-workers[66] have compiled thermodynamic data on the protonation and metal complexation of polyamines that establish that the first and second protonations of **3** and **4** occur mainly on the primary nitrogens.[67] Moreover the first protonation of spermidine occurs at the aminobutyl end (N^8) whose greater basicity is likely due to the "negligible transmission of inductive effects" between amines spanning the tetramethylene chain. Since N^1 and N^4 are only three carbons apart, their basicities are decreased by mutual electron withdrawal. The primary amine groups of **3** are also more reactive for steric reasons, an important feature when bulkier electrophiles

Scheme II[a]



[a] (a) EtOH, *p*-TsOH, Δ, 77%; (b) CH_2=CHCN, EtOH, 87%; (c) $NaBH_3CN$, 88%, (d) $NaBH_4$–$CoCl_2$, 84%; (e) (e) Bu_4NOH, 95%; (f) catecholborane-pyridine, 65–70% (to 31).

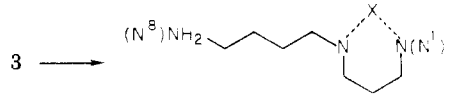
Table I

polyamine	pK_as
spermine[a]	10.8, 10.02, 8.85, 7.96
spermidine[a]	10.89, 9.81, 8.34
putrescine[b]	11.15, 9.71
$NH_2(CH_2)_3NH_2$[b]	10.94, 9.03
$NH_2CH_2CH_2NH_2$[b]	10.7, 7.5

[a] Reference 67. [b] Reference 69.

such as acylating agents are employed.[68] Table I presents some equilibrium pK_a values for representative polyamines that illustrate this important electronic effect. Clearly, a synthetic method for differentiating the two end groups of spermidine was required.

To us, the 1,3 arrangement of N^1 and N^4 suggested that a number of strain-free, cyclic, six-center derivatives might be formed having the general representation **35**. If reversible, this process would temporarily block



35

two of the three nitrogens and leave the 8-amino group (N^8) free. Since one atom or functional group "X" would serve as a double blocking agent, the overall differentiation would involve a minimum of protecting group manipulations.

Obvious candidates for this approach included (1) metal chelates having X = Ni^{2+}, Cu^{2+}, or Pt^{2+}, (2) metallocycles with X = R_2Sn or R_2Si, (3) hexahydropyrimidines from interaction of the 1,3-diamine with aldehydes or ketones, e.g., X = PhCH or $(CH_3)_2C$, and (4) oxidized versions of **3** such as a cyclic amidine (X = —CH=) or urea (X = >CO). All rely on the well-known principle that a six-membered ring, either chelated or covalently bonded, should form at a faster rate

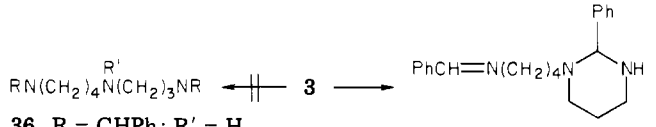
(60) S. M. Kupchan, H. P. J. Hintz, R. M. Smith, A. Karim, M. W. Cass, W. A. Court, and M. Yatagai, *J. Org. Chem.*, **42**, 3660 (1977).
(61) D. B. Collum, S.-C. Chen, and B. Ganem, *J. Org. Chem.*, **43**, 4393 (1978).
(62) J. S. McManis and B. Ganem, *J. Org. Chem.*, **45**, 2041 (1980).
(63) J. S. McManis, Ph.D. Thesis, Cornell University, 1980.
(64) H. H. Wasserman, R. P. Robinson, and H. Matsuyama, *Tetrahedron Lett.*, **21**, 3493 (1980).
(65) H. Yamamoto and K. Maruoka, *J. Am. Chem. Soc.*, **103**, 6133 (1981).
(66) B. N. Palmer and H. K. J. Powell, *J. Chem. Soc., Dalton Trans.*, 2086, 2089 (1974).
(67) M. Gold and H. K. J. Powell, *J. Chem. Soc., Dalton Trans.*, 230 (1976).
(68) H.-P. Husson, C. Poupat, and P. Potier, *C. R. Hebd. Seances Acad. Sci., Ser. C*, **276**, 1039 (1973).
(69) "CRC Handbook of Chemistry and Physics", 52nd ed., R. C. Weast, Ed., Chemical Rubber Co., Cleveland, Ohio, 1971–72), p D117.

(and generally be more stable) than the corresponding seven-membered counterparts comprising N^4 and N^8. After functionalization of N^8 in **35** and removal of "X", further chemistry at N^1 and than at N^4 would be feasible.

Metal chelates of polyamines seemed an attractive possibility at first, since the ionic blocking group might readily be excised by precipitation of an insoluble sulfide salt. Hanessian and others have utilized such chelates between pairs of amino and hydroxy groups to achieve selective chemical modifications of the aminoglycoside antibiotics.[70] Unfortunately tri- and tetraamines tend to wrap tightly around metal ions: even a seven-membered chelate ring contributes substantially to the stability of both Cu^{2+} and Pt^{2+} complexes with spermidine.[66,67] Preliminary experiments with $CuCl_2$ and $NiCl_2$-diphos convinced us that an effective solution lay elsewhere.

Cyclic Ureas

Efforts to prepare the six-membered cyclic urea of **3** led us at first to investigate the interaction of spermidine with aldehydes to give a bis(imine), **36**. Unlike



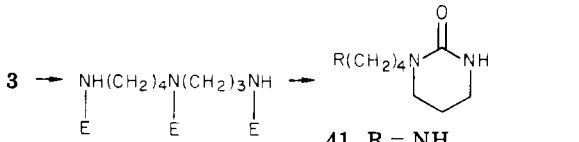
36, R = CHPh; R' = H
37, R = CHPh; R' = CO_2CH_3
38, R = H_2; R' = CO_2CH_3

39

propanediamine, which beautifully formed a bis(imine) with benzaldehyde, spermidine was transformed to **39** in high yield. This outcome thus foiled our attempts to make urethane **37**, a projected urea precursor via **38**, but it offered the first indication of spermidine's powerful affinity for aldehydes. One-to-one mixtures of **3** and C_6H_5CHO furnished **39** (50%) and recovered **3** (50%); moreover, both the imine and heterocyclic groups in **39** hydrolyzed at comparable rates in dilute acid. The fact that anisaldehyde, *p*-nitrobenzaldehyde, furfural, and heptanal all behaved similarly attested to the thermodynamic stability of such 2:1 adducts.

While structure **39** was not the expected product, we recognized that addition of a two-carbon acetate unit to its imine bond would afford the previously prepared triamino acid **30** (Scheme II) after mild hydrolysis of the heterocyclic ring. With malonic acid, this condensation indeed furnished our key intermediate, but in impractically low yields. Reaction of **39** with *tert*-butyl lithioacetate failed altogether.

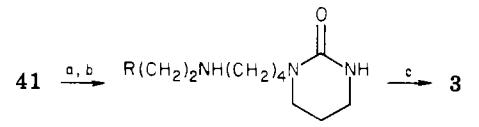
Ultimately urea **41** (nicknamed "urea-protected spermidine") was made by an exceedingly simple method adapted from the biotin synthesis of Confalone et al.[71] Exhaustive acylation of **3** with methyl chloroformate produced the viscous tricarbomethoxyspermidine **40**. In aqueous $Ba(OH)_2$ at reflux, **4**



40, E = CO_2CH_3

41, R = NH_2
42, R = phthalimido

Scheme III[a]

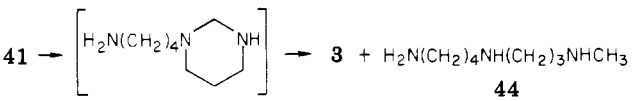


45, R = CN; **46**, R = CH_2NH_2

[a] (a) CH_2=CHCN, C_6H_6, 95%; (b) BH_3-THF, room temperature, 81% (to **46**); (c) $NH_2(CH_2)_3NH_2$ (100 equiv), 140 °C, 65%.

formed **41** as a waxy solid, mp 44–45 °C, in 92% overall yield from spermidine.[72] Unlike the native polyamine, this urea was mobile in a silica gel thin-layer chromatogram; it also gave the characteristic blue color in response to ninhydrin spray reagent. The structure of **41** was unambiguously confirmed by comparison of its phthalimide derivative **42** (mp 179.5–180.5 °C) with an authentic sample made by alkylating trimethyleneurea with *N*-(4-bromobutyl)phthalimide. Urea **41** no doubt arises by preferential hydrolysis and decarboxylation of the terminal urethanes in **42** followed by in situ cyclization of **38**.

Before undertaking some selective reactions of **41**, we needed an efficient means of removing the carbonyl blocking group. Cyclic ureas are extraordinarily resistant to hydrolysis in acid or base, and **41** proved to be no exception. We therefore designed a process called "urea exchange" whereby heating **41** in a large excess of some low-boiling, inexpensive diamine would release the polyamine and concomitantly form a water-soluble, solvent-derived urea. This plan worked best with 1,3-propanediamine and considerably less well with ethylenediamine, which is a poorer nucleophile (Table I). By use of lithium aluminum hydride,[73] the reduction of **41** in THF at reflux furnished spermidine and N^1-



43

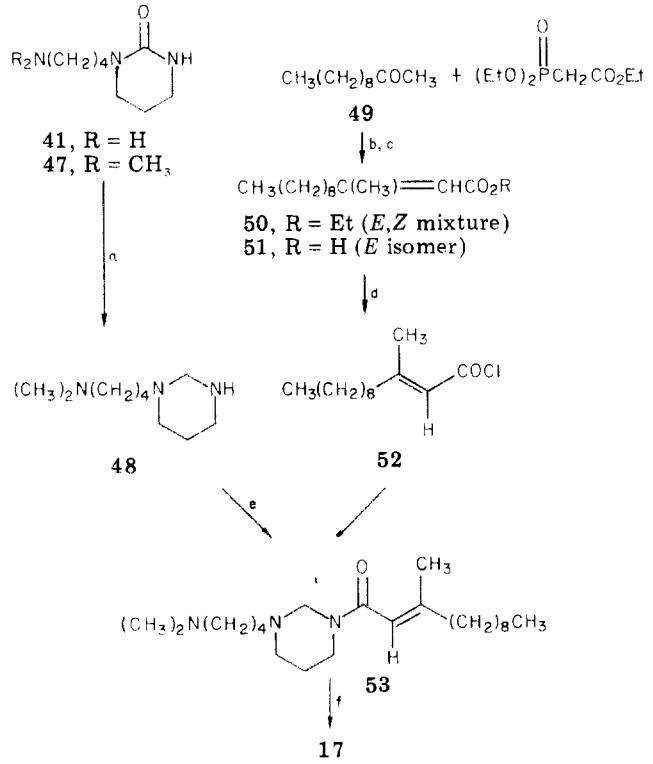
methylspermidine (**44**) in a 4:1 ratio (85% yield after hydrolysis), presumably via intermediate **43**. Other chemistry of **43** will be presented in a later section, but its overreduction exclusively to **44** constituted the first selective polyamine alkylation we know of. Its regiochemistry was consistent with Benkovic's finding that *gem*-diamines formed iminium ions by preferential expulsion of the less basic amine leaving group.[74]

Because **3** and **44** were difficult to separate, deprotection of **41** by urea exchange became the preferred method and enabled us to achieve the first rationally designed synthesis of spermine from spermidine depicted in Scheme III. Besides providing a straightforward route for making monoradiolabeled samples of **4** from **3**, Scheme III illustrates how a protected spermidine **41** can give rise to a unique protected spermine like **45** or **46** capable of undergoing selective reactions at all four nitrogens.

For many of the synthetic exploits we envisioned, urea **41** was almost too effective a blocking group; its removal from complex polyamine conjugates might well destroy other functionality. It proved, however, ideally

(70) S. Hanessian and G. Patil, *Tetrahedron Lett.*, 1031, 1035 (1978).
(71) P. N. Confalone, G. Pizzolato, and M. R. Uskokovič, *J. Org. Chem.*, **42**, 135 (1977).
(72) K. Chantrapromma, J. S. McManis, and B. Ganem, *Tetrahydron Lett.*, **21**, 2605 (1980).
(73) (a) E. M. Wilson, *Tetrahedron*, **21**, 2561 (1965); (b) R. F. Evans, *Aust. J. Chem.*, **20**, 1643 (1967).
(74) G. Moad and S. J. Benkovic, *J. Am. Chem. Soc.*, **100**, 5495 (1978).

Scheme IV[a]



[a] (a) $LiAlH_4$, 1.1 equiv, 57%; (b) NaH, THF, 90%; (c) 10% NaOH, CH_3OH-H_2O (to 51); (d) $SOCl_2$, 67%; (e) Na_2CO_3, H_2O, CH_2Cl_2, 82%; (f) HCO_2H, Δ, 96%.

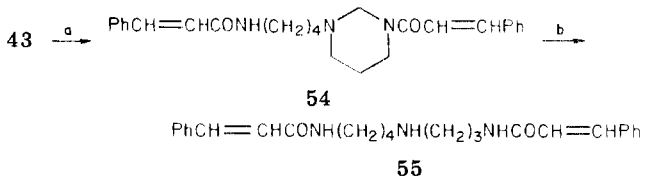
suited for an approach to the cytotoxic coral metabolite **17** from *Sinularia brongersmai* as outlined in Scheme IV. One unusual aspect worth noting was the dual purpose served by hexahydropyrimidine intermediate **48** both as a protecting device and as a latent *N*-methyl function.[72] Eschweiler–Clarke methylation of **41** furnished **47** in 80% yield. Upon reduction with $LiAlH_4$, urea **47** was transformed to *gem*-diamine **48**, thus setting the stage for a regioselective acylation at N^1 with fatty acid chloride **52**. Reduction of the heterocycle in **53** proceeded as planned,[74] using the very mild reducing agent formic acid to afford trimethylated spermidine **17** in pure form. Catalytic hydrogenation of **17** (Pd–C, ethyl acetate) quantitatively furnished its dihydro derivative. Together these samples conclusively established the identity of **17**, which had never been obtained pure from natural sources.

The Formaldehyde Solution

The geminal diamine **43** arising from $LiAlH_4$ reduction of **41** qualifies as a "protected spermidine" in its own right: the temporary heterocycle creates an array of primary, secondary, and tertiary amines that, given the strong mutual interactions of N^1 and N^4, should be easily distinguished chemically. When we first prepared **43** from **41** we were surprised at its resistance to acid; heating in 10% aqueous HCl was necessary to regenerate spermidine. Similar, five-membered imidazolidines of formaldehyde are important intermediates of one-carbon metabolism during interconversion of the coenzymes tetrahydrofolic acid and 5,10-methylenetetrahydrofolate.[75] In view of this, and despite the

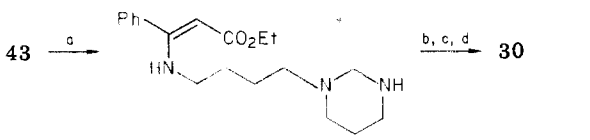
(75) S. J. Benkovic, P. A. Benkovic, and R. Chrzanowski, *J. Am. Chem. Soc.*, **92**, 523 (1970).

Scheme V[a]



[a] (a) PhCH=CHCOCl, Et_3N; (b) $CH_2(CO_2H)CO_2Et$, $C_5H_{11}N$, EtOH, 85% from **43**.

Scheme VI[a]



[a] (a) PhC≡CCO_2Et, EtOH, reflux; (b) $CH_2(CO_2H)CO_2Et$, $C_5H_{11}N$, EtOH; (c) $NaBH_3CN$, 43% from **43**; (d) Bu_4NOH

overwhelming trends observed earlier with aldehydes, we decided to attempt the direct preparation of **43** from **3** and formaldehyde. In the event, simply mixing **3** with 37% formalin solution (0.98 equiv) in water furnished pure **43** in 95% yield after $CHCl_3$ extraction! Perhaps the aqueous instability of formaldehyde imines explains this aldehyde's unique behavior with **3**; excess HCHO generated an amorphous, uncharacterized product that was best avoided. Whatever the reason, we had in hand a promising new protected polyamine that could be trivally prepared in virtually unlimited amounts.

The well-defined order of reactivity in **43** can be exploited in a number of highly regioselective acylations and alkylations. For the synthesis of the alkaloid maytenine (**55**), a constituent of *Maytenus chuchuhuasha*,[68,76,77] 2 equiv of cinnamoyl chloride transformed **43** to **54**. Since extensive deacylation at N^1 took place during acidic hydrolysis of the protecting group, an alternative method was devised. The chemistry of free[73] and acylated[78] *gem*-diamines often parallels that of the corresponding imines so that the classical Knoevenagel condensation offered a prospective solution. In fact gently heating **54** with ethyl hydrogen malonate and piperidine in ethanol furnished maytenine in 85% yield (Scheme V).[62,68] Likewise free spermidine could be regenerated from **43** under similar conditions, making possible yet another spermidine → spermine route akin to that shown in Scheme III.

At last it seemed that we might realize our initial goal of a biomimetic synthesis of celacinnine (**32**) directly from spermidine (Scheme VI). Although ethyl cinnamate was inert to **43** and other simple primary amines, a smooth 1,4 addition occurred between **43** and ethyl phenylpropiolate to furnish ester **56**. Deblocking as before followed by enamine reduction eventually afforded the same triamino carboxylate salt **30** (Scheme II) utilized in our earlier, less convergent approach.[62]

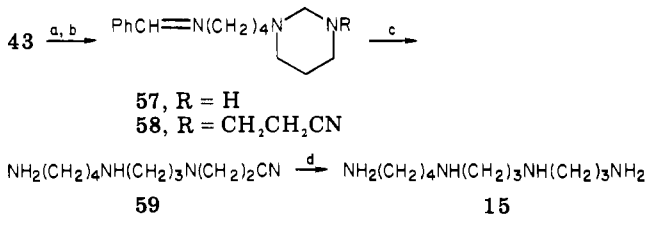
It was at this stage of our work that Oshima reported the structure of a rare, new tetraamine, thermospermine

(76) G. Englert, K. Klinga, R. Hamet, E. Schlittler, and W. Vetter, *Helv. Chim. Acta*, **56**, 474 (1973).

(77) E. Schlittler, U. Spitaler, and N. Weber, *Helv. Chim. Acta*, **56**, 1097 (1973).

(78) The mechanism of hydrolysis of these species has recently been studied: G. M. Loudon, M. R. Almond, and J. N. Jacob, *J. Am. Chem. Soc.*, **103**, 4508 (1981).

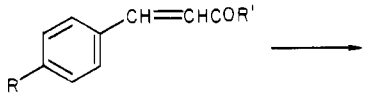
Scheme VII[a]



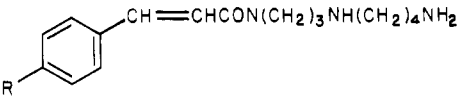
[a] (a) C_6H_5CHO (1.0 equiv), C_6H_6, room temperature; 100% (b) CH_2=CHCN (1.1 equiv), EtOH, room temperature; 99%; (c) 2N HCl-CH_3OH, 77 °C, 8 h, 50%; (d) $NaBH_4$, $CoCl_2$, CH_3OH, 70%.

(15).[43] Retrosynthetic analysis suggested that the molecule might swiftly be assembled by a selective alkylation of N^1 in formaldehyde adduct **43**. N^8 was first quantitatively incapacitated as the *N*-benzylidene derivative **57** (Scheme VII). Despite its diminished nucleophilicity, the free secondary nitrogen in **57** added efficiently to acrylonitrile in a 1,4 fashion to afford cyano imine **58**. (We have since noted that less reactive Michael acceptors, e.g., PhC≡CCO_2Et, were inert to **57**, although acylations generally work well.)[79] It was now appropriate to hydrolyze both the imine and *gem*-diamine functions simultaneously with acid, liberating triaminonitrile **59**. Reduction of **5** with the unusual system[80,81] $NaBH_4$–$CoCl_2$ smoothly produced thermospermine in multigram quantities. An overall yield of 31% from **3** underscored the practicality of this approach.

Benzylidene derivative **57** is also the cornerstone of one plan for glycocinnamoylspermidine synthesis, which relies on acylation at N^1 with a premade glycoside from *p*-coumaric acid (**60**) and a suitably acetylated tri-



60, R, R' = OH
61, R = OCH_3; R' = Cl



62, R = OCH_3
5–7 R = -O-trisaccharide

saccharide. In a model system for the natural products **5–7**, *p*-methoxycinnamoyl chloride (**61**) combined with **57** (Et_3N, THF) to give substituted cinnamoylspermidine **62** in 50% yield after deblocking.

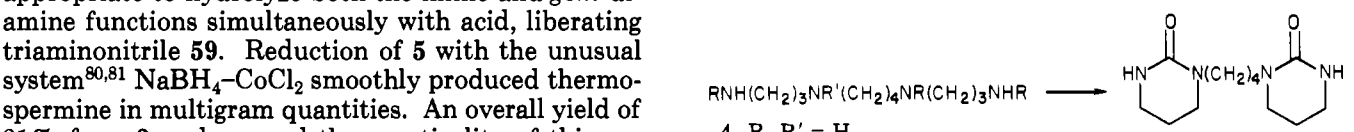
Both N^1-acetyl and N^8-acetylspermidine are important urinary metabolites that are hard to obtain pure.[30] Reaction of **57** and **43** with *N,N',N'',N'''*-tetraacetylglycoluril[82] and acetyl chloride, respectively, now provides each isomer free of the other.

Clearly, spermidine derivative **43** has played a pivotal part in the research reported here and proven itself of value in many different synthetic undertakings. The fact that formalin, a principal component of ordinary embalming fluid, gave rise to such useful cyclic structures seemed oddly in keeping with the thanatotic connotations of much polyamine nomenclature.

Extensions to Higher Polyamines

Many polyamines found in nature contain the 1,3-diaminopropyl synthon, and some early experiments at Cornell suggested that temporary, 6-membered intermediates like urea- or formaldehyde-protected spermidine could be generated in more complex structures. Neither 1,4- nor 1,5-diamines displayed competing ring-forming processes: putrescine and cadaverine altogether failed to form either type of (seven- or eight-membered) derivative and were recovered unchanged. Moreover *N*-(aminopropyl)cadaverine, $NH_2(CH_2)_3NH(CH_2)_5NH_2$, behaved just like spermidine to afford the expected trimethyleneurea and hexahydropyrimidine in high yield. This information later proved important in establishing a microscale polyamine analytical method (vide infra).

It was especially gratifying to discover that our conception of pairwise nitrogen protection applied equally well to spermine (**4**), which displays two 1,3-diamine moieties. Thus, **4** afforded bis(urea) **66** from hydrolysis of its tetrakis(*N*-methoxycarbonyl)derivative **63**. It



4, R, R' = H
63, R, R' = CO_2CH_3
64, R = H; R' = $CH_2CH_2CH_2NH_2$
65, R = CO_2CH_3, R' = $CH_2CH_2CH_2NHCO_2CH_3$

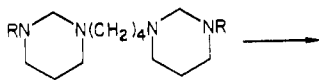
66

was, in fact, by application of this technique that commercial, technical grade spermine was ultimately freed of its impurity.[83] The contaminant, N^4-(γ-aminopropyl)spermine (**64**), arose from trialkylation of putrescine with acrylonitrile during the industrial synthesis of **4**. Since it formed an acid-soluble tetraurethane **65**, the impurity was readily separated from **63**. Samples of high-purity spermine could be obtained by applying the urea exchange reaction to **66**. In contrast to its spermidine counterpart **41**, urea **66** was a high-melting (mp 225–228 °C), neutral compound that gave no color reaction to ninhydrin spray on a thin-layer chromatogram.

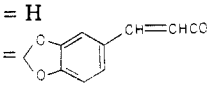
Spermine also cyclized with formalin solution (1.9 equiv) to bis(hexahydropyrimidine) **67** (mp 82–83 °C), a "protected spermine" that effectively sealed off both internal nitrogens against electrophilic reagents. Our finding coincided serendipitously with the disclosure of kukoamine A's structure (**20**) in 1980 and prompted the total synthesis of this antihypertensive agent shown in Scheme VIII.[84] (Methylenedioxy)caffeoyl chloride best acylated **67** and furnished pure **68** as an amorphous solid. Their purpose served, the *gem*-diamine heterocycles were disbanded by the Knoevenogel reaction and the resulting caffeoylspermine **69** was reduced with PtO_2–H_2–HOAc to bis(amide) **71** in quantitative yield. Alternatively, formic acid reduction of **68** and its analogs gave the anticipated N^4,N^8-dimethyl-1,12-diacylspermines **70**. Cleavage of the two methylenedioxy rings in **71** with BCl_3 completed the synthesis of **20** in

(79) C. M. Tice, Unpublished results.
(80) T. Satoh, S. Suzuki, Y. Suzuki, Y. Miyaji, and Z. Imai, *Tetrahedron Lett.*, 4555 (1969).
(81) The mechanism of this complex reducing agent will be described in a forthcoming publication.
(82) Ch. Hase and D. Kuhling, *Liebigs Ann. Chem.*, 95 (1975).
(83) Pure spermine can be purchased from Ames Laboratories, Milford CT.
(84) K. Chantrapromma and B. Ganem, *Tetrahedron Lett.*, **22**, 23 (1981).

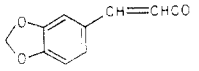
Scheme VIII

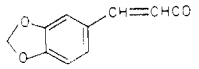


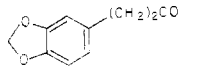
67, R = H

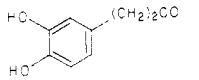
68, R = 

$RNH(CH_2)_3NR'(CH_2)_4NR'(CH_2)_3NHR$

69, R' = H, R = 

70, R' = CH_3 R = 

71, R' = H, R = 

20, R' = H, R = 

62% overall yield. This work has made possible an extensive evaluation of structure–activity relationships in **20**, resulting in many highly promising therapeutic leads.

The most common polyamines **1–4** are often found together in the blood and urine of higher primates, and research into their clinical significance has spawned a variety of qualitative and quantitative analytic methods for their detection.[8,30] One of the most powerful techniques involves dansylation for fluorometric TLC or HPLC assay.[85] In attempting to design a new urinalysis technique for specifically monitoring spermine/spermidine ratios, we observed that very dilute solutions of **1–4** could be cyclized in one step with $ClCO_2CH_3$ and $Ba(OH)_2$ and then assayed by paper chromatography. The simple diamines were unaffected, spermidine appeared as a ninhydrin-positive, polar urea **41** and spermine formed the characteristic bis(urea) **66**, which migrated to the top of the chromatogram. Since nanomolar amounts were easily visualized, a few milliliters of urine from a healthy individual would suffice for detection. Although not as sensitive as dansylation, perhaps under special circumstances this derivatization may someday complement existing analytical protocols.

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(85) M. M. Abdel-Monem, K. Ohno, N. E. Newton, and C. E. Weeks in ref 8, Vol. 2, p 37.