$Cu_4O_3(O^iPr)_2$ unit capped on two opposite ends by $Zr_2(O^iPr)_8$ units. At the crystallographic center of symmetry is an unusual four-coordinate *planar* O^{2-} ion. Only two other examples of this geometry are known.[10,11] An alternative description of the structure is that two face-shared bioctahedral $Zr_2O(O^iPr)_8^{2-}$ units bind through a pseudotetrahedral μ-O and two terminal O^iPr groups to a central planar $Cu_4O(O^iPr)_2^{4+}$ unit. This view leads to the idea that the $Zr_2(O^iPr)_8O^{2-}$ unit is a template (via $2O^iPr$ and the oxide) for growth of the planar ribbon of composition $Cu_4O(O^iPr)_2^{4+}$. Growth of this ribbon into a sheet [i.e., growth perpendicular to the $(\mu_4\text{-}O)_3$ direction] is prevented by the isopropyl groups on the oxygens which bridge the coppers.

Spectroscopic analysis confirms the chemical formula and structure. IR spectra lack any O-H stretches, thus excluding the presence of hydroxyl or coordinated alcohol. The NMR spectra[12] of **1** lack the expected shifting and broadening associated with paramagnetic species. The methine region of the $^{13}C\{^1H\}$ NMR spectrum shows that the solid-state structure is maintained in solution: the expected five chemical shifts with approximately the correct integral ratio of 2:2:2:2:1 are found.[12] This conclusion is further supported by the methyl group ^1H and ^{13}C NMR peaks. These are sufficiently complex to indicate the retention of the solid-state structure (with diastereotopic methyls) in solution.

On the basis of the lack of paramagnetic characteristics in the NMR spectra, the magnetic susceptibility was investigated. This reveals that, while there are unpaired electron spins, these spins are coupled antiferromagnetically. The solution magnetic susceptibility (Evans method, 295 K, THF) yields a μ_{eff} of 0.9 μ_B/Cu. A variable-temperature (56-230 K) solid-state study shows the μ_{eff} to remain clearly constant at 1.1 μ_B/Cu from 230 to 100 K and then drop to 0.4 μ_B/Cu by 56 K. A mononuclear Cu^{2+} ion would have μ_{eff} = 1.73 μ_B. Our initial exploration of the parameter space of the three J values (two J_{cis} and one J_{trans}) of a Heisenberg Hamiltonian model appropriate to a centrosymmetric Cu_4^{8+} unit shows a singlet ground state. There are another singlet, a set of three triplets, and a quintet state at higher energies; one or more of the triplets become appreciably populated by 100 K, causing the rise in μ_{eff} from 56 to 100 K, but up to now it has not been possible to simulate the magnetic susceptibility data, and further work is in progress. The μ_{eff} fails to rise further even at 300 K because a quintet state lies too high to be significantly populated.

This heterometallic oxo alkoxide contains a planar Cu_4 array previously unknown in the chemistry of Cu(II). This work suggests that electropositive partner metals in heterometallic species can "guide" the growth of Cu/O patches which mimic some of the structural features of high T_c superconductors.[13] We believe that the thermodynamically favored unit (i.e., Zr_2X_9) of a "partner metal" in a heterometallic alkoxide can serve as a structural template for the planar Cu_4O array. In the present case, three colinear donor sites (i.e., one terminal alkoxide on each Zr together with the μ-O^{2-}) of two $Zr_2(O^iPr)_8O^{2-}$ units are complementary to the growth of a copper(II) oxide "ribbon" *two* coppers wide. Further growth along the ribbon direction (i.e., the O5-O5' axis) would require insertion of the repeat unit $Cu_2O(O^iPr)_2$.

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Supplementary Material Available: A table of positional and thermal parameters for compound **1** (1 page). Ordering information is given on any current masthead page.

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General Method for Incorporation of Modified N^ω-Cyanoguanidino Moieties on Selected Amino Functions during Solid-Phase Peptide Synthesis

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In peptide chemistry, incorporation of unusual or unnatural amino acids into bioactive peptides is an important tool in structure-activity relationship (SAR) studies and in the ultimate development of more stable and effective analogues.[1] It is desirable that unusual amino acids be easily prepared or readily available from optically active amino acids through derivatization and that the method be compatible with solid-phase peptide synthesis (SPPS). We describe herein a general method for the preparation of N^ω-cyano-$N^{\omega'}$-alkyl- or -arylhomoarginine-containing peptides as well as heterocyclic N^ϵ-lysine derivatives via modification of the ϵ-amino of lysine residues in orthogonally protected, resin-bound peptides.

In studies directed toward the development of potent and long-acting gonadotropin-releasing hormone[2] (GnRH) analogues, extensive use has been made of unusual amino acid substitutions to improve its therapeutic index.[3] Substitution of basic amino acids such as D-Arg in position 6 significantly increased potency of GnRH antagonists which contained a hydrophobic aromatic N-terminus.[4] Unfortunately this structural combination has been implicated in the deleterious histamine-mediated flare and wheal response seen when the peptides were injected subcutaneously in rats.[5] Our approach to this problem was to attenuate the basicity of various guanidino-containing residues of active analogues by modifying the guanidine moiety with an electronegative N^ω-cyano substituent.[6] This strategy proved successful in the development of the potent guanidino-containing histamine H_2 receptor antagonists (of which cimetidine is a member) where introduction of electron-attracting substituents such as cyano or nitro groups improved the effectiveness of the analogues in inhibiting gastric acid secretion.[7]

Peptides were synthesized by standard SPPS methodology either manually or on a Beckman 990 peptide synthesizer.[8] The ϵ-amino groups of lysine residues to be modified were protected as the (9*H*-fluorenylmethoxy)carbonyl (Fmoc) derivatives. The fully assembled peptide resin was treated with a 20% piperidine/DMF solution (10 min) to remove the Fmoc from the lysine side chain(s) followed by addition of diphenyl cyanocarbonimidate (PCI) in DMF at room temperature (Scheme I) to form intermediate A.[9]

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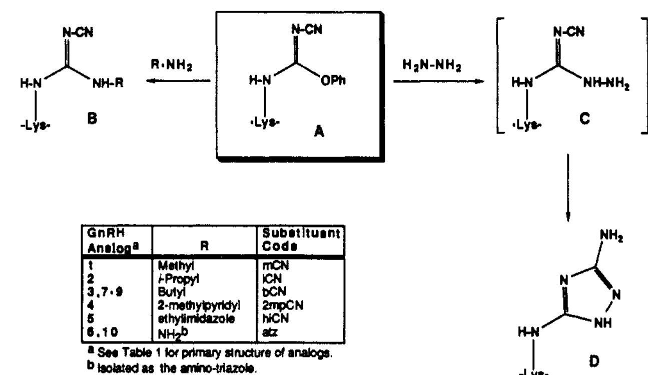
Table I. Characterization of Modified N^{ω}-Cyanohomoarginine GnRH Antagonists

	Compounds[a]	RT (min, %CH_3CN)[b]	$[\alpha]_D$[c]
1	[Ac-DNal[1],DCpa[2],DPal[3],HAr[5](mCN),DHAr[6](mCN),ILys[8],DAla[10]]-GnRH	4.00 (32.7)	-29.4
2	[,HAr[5](iCN),DHAr[6](iCN),ILys[8],DAla[10]]-GnRH	4.68 (35.4)	-28.2
3	[,HAr[5](bCN),DHAr[6](bCN),ILys[8],DAla[10]]-GnRH	4.11 (37.2)	-26.4
4	[,HAr[5](2mpCN),DHAr[6](2mpCN),ILys[8],DAla[10]]-GnRH	3.77 (27.6)	-24.8
5	[,HAr[5](hiCN),DHAr[6](hiCN),ILys[8],DAla[10]]-GnRH	3.91 (27.6)	-25.3
6	[,Lys[5](atz),DLys[6](atz),ILys[8],DAla[10]]-GnRH	3.72 (27.0)	-34.2
7	[,NicLys[5],DNicLys[6],HAr(bCN)[8],DAla[10]]-GnRH	3.77 (36.0)	-32.9
8	[,DTrp[3],DHAr(bCN)[6],HAr(bCN)[8],DAla[10]]GnRH	4.28 (51.0)	-30.7
9	[,DHAr[3,6](bCN),Lys[5](bCN),ILys[8],DAla[10]]-GnRH	4.04 (45.6)	-30.8
10	[,DLys[3](atz),Arg[5],DNal[6],DAla[10]]-GnRH	4.39 (39.6)	-36.5

[a] IUPAC rules are used for nomenclature except for the following: Ac = acetate; Nal = 3-(2-naphthyl)alanine; Cpa = 4-chlorophenylalanine; Pal = 3-(3-pyridyl)alanine; ILys = N^{ϵ}-isopropyllysine; mCN = N^{ω}-cyano-$N^{\omega'}$-methyl; eCN = N^{ω}-cyano-$N^{\omega'}$-ethyl; iCN = N^{ω}-cyano-$N^{\omega'}$-isopropyl; bCN = N^{ω}-cyano-$N^{\omega'}$-butyl; 2mpCN = N^{ω}-cyano-$N^{\omega'}$-2-methylpyridyl; hiCN = N^{ω}-cyano-$N^{\omega'}$-ethylimidazole; Lys(atz) = N^{ϵ}-5'-(3'-amino-1H-1',2',4'-triazolyl)lysine. [b] RT = retention time in minutes, % CH_3CN. Peptides (10 μg/10 μL) dissolved in 0.1% TFA were applied to a Vydac C_{18} column (5 μm, 300-Å pore size; 4.5 × 250 mm) under isocratic conditions, 0.1% TFA/H_2O with % CH_3CN shown, at a flow rate of 2.0 mL/min. UV detection was 0.1 AUFS at 210 nm. [c] c = 1. 50% HOAc/H_2O.

Scheme I



After the PCI coupling was complete (negative ninhydrin test, from 2 to 12 h), an amine, RNH_2, in DMF was then added and the mixture was stirred for 2–4 days. The peptide was then cleaved (anhydrous HF/1% anisole for 1.5 hr at 0 °C) and purified to greater than 95% purity using reverse-phase HPLC.[10] A variety of amines including straight-chain and branched amines reacted readily with the intermediate A to give N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylhomoarginine side chains as typified by B. On the other hand, reaction of A with hydrazine afforded the heterocyclic amino acid derivative D which resulted from initial attack of the bifunctional hydrazine nucleophile at the imidate carbon to give C followed by cyclization at the nitrile carbon atom to yield D. This procedure thus allowed entry into an unusual class of substituted lysine or ornithine amino acids possessing a heterocyclic ring system. The yields of modified cyanoguanidino compounds were comparable to that obtained for unsubstituted peptides, suggesting that derivatization was essentially quantitative.

As summarized in Table I, peptides containing N^{ω}-cyano-$N^{\omega'}$-alkylhomoarginine residues (**1–3**, **7–9**) or N^{ω}-cyano-$N^{\omega'}$-arylhomoarginine residues (**4** and **5**) as well as the N^{ϵ}-(aminotriazolyl)lysine derivatives (**6** and **10**) have been synthesized according to the procedure outlined above.[11] During the course of our studies, it was found that the peptides prepared with trifluoroacetate (TFA) as the counterion decomposed (from ≥95% to 50–70% purity by HPLC) over a 2–6-month period when stored at 0 °C as lyophilized powders that were found to contain as much as 17% TFA by weight (ca. 3 mequiv as determined by HPLC). Isolation of the decomposition products, followed by characterization using mass spectrometry, revealed that hydrolysis of the nitrile had occurred under the acidic conditions induced by TFA (present as the counterion or in excess) in the presence of water of hydration.[12] Repurification and isolation of the peptides as the acetate salts (ca. 0.5 mequiv as determined by HPLC) resulted in compounds stable to hydrolysis (as powders at 0 °C) presumably due to the lower acidity of this counterion. It should be noted that the N^{ϵ}-(aminotriazolyl) derivatives (entries 6 and 10) are stable as TFA salts, consistent with the proposed mode of decomposition for the cyanoguanidino peptides.

In summary, we have successfully demonstrated the applicability of a general synthetic method for synthesizing modified lysine residues on a resin-bound peptide as evidenced by the numerous examples of N^{ω}-cyano-$N^{\omega'}$-alkyl- or -arylhomoarginine- and N^{ϵ}-lysine-modified GnRH analogues prepared in our laboratory.[13] Introduction of these pI altering cyanoguanidino and N^{ϵ}-(aminotriazolyl)lysine residues can be used to begin to assess the role of the basic residues in bioactive peptides such as GnRH. Furthermore, the modifications may also confer enzymatic stability to guanidino- and lysine-containing peptides. The flexibility of this method, combined with its procedural simplicity and good yields, enhances its applicability to peptide synthesis. Preliminary studies on the effect of these modifications on GnRH bioactivity (including antiovulatory activity, binding affinity, and histamine release) have yielded encouraging results, and efforts are continuing to fully characterize the biological effects of these analogues[14] as well as the application of this chemistry to the modification of free peptides and proteins in solution.

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(12) It is proposed that the hydrolysis is acid catalyzed and results in the formation of the corresponding guanylurea. This proposal is based on detailed mass spectral analysis of the degradation products of compound **5** and the fact that cimetidine, the cyanoguanidino-containing H_2 antagonist, was similarly hydrolyzed in 5 days in dilute HCl at room temperature. (Metabolic studies, however, indicated cimetidine to be excreted essentially unchanged.[7a])

(13) All compounds gave satisfactory HPLC, mass spectra, and amino acid analyses (new amino acids were not characterized and were not detected by standard amino acid analysis). The FTIR spectra of compound **5** exhibited an absorbance at 2165 cm^{-1}, indicating the presence of the cyano group, which was absent in compound **6**.

(14) Manuscript in preparation.

of Point Loma Nazarene College, San Diego, CA, for the use of the Nicolet 710 FTIR instrument. High-resolution FAB mass spectra were obtained from Dr. Terry Lee, Beckman Research Institute of the City of Hope, Duarte, CA.

Biosynthetic Studies of Marine Lipids. 32.[1] The Missing Step in Sterol Cyclopropyl Biosynthesis: Enzymatic Desaturation of 24(*S*)-Ethylcholesterol

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Marine sponges of the order Haplosclerida[2] contain many examples of the most unusual marine sterols: those containing cyclopropanes and cyclopropenes in the side chain (**1–4**).[3] In addition, the unusual acyclic side chain sterols, ficisterol (**5**)[4] and 26(29)-dehydroaplysterol (**6**),[5] which can formally be considered to arise through acid-catalyzed ring opening of the cyclopropyl sterols,[6] cooccur in these same sponges. Recently, through the degradation of biosynthetically radiolabeled petrosterol (**1**),[7] we demonstrated evidence for a unified biosynthetic scheme, involving a highly stereospecific rearrangement (Figure 1).[8] The source of the protonated cyclopropane[9] intermediate (**7**) has, until now, been unclear.

In feeding experiments with [3-^{3}H]dihydrocalysterol (**2**), no conversion to petrosterol (**1**) was detected, thereby ruling out direct enzymatic protonation.[7a] It was thought that *S*-adenosylmethionine (SAM) methylation of 24-methylenecholesterol (**8**, Figure 2), followed by isomerization of the tertiary carbonium ion (**9**) to the requisite secondary carbonium ion at C-23 (**10**), might be the enzymatic step leading to the cyclopropyl sterols (**1–4**).[7,8] However, when this was tested experimentally with [^{3}H]SAM in a cell-free extract of *Cribrochalina vasculum*, we found that 24-methylenecholesterol (**8**) was converted only to clerosterol (**11**, Figure 3).[10]

The finding that clerosterol (**11**) is the product of 24-methylenecholesterol (**8**) biomethylation in cyclopropyl sterol producing sponge suggested to us the intermediacy of the a priori unlikely clionasterol (**12**) in the biosynthesis of the cyclopropyl sterols (Figure 3). The isolation of a sterol bearing a 23-hydroxy side chain (**13**, R = Me) in a yeast mutant incapable of desaturation at the 22-position[11] suggests that the cyctochrome P-450

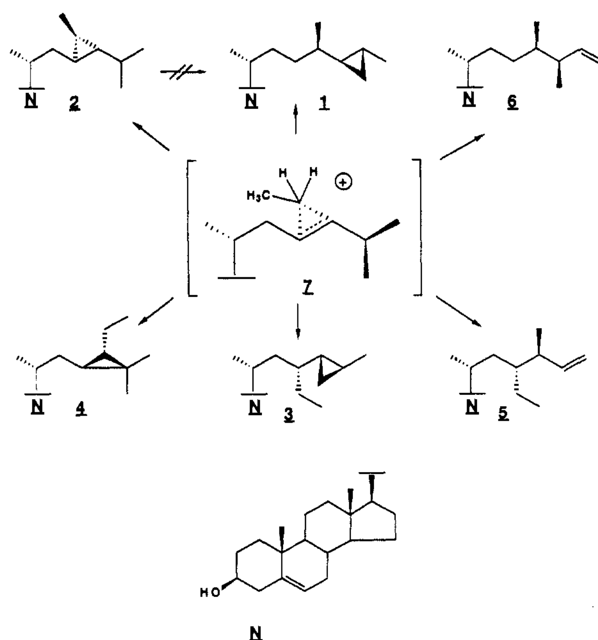

Figure 1. Unified biosynthetic pathway based on the intermediacy of the protonated cyclopropane 7.[8]

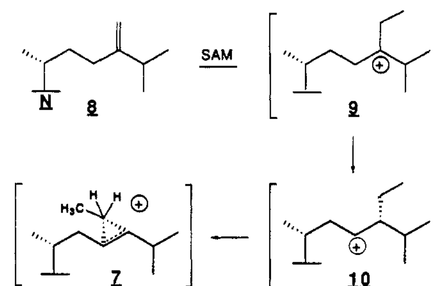

Figure 2. Possible route to the protonated cyclopropane intermediate 7.

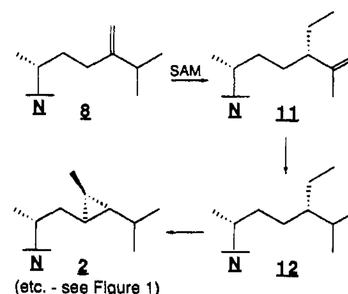

Figure 3. Biosynthetic pathway to sponge cyclopropyl sterols.

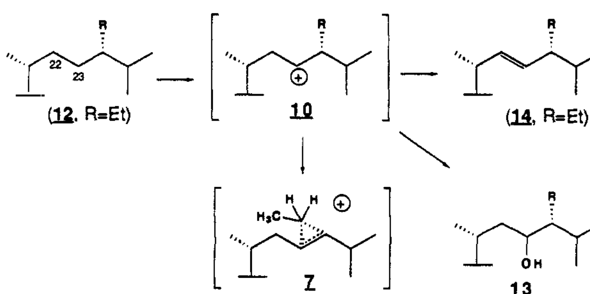

Figure 4. Products of the Δ^{22}-desaturase.

Δ^{22}-desaturase[12] initiates the formation of the double bond by deprotonation at C-23 (Figure 4). The secondary carbonium ion (**10**) thus formed can eliminate a proton directly from C-22 to produce the Δ^{22} double bond (e.g., **14**), rearrange via the pro-

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