Ammonia/methanol was also used as a solvent to farnesylate KCVLS; however, yields were lower and the peptide was less pure than for ammonia.

Du Vigneaud et al.[26] first described alkylation of cysteine in liquid ammonia. They generated the sodium thiolate by in situ reduction of cystine with sodium and then added electrophilic alkyl halides. We found cysteine to be readily alkylated by farnesyl chloride in ammonia by simply mixing the amino acid and the electrophile. The use of ammonia or methanol/ammonia[27] affords a mild regioselective procedure (note that KCVLS contains free amino, hydroxyl, and carboxylate moieties) for formation of farnesyl cysteinyl thioethers. We have used this procedure to construct farnesylated peptides needed for assays of protein prenyltransferase activity in yeast[5] and as authentic samples for structure determinations of prenylated proteins.[16] We are currently extending the study to other isoprene units and peptides.

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(25) ^{13}C NMR (75, MHz, $CDCl_3$): δ 168.4, 140.8, 135.3, 131.2, 124.2, 123.5, 118.7, 53.5, 52.4, 39.7, 39.7, 31.2, 29.5, 26.8, 26.5, 25.8, 17.8, 16.2, and 16.1 ppm.

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Efficient Method for Regioselective Isoprenylation of Cysteine Thiols in Unprotected Peptides

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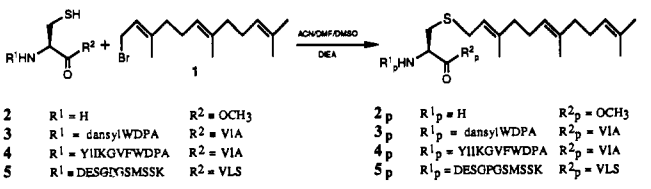
Isoprenylation, a recently discovered posttranslational modification of proteins,[1] has been found to be crucial for both membrane association and transforming activity of oncogenic *ras* proteins[2-4] in which the isoprene unit is located at the C-terminal cysteine.[5,6] The isoprene moiety on yeast a-factor mating hormone was identified unambiguously as the C_{15} farnesyl group by ^1H NMR spectroscopy and mass spectrometry,[7] and more recently, proteins of HeLa cells were reported to be geranylgeranylated (C_{20} unit).[8]

The chemical synthesis of farnesylated peptides by alkylation of the sulhydryl group with farnesyl bromide in 50% DMF in the presence of MgO has been documented in the literature;[9,10] however, we have had little success in preparing various isoprenylated peptides by the reported procedure. In this communication, we describe an improved and efficient method for regioselective isoprenylation of cysteine thiols in unprotected peptides.

Scheme I



2	R^1 = H	R^2 = OCH_3	2_p	R^1_p = H	R^2_p = OCH_3
3	R^1 = dansylWDPA	R^2 = VIA	3_p	R^1_p = dansylWDPA	R^2_p = VIA
4	R^1 = YIIKGVFWDPA	R^2 = VIA	4_p	R^1_p = YIIKGVFWDPA	R^2_p = VIA
5	R^1 = DESGPGSMSSK	R^2 = VLS	5_p	R^1_p = DESGPGSMSSK	R^2_p = VLS

The model system, cysteine methyl ester (**2**) containing an amino and a thiol nucleophile, in acetonitrile (ACN) and dimethylformamide (DMF) was treated with 4 equiv of farnesyl bromide[11] (**1**) (Scheme I) at 0 °C in the presence of diisopropylethylamine (DIEA). The resulting S-farnesylated cysteine methyl ester ($\mathbf{2}_p$) (Scheme I), obtained in 90% yield, appeared as a single regioisomer by ^1H NMR analysis,[12] displaying the reported chemical shift for the C-1 allylic protons at 3.2 ppm.[3] In addition, a positive quantitative ninhydrin result supports the structure of $\mathbf{2}_p$. The farnesylation reaction was found to be very dependent on the quality of the farnesyl bromide,[13] which is sensitive to decomposition upon silica gel purification and, therefore, was used directly without further purification.

Successful mono- and regioselective farnesylation of unprotected peptides with farnesyl bromide depends on the solvent system, temperature, pH, amount of the farnesyl bromide, and peptide concentration. Among various solvents,[14] we have found that use of a mixed solvent system, DMSO/DMF/ACN, provided the maximal solubility for most peptides. Although several competing nucleophiles are present in the unprotected peptide, such as carboxylate anion, free amino group, phenolic anion, etc., we have found that farnesyl bromide reacts exclusively with the sulfhydryl anion of the unprotected peptide at pH 10-12 at 0 °C to afford the desired S-alkylated adduct. The pH of the reaction mixture was maintained by addition of 6-9 equiv of DIEA (measured on moist pH paper). Three to four equivalents of farnesyl bromide with 0.01-0.02 M concentration of the peptide was found to represent optimal conditions. Use of greater amounts of farnesyl bromide (>10 equiv) and higher peptide concentrations (>0.06 M) often generate multifarnesylated adducts. The formation of the disulfide dimer was significantly suppressed by degassing of the reaction solution followed by sparging with argon.

In a typical reaction, the peptide (dansyl)WDPACVIA[15] (**3**) (Scheme I) was treated with 3 equiv of farnesyl bromide in the mixed solvent DMSO/DMF/ACN (3:3:1) at a concentration of 0.01 M in the presence of 8 equiv of DIEA at 0 °C under an argon atmosphere for 3 h, to afford the desired *S*-farnesyl regioisomer (dansyl)WDPAC(far)VIA ($\mathbf{3}_p$) (Scheme I), in 86% yield. The reaction mixture was quenched with acetic acid at 0 °C to bring the pH to around 4 and then lyophilized followed by reversed-phase (C_{18}) HPLC purification. The reaction is monitored by reversed-phase (C_{18}) HPLC with photodiode array detection[16] from which the characteristic UV spectrum of each individual peak in the chromatogram is used to determine the progress of the reaction and to identify the mono- and multifarnesylated adducts. The structure of peptide $\mathbf{3}_p$ was characterized by amino acid analysis

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(12) ^1H NMR spectral data of $\mathbf{2}_p$ (400 MHz, $CDCl_3$): δ 1.6 (s, 6 H), 1.67-1.68 (t, 6 H), 1.83-2.13 (m, 8 H), 2.28 (br, 2 H), 2.67 (dd, 1 H), 2.89 (dd, 1 H), 3.18 (m, 2 H), 3.63 (dd, 1 H), 3.74 (s, 3 H), 5.10 (m, 2 H), 5.23 (t, 1 H).

(13) Farnesyl bromide is stable at -20 °C for several months.

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and fast atom bombardment mass spectroscopy, which showed an M + 1 molecular peak at m/z 1311.8 (calculated = 1312.1). These studies were also extended to the longer peptides, YIIKGVFWDPACVIA (**4**) and DESGPGSMSSKCVLS[15] (**5**) (Scheme I), both of which were farnesylated under similar reaction conditions to afford the desired products $\mathbf{4}_p$ and $\mathbf{5}_p$ (Scheme I) in 84% and 85% yield,[17] respectively.

This method has provided the first practical method for regioselective isoprenylation of cysteine thiols in unprotected peptides and should permit systematic studies of regioselective isoprenylation of cysteine thiols in peptides and proteins. Several farnesylated peptides prepared by this method have been used to assay the activity of protein prenyltransferase in yeast.[4] A variety of different isoprenylating reagents are now under investigation in our laboratory.

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(17) The structures of peptides $\mathbf{4}_p$ and $\mathbf{5}_p$ were identified by amino acid analysis and fast atom bombardment mass spectroscopy, which gave an M + 1 peak at m/z 1899.2 (calculated = 1898.4) for peptide $\mathbf{4}_p$ and m/z 1687.2 (calculated = 1687.0) for peptide $\mathbf{5}_p$.

Clavepictines A and B: Cytotoxic Quinolizidines from the Tunicate *Clavelina picta*[1]

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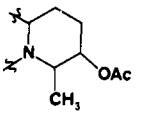
In our investigations of the chemistry and biological significance of the secondary metabolites of Bermudian tunicates,[3] we found cytotoxicity (KB: IC_{50} = 12 μg/mL) and antimicrobial activity in the organic soluble extracts of *Clavelina picta*. From substantial collections (1-2 kg) made in the summer of 1984 and the spring of 1987, we have found the first quinolizidines from a tunicate and have evaluated the cytotoxicity of these novel compounds.

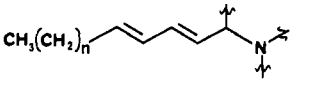
Clavepictine A was obtained from the extracts by a sequence of solvent partitioning ($CHCl_3$ vs 40% aqueous MeOH), gel permeation chromatography (Bio-Beads S-X4 and Sephadex LH-20), reversed-phase low-pressure chromatography (C_{18}, H_2O–MeOH gradient), and centrifugal countercurrent chromatography (CCC; hexane–CH_2Cl_2–CH_3CN, 10:3:7) as a colorless oil.[4] It could also be obtained naturally as the hydrochloride salt (IR 2500 cm^{-1}; positive Beilstein test). High-resolution MS provided the molecular formula $C_{22}H_{37}NO_2$, requiring five sites of unsaturation. One was readily attributed to an acetate ester (IR 1730 cm^{-1}; ^1H NMR δ 2.05, 3 H, s; MS m/z 305 (M^+ - 42). Two more comprised a conjugated heteroannular diene (λ_{max} 232 nm), leaving two rings to be placed in the molecule.

The ^{13}C NMR spectrum revealed the expected acetate carbonyl and four protonated olefinic carbons, but also contained four signals for methines bearing hetero atoms. One (δ 73.4) obviously carried the acetate; therefore, the remaining three (δ 58.0, 53.0, 49.1) all bore nitrogen. All the remaining carbons were methylenes or methyls, indicating that all branch or juncture points were attached to hetero atoms. Noteworthy was the presence of two methyl groups besides the acetate methyl. (See Table I.)

Two part structures could be constructed without difficulty. A nitrogen-bearing methine (δ 3.5) was found to be coupled to a methyl group (δ 1.09) and to the acetate-bearing methine (δ 4.8). A sequence of two methylenes was found to connect the acetate-bearing methine to a nitrogen-bearing methine at δ 3.08, completing part structure **1a**. The second part structure began with the third nitrogen-bearing methine (δ 3.8), which was coupled to a terminus of the diene system (δ 5.65). As expected from the UV and ^{13}C NMR data, the olefinic protons were sequentially connected on adjacent carbons and the opposite terminus was coupled to an allylic methylene at δ 2.0, which was coupled into a methylene envelope at δ 1.2. Irradiation at δ 1.2 revealed a terminal methyl group at δ 0.9, giving part structure **1b**.

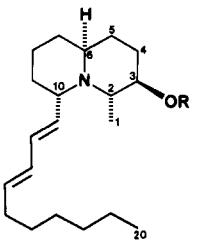


1a



1b

All that remained was to define the size of the second ring or the length of the alkadienyl side chain. Mass spectral cleavage of the alkadienyl chain would provide the necessary evidence, but fragmentation α to the nitrogen would require rupture of an sp^2–sp^3 carbon–carbon bond. This ion (m/z 210) was weak in the mass spectrum of clavepictine A, but catalytic reduction (Pd/C) gave a tetrahydro derivative whose mass spectrum featured a base peak at m/z 210, corresponding to loss of an alkyl chain from a quinolizidine nucleus bearing methyl and acetoxyl substituents. Thus, the gross structure of clavepectine A had to be **1**.



1 R = Ac
2 R = H

The relative stereochemistry of **1** was gleaned from NOE experiments. Irradiation of the methyl group (C-1) attached to the α-methine (C-2) elicited responses from its vicinal neighbor (H-2) and the ring-juncture α-methine (H-6), indicating a 1,3-diaxial relationship of the methyl group and H-6. The small coupling constant between H-2 and H-3, the acetate-bearing methine, and the observation of an NOE between the two suggested that they were trans-diequatorial, putting the acetoxyl substituent in an axial position. No NOE was observed between H-6 and H-10, the third α-methine, but one was observed between H-10 and H-12, suggesting that the alkadienyl side chain was positioned so as to bisect the "plane" of the attached ring. Moreover, an NOE was revealed between H-2 and H-11. These data could best be accommodated by a cis ring juncture in the quinolizidine, an equatorial alkadienyl substituent, and as noted above, axially disposed methyl and acetoxyl groups.

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(4) $[\alpha]_D$ -75.6° (*c* 0.7, CH_2Cl_2); IR ν_{max} (neat) 3015, 2932, 2861, 1735, 1453, 1375, 1252, 1163, 1130, 1105, 1039, 992 cm^{-1}; UV λ_{max} (EtOH) 230 nm (ε = 19 000); EIHRMS m/z 347.2831 ($C_{22}H_{37}NO_2$ requires 347.2824); EIMS m/z (relative intensity) 347 (M^+, 6), 332 (9), 288 (9), 260 (100), 210 (4), 150 (7).

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