# Articles

## A Novel Potassium-Binding Hydrolysis Product of Ascidiacyclamide: A Cyclic Octapeptide Isolated from the Ascidian *Lissoclinum patella*

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A new octapeptide has been isolated and characterized as its potassium complex from acidic aqueous methanol solutions of ascidiacyclamide, a cyclic peptide isolated from the ascidian *Lissoclinum patella*. Crystals suitable for X-ray structure determination were orthorhombic, space group $P2_12_12_1$, with $a = 15.442(3)$ Å, $b = 36.167(1)$ Å, $c = 9.567(3)$ Å, $V = 5343(2)$ Å$^3$, $Z = 4$, and $R = 0.069$. The structure consists of the macrocyclic cation, three perchlorate anions, and two water molecules. The macrocyclic ring of ascidiacyclamide remains intact, but two oxazoline rings have been opened to form two amino lactones, with the amine protonated. A potassium ion is bound to the N atoms of the thiazole rings (K(1)···N(1), 2.47(2) Å; K(1)···N(5), 2.50(2) Å) and to the adjacent O (amide) atoms (K(1)···O(1), 2.34(1) Å; K(1)···O(5), 2.34(1) Å). Two perchlorate anions, located on either side of the plane of the macrocyclic ring, are weakly associated with the potassium cation (K(1)···O(10), 2.72(3) Å; K(1)···O(16), 2.48(3) Å). This cyclic octapeptide reacts with copper(II) to give a purple solution ($\lambda_{max}$ 590 nm).

### Introduction

The putative relationship between marine secondary metabolites and their capacity for metal ion complexation has recently been reviewed.[2] Notwithstanding the demonstrated capacity of some marine organisms to concentrate a range of metal ions *in vivo*,[3−7] there is little evidence that the marine secondary metabolites are involved in that role. There are a few examples of metabolites isolated as their respective metal complexes, and there is little evidence of selectivity exhibited by these compounds toward particular metal ions.[2] Despite the remarkable chemical and structural diversity of the secondary metabolites, their roles are not immediately apparent; is it the *in vivo* sequestration and transport of metal ions, the provision of a convenient template for biological assembly of the metabolites, or functions associated with the biological activity of the compounds?[2]

Our interest has been directed toward the capacity of the marine secondary metabolites to coordinate to metal ions. The characterization of copper complexes of ascidiacyclamide (**1**) and patellamide D (**2**), cyclic peptides isolated from the ascidian *Lissoclinum patella*, has been reported.[8,9] (The molecules referred to in this paper are described in Figure 1.) As well, a novel silver(I) complex with the chemically synthesized cyclic peptide westiellamide, previously isolated from the ascidian *Lissoclinum bistratum* and from a terrestrial source, has been reported.[10−13] As part of the study of the complexation reactions of ascidiacyclamide, we have isolated a potassium complex of a cyclic peptide derived from ascidiacyclamide after acid hydrolysis of the oxazoline rings. (Figure 2 is a schematic drawing of the potassium complex, with full atom-numbering scheme.) This paper reports the characterization by X-ray crystallography of the potassium complex of hydrolyzed ascidiacyclamide, a possible biological precursor to the cyclic peptide ascidiacyclamide. In addition, preliminary spectroscopic analysis of copper(II) complexes of this ligand is presented.

### Experimental Section

Circular dichroism spectra were recorded with a Jobin-Yvon Dichrographe III spectrometer (1 mm quartz cell, sensitivity $2 \times 10^{-5}$ or $5 \times 10^{-6}$). Ultraviolet/visible spectra were recorded with a Beckman DU7500 diode array spectrophotometer. Electrospray ionization mass spectra were recorded with an API-111 triple-quadrupole mass spectrometer (PE/Sciex, Thornhill, Ontario, Canada) (orifice potential

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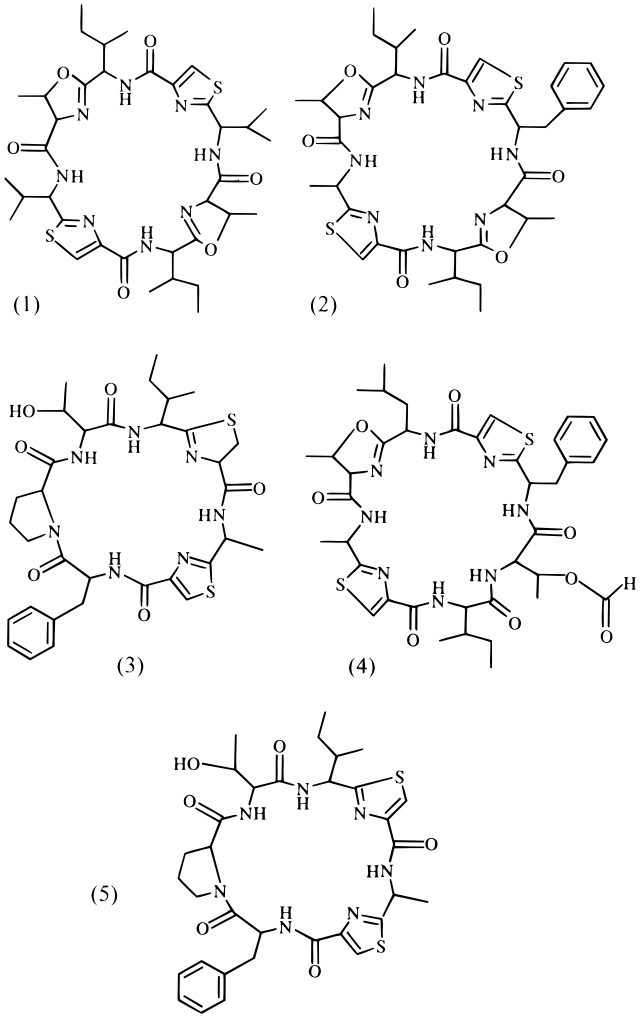


**Figure 1.** Structures of cyclic peptides.

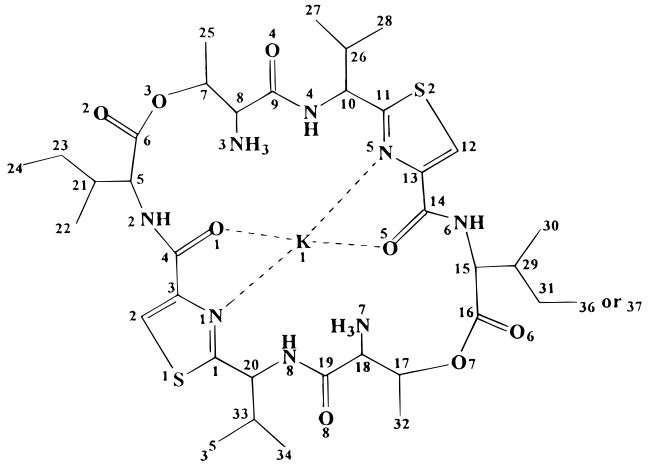

**Figure 2.** Schematic diagram of the $K^+$ complex of hydrolyzed ascidiacyclamide.

difference (OR) = 120 V), as described previously.[8] Ascidiacyclamide was isolated and purified as described previously.[14]

**$K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3 \cdot 2H_2O$.** A 95% methanol solution (0.005 M $HClO_4$, 0.1 M $Et_4NClO_4$) of ascidiacyclamide (1 mM) was maintained at 25 °C during the course of a potentiometric titration (0.1 M $NEt_4OH$).[15] At the completion of the titration, an aliquot of solution (containing ca. 5 mg of ligand) was taken to dryness in a vacuum desiccator. A crystalline sample, which contained $Et_4NClO_4$, was isolated. The solid was extracted with a small volume (1 mL) of $CHCl_3$, the undissolved $Et_4NClO_4$ was discarded, and the resulting solution was again taken to dryness. The procedure was repeated five times. The resulting product was dissolved in methanol and maintained at 25 °C in an atmosphere of ether. Small colorless crystals were subsequently isolated. A methanol solution of this product exhibited a negative circular dichroism ($\Delta\epsilon_{238} = -8.0\ M^{-1}\ cm^{-1}$).

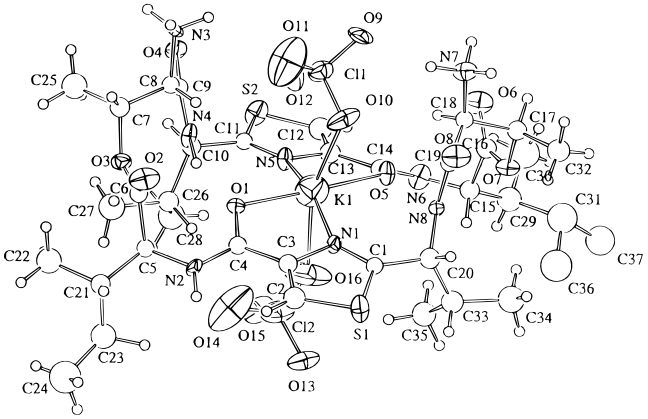

**Figure 3.** ORTEP plot of the $K^+$ complex of hydrolyzed ascidiacyclamide.

**X-ray Structure Determination.** For diffractometry a crystal (thin, colorless needle) was mounted on a glass fiber with cyanoacrylate resin. Lattice parameters at 21 °C were determined by least-squares fits to the setting parameters of 25 independent reflections, measured and refined on an AFC-7 four-circle diffractometer employing graphite monochromated Cu Kα radiation. Intensity data were collected in the range $1 < \theta < 60°$. Data reduction and application of Lorentz, polarization, absorption (empirical $\psi$ scan), and decomposition corrections were carried out using the teXsan system.[16] The structure was solved by direct methods using SHELXS-86,[17] and the solution was extended by difference Fourier methods. Hydrogen atoms were included at calculated sites with fixed isotropic thermal parameters. All other atoms with the exception of the C atoms and N(8) were refined anisotropically. Full-matrix least-squares methods were used to refine an overall scale factor and positional and thermal parameters. Neutral atom scattering factors were taken from Cromer and Waber.[18] Anomalous dispersion effects were included in $F_c$;[19] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[20] The values for the mass attenuation coefficients were those of Creagh and Hubbell.[21] All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corp., and plots were drawn using ORTEP.[22] The atom-numbering scheme is given in Figure 3. Crystal data are reported in Table 1, final atomic coordinates are listed in Table 2, and listings of bond lengths and angles are given in Tables 3 and 4. A complete table of crystal data, non-hydrogen atom thermal parameters, and hydrogen atom coordinates and thermal parameters (Tables S1−S3) are available as supporting information.

**EPR Spectroscopy.** X-band, ~9.2 GHz EPR spectra, recorded as the first derivative of absorption, were obtained using a Bruker ESP300E

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**Table 1.** Crystal Data for $K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3{\cdot}2H_2O$

| | |
|---|---|
| formula | $C_{36}H_{62}Cl_3KN_8O_{22}S_2$ |
| fw | 1168.52 |
| crystal system | orthorhombic |
| space group | $P2_12_12_1$ |
| *a*, Å | 15.442(3) |
| *b*, Å | 36.167(1) |
| *c*, Å | 9.567(3) |
| *V*, Å$^3$ | 5343(2) |
| temp, K | 294 |
| $d_{calcd}$, g cm$^3$ | 1.453 |
| *Z* | 4 |
| $\mu$ (Cu K$\alpha$), cm$^{-1}$ | 36.558 |
| $\lambda$, Å | 1.5418 |
| $R^a$ | 0.069 |
| $R_w{}^b$ | 0.071 |

$^a$ $R = \sum||F_o| - |F_c||/\sum|F_o|$. $^b$ $R_w = (\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2)^{1/2}$, $w = 1/(\sigma^2(F_o))$.

EPR spectrometer. A rectangular $TE_{102}$ cavity was used to measure X-band EPR spectra. Version 3.02 of Bruker's esp300e software was employed for data collection. A flowthrough cryostat in conjunction with a Eurotherm (B-VT-2000) variable temperature controller provided temperatures of 120−140 K at the sample position in the cavity. Calibration of the microwave frequency and the magnetic field was performed with an EIP 548B microwave frequency counter and a Bruker 035M gaussmeter. Simulation of the monomeric copper(II) EPR signal, measured as a function of magnetic field and at a constant frequency, was performed as described previously.[9] Computer simulations of the EPR spectra arising from mononuclear copper(II) were refined using an automatic nonlinear least-squares fitting program (epr50fit.f) running on a SUN SPARC 10/30 workstation.

## Results and Discussion

Crystals suitable for X-ray structural analysis were isolated from a methanol solution of ascidiacyclamide which had been treated with dilute aqueous acid. The structure of the product, identified as the potassium salt of hydrolyzed ascidiacyclamide, consisted of the macrocyclic cation, three perchlorate anions, and two water molecules. The structural analysis confirms that the ascidiacyclamide has been hydrolyzed. The macrocyclic ring remains intact, but the two oxazoline rings have each been opened to form an amino lactone. The amine groups formed by the hydrolysis are protonated. The crystal structure reveals an increase in flexibility of the cyclic ring structure, resulting in the rotation of the carbonyl oxygen atoms O(5) and O(1) toward the center of the ring. A potassium cation is bound to the N atoms of the thiazole rings and to the adjacent O (amide) atoms. Two perchlorate anions are weakly associated with the potassium cation.

The $K^+{\cdots}O(=C)$ distances (K(1)···O(1), 2.34(1) Å; K(1)···O(5), 2.34(1) Å) are shorter than those observed for similar $K^+{\cdots}O{=}C$ contacts. For example, for the complex K[18-crown-6][*closo*-3,3-$(PPh_3)_2$-3,1,2-$RhC_2B_9H_{11}$]·$C_4H_8O{\cdot}H_2O$, in which the $K{\cdots}O_{ether}$ distances are 2.754−2.815(6) Å, the coordination sphere of the potassium is completed by interaction with the oxygen from a methyl ethyl ketone solvate (K···O, 2.688(8) Å);[23] similarly the complex [18] < $O_5$-(2,2′)benzophenono·$2_4$-coronand-5 > KNCS displays a $K^+{\cdots}O{=}C$ contact of 2.587(2) Å.[24] More interestingly, the potassium complexes of valinomycin and the macrotetrolide nonactin display $K^+{\cdots}O{=}C$ contacts ranging from 2.73 to 2.81 Å.[25−28] The $K^+$ is also associated with the nitrogen atom of the thiazole rings of the hydrolyzed ascidiacyclamide (K(1)···N(1), 2.47(2) Å; K(1)···N(5), 2.50(2) Å). There are examples of similar but longer interactions between a potassium cation with the nitrogen donors of heterocyclic rings. For example, in a series of macrocyclic polyether diester ligands containing a proton-ionizable triazole subcyclic unit, the $K^+{\cdots}N$ interactions were observed to vary from 2.862(5) to 2.68 Å.[29,30] As well, in the molecules $[K_2(pyr)_4(OEP)]$ (pyr = pyridine, OEP = octaethylporphyrin) and $[K_2Pc(18\text{-crown-}6)_2]$ (Pc = phthalocyanine), the K···N contacts range from $2.770(7)_{av}$ Å (K···N, OEP) to $2.989(9)_{av}$ Å (K···N, pyridine)[31] and $2.91(2)_{av}$ Å (K···N, Pc),[32] respectively.

The crystal structure of the hydrolyzed complex shows that the cyclic peptide backbone is different from that observed in ascidiacyclamide. The structure of ascidiacyclamide shows that the cyclic peptide chain consists of alternating D-valine and L-isoleucine side chains with thiazole and oxazoline rings located alternately at the corner of the 24-azacrown-8 macrocyclic ring structure.[33−35] The molecule takes on a saddle-shaped conformation with isoleucine and valine side chains projecting above and below the macrocyclic plane containing the thiazole and oxazoline rings at the corners, a structural arrangement classified as a type II conformation.[33−36] A similar structure has been reported for patellamide A.[37] In the hydrolyzed compound described here, the valine and isoleucine residues form the corners of another saddle where the methyl and protonated amine groups (C(25), C(32), N(3), and N(7)) form the apex of the saddle points. The saddle shape is maintained by the rigid thiazole rings (C(14)−C(13)−N(5)−C(11), 178.2°; C(13)−N(5)−C(11)−C(10), 175.1°) and features hydrophilic and hydrophobic sides, with the uncomplexed carbonyl oxygens and the two protonated amines (O(2), O(4), O(6), O(8), N(3), and N(7)) facing in one direction, while valine and isoleucine side chains are orientated in the opposite direction. The two perchlorate anions are on either side of the plane of the macrocyclic ring defined by C(14)−C(13)−N(5)−C(11)−C(10) and C(4)−C(3)−N(1)−C(1)−C(20), with K(1)···O(10) 2.72(3) Å and K(1)···O(16) 2.48(3) Å. One of the perchlorate anions appears to be within a cleft defined by N(3) and N(7), the interaction between the anion and the $K^+$ cation possibly assisted by hydrogen bonding to the protonated amines (N(7)-H···O(9), 2.20 Å; N(3)-H···O(11), 2.43 Å). The longer $K^+{\cdots}(ClO_4^-)$ interaction is present on what might be expected to be the less hydrophobic cleft, that defined by O(2), O(4), O(6), O(8), N(3), and N(7).

As the crystal was isolated from a methanol solution of $Et_4$-

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**Table 2.** Positional and Thermal Parameters for $K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3 \cdot 2H_2O$

| atom | *x* | *y* | *z* | $B_{eq}$ (Å$^2$) | atom | *x* | *y* | *z* | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|---|---|---|---|---|
| K(1) | 0.7721(5) | 0.1263(2) | 1.0069(8) | 9.4(4) | C(1) | 0.668(1) | 0.1507(5) | 0.686(2) | 2.5(4) |
| Cl(1) | 0.7453(5) | 0.2147(2) | 1.1627(6) | 4.9(3) | C(2) | 0.801(1) | 0.1617(6) | 0.561(2) | 4.3(5) |
| Cl(2) | 0.8414(6) | 0.0421(2) | 0.8401(8) | 6.8(4) | C(3) | 0.811(1) | 0.1538(5) | 0.695(2) | 3.0(4) |
| Cl(3) | 0.3725(6) | 0.2117(3) | 0.4909(8) | 8.1(5) | C(4) | 0.894(1) | 0.1508(5) | 0.770(2) | 2.9(4) |
| S(1) | 0.6922(4) | 0.1617(2) | 0.5175(5) | 5.4(3) | C(5) | 1.048(1) | 0.1508(5) | 0.772(2) | 2.7(4) |
| S(2) | 0.8687(4) | 0.0621(2) | 1.4449(6) | 4.8(3) | C(6) | 1.055(1) | 0.1850(6) | 0.861(2) | 2.9(4) |
| O(1) | 0.8961(8) | 0.1504(3) | 0.901(1) | 3.1(6) | C(7) | 1.082(1) | 0.2042(5) | 1.097(2) | 3.5(5) |
| O(2) | 1.041(1) | 0.2146(3) | 0.828(1) | 5.2(8) | C(8) | 0.997(1) | 0.1995(5) | 1.178(2) | 2.7(4) |
| O(3) | 1.0839(8) | 0.1756(3) | 0.993(1) | 3.3(7) | C(9) | 0.986(1) | 0.1650(5) | 1.262(2) | 2.5(4) |
| O(4) | 0.9907(9) | 0.1646(4) | 1.387(1) | 4.3(7) | C(10) | 0.974(1) | 0.0971(5) | 1.247(2) | 3.1(4) |
| O(5) | 0.6503(7) | 0.1023(4) | 1.121(1) | 3.6(7) | C(11) | 0.888(1) | 0.0852(5) | 1.290(2) | 2.5(4) |
| O(6) | 0.494(1) | 0.1106(4) | 1.321(1) | 5.6(9) | C(12) | 0.764(1) | 0.0568(6) | 1.399(2) | 4.6(5) |
| O(7) | 0.4464(8) | 0.1120(3) | 1.099(1) | 3.1(6) | C(13) | 0.747(1) | 0.0734(5) | 1.273(2) | 3.7(4) |
| O(8) | 0.522(1) | 0.2067(4) | 0.886(1) | 5.2(9) | C(14) | 0.663(1) | 0.0764(6) | 1.205(2) | 3.4(5) |
| O(9) | 0.677(1) | 0.2250(6) | 1.247(2) | 9(1) | C(15) | 0.517(1) | 0.0577(5) | 1.175(2) | 3.5(4) |
| O(10) | 0.711(1) | 0.1959(8) | 1.051(2) | 15(2) | C(16) | 0.483(1) | 0.0961(6) | 1.207(2) | 3.7(5) |
| O(11) | 0.797(2) | 0.2437(6) | 1.120(3) | 15(2) | C(17) | 0.426(1) | 0.1501(5) | 1.117(2) | 3.4(4) |
| O(12) | 0.800(1) | 0.1931(7) | 1.238(2) | 12(2) | C(18) | 0.511(1) | 0.1723(5) | 1.099(2) | 2.7(4) |
| O(13) | 0.801(1) | 0.0260(4) | 0.720(2) | 6(1) | C(19) | 0.537(1) | 0.1768(6) | 0.947(2) | 2.9(4) |
| O(14) | 0.917(2) | 0.0644(6) | 0.797(2) | 14(2) | C(20) | 0.576(1) | 0.1430(5) | 0.724(2) | 3.7(5) |
| O(15) | 0.875(1) | 0.0141(4) | 0.924(2) | 6(1) | C(21) | 1.125(1) | 0.1466(5) | 0.671(2) | 3.5(4) |
| O(16) | 0.788(2) | 0.0644(7) | 0.900(2) | 15(2) | C(22) | 1.211(1) | 0.1502(7) | 0.745(3) | 5.6(6) |
| O(17) | 0.451(2) | 0.197(1) | 0.433(2) | 18(3) | C(23) | 1.126(2) | 0.1085(7) | 0.603(3) | 5.7(6) |
| O(18) | 0.367(2) | 0.2476(8) | 0.468(3) | 16(2) | C(24) | 1.191(2) | 0.1038(8) | 0.485(3) | 9.2(8) |
| O(19) | 0.319(2) | 0.192(1) | 0.419(4) | 24(4) | C(25) | 1.158(2) | 0.1965(7) | 1.194(2) | 5.9(6) |
| O(20) | 0.382(2) | 0.2043(6) | 0.640(2) | 12(2) | C(26) | 1.017(1) | 0.0689(5) | 1.144(2) | 3.7(5) |
| O(21) | 0.101(1) | 0.2389(5) | 0.508(2) | 10(1) | C(27) | 1.111(2) | 0.0801(7) | 1.103(2) | 6.2(6) |
| O(22) | 0.851(1) | 0.2477(6) | −0.145(3) | 13(2) | C(28) | 1.017(2) | 0.0304(7) | 1.200(3) | 6.4(6) |
| N(1) | 0.735(1) | 0.1480(4) | 0.770(1) | 2.7(7) | C(29) | 0.454(2) | 0.0273(6) | 1.221(2) | 5.4(6) |
| N(2) | 0.967(1) | 0.1518(5) | 0.700(1) | 3.1(8) | C(30) | 0.427(2) | 0.0299(8) | 1.365(3) | 8.1(8) |
| N(3) | 0.986(1) | 0.2303(4) | 1.276(2) | 3.1(8) | C(31) | 0.366(2) | 0.0324(8) | 1.139(3) | 8.2(8) |
| N(4) | 0.947(1) | 0.1338(4) | 1.185(1) | 3.0(8) | C(32) | 0.356(1) | 0.1601(6) | 1.018(2) | 4.6(5) |
| N(5) | 0.820(1) | 0.0898(4) | 1.214(2) | 2.9(8) | C(33) | 0.544(1) | 0.1081(6) | 0.673(2) | 4.4(5) |
| N(6) | 0.600(1) | 0.0528(4) | 1.238(8) | 4(1) | C(34) | 0.446(2) | 0.1007(7) | 0.694(3) | 6.3(7) |
| N(7) | 0.505(1) | 0.2093(4) | 1.158(2) | 4(1) | C(35) | 0.595(1) | 0.0748(6) | 0.726(2) | 5.2(6) |
| N(8) | 0.5632(9) | 0.1456(4) | 0.882(1) | 2.2(3) | C(36) | 0.378(4) | 0.026(2) | 1.001(7) | 9(1) |
| | | | | | C(37) | 0.306(3) | −0.000(1) | 1.149(6) | 8(1) |

$NClO_4$, the potassium must have been present as an impurity in either the methanol or the tetraalkylammonium salts. Electrospray ionization mass spectrometry of the methanol solution from which the potassium complex was obtained shows peaks attributable to $[ascidH_4 + H^+]^+$ (758), $[ascidH_4 \cdot H_2O + H^+]^+$ (776) (this peak may be attributed also to ascidiacyclamide with one oxazoline ring hydrolyzed), [hydrolyzed ascidiacyclamide $+ H^+]^+$ (794), and [hydrolyzed ascidiacyclamide $+ K^+]^+$ (832). We have previously reported intact molecular ion adducts of $Na^+$ and $K^+$ with ascidiacyclamide, although no crystalline material has been isolated.[8] The inclusion of alkali metals within the macrocyclic cavity of marine secondary metabolites has been observed previously.[2] In the case of the bryostatins, macrocyclic lactones isolated from the marine bryozoan *Bugula neritina*, secondary ion mass spectrometry employing an alkali metal iodide[38] has been employed to detect molecular ions, for example $[M + Na^+]^+$ [39−43] and $[M + Li^+]^+$. [44,45] Likewise FABMS, in the presence of alkali metal ions, of the macrolides laulimalide and isolaulimalide isolated from the sponge *Hyattella sp*. and a nudibranch predator (*Chromodoris lochi*), respectively, indicated $[M + K^+]^+$ and $[M + Cs^+]^+$ molecular ions,[46] and similar methodology with the iejimalides C and D, antineoplastic 24-membered macrolide sulfates from the ascidian *Eudistoma* cf. *rigida*, indicated $[M + Na^+]^+$ molecular ions, while negative ion FABMS afforded $[M + Na^+ - 2H^+]^-$ ions.[47] In each of these examples, FABMS suggests an association between the alkali metal ion and the marine secondary metabolite, but there is no evidence that the alkali metal is within the macrocyclic cavity. In contrast, the aplasmomycins (A−C) isolated from *Streptomyces griseus* have been characterized crystallographically and shown to contain boron, and in the case of aplasmomycin C a sodium ion is located in the macrocyclic cavity.[48] All three aplasmomycins bind alkali metal ions, and transport of $K^+$ by aplasmomycins A and B has been demonstrated.[49,50]

Preliminary results suggest that the hydrolyzed ascidiacyclamide reacts readily with transition metal ions. Previously we have shown that reaction of copper(II) nitrate with ascidiacyclamide resulted in a blue crystalline complex characterized as $[Cu_2(ascidH_2)(1,2\text{-}\mu\text{-}CO_3)(H_2O)_2]$ with the metal ions coordinated by three nitrogen donors, one each from an oxazoline, a thiazole, and a deprotonated amide with a water molecule and

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**Table 3.** Bond Lengths (Å) for $K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3 \cdot 2H_2O$

| | | | |
|---|---|---|---|
| K(1)−O(1) | 2.34(1) | K(1)−O(5) | 2.34(1) |
| K(1)−O(10) | 2.72(3) | K(1)−O(16) | 2.48(3) |
| K(1)−N(1) | 2.47(2) | K(1)−N(5) | 2.50(2) |
| Cl(1)−O(9) | 1.37(2) | Cl(1)−O(10) | 1.38(2) |
| Cl(1)−O(11) | 1.38(3) | Cl(1)−O(12) | 1.35(2) |
| Cl(2)−O(13) | 1.43(2) | Cl(2)−O(14) | 1.47(3) |
| Cl(2)−O(15) | 1.40(2) | Cl(2)−O(16) | 1.29(3) |
| Cl(3)−O(17) | 1.44(4) | Cl(3)−O(18) | 1.32(3) |
| Cl(3)−O(19) | 1.29(4) | Cl(3)−O(20) | 1.45(2) |
| S(1)−C(1) | 1.70(2) | S(1)−C(2) | 1.72(2) |
| S(2)−C(11) | 1.73(2) | S(2)−C(12) | 1.69(2) |
| O(1)−C(4) | 1.25(2) | O(2)−C(6) | 1.13(2) |
| O(3)−C(6) | 1.39(2) | O(3)−C(7) | 1.44(2) |
| O(4)−C(9) | 1.20(2) | O(5)−C(14) | 1.25(2) |
| O(6)−C(16) | 1.22(3) | O(7)−C(16) | 1.31(2) |
| O(7)−C(17) | 1.43(2) | O(8)−C(19) | 1.25(2) |
| N(1)−C(1) | 1.31(2) | N(1)−C(3) | 1.39(2) |
| N(2)−C(4) | 1.31(2) | N(2)−C(5) | 1.43(2) |
| N(3)−C(8) | 1.47(2) | N(4)−C(9) | 1.36(2) |
| N(4)−C(10) | 1.46(2) | N(5)−C(11) | 1.28(2) |
| N(5)−C(13) | 1.40(3) | N(6)−C(14) | 1.32(3) |
| N(6)−C(15) | 1.44(3) | N(7)−C(18) | 1.46(2) |
| N(8)−C(19) | 1.35(2) | N(8)−C(20) | 1.52(2) |
| C(1)−C(20) | 1.49(3) | C(2)−C(3) | 1.33(3) |
| C(3)−C(4) | 1.47(3) | C(5)−C(6) | 1.50(3) |
| C(5)−C(21) | 1.54(3) | C(7)−C(8) | 1.53(3) |
| C(7)−C(25) | 1.52(3) | C(8)−C(9) | 1.49(3) |
| C(10)−C(11) | 1.46(3) | C(10)−C(26) | 1.56(3) |
| C(12)−C(13) | 1.37(3) | C(13)−C(14) | 1.46(3) |
| C(15)−C(16) | 1.52(3) | C(15)−C(29) | 1.53(3) |
| C(17)−C(18) | 1.55(3) | C(17)−C(32) | 1.47(3) |
| C(18)−C(19) | 1.52(3) | C(20)−C(33) | 1.45(3) |
| C(21)−C(22) | 1.51(3) | C(21)−C(23) | 1.52(3) |
| C(23)−C(24) | 1.53(4) | C(26)−C(27) | 1.56(3) |
| C(26)−C(28) | 1.49(3) | C(29)−C(30) | 1.44(4) |
| C(29)−C(31) | 1.58(4) | C(31)−C(36) | 1.35(7) |
| C(31)−C(37) | 1.50(6) | C(33)−C(34) | 1.55(3) |
| C(33)−C(35) | 1.53(3) | O(2)−C(6) | 1.13(2) |

the oxygen atom of a bridging carbonate anion completing the coordination sphere of each metal.[8] Extensive EPR and CD studies in solution indicated that the final carbonate complex was formed as the final product after a series of equilibria were established.[8] Preliminary studies of the interaction in solution of copper(II) with the hydrolyzed ascidiacyclamide indicate quite different chemistry. The solutions in this case are purple in color and display visible spectra (1:1 (ligand:copper) $\lambda_{max}$ 590 nm ($\epsilon$ 95 $M^{-1}$ $cm^{-1}$) 1:2 (ligand:copper) $\lambda_{max}$ 590 nm ($\epsilon$ 210 $M^{-1}$ $cm^{-1}$)) and circular dichroism spectra (1:1 (ligand:copper) $\lambda_{max}$ 354 and 640 nm ($\Delta\epsilon$ −0.4 and −0.6 $M^{-1}$ $cm^{-1}$, respectively); 1:2 (ligand:copper) $\lambda_{max}$ 342 and 656 nm ($\Delta\epsilon$ 0.9 and −1.5 $M^{-1}$ $cm^{-1}$, respectively)). This may be the result of an increase in macrocycle flexibility after hydrolysis of ascidiacyclamide, allowing a fourth nitrogen atom to bind or more likely permitting the thiazole sulfur to bind to copper(II). The EPR spectra of these species suggest that for a ligand:metal ratio of 1:1, a single monomeric copper(II) complex is formed in solution ($g_z$ 2.244, $g_x$ 2.068, $g_y$ 2.068; $A_z$ 176.5 × $10^{-4}$ $cm^{-1}$, $A_x$ 17.3 × $10^{-4}$ $cm^{-1}$, $A_y$ 17.3 × $10^{-4}$ $cm^{-1}$), while computer simulations of EPR spectra suggest that for a 1:2 ratio, two monomeric copper(II) species were present. (Species 1: $g_z$ 2.270, $g_x$ 2.070, $g_y$ 2.070; $A_z$ 153.7 × $10^{-4}$ $cm^{-1}$, $A_x$ 12.1 × $10^{-4}$ $cm^{-1}$, $A_y$ 12.1 × $10^{-4}$ $cm^{-1}$. Species 2: $g_z$ 2.225, $g_x$ 2.055, $g_y$ 2.055; $A_z$ 166.2 × $10^{-4}$ $cm^{-1}$, $A_x$ 18.8 × $10^{-4}$ $cm^{-1}$, $A_y$ 18.8 × $10^{-4}$ $cm^{-1}$.[51]) Exposure of the solutions of these copper(II) complexes to air did not change the color or EPR spectra, suggesting that carbon dioxide is not sequestered in solution, as observed for the copper complexes of ascidiacyclamide and patellamide D.[8,9]

(51) van den Brenk, A. L.; Hanson, G. R.; Fairlie, D. P.; Gahan, L. R. Unpublished results.

**Table 4.** Bond Angles (deg) for $K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3 \cdot 2H_2O$

| | | | |
|---|---|---|---|
| O(1)−K(1)−O(5) | 178.1(5) | O(1)−K(1)−O(10) | 90.4(6) |
| O(1)−K(1)−O(16) | 94.5(7) | O(1)−K(1)−N(1) | 70.9(5) |
| O(1)−K(1)−N(5) | 107.4(5) | O(5)−K(1)−O(10) | 89.5(6) |
| O(5)−K(1)−O(16) | 86.3(7) | O(5)−K(1)−N(1) | 110.9(5) |
| O(5)−K(1)−N(5) | 70.9(5) | O(10)−K(1)−O(16) | 159.3(8) |
| O(10)−K(1)−N(1) | 76.6(6) | O(10)−K(1)−N(5) | 118.1(6) |
| O(16)−K(1)−N(1) | 86.0(7) | O(16)−K(1)−N(5) | 79.5(6) |
| N(1)−K(1)−N(5) | 165.4(6) | O(9)−Cl(1)−O(10) | 107(1) |
| O(9)−Cl(1)−O(11) | 114(1) | O(9)−Cl(1)−O(12) | 109(1) |
| O(10)−Cl(1)−O(11) | 112(2) | O(10)−Cl(1)−O(12) | 112(2) |
| O(11)−Cl(1)−O(12) | 104(2) | O(13)−Cl(2)−O(14) | 110(1) |
| O(13)−Cl(2)−O(15) | 109(1) | O(13)−Cl(2)−O(16) | 110(1) |
| O(14)−Cl(2)−O(15) | 105(1) | O(14)−Cl(2)−O(16) | 107(2) |
| O(15)−Cl(2)−O(16) | 116(1) | O(17)−Cl(3)−O(18) | 111(2) |
| O(17)−Cl(3)−O(19) | 98(2) | O(17)−Cl(3)−O(20) | 103(2) |
| O(18)−Cl(3)−O(19) | 114(2) | O(18)−Cl(3)−O(20) | 110(2) |
| O(19)−Cl(3)−O(20) | 119(2) | C(1)−S(1)−C(2) | 89.2(9) |
| C(11)−S(2)−C(12) | 90(1) | K(1)−O(1)−C(4) | 115(1) |
| C(6)−O(3)−C(7) | 117(1) | K(1)−O(5)−C(14) | 117(1) |
| C(16)−O(17)−C(17) | 115(1) | K(1)−O(10)−Cl(1) | 116(1) |
| K(1)−O(16)−Cl(2) | 144(2) | K(1)−N(1)−C(1) | 140(1) |
| K(1)−N(1)−C(3) | 109(1) | C(1)−N(1)−C(3) | 110(1) |
| C(4)−N(2)−C(5) | 121(1) | C(9)−N(4)−C(10) | 122(1) |
| K(1)−N(5)−C(11) | 139(1) | K(1)−N(5)−C(13) | 108(1) |
| C(11)−N(5)−C(13) | 112(2) | C(14)−N(6)−C(15) | 119(2) |
| C(19)−N(8)−C(20) | 123(1) | S(1)−C(1)−N(1) | 115(1) |
| S(1)−C(1)−C(20) | 119(1) | N(1)−C(1)−C(20) | 126(2) |
| S(1)−C(2)−C(3) | 110(2) | N(1)−C(3)−C(2) | 115(2) |
| N(1)−C(3)−C(4) | 119(2) | C(2)−C(3)−C(4) | 126(2) |
| O(1)−C(4)−N(2) | 119(2) | O(1)−C(4)−C(3) | 120(2) |
| N(2)−C(4)−C(3) | 120(2) | N(2)−C(5)−C(6) | 108(2) |
| N(2)−C(5)−C(21) | 112(1) | C(6)−C(5)−C(21) | 113(2) |
| O(2)−C(6)−O(3) | 123(2) | O(2)−C(6)−C(5) | 127(2) |
| O(3)−C(6)−C(5) | 110(2) | O(3)−C(7)−C(8) | 107(1) |
| O(3)−C(7)−C(25) | 106(2) | C(8)−C(7)−C(25) | 110(2) |
| N(3)−C(8)−C(7) | 110(1) | N(3)−C(8)−C(9) | 106(1) |
| C(7)−C(8)−C(9) | 118(2) | O(4)−C(9)−N(4) | 123(2) |
| O(4)−C(9)−C(8) | 123(2) | N(4)−C(9)−C(8) | 114(2) |
| N(4)−C(10)−C(11) | 112(1) | N(4)−C(10)−C(26) | 110(1) |
| C(11)−C(10)−C(26) | 112(2) | S(2)−C(11)−N(5) | 114(1) |
| S(2)−C(11)−C(10) | 122(1) | N(5)−C(11)−C(10) | 124(2) |
| S(2)−C(12)−C(13) | 111(2) | N(5)−C(13)−C(12) | 113(2) |
| N(5)−C(13)−C(14) | 121(2) | C(12)−C(13)−C(14) | 127(2) |
| O(5)−C(14)−N(6) | 121(2) | O(5)−C(14)−C(13) | 119(2) |
| N(6)−C(14)−C(13) | 120(2) | N(6)−C(15)−C(16) | 109(2) |
| N(6)−C(15)−C(29) | 111(2) | C(16)−C(15)−C(29) | 113(2) |
| O(6)−C(16)−O(7) | 125(2) | O(6)−C(16)−C(15) | 122(2) |
| O(7)−C(16)−C(15) | 113(2) | O(7)−C(17)−C(18) | 107(1) |
| O(7)−C(17)−C(32) | 109(2) | C(18)−C(17)−C(32) | 115(2) |
| N(7)−C(18)−C(17) | 112(2) | N(7)−C(18)−C(19) | 107(1) |
| C(17)−C(18)−C(19) | 112(2) | O(8)−C(19)−N(8) | 124(2) |
| O(8)−C(19)−C(18) | 120(2) | N(8)−C(19)−C(18) | 115(2) |
| N(8)−C(20)−C(1) | 111(2) | N(8)−C(20)−C(33) | 110(2) |
| C(1)−C(20)−C(33) | 114(2) | C(5)−C(21)−C(22) | 112(2) |
| C(5)−C(21)−C(23) | 111(2) | C(22)−C(21)−C(23) | 106(2) |
| C(21)−C(23)−C(24) | 115(2) | C(10)−C(26)−C(27) | 113(2) |
| C(10)−C(26)−C(28) | 112(2) | C(27)−C(26)−C(28) | 110(2) |
| C(15)−C(29)−C(30) | 114(2) | C(15)−C(29)−C(31) | 109(2) |
| C(30)−C(29)−C(31) | 103(2) | C(29)−C(31)−C(36) | 110(3) |
| C(29)−C(31)−C(37) | 114(3) | C(36)−C(31)−C(37) | 91(4) |
| C(20)−C(33)−C(34) | 116(2) | C(20)−C(33)−C(35) | 113(2) |
| C(34)−C(33)−C(35) | 109(2) | N(3)−C(8)−C(9) | 106(1) |

The observed incorporation of potassium and the preliminary results with copper(II) indicate that the hydrolysis has a profound effect on the complexation behavior of this ligand, compared to ascidiacyclamide. Hydrolysis of the oxazoline contributes to a release in steric strain in the cyclic ring, although the major planarity imposed by the conjugation of the thiazole rings and associated amides, as observed in ascidiacyclamide itself, is retained.[35,36,52,53] The hydrolysis of an oxazoline ring could lead to two products. Acid-induced hydrolysis involves

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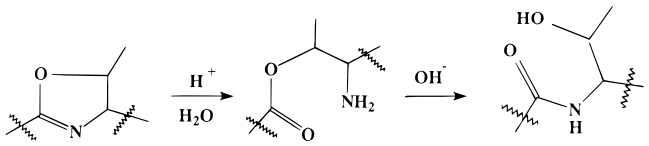
**Scheme 1.** Acid-Induced Ring Opening of Oxazoline Rings To Produce, Initially, an Amino Lactone and, Subsequently, the Threonine-Containing Residue




nucleophilic addition of water across the oxazoline double bond to form an amino lactone; subsequent addition of base causes nucleophilic addition−elimination to the threonine-containing product (Scheme 1).[54] The amino lactone only has been isolated in this work. The possibility of acid-induced ring opening of the oxazoline ring has been mentioned previously with respect to the methodology utilized in the synthesis of ascidiacyclamide.[55] Hydrolysis of patellamides A−C to acyclic tripeptide *N*-acetyl methyl esters, where the oxazoline ring is converted to serine or threonine, has been observed.[56] Acid hydrolysis of lissoclinamide-2 in 5% $H_2SO_4$/MeOH for 1 h also opened an oxazoline ring to give an amino lactone product which underwent transacylation to an amide alcohol after stirring in base.[57] The final product was identical with prelissoclinamide-2 (**3**) which was extracted from *L. patella*. Prelissoclinamide-2 is one example of a group of metabolites such as prepatellamide-B-formate (**4**) and preulicyclamide (**5**) in which the threonine residue has not cyclized to an oxazoline. These metabolites were suggested to be biosynthetic intermediates in the peptide pathway rather than degradation products.[57]

There are a number of consequences of the hydrolysis of the oxazoline rings in the ascidiacylamide structure. The most obvious one is the increase in flexibility of the cyclic structure. This will affect coordination to transition metal ions and may also influence biological activity since the oxazoline ring has been proposed to be necessary for the cytotoxic activity of the peptide.[33,58] These aspects of the chemistry of the macrocycle are under investigation.

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**Supporting Information Available:** Crystal data for $K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3 \cdot 2H_2O$ (Table S1), thermal parameters for $K(C_{36}H_{58}N_8O_8S_2)(ClO_4)_3 \cdot 2H_2O$ (Table S2), and hydrogen positional and thermal parameters (Table S3) (5 pages). Ordering information is given on any current masthead page.

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