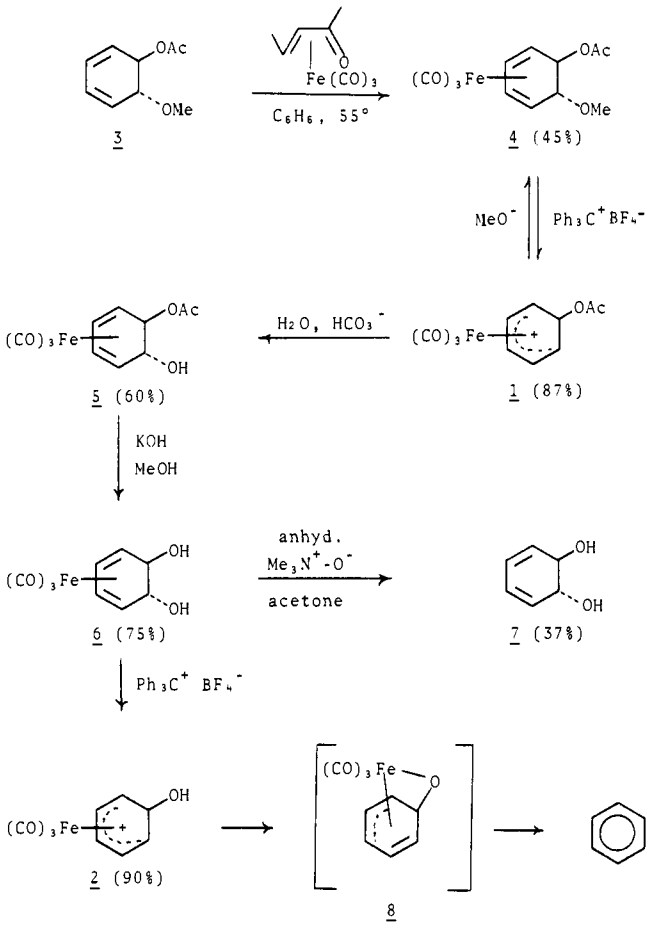
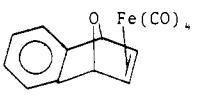


tendency to decompose on exposure to the atmosphere. When **2** is dissolved in acetone-$d_6$ containing a trace of water or in $CD_3OD$, effervescence is observed and, within 60 min, the salt has undergone quantitative conversion to benzene. No phenol or $Fe(CO)_3$ complex of cyclohexadienone is observed. We assume the decomposition occurs via **8**. A similar intermediate has been suggested for the formation of naphthalene from **9**.[13]



**Acknowledgment.** Financial support from the National Institutes of Health, Grant No. 1R01-GM19103, is gratefully acknowledged.

## References and Notes


(1) D. M. Jerina and J. W. Daly, *Science*, **185**, 573 (1974); T. C. Bruice and P. Y. Bruice, *Acc. Chem. Res.*, **9**, 378 (1976).

(2) Readily prepared in 70% yield on the 80-g scale by the addition of methoxide to oxepin–benzene oxide and acetylation of the methoxy alcohol.[3]

(3) A. M. Jeffrey, H. J. C. Yeh, D. M. Jerina, R. M. DeMarinis, C. H. Foster, D. E. Piccolo, and G. A. Berchtold, *J. Am. Chem. Soc.*, **96**, 6929 (1974).

(4) M. Brookhart, G. W. Koszalka, G. O. Nelson, G. Scholes, and R. A. Watson, *J. Am. Chem. Soc.*, **98**, 8155 (1976).

(5) Reaction of **3** with $Fe_3(CO)_{12}$ gave **4** in 30% yield.

(6) Satisfactory analytical data have been obtained for **1** and **2**. Complexes **4**, **5**, and **6** are obtained as oils that undergo slow decomposition at room temperature. Spectral data for **1**, **2**, and **4**–**6** follow. **1**: IR (KBr) 2110, 2060, 1975, 1730, 1235, 1050 $cm^{-1}$; UV max (EtOH) 216 nm (sh) ($\epsilon$ 24 800); NMR ($CF_3CO_2H$) $\delta$ 2.47 (s, 3 H), 4.26 (br d, 2 H, $J$ = 7 Hz), 5.18 (br d, 1 H), 6.05 (br t, 2 H, $J$ = 6 Hz), 7.36 ppm (br t, 1 H, $J$ = 6 Hz). **2**: IR (Nujol mull) 2120, 2070, 1310, 1240, 1165, 1070 $cm^{-1}$; NMR ($CF_3CO_2H$) $\delta$ 4.23 (d, 2 H, $J$ = 7 Hz), 4.62 (s, 1 H), 5.95 (t, 2 H, $J$ = 6 Hz), 7.30 ppm (t, 1 H, $J$ = 6 Hz). **4**: IR ($CHCl_3$) 2060, 1985 (br), 1728, 1371, 1240 $cm^{-1}$; UV max (EtOH) 216 nm ($\epsilon$ 14 000), 239 (8600); NMR ($CDCl_3$) $\delta$ 2.12 (s, 3 H), 3.00 (m, 2 H), 3.26 (s, 3 H), 3.64 (m, 1 H), 4.68 (m, 1 H), 5.50 ppm (m, 2 H). **5**: IR ($CHCl_3$) 2060, 1985, 1720, 1374, 1250, 1030 $cm^{-1}$; UV max (EtOH) 216 nm ($\epsilon$ 18 600), 244 (sh) (3800); NMR ($CDCl_3$) $\delta$ 2.13 (s, 3 H), 2.72–3.16 (m, 2 H), 3.36 (br, s, 1 H, exchanged with $D_2O$), 3.95 (m, 1 H), 4.38 (br s, 1 H), 5.51 ppm (m, 2 H). **6**: IR ($CHCl_3$) 3590, 2060, 1985, 1005 $cm^{-1}$; NMR (acetone-$d_6$) $\delta$ 2.93–3.2 (m, 3 H, 1 exchanged with $D_2O$), 3.63 (br s, 1 H), 3.82 (m, 1 H), 3.94–4.36 (m, 1 H, exchanged with $D_2O$), 5.56 ppm (m, 2 H).

(7) T. H. Whitesides, R. W. Slaven, and J. C. Calabrese, *Inorg. Chem.*, **13**, 1895 (1974).

(8) A. J. Birch and I. D. Jenkins in "Transition Metal Organometallics in Organic Synthesis", Vol. 1, H. Alper, Ed., Academic Press, New York, N.Y., 1976, p 1–82.

(9) Nucleophilic addition to $\eta^5$-(1,3-cyclohexadienyl)iron tricarbonyl cation under normal conditions affords the exo product resulting from kinetic control, but exceptions have been observed.[8] Addition of methoxide affords the exo product, but, under conditions of equilibration, isomerization to an equilibrium mixture (35% exo:65% endo) is observed.[10] Similar results are observed with hydroxy,[8] ethoxy,[10] and malononitrile[10] adducts.

(10) K. E. Hine, B. F. G. Johnson, and J. Lewis, *J. Chem. Soc., Chem. Commun.*, 81 (1975).

(11) D. J. Harris and V. Snieckus, *J. Chem. Soc., Chem. Commun.*, 844 (1976); Y. Shvo and E. Hazum, *ibid.*, 336 (1974); R. Aumann and H. Averbeck, *J. Organomet. Chem.*, **85**, C4 (1975).

(12) Removal of the exo substituent is favored for steric reasons: A. L. Burrows, B. F. G. Johnson, J. Lewis, and D. G. Parker, *J. Organomet. Chem.*, **127**, C22 (1977).

(13) L. Lombardo, D. Wege, and S. P. Wilkinson, *Aust. J. Chem.*, **27**, 143 (1974).


**Robert W. Ashworth, Glenn A. Berchtold***
*Department of Chemistry*
*Massachusetts Institute of Technology*
*Cambridge, Massachusetts 02139*
*Received April 18, 1977*

## Fluorescence from 2,2′-Bipyridine. Evidence for Covalent Hydrate Formation[1]

*Sir:*

There are many tantalizing reports in the literature concerning anomalous properties of 2,2′-bipyridine (bipy) and its coordination complexes in aqueous solution.[2] Recently, a proposal has been made about possible formation of covalent hydrates by reaction of $H_2O$ or $OH^-$ with coordinated or free bipy to account for these anomalies.[3] Some consideration has been given to the role these species may play in the ground-state chemistry of tris-bipy complexes of transition metals,[4-6] and there is reason to believe that covalent hydration is implicated in some excited-state chemistry;[7] a covalent hydrate intermediate has been proposed in the photochemistry of pyridine.[8] However, up to this time, direct evidence for the formation of covalent hydrates of bipy has not been advanced, although covalent hydrate formation is well established for 1,3-diazanaphthalenes and other aromatic N heterocycles.[9] In this paper we report spectral (absorption and emission) evidence for such species.

The ground-state absorption spectra of bipy free base (B), monocation ($BH^+$), and dication ($BH_2^{2+}$) and their associated acid–base equilibrium constants in aqueous solution ($pK_a(BH_2^{2+}) = -0.5$; $pK_a(BH^+) = 4.5$) are well documented.[2b,10] The first strong absorption band of B with $\lambda_{max}$ 281 nm in aqueous solution has been assigned as a $\pi$–$\pi^*$ transition.[11] An additional unassigned band at 306 nm, seen only in aqueous solution, has also been reported.[12] Figure 1 shows the absorption spectrum of bipy in neat $CH_3CN$ and in an $H_2O$-rich $CH_3CN$ solution at constant [bipy].[13] Because the absorption band in the 308-nm region is seen only in the presence of $H_2O$, in neat or mixed aqueous solutions ($CH_3CN/H_2O$, $CH_3OH/H_2O$, $DMF/H_2O$), rather than showing a red shift in nonhydrogen bonding and nonpolar solvents,[15] it cannot be assigned as an n–$\pi^*$ transition of B.

No fluorescence is detected from solutions of bipy in neat DMF, $CH_3CN$, benzene, cyclohexane, methanol, or ethanol. In neat $H_2O$ or a neutral solution of bipy in $CH_3CN/H_2O$ (or $CH_3OH/H_2O$ or $DMF/H_2O$), a strong, structured emission is observed with $\lambda_{max}$ 328 nm (Figure 2, lower curve) which increases in intensity with increasing mole fraction of $H_2O$.

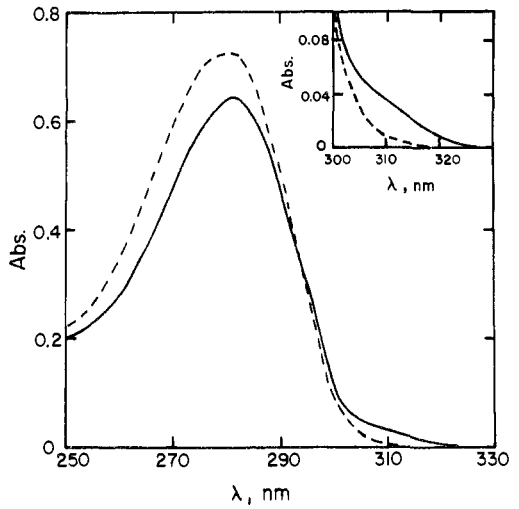



**Figure 1.** Absorption spectrum of 2,2′-bipyridine in neat $CH_3CN$ (- - -) and $CH_3CN/H_2O$ with mole fraction of $H_2O$ 0.921 (—); [bipy] is constant, $5 \times 10^{-5}$ M; cell length 1.0 cm.

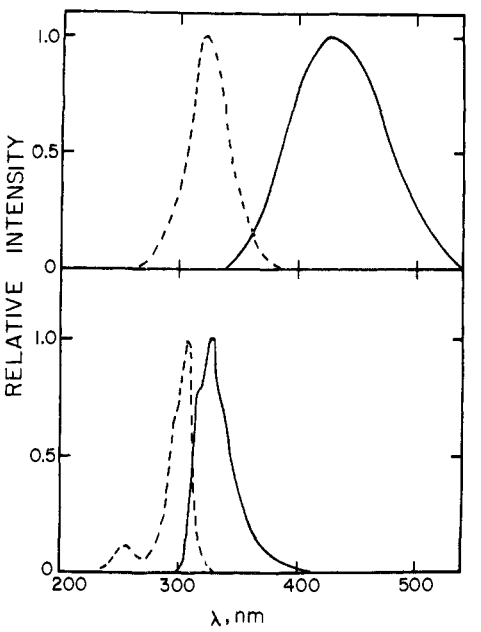



**Figure 2.** Emission (—) and excitation (- - -) spectra (uncorrected) of bipy in neat $H_2O$ solution: Upper, pH 1.7; lower, pH 8.2. The maxima of all spectra are normalized to a relative intensity of unity.

Conversely, the presence of $CH_3CN$ in a neutral solution of bipy in $H_2O$ causes a diminution of the emission intensity (relative to that in neat $H_2O$) which decreases with decreasing mole fraction of $H_2O$. This emission is always accompanied by the presence of the absorption band at 308 nm. The excitation spectrum of this emission, also shown in Figure 2, consists of a peak at 308 nm and a small shoulder at 295 nm; no peak could be detected in the excitation spectrum corresponding to the 281-nm transition in the absorption spectrum of B. These results are independent of the purification techniques used for the materials (bipy, crystallization from ethanol, ethereal wash, air drying, sublimation three times; $H_2O$, distillation from $KMnO_4$, Millipore conductance train).

In water solution, the emission intensity, $I_f$, is a linear function of [bipy]. $I_f$ is pH-dependent having a maximum at pH ~5 and decreasing with increasing pH; in sufficiently alkaline solution (pH >12.4) the emission cannot be detected. This decrease in $I_f$ is accompanied by the disappearance of the 308-nm absorption band. In the presence of phosphate buffer, $1/I_f$ is a linear function of $[HPO_4^{2-}]$; at constant $[HPO_4^{2-}]$, $1/I_f$ is a linear function of $[OH^-]$. The functional dependence of $I_f$ on pH is not affected by the presence of $O_2$.

To summarize, solutions of bipy in neat $H_2O$ or mixed aqueous solutions show a ground-state absorption band in the 308-nm region which is the origin of an emission band in the 328-nm region. Both absorption and emission require the presence of $H_2O$ and are removed by base.

This emission does not arise from a photoproduct because (1) $I_f$ does not change with time of exposure to the exciting source and (2) photolysis of B in $H_2O$ (pH 4.8) at 280 nm does not affect $I_f$. Emission from a ground-state dimer of B is eliminated because (1) emission is observed with [B] as low as $10^{-6}$ M, and (2) $I_f$ is a linear function of [bipy]. The emission is not due to the formation of a ground-state H-bonded species because (1) it does not occur in methanol or ethanol, and (2) the species absorbing at 308 nm is removed by the addition of modest amounts of base. The emission does not arise from $BH^+$; the emission ($\lambda_{max}$ 335–340 nm) and excitation ($\lambda_{max}$ 297 nm) spectra of that species have been observed in acidified neat $CH_3CN$ as well as in acidic ($[H^+] > 1$ M) $H_2O$ solutions.

We attribute the 328-nm emission and 308-nm absorption bands to the presence of a covalent hydrate formed from the addition of $H_2O$ to B according to the equilibrium

$$B + H_2O \rightleftarrows B{\cdot}H_2O \qquad (1)$$

The 328-nm emission is assigned as fluorescence from the lowest excited singlet state of $B{\cdot}H_2O$. It should be noted that the exact structure of the species written as $B{\cdot}H_2O$ is not known. Studies of covalent hydration of similar aromatic N heterocycles have shown[9] that (1) after the initial addition reaction the added $H_2O$ may migrate to other sites in the ring, (2) more than one $H_2O$ molecule may add to the same ring, and (3) all of the possible hydrated species, including protonated forms, are in equilibrium with anhydrous forms.

The disappearance of the 308-nm absorption band upon addition of $OH^-$ is not accompanied by any change in the extinction coefficient of the 281-nm band of B within the experimental precision of 3%. We therefore conclude that $K_1 = [B{\cdot}H_2O]/[B] \leq 0.03$ and that the 308-nm absorption of $B{\cdot}H_2O$ has an extinction coefficient of $\sim 10^4$ $M^{-1}$ $cm^{-1}$. This extent of covalent hydrate formation is not unusual.[9]

The effect of base on the emission and absorption bands of $B{\cdot}H_2O$ is caused by the general base reaction

$$B{\cdot}H_2O + A^- \rightleftarrows B{\cdot}OH^- + HA \qquad (2)$$

where $B{\cdot}OH^-$ is nonfluorescent and $A^- = HPO_4^{2-}$ or $OH^-$. No spectral evidence is seen for $B{\cdot}OH^-$ which is assumed to be at a very low concentration owing to the re-formation of B in reaction 3.

$$B{\cdot}OH^- \rightleftarrows B + OH^- \qquad (3)$$

$I_f$ also diminishes as the solution of bipy in $H_2O$ is made more acidic than pH 5. At pH 2 $I_f$ has diminished sufficiently to reveal a weaker emission (~1000 × less intense) at 425 nm (Figure 2, upper curve) with an excitation spectrum ($\lambda_{max}$ 320 nm) that does not correspond to the absorption spectrum of $BH^+$ ($\lambda_{max}$ 301 nm). This emission intensity increases linearly with [bipy] and is found only in neat $H_2O$ or mixed aqueous solutions. In this case, however, no separate absorption band could be resolved from the $BH^+$ ground-state absorption. The 425-nm emission is assigned as fluorescence from the protonated covalent hydrate, $BH{\cdot}(H_2O)^+$. The determination of the exact nature of this species and its excited- or ground-state equilibria is complicated by the interlocking of the $BH^+$–B equilibrium.

In conclusion, the existance of covalent hydrates of bipy free base and monocation is established. The implications of the

presence of covalent hydrates for the ground- and excited-state chemistry of bipy and other aromatic N heterocycles and their coordination complexes cannot be ignored.

## References and Notes


(1) Research supported by National Science Foundation through Grant No. CHE76-21050.
(2) (a) A. Jensen, F. Basolo, and H. M. Neuman, *J. Am. Chem. Soc.*, **80**, 2354 (1958); (b) R. H. Linnell and A. Kaczmarczyk, *J. Phys. Chem.*, **65**, 1196 (1961); (c) A. A. Schilt, *Anal. Chem.*, **35**, 1599 (1963).
(3) R. D. Gillard, *Coord. Chem. Rev.*, **16**, 67 (1975).
(4) M. Maestri, F. Bolletta, N. Serpone, L. Moggi, and V. Balzani, *Inorg. Chem.*, **15**, 2048 (1976).
(5) C. Creutz and N. Sutin, *Proc. Natl. Acad. Sci.*, **72**, 2858 (1975).
(6) G. Nord and O. Wernberg, *J. Chem. Soc., Dalton Trans.*, 866 (1972).
(7) J. F. Endicott, Microsymposium on Photochemistry and Photophysics of Coordination Compounds, Ferrara, Italy, July 1976.
(8) J. Joussot-Dubien and J. Houdard-Pereyre, *Bull. Soc. Chim. Fr.*, 2619 (1969); K. E. Wilzbach and D. J. Rausch, *J. Am. Chem. Soc.*, **94**, 2178 (1970).
(9) A. Albert, *Adv. Heterocycl. Chem.*, **20**, 117 (1976).
(10) F. H. Estheimer and O. T. Benfey, *J. Am. Chem. Soc.*, **78**, 5309 (1956); K. Nakamoto, *J. Phys. Chem.*, **64**, 1420 (1960); W. A. E. McBryde, *Can. J. Chem.*, **43**, 3472 (1965).
(11) (a) Y. Gondo, *J. Chem. Phys.*, **41**, 3928 (1964); (b) G. M. Badger and I. S. Walker, *J. Chem. Soc.*, 122 (1956); (c) L. Gil, E. Moraga, and S. Bunel, *Mol. Phys.*, **12**, 333 (1967).
(12) T. M. Spotswood and C. I. Tanzer, *Aust. J. Chem.*, **20**, 1227 (1967).
(13) The difference in the absorbance of the 281-nm band is the result of the dependence of $\epsilon$ upon solvent medium.[14]
(14) I. B. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules", Academic Press, New York, N.Y., 1971.
(15) M. Kasha, *Discuss. Faraday Soc.*, **9**, 14 (1950).



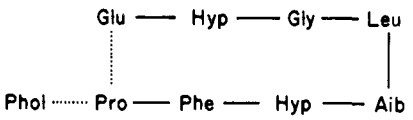
**Marian S. Henry, Morton Z. Hoffman***
*Department of Chemistry, Boston University*
*Boston, Massachusetts 02215*

*Received December 27, 1976*

## Structure of Antiamoebin I from High Resolution Field Desorption and Gas Chromatographic Mass Spectrometry Studies

*Sir:*

The antibiotic antiamoebin was reported in 1968[2a] to be produced by *Emericellopsis poonensis* Thirum., *E. synnematicola* Mathur and Thirum., and *Cephalosporium pimprina* Thirum., and to be active against protozoa and helminths.[2b] Subsequently, its structure was assigned as **1**, a cyclic octapeptide linked to phenylalaninol.[3] Oleic, linoleic, and palmitic acids were also indicated to be a part of the molecule.[4] We have now shown that antiamoebin is a mixture of two closely related compounds, antiamoebins I (~98%) and II (~2%), separable by countercurrent distribution in the system $CHCl_3$-$C_6H_6$-MeOH-$H_2O$, 30:30:46:14,[3] in which we find $K_I = 5.7$ and $K_{II} = 2.1$, and that the structures of these antibiotics are actually quite different from **1**.

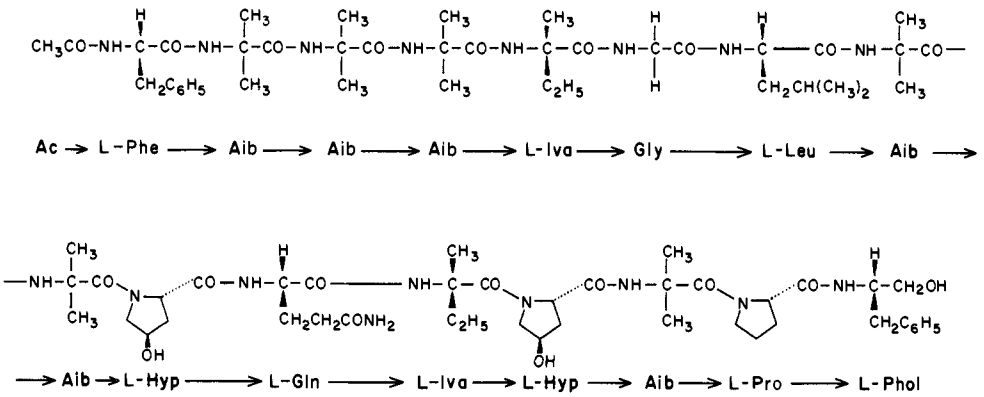



**1**

We assign here structure **2** to antiamoebin I, employing gas chromatography–high resolution electron impact mass spectrometry (GC-HREIMS) and high resolution field desorption mass spectrometry (HRFDMS) as principal structural tools. We also propose the name peptaibophol (defined as peptide antibiotics containing phenylalaninol and several moles of $\alpha$-aminoisobutyric acid as well as other amino acids) for this class of antibiotics, which we have recently shown includes emerimicins II, III, and IV,[5] zervamicins I and II,[5b] and alamethicins I and II,[6] as well as antiamoebins I and II. Two other antibiotics, suzukacillin[7] and samarosporin,[8] also belong to this class, as does (probably) stilbellin.[9]

Antiamoebin I, mp 194–196 °C (MeOH-$H_2O$), $[\alpha]^{25}_D$ +17.8° (*c* 2.1, MeOH), gives microanalyses agreeing with the molecular formula $C_{82}H_{127}N_{17}O_{20}\cdot 2H_2O$ (mol wt (anhydrous), 1669), while the cationated molecular ion[10] is found at *m/e* 1692 (M + Na) in the field desorption mass spectrum (FDMS).[11] Acetylation of antiamoebin I with acetic anhydride–pyridine gave antiamoebin I triacetate, whose FDMS showed ions at *m/e* 1818.9596 (matched vs. the peak at *m/e* 1720.9514 ($C_{34}H_{18}F_{56}N_3O_6P_3$) in the FDMS of bis(dodecafluoroheptoxy)tetrakis(octafluoropentoxy)cyclotriphosphazene),[11c] 1758, and 1698, corresponding to $C_{88}H_{133}N_{17}O_{23}Na$ (M + Na), M + Na − HOAc, and M + Na − 2HOAc, respectively. Cationated molecular ions (M + Na) for the tripropionate and tributyrate were found by FDMS at 1860 and 1902, respectively, while those for the lithium and potassium conjugates of the triacetate were found at *m/e* 1802 and 1834, respectively.[10]

Hydrolysis of antiamoebin I with 6 N hydrochloric acid gave a mixture of amino acids but no fatty acids. Analysis of the hydrolysis mixture was carried out by FDMS followed by HRFDMS (employing a variety of reference standards described elsewhere),[6] which gave M + H ions at *m/e* 76.0409 ($C_2H_6NO_2$, Gly), 104.0714 ($C_4H_{10}NO_2$, Aib, $\alpha$-aminoisobutyric acid;[12,13] cf. seq.), 116.0721 ($C_5H_{10}NO_2$, Pro), 118.0883 ($C_5H_{12}NO_2$, Iva, isovaline, $\alpha$-amino-$\alpha$-methylbutyric acid;[14,15] cf. seq.), 132.0654 ($C_5H_{10}NO_3$, Hyp), 132.1012 ($C_6H_{14}NO_2$, Leu), 148.0604 ($C_5H_{10}NO_4$, Glu), 152.1078 ($C_9H_{14}NO$, Phol, phenylalaninol; cf. seq.), and 166.0849 ($C_9H_{12}NO_2$, Phe); by amino acid analysis (Beckman/Spinco, Model 120), which indicated 6–7 mol of Aib, 2




**2**