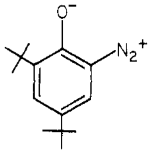
quantitatively to give two isomers **5a,b**[10] (**5a**/**5b** = 6:4) of a methanol addition product. Upon warming, **5a,b** gave several decomposition products including **3** (30–64%). However, when Ph_2S was added to the solution immediately after irradiation at −78 °C and the solution subsequently warmed to room temperature, **3** was formed exclusively along with Ph_2SO,[11] indicating that oxygen-atom transfer occurred from **5a,b** to Ph_2S upon warming.[12] On the basis of this evidence, together with NMR data, it is reasonable to assume that the oxidation of **2** leads to a carbonyl oxide which reacts with MeOH to give both isomers of the α-ketomethoxy hydroperoxides **5a,b.**[4]

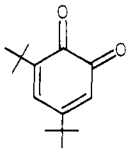
Thermolysis of **2** under N_2 in benzene afforded **6** (~40%)[13] along with some reversion to **1**. However, when the decomposition of **2** was catalyzed by $Pd(OAc)_2$ at room temperature, under N_2, **6** was obtained quantitatively. The formation of **6** is surprising, and must result from a deep-seated rearrangement, but the structure is secure.[13]

The photochemistry of **2** was also examined. Direct irradiation of **2** in MeOH at 0 °C using a tungsten lamp (no filter) resulted in the formation of the unusual rearranged peroxide **7** (95%).[14] Wolff rearrangement, which is a favorable process in most α-diazo ketones,[15] is not operative in this case.

The initial formation of **2** was unexpected since diazo groups are usually very susceptible to the attack of $^1O_2$.[1d–i] Stable endoperoxides have been shown to be derived from nonaromatic, polyaromatic, and vinyl aromatic systems,[16] but we expected the ketodiazodiene system to be sufficiently deactivated by the electron-withdrawing substituents to be unreactive. Photoreaction of 3,5-*tert*-butyl-*o*-benzoquinone **8** under similar conditions did not give any oxidation products. The results suggest an important role of the diazo group for the formation of **2**. The fact that there are considerable low-field shifts of the ring protons of **1** compared to those of **8** in the ^1H NMR[17] suggest a significant contribution of the resonance structure **1′**, which would be expected to deactivate the diazo group relative to the ring toward attack by $^1O_2$. The importance of structure **1′** has also been suggested in the thermal reactions of *o*-diazoquinones with ketenes and diazo compounds.[18] Futher investigation of the reaction as well as the chemistry of these peroxides is now in progress.



1′



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Acknowledgment. This work was supported by NIH Grant GM 20080.

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(10) After complete photooxygenation of **1** or **2** in CH_2Cl_2–MeOH (1:1) at −78 °C, the solvent was carefully removed at 0 °C. The residue was dissolved in $CDCl_3$ and transferred to an NMR tube. The spectrum was taken at different temperatures. ^1H NMR spectrum showed the formation of two products **5a,b** (ratio **5a**/**5b**=6:4). No other products were observed. ^1H NMR of **5a**: δ ($CDCl_3$, −50 °C) 1.06 (9 H, s), 1.09 (9 H, s), 3.36 (3 H, s), 4.84 (1 H, br), 4.98 (1 H, d, J = 2.0 Hz), 6.07 (1 H, d, J = 2.0 Hz). ^1H NMR of **5b**: δ 1.01 (9 H, s), 1.06 (9 H, s), 3.48 (3 H, s), 5.17 (1 H, d, J = 2.0 Hz), 6.10 (1 H, d, J = 2.0 Hz), 10.4 (1 H, br). The peaks at 4.8 and 10.4 ppm shifted to higher field with increasing temperature. Compound **5b** is less stable and decomposed more rapidly than **5a** upon warming to room temperature.

(11) Endoperoxides **2–4** did not oxidize Ph_2S under the conditions.

(12) For oxygen-atom transfer reactions by α-alkoxy hydroperoxides; see: (a) Rebek, J.; McCready, R.; Wolak, R. *J. Chem. Soc., Chem. Commun.* **1980**, 705. (b) Rebek, J. *Heterocycles* **1981**, *15*, 517.

(13) Compound **6**: mp 80 °C; ^1H NMR δ ($CDCl_3$), 1.30 (9 H, s), 1.36 (9 H, s), 6.30 (1 H, s), 10.30 (1 H, s); ^{13}C NMR δ ($CDCl_3$), 28.1 (q), 30.3 (q), 37.1 (s), 37.6 (s), 100.5 (d), 118.1 (s), 163.3 (s), 171.1 (s), 175.2 (s), 192.4 (d); IR (cm^{-1}) 2820, 2720, 1710, 1690, 1620; *mass spectrum*, *m/e* 236; UV (MeOH) λ_{max} 318 mm (ϵ 6300). The exact structure of **6** was established by X-ray crystal analysis: Ryang, H.-S.; Dobrowolsky, D.; Foote, C. S., to be published.

(14) Compound **7**: mp 111 °C dec; ^1H NMR ($CDCl_3$), 1.08 (9 H, s), 3.10 (1 H, dd, J = 2.9, 0.7 Hz), 3.50 (3 H, s), 5.06 (1 H, d, J = 2.9 Hz), 6.09 (1 H, d, J = 0.7 Hz); mass spectrum, *m/e* 224 (M − 44), 208; IR (Nujol) 1760, 1600 cm^{-1}; positive peroxide test with KI/HCl. Ando et al. have isolated this cyclic peroxide from photooxygenation of **1** in MeOH and determined the structure by X-ray crystallography.[19] The above analytical data for **7** are identical with those reported by them.

(15) (a) Kirmse, W. "Carbene Chemistry", 2nd ed.; Acadamic Press: New York, 1971. (b) Meier, H.; Zeller, K.-P. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 32.

(16) (a) Frimer, A. A. *Chem. Rev.* **1979**, *79*, 359. (b) Saito, I.; Matsuura, T. In "Singlet Oxygen"; Wasserman, H. H., Murray, R. W., Eds; Academic Press: New York, 1979; p 511.

(17) ^1H NMR of **1**: δ ($CDCl_3$) 1.25 (9 H, s), 1.35 (9 H, s), 7.49 (1 H, d, J = 2.0 Hz), 7.56 (1 H, d, J = 2.0 Hz). ^1H NMR of **8**: δ ($CDCl_3$) 1.19 (9 H, s), 1.23 (9 H, s), 6.20 (1 H, d, J = 2.3 Hz), 6.87 (1 H, d, J = 2.3 Hz).

(18) (a) Huisgen, R.; Gleischmann, R. *Liebigs Ann. Chem.* **1959**, *623*, 47. (b) Ried, W.; Wagner, K. *Ibid.* **1965**, *681*, 45.

(19) We thank Professor Ando for a prepublication copy of his manuscript: Ando, W.; Miyazaki, H.; Veno, K.; Nakanishi, H.; Sakurai, T.; Kobayashi, K. *J. Am. Chem. Soc.*, preceding paper in this issue.

Dinucleating Octaaza Macrocyclic Ligands from Simple Imine Condensations

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Macrocyclic ligands which are capable of incorporating two metal ions[1] offer the possibility of studying unusual electronic and chemical properties which depend upon proximity of two metal centers. An advantage of macrocyclic systems for this type of investigation is that variation of ring size or other geometric constraints should allow the separation and disposition of the two metal ions to be controlled in a systematic manner. In this paper we describe a series of such ligands which have been obtained in high yields from simple imine condensation reactions and have been characterized by field desorption mass spectrometry and X-ray structure determination.

We have reported[2] that under appropriate conditions the dialdehyde **1a** can be condensed with a range of diamines **2** to give tetraaza macrocycles **3** with a wide range of ring sizes. These reactions proceed without addition of "metal-ion templates",[3] provided that reaction conditions and solvents are selected which allow the free ligands to separate from solution before they can undergo conversion to species which are less soluble or thermodynamically more stable. It was noted,[2] for example, that on prolonged heating in methanol, **3a** is converted to a species of higher relative molecular mass (m_r). We have now characterized a number of the higher m_r materials obtained from condensations under conditions defined in Scheme I and shown them to be an

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(1) (a) Groh, S. E. *Isr. J. Chem.* **1976**, *15*, 227–307. (b) Fenton, D. E.; Lintvedt, R. L. *J. Am. Chem. Soc.* **1978**, *100*, 6367–6372. (c) Fenton, D. E.; Bresciani-Pahor, N.; Calligaris, M.; Nardin, G.; Randaccio, L. *J. Chem. Soc., Chem. Commun.* **1979**, 39–40. (d) Gagnê, R. R.; Henling, L. M.; Kistenmacher, T. J. *Inorg. Chem.* **1980**, *19*, 1226–1231. (e) Burnett, M. G.; McKee, V.; Nelson, S. M.; Drew, M. G. B. *J. Chem. Soc., Chem. Commun.* **1980**, 829–831. (f) Kahn, O.; Morgenstern-Badarau, I.; Audiere, J. P.; Lehn, J. M.; Sullivan, S. A. *J. Am. Chem. Soc.* **1980**, *102*, 5936–5938. (g) Coughlin, P. K.; Lippard, S. J.; Martin, A. E.; Bulkowski, J. E. *J. Am. Chem. Soc.* **1980**, *102*, 7616–7617.

(2) Owston, P. G.; Peters, R.; Ramsammy, E.; Tasker, P. A.; Trotter, J. *J. Chem. Soc., Chem. Commun* **1980**, 1218–1220.

(3) (a) Melsom, G. A. "Co-ordination Chemistry of Macrocyclic Compounds"; Plenum Press: New York, 1979; Chapter 2. (b) Green, M.; Smith, J.; Tasker, P. A. *Inorg. Chim. Acta* **1971**, *5*, 17–24. (c) Black, D. St. C.; Bos Vanderzalm, C. H.; Wong, L. C. H. *Aust. J. Chem.* **1979**, *32*, 2303–2311 and references therein.

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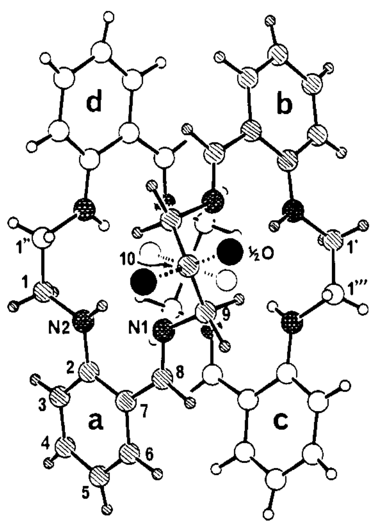


Figure 1. Tetraimine **5b**, showing the disorder of the hydroxyl groups about the 2-fold axis which passes through carbon atoms C(10). Another crystallographic C_2 axis passes through the midpoints of the C(1)–C(1) bonds. Shaded C and H atoms are in the half of the molecule nearer the viewer.

interesting new class of potentially octadentate macrocycles **5**.

These high m_r compounds, which were obtained (see Scheme I) from reactions of the diamines **2a–c**, are relatively insoluble and involatile, and cryoscopic methods and electron-impact mass spectrometry could not be used to determine their relative molecular masses. However, the samples showed simple field desorption (FD) mass spectra,[4] giving molecular ions compatible with the [2 + 2] condensation products:[5] **5a**, m/e 584; **5b**, m/e 645 $(M + 1)^+$; **5c**, m/e 697 $(M - 1)^+$. No fragmentation products were observed in the range m/e 400–800.

FDMS also proved useful in identifying compounds which were present when mixtures of products were obtained from similar condensation reactions. For example, a mixture (ca. 1:4) of the diimine **4** and the tetraimine **6** [m/e 307 $(M + 1)^+$ and m/e 613 $(M + 1)^+$, respectively] was obtained from the condensation of the trimethylene-bridged dialdehyde **1b** and 1,2-diaminoethane (1:1.1, 4 h in 15-cm^3 refluxing $CHCl_3$). These substrates have been used previously,[6] but under conditions of high dilution, to prepare the diimine **4**.

An X-ray structure determination[7] confirmed the presence of a 30-membered ring in **5b** (see Figure 1). The inner great ring has a configuration which effectively creates two "N_4" donor sets (from the *o*-iminoanilino units of a with d and b with c, see Figure 1), in the two halves of the molecule. Such an arrangement would cause close approximation of two metal ions in a dinculear complex, because the centroids of the two donor sets are separated by only 3.44 Å. A similar ligand geometry is found[8] in a dicopper complex of the smaller ring **5a** in which the two copper ions are forced to adopt a very short Cu–Cu bond [2.445 (5) Å].

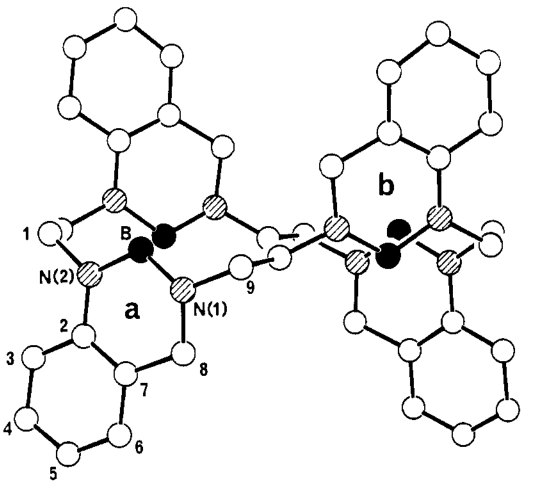
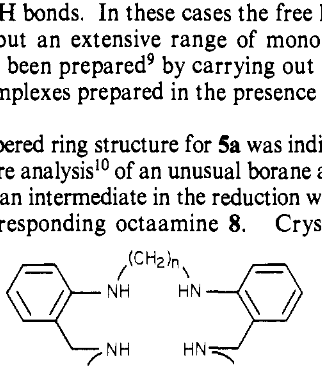


Figure 2. The 20-membered B–N heterocycle **7**.

A crystallographic 2-fold axis passes through the carbon atoms having the hydroxyl substituents, and the oxygen atoms are statistically disordered with half-occupancies of the sites shown in Figure 1. Consequently, the structure determination does not allow a distinction to be made between meso and racemic isomeric forms for **5b**.

We conclude that ease of isolation of the metal-free macrocycles **5** is dependent on the presence of strong *intra*molecular hydrogen bonding in the *o*-iminoanilino units. The geometry of the *o*-iminoanilino unit in **5b** is very similar to that found[2] in the related tetraaza macrocycles **3**. Reactions of 2,6-diacetylpyridine with certain diamines have also been observed[9] to give large ring ligands by [2 + 2] condensations,[3a] but for these systems the new chelate rings are of the α-diimine type and do not have the facility to form *intra*molecular H bonds. In these cases the free ligands have not been isolated, but an extensive range of mono- and binuclear complexes have been prepared[9] by carrying out transmetalation reactions on complexes prepared in the presence of certain metal ion templates.

The 28-membered ring structure for **5a** was indirectly confirmed by x-ray structure analysis[10] of an unusual borane adduct (**7**) which was obtained as an intermediate in the reduction with borane/THF to give the corresponding octaamine **8**. Crystals of **7** which separated from a suspension of **5a** in borane/THF gave a sharp infrared absorption band at 2510 cm^{-1}, a region in whcih B–H stretching modes are known to occur. They were found to be surprisingly stable to hydrolysis, and X-ray diffraction data were



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[4] Spectra were obtained on a KRATOS MS50 spectrometer, operated at 8-kV accelerating voltage and with a potential difference of 10 kV between the emitter and extractor plate. Spectra, recorded on oscillograph paper, were calibrated with the EI spectrum of Fomblin oil (Henning, J.; Lotz, H. *Vacuum* **1977**, *27*, 171–175). High-temperature activated emitters were loaded by dipping into either a solution (**5c** and **4/6**) or a suspension (**5a,b,8a**) in CH_2Cl_2. Emitter heating currents in the range 15–21 mA were required to desorb these compounds.

[5] The hydrogenated octaaza macrocycle (**8**, R = $(CH_2)_2$, $n = 2$) and a related "N_4O_4" system (with phenoxo groups replacing the anilino units: Lindoy, L. F. et al., unpublished results) were also identified by FDMS measurements [m/e 592 $(M)^+$ and 597 $(M + 1)^+$, respectively].

[6] Black, D. St. C.; Hartshorn, A. J.; Horner, M.; Hunig, S. *Aust. J. Chem.* **1977**, *30*, 2493–2514.

[7] Crystal data for **5b**: 7,8,9,16,17,18,19,26,27,28,35,36,37,38-tetradecahydro-8,27-dihydroxytetrabenz[*e,n,t,c'*]-1,4,8,12,16,19,23,27-octaazacyclotricosine; $C_{38}H_{44}N_8O_2$; M_r 644.8; orthorhombic; space group *Fddd*; $a = 33.076$ (3), $b = 17.459$ (2), $c = 11.757$ (3) Å; $U = 6789.3$ Å^3; $Z = 8$, $D_c = 1.26$ g cm^{-3}, μ(Mo Kα) = 0.45 cm^{-1}; 2047 intensities were recorded on a Philips PW1100 four-circle diffractometer, and merged to give 701 unique observed reflections [$F > 6\sigma(F)$]. The residuals are $R = 0.060$ and $R_w = \sum w^{1/2}\Delta/\sum w^{1/2}|F_o| = 0.069$. The structure was solved by direct methods and refined by the full-matrix least-squares method.

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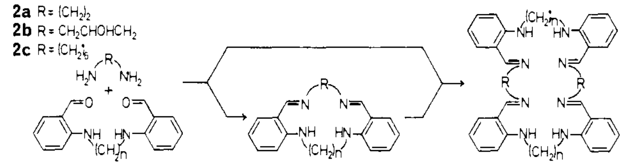
[9] (a) Drew, M. G. B.; Knox, C. C.; Nelson, S. M. *J. Chem. Soc., Dalton Trans.* **1980**, 942–948. (b) Drew, M. G. B.; McFall, S. G.; Nelson, S. M.; Waters, C. P. *J. Chem. Res., Synop.* **1979**, 16–17. (c) Drew, M. G. B.; McCann, M.; *J. Chem. Soc., Chem. Commun.*, **1979**, 481–482.

[10] Crystal data for **7**: $C_{36}H_{44}B_4N_8$; M_r 632.1, orthorhombic; space group *Pccn*; $a = 20.162$ (2), $b = 10.543$ (2), $c = 16.469$ (2) Å; $U = 3501.4$ Å^3; $Z = 4$; $D_c = 1.20$ g cm^{-3}; μ(Mo Kα) = 0.39 cm^{-1}; 3469 intensities were recorded on a Philips PW1100 four-circle diffractometer and merged to give 1099 unique observed reflections [$F > 5\sigma(F)$]. The residuals are $R = 0.094$, $R_w = \sum w^{1/2}\Delta/\sum w^{1/2}|F_o|] = 0.096$. The structure was solved by direct methods and refined by the full-matrix least squares method.

Scheme I[a]



1a n = 2
1b n = 3

	n	R
3a	2	$(CH_2)_2$
3b	2	$CH_2CHOHCH_2$
3c	2	$(CH_2)_6$
4	3	$(CH_2)_2$

	n	R	method	yield/%
5a	2	$(CH_2)_2$	A,B	96,79
5b	2	$CH_2CHOHCH_2$	A,B	85,80
5c	2	$(CH_2)_6$	C	50
6	3	$(CH_2)_2$	-	-

[a] Reaction conditions are (A) 1a (2 mmol) + **2** (2.2 mmol) in MeOH (40–50 cm^3) refluxed 6 h and a further 24 h after addition of $CHCl_3$ (5 cm^3); (B) **3** heated 24 h in refluxing MeOH containing acetic acid (1 mol %); (C) 1a (10 mmol) + 2c (11.5 mmol) in EtOH (50 cm^3) refluxed for 1.5 h, filtered, and set aside for 12 h.

collected without special precautions to protect the crystals from atmospheric moisture. Structure determination showed this material to be tetrabenz[*a*,*g*,*o*,*u*]-1,5,8,12,15,19,22,26-octaaza-29,30,31,32-tetraborapentacyclo[$1^{1,5}.1^{8,12}.1^{15,19}.1^{22,26}$]dotriacontane in which a B–H unit has been incorporated between each pair of *o*-iminoanilino nitrogen to give the unusual 20-membered B–N heterocycle shown in Figure 2. The two halves of the molecule are related by a crystallographic 2-fold axis perpendicular to the best plane through the inner great ring.

The isolation of the potentially octadentate macrocycles **5** and **8** presents the interesting possibility of preparing a series of dinuclear complexes in which the separation and disposition of the two metal ions is controlled by ring sizes and other geometric constraints in the ligands.

Acknowledgment. We thank the Science Research Council (U.K.) for studentships (to R.P., P.M.J., and K.P.D.) and diffractometer equipment and computing facilities. P.A.T. thanks the Regents of the University of California for support during a period of leave spent at the Irvine Campus.

Supplementary Material Available: Fractional coordinates, thermal parameters, bond distances, bond angles and observed and calculated structure factors for compounds **5b** and **7** (7 pages). Ordering information is given on any current masthead page.

Assignment of the Nitrogen-15 Nuclear Magnetic Resonances of Biotin and Unequivocal Synthesis Of (+)-[1-^{15}N]Biotin[1]

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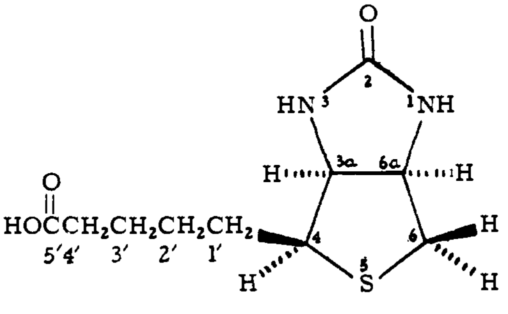
(+)-Biotin (**1**) is an essential cofactor for several enzymes involving carboxylation and carbonyl-exchange reactions.[2] It participates in the fixation, activation, and transfer of carbon dioxide by forming an *N*-carboxybiotin in which N1 was established as the point of attachment. The chemical reactivity of the two ureido nitrogens of **1** is strikingly different. Thus, N1- and N3-acylated products in an 100:7 ratio resulted from the reaction of methyl chloroformate with biotin methyl ester.[4a] The low proportion of the N3 product was attributed to steric hindrance to the approach of the reagent to N3 because of the presence of the carboxylbutyl side chain. A similar argument was employed[4b] to account for the difference in exchange rates for the ureido protons with ethanol.

Table I. ^{15}N NMR Chemical Shifts and Coupling Constants for Biotin and Model Compounds

compd (concn, M)	solvent	$\delta\,^{15}N$ ($^1J_{NH}$, Hz) N1	$\delta\,^{15}N$ ($^1J_{NH}$, Hz) N3
2-imidazolidinone (**3**) (1)	H_2O	294.0	294.0
(1.8)	H_2O	294.5	294.5
(1)	0.1 N $NaHCO_3$	294.3	294.3
(2)	$(CH_3)_2SO$	296.7 (93)	296.7 (93)
desthiobiotin (**2**) (saturated soln)	0.1 N $NaHCO_3$	276.8	282.5
(0.7)	$(CH_3)_2SO$	279.3 (90)	285.0 (91)
biotin (**1**) (saturated soln)	0.1 N $NaHCO_3$	283.6	292.6
(0.3)	$(CH_3)_2SO$	285.6 (94)	294.7 (92)



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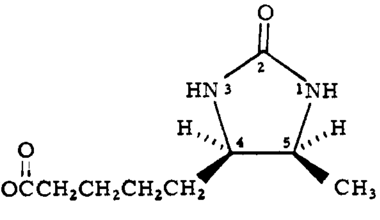
Because of the lack of sensitive physical methods to distinguish between the two nitrogens of **1** in solution, we have examined the ^{15}N NMR spectra in the hope of providing a useful probe for determining how protein systems interact with biotin.

The ^{15}N chemical shifts[5] and one-bond NH coupling constants for biotin and two model compounds, desthiobiotin (**2**), and 2-imidazolidinone (**3**), are given in Table I. For both biotin and desthiobiotin, two well-resolved ^{15}N resonances are observed in the proton-decoupled spectra. The two resonances in desthiobiotin



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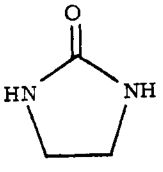

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[†] Contribution No. 6345 from these laboratories.

(1) This work was supported at the University of Illinois by Research Grants CHE-76-23543 and CHE-79-22001 from the National Science Foundation and at the California Institute of Technology by the National Science Foundation and the U.S. Public Health Service, Research Grant GM-11072 from the Division of General Medical Sciences.

(2) For reviews of biotin enzymes, see: (a) Woods, H. G.; Barden, R. E. *Annu. Rev. Biochem.* **1977**, *46*, 385–413. (b) Lynen, F. L. *CRC Crit. Rev. Biochem.* **1979** *7*, 103–119.

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(4) (a) Knappe, J.; Ringlemann, E.; Lynen, F. *Biochem. Z.* **1961**, *335*, 168–176. (b) Glasel, J. A. *Biochemistry* **1966**, *5*, 1851–1855.

(5) The ^{15}N NMR spectra were recorded on a Bruker WH-180 Fourier-transform quadrature-detection spectrometer operating at 42 kG (18.25 MHz for ^{15}N and 180 MHz for ^{1}H) with 25-mm sample probe. Nitrogen chemical shifts are reported in ppm upfield of 1 M $H^{15}NO_3$.

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