and acid (mostly benzoic acid) were obtained by heating the silica with 10% H_2SO_4 at 50 °C for 4 h. Yields were determined by GLC using on-column injection, H_2 carrier gas, a flame ionization detector, temperature programming, and a dibenzyl internal standard. The column was a 30 m × 0.25 mm i.d. quartz capillary with a 0.25-μm film thickness. The packing was a DB-1701 bonded phase consisting of polysilanes with 86% dimethyl and 14% cyanopropyl substituents. Identification was by retention times, spiking with authentic samples, and low-resolution GC/mass spectrometry.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research.

Registry No. Benzoyl peroxide, 94-36-0.

A Convenient Synthesis of a Macrocyclic Dioxo Pentaamine and X-ray Crystal Structure of Its Monohydrazoic Acid Salt

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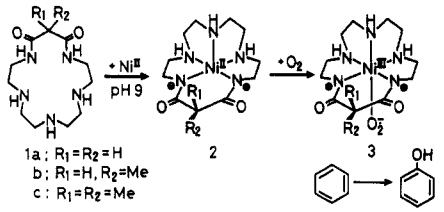
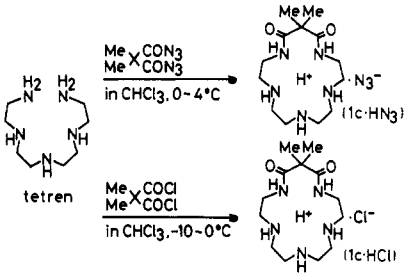
Received February 16, 1989

Sixteen-membered dioxo pentaamines **1a** and **1b** form a square-pyramidal complex **2** with high spin Ni^{II},[1,2] which possesses just the right redox potential ($E°$) of +0.24 V (vs SCE in H_2O) for Ni^{II}/Ni^{III} in **2** to permit interaction with O_2, yielding **3**,[3] whereupon the Ni^{II}-bound O_2 becomes activated so as to directly oxygenate benzene into phenol;[4]



1a (as monoprotonated species) also becomes a receptor molecule for anions (e.g. phosphate or ATP) at neutral pH.[5] Despite such novel properties, the ligand synthesis involving condensation of dimethyl (or diethyl) malonate with 1,11-diamino-3,6,9-triazaundecane (**tetren**) is very tedious, requiring more than a few weeks in refluxing methanol.

We now have discovered a much more practical synthetic route to **1c** (with $R_1 = R_2 = CH_3$), whose Ni^{II} complex **2c** shows an even lower $E°$ value of +0.17 V vs SCE,[6] indicating more promising properties for O_2 activation. The new synthesis is depicted as follows: The cyclization took place within a few days. The crystalline products, obtained after purification by silica gel chromatography (eluent, CH_2Cl_2–CH_3OH–28% aqueous NH_3 = 100:20:1 in volume), followed by recrystallization from CH_3CN/CH_3OH, were formulated as **1c**·HX on the basis of X-ray



crystal analysis or the pH-metric titrations. The yields of **1c**·HN_3 and **1c**·HCl were 16% and 7%, respectively. These sticking HX were removed only after passing through a strong anion exchange resin column (Amberlite IRA-400) to yield free **1c**, which shows two different modes of ν_{CO} (KBr pellet) at 1663 and 1640 cm^{-1}. The IR spectrum (KBr pellet) for **1c**·HN_3 shows the similar C=O stretchings at 1663 and 1637 cm^{-1}, through a little wider. A N_3^- stretching peak occurs at 2037 cm^{-1}, appreciably deviated from 2110 cm^{-1} of ionic N_3^- (NaN_3) to suggest some interaction of N_3^- with **1c**·H^+. The pH-titration of **1c**·HN_3 has established the pK_a values of 9.4, 8.4, and <3 for the three secondary amines and a normal value[7] of 4.7 for the azide ion at I = 0.2 ($NaClO_4$) and 35 °C. Meanwhile, the free base **1c** showed the pK_a of 9.4, 8.4, and <3.

We were particularly interested in the structure of the **1c**·HN_3 product, since there were few examples[8] of HN_3 polyamine salts structurally characterized, and we were curious about where its H^+ is coordinating to (or which amine is the most basic) and how the N_3^- exist in the monoprotonated macrocycle that can often become an anion receptor.[5] We have thus conducted an X-ray crystal structure analysis of **1c**·HN_3.

The perspective view of **1c**·HN_3 with the atom-numbering system is shown in Figure 1. The complex consists of two macrocyclic cations and two N_3^- anions, which, respectively, are related by inversion point in the crystal. The difference electron density map has revealed that *the protonation is occurring at the N(4) atom*, which might be the most basic nitrogen in **1c**. The ionic proton serves an intraannular hydrogen bonding with amide O(18) pointing into the macrocyclic hole. As a result, this amide hydrogen HN(1) is exposed to N_3^-, which is of an ionic nature in view of the equidistances (1.136 Å) of the two N–N bondings. The N_3^- anion strongly hydrogen bonds with three neutral NH's; N(21′)···HN(1) 2.00 Å, N(23)···HN(7) 2.21 Å, and N(23)···HN(13) 2.07 Å (see Table I), which would be energetically more favorable than a direct interaction with the ionic H^+N(4), as revealed by the longer distance 3.16 Å of N(23)···H^+N(4). We now assign the C=O stretchings of 1663 and 1637 cm^{-1} to the non-hydrogen bonding amide C(14)=O(17) and the hydrogen bonding amide C(16)=O(18), respectively.

The measurements of the ^1H NMR chemical shifts of **1c** in D_2O by varying pH (pD = 12–2, see the Experimental

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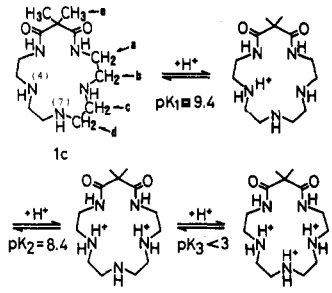
Table I. Intra- and Intermolecular Hydrogen Bonds

	bond lengths, Å	bond angle, deg
Intramolecular Hydrogen Bond		
$^{+}$N(4)–H···O(18)	N···O 2.907 (3) N–H 0.90 (3) H···O 2.14 (3)	N–H···O 142
Intermolecular Hydrogen Bonds		
N(1)–H···N(21′)	N···N 2.832 (4) N–H 0.91 (3) H···N 2.00 (3))	N–H···N 152
N(7)–H···N(23) (x, y, z)	N···N 3.066 (4) N–H 0.93 (3) H···N 2.21 (3)	N–H···N 153
N(13)–H···N(23) (x, y, z)	N···N 2.915 (4) N–H 0.88 (3) H···N 2.07 (3)	N–H···N 161
N(4)–H···N(10′) (–x, $-^1/_2$ + y, $^3/_2$ – z)	N···N 2.756 (3) N–H 1.04 (3) H···N 1.72 (3)	N–H···N 152

Scheme I. Sequence of Protonation to 1c



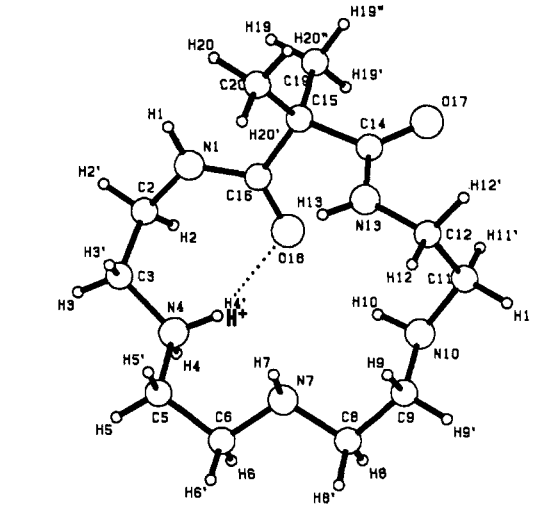
Section) indicate the most dramatic shift of H_d upon the third protonation, confirming that the N(7) atom is indeed the least basic site in solution. We have thus established the sequence of protonation as depicted in Scheme I. The ^{1}H NMR spectrum of **1c**·HX in D_2O was identical with that of **1c** in pD 8.9 solution, indicating little interaction of N_3^- with **1c**·H^+ in aqueous solution.

The most interesting fact in this study is that, along with the discovery of the practical synthetic route to **1c**, *the proton affinity in the macrocyclic dioxo pentaamine is determined primarily by its easiness to form intramolecular hydrogen bondings*. Without such effects of intramolecular hydrogen bondings, one would expect N(7), the furthest amine from the electron-withdrawing amides, to be most basic. Such a mode of protonation may be rather common in biological systems containing peptides. The present result may also call for conception of cooperative nonionic hydrogen bondings in designing or understanding anion recognition molecular devices in apolar environments.[9,10]

Experimental Section

^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a JEOL GX-400 spectrometer employing D_2O as the solvent and DSS as internal standard at 35 °C. IR and mass spectra were obtained on a Shimadzu FTIR-4200 and on a JEOL JMS-01SG-2, respectively. Elemental analysis was performed on a Yanagimoto CHN-Corder. Column chromatography was carried out on silica gel (Wakogel C-300). The pH-metric determination was conducted in a usual manner as described before.[11]

Synthesis of 15,15-Dimethyl-1,4,7,10,13-pentaaza-14,16-dioxocyclohexadecane (Dimethyldioxo[16]aneN_5·HX, 1c·HX).



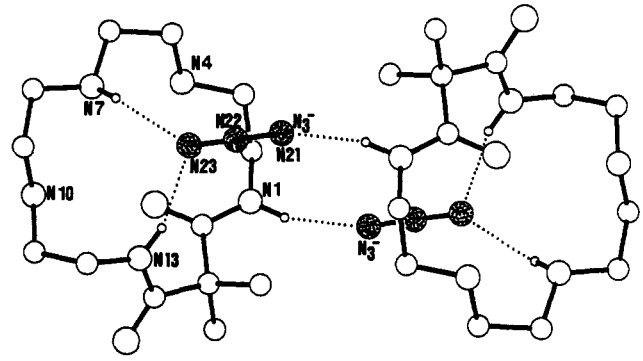


Figure 1. Structure of **1c**·HN_3: an upper figure shows intramolecular hydrogen bond between ionic amine proton and amide oxygen (N_3^- ion was omitted for clarity); lower figures show intermolecular hydrogen bonds with bridged N_3^- ions (nonbonding hydrogens were omitted for clarity).

A solution of dimethylmalonic acid (1.0 g, 7.6 mmol) in 15 mL of $SOCl_2$ was refluxed for 2 h. After evaporation of the solvent in vacuo, the residue was dissolved in 20 mL of dry $CHCl_3$. The solution was added dropwise into a 50 mL of 1 M NaN_3 aqueous solution with stirring at 0 °C for 1 h. After the $CHCl_3$ phase was dried with Na_2SO_4, 1,11-diamino-3,6,9-triazaundecane (**tetren**, 1.3 g, 6.9 mmol) and triethylamine (1.4 g, 14 mmol) were added into the solution at 0 °C. After 40 h at 4 °C, the solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography (eluent; CH_2Cl_2–MeOH–28% aqueous NH_3 = 100:20:1 in volume). The colorless crystals of 1,4,7,10,13-pentaza-15,15-dimethyl-14,16-dioxocyclohexadecane·HN_3, **1c**·HN_3 (0.37 g, 16% yield) were obtained after recrystallization from CH_3CN–CH_3OH; mp 153 °C dec. Anal. Calcd for $C_{13}H_{28}N_8O_2$: C, 47.54; H, 8.59; N, 34.12. Found: C, 47.34; H, 8.64; N, 33.86. IR (KBr pellet): $\nu_{N=N}$ 2037, ν_{CO} 1663, 1637 cm^{-1} for **1c**·HN_3, ν_{CO} 1663, 1640 for **1c**. ^{1}H NMR (D_2O): δ **1c** (pD = 12) 3.40 (m, H_a), 2.73 (m, H_b), 2.67 (br s, H_c, H_d), 1.41 (s, H_e); **1c**·H^+ (pD = 8.9) 3.46 (m, H_a), 2.95 (m, H_b), 2.91 (m, H_c), 2.89 (m, H_d), 1.43 (s, H_e); **1c**·$2H^+$ (pD = 6.5) 3.55 (m, H_a), 3.17 (m, H_b), 3.09 (m, H_c), 2.92 (m, H_d), 1.46 (s, H_e); **1**·$3H^+$ (pD = 2.0) 3.70 (m, H_a), 3.41 (m, H_b), 3.67 (m, H_c), 3.65 (m, H_d), 1.54 (s, H_e). Only four ^{1}H NMR signals for methylene protons (H_{a-d}) of **1c**·H^+ were observed, so that the proton exchange reaction between N(4) and N(10) is sufficiently fast in aqueous solution at 35 °C. ^{13}C NMR (**1c**·HN_3 in D_2O): δ 24.83, 40.60, 48.76, 48.97, 50.57, 53.36, 179.93. The **1c**·HCl was synthesized in a similar manner without addition of NaN_3 with yield 7%, mp 207 °C dec. Only after **1c**·HX was passed through a strong anion exchange resin column (Amberlite IRA-400) was the free **1c** obtained as colorless crystals, recrystallized form CH_3CN; mp 159 °C, M^+ (m/e) 285.

X-ray Crystal Study. Intensities of 3087 unique reflections in the region of $2\theta < 130°$ were measured on Rigaku AFC-5 diffractometer using Cu K_α radiation and corrected for absorption effects by use of North's method.[12] The structure was solved

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by the direct method and refined by block-diagonal least-squares technique on FACOM M-340R computer to R and R_w = 0.045 and 0.066, respectively, for 2472 observed reflections with $|F_o| > 3\sigma(F_o)$. Crystal data for **1c**·HN_3: $C_{13}H_{28}N_8O_2$, M = 328.4, monoclinic, space group $P2_1/c$, a = 8.710 (1) Å, b = 9.819 (1) Å, c = 21.635 (3) Å, β = 101.97 (1)°, V = 1809.9 (4) Å^3, Z = 4, D_c = 1.205 g cm^{-3}.

Acknowledgment. The present study was supported by Grant-in-Aid for Special Project Research No. 60119003 for E.K. from the Ministry of Education.

Supplementary Material Available: Atomic coordinates, temperature factors, bond lengths, and bond angles for **1c**·HN_3 (4 pages); structure factors for dimethyldioxo[16]aneN_5·HN_3 (20 pages). Ordering information is given on any current masthead page.

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Studies on the Reactivity of Bicyclomycin with Nucleophilic Amino Acid Derivatives†,1

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Received February 17, 1989

Bicyclomycin (1) is a clinically useful antibiotic marketed under the trade name Bicozamycin. Its unique structure and broad spectrum of antimicrobial activity have contributed to the increasing interest in this drug.[2-15] Biochemical studies indicate that 1 binds with select bacterial inner-membrane proteins (i.e., sulfhydryl-containing residues) leading to the disruption of cell wall growth.[5] Controversy exists as to whether drug activation occurs by an initial chemical[6-11] or enzyme-mediated[7,12] process prior to binding with the biological substrate. Recent studies[9-11] in our laboratory on the chemical reactivity of bicyclomycin with simple thiols and amines have provided support for the former scenario. These investigations led to the discovery of an extraordinary transformation in which drug modification proceeded with the generation of piperidinetrione **5** and loss of ammonia (Scheme I).[10,11] The facility of this reaction and the unusual structural properties of **5** led us to speculate that this process may be necessary for complete drug function and that **5** may serve as an efficient trap for additional nucleophiles present at the receptor site.[10,11] In this study, the chemical reactivity of bicyclomycin with nucleophilic amino acid derivatives is examined. We report the *first* examples of the reaction of 1 with cysteine derivatives. Drug modification proceeded rapidly at intermediate pH values to generate sulfide **5**. Special attention is also drawn to the susceptibility of piperidinetrione **5** toward nucleophilic attack by select amino acid derivatives.

Results and Discussion

Treatment of 1 with ethyl mercaptan (**3a**), *N*-acetyl-L-cysteine methyl ester (**3b**), and *N*-acetyl-L-cysteine *N'*-methylamide[16] (**3c**) in 3:1 tetrahydrofuran–water mixtures ("pH" 7.7–8.7) led to the formation of the C(5a)-sulfide adducts **6a–c**, respectively, along with unreacted bicyclomycin.[17,19] The identity of the two cysteine adducts **6b** and **6c** were established by comparison of the ^{1}H and ^{13}C NMR spectral data with that of **6a** (Table I). An X-ray crystallographic structure of **6a** has been previously described.[10] Sulfides **6a–c** reacted with N_α-acetyl-L-lysine *N'*-methylamide (**7**) (methanol, 45 °C, 16–24 h) to yield **9a–c**.[20] The ^{13}C NMR spectra of **9b** and **9c** indicated that the lysine-mediated process proceeded to give essentially a single compound, while a second minor product was detected in the reaction of **6a** with **7**. FAB mass spectra for **9a–c** exhibited a molecular ion peak corresponding to a 1:1 adduct. Important structural information concerning the site of lysine substitution in **9** was derived from careful inspection of the ^{1}H, COSY, and ^{13}C NMR spectra of each product (Table I). Significantly, a resonance is observed in the ^{1}H and COSY spectra for a C(5) methine proton suggesting that the amine-mediated reaction had proceeded at C(6) in **5** (**6**) with cleavage of the C(6)–C(5) bond. In agreement with this contention, the C(6) resonance in **9** appears significantly upfield (Δ ppm ~33) from the corresponding signal in **6**. Comparison of the ^{1}H and ^{13}C NMR spectral data for **6** and **9** indicated that the site of attachment of the triose group to the remaining molecule differs in these two adducts. In the ^{1}H NMR of **9** a downfield shift (Δ ppm ~0.5) of the C(1') methine proton

† Dedicated to the memory of Professor E. T. Kaiser.

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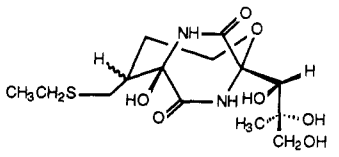

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(19) The amount of bicyclomycin recovered in these reactions ranged from 8 to 27%. A comparable result was observed upon treatment of 1 with **3c** in 9:1 methanol–water mixtures. TLC analysis of the reaction prior to workup indicated the presence of **6c** along with a small amount of 1.
(20) TLC analysis prior to workup indicated that the reaction was complete.

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