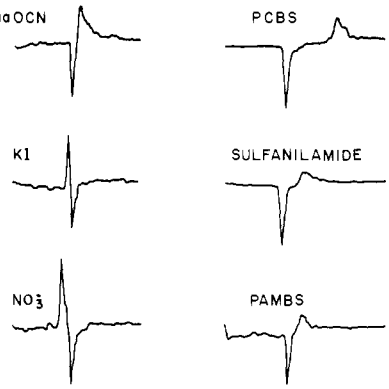

Figure 2. Effects of inhibitors on chemical shift of the ^{13}C NMR resonance of the enriched carboxylate of CmHCAB at pH 7.9 ± 0.1. Each spectrum is the *difference* generated by subtracting the spectrum of the uninhibited enzyme (negative peak marks position of enriched carbon signal) from the spectrum taken in the presence of the indicated inhibitor (positive peak indicates new position of enriched carbon signal). Spectral width shown is 14.2 ppm, negative peak is at 174.54 ppm, and shifts range from a downfield 0.83 ppm for nitrate to an upfield 3.88 ppm for *p*-carboxybenzenesulfonamide (PCBS). PAMBS is *p*-aminomethylbenzenesulfonamide. Spectra are proton decoupled.

histidine 200 (deprotonation of the nitrogen not bearing the carboxymethyl group). The 1.9 ppm downfield shift on deprotonation associated with this ionization is comparable to the 3.1ppm downfield shift observed[10] in the corresponding carboxyl group of N^{τ}-carboxymethyl-L-histidine upon deprotonation of its imidazole ring. The 9.2 inflection is tentatively assigned to the ionization of the zinc-bound water ligand. The downfield shift on deprotonation is consistent with the removal of a positive charge from the vicinity of the carboxylate.[11] Note that there is evidence to rule out a direct inner-sphere coordination of the carboxyl to the metal, at least when Co(II) replaces the zinc.[9] Support for this assignment also comes from observations on the pH dependence of the spectral changes of Co(II) substituted CmHCAB[9] and from pH perturbations of NMR signals of C-2 protons of histidines tentatively assigned to the three zinc ligands in a 270-MHz study[12] of HCAB and CmHCAB. It is worth noting that in this latter study a titrating resonance with pK_a of 6.14 was tentatively assigned to histidine 200 of *native* HCAB. Thus our observations appear to lend support to this assignment of the ^1H NMR study.[13] Our present findings also provide an excellent basis for identifying the group with pK_a of 6.1 recently found by Whitney to influence iodide inhibitor binding in CmHCAB[14] and to affect the visible spectrum of Co^{II}CmHCAB.[9,14]

The probe gives additional promise of providing *microscopic* ionization constants in complexes of the enzyme with inhibitors and substrates. Figure 2 shows that the chemical shift at pH 7.9 is very sensitive to inhibitor binding at the active site. Two main types of carbonic anhydrase inhibitors are represented, the monoanions and the aromatic sulfonamides. Studies are currently in progress to resolve the observed shifts of Figure 2 in terms of contributions from intrinsic changes of the chemical shift of the various ionization states and contributions from alterations of the pK_a values. This and many other aspects of the active site properties are currently being pursued using this promising probe, and attempts are being made to similarly label other histidines in both the B and C isoenzymes using ^{13}C enriched reagents.

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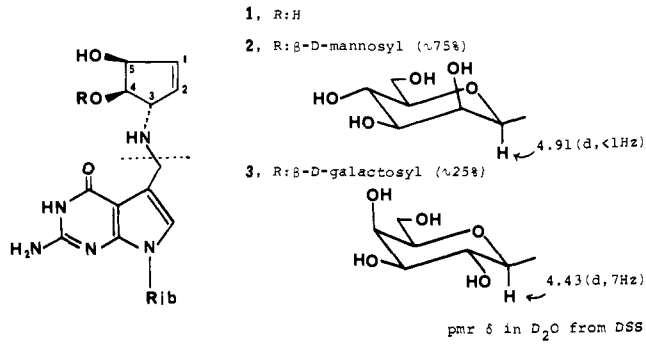
The Structure of Q* Nucleoside Isolated from Rabbit Liver Transfer Ribonucleic Acid

Sir:

The Q nucleoside which is located in the first position of the anticodon of *E. coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ has been assigned structure **1**;[1,2] the relative stereochemistry of the cyclopentene substituents have been determined as 3,4-trans and 4,5-cis on the basis of NMR comparisons with synthetic models.[3] We have recently shown that the tRNA's of various animals contain a new nucleoside Q*, and that the Q* content is generally more abundant than that of Q.[4] In particular it is to be noted that hepatoma cells have larger quantities of Q* as compared to normal cells.[4] In the following, we show that Q* nucleoside isolated from rabbit liver consists of a mixture of **2** (major) and **3** (minor); it is the first tRNA nucleoside to contain a sugar moiety in the side chain,[5] and is the most structurally complex nucleoside thus far known.



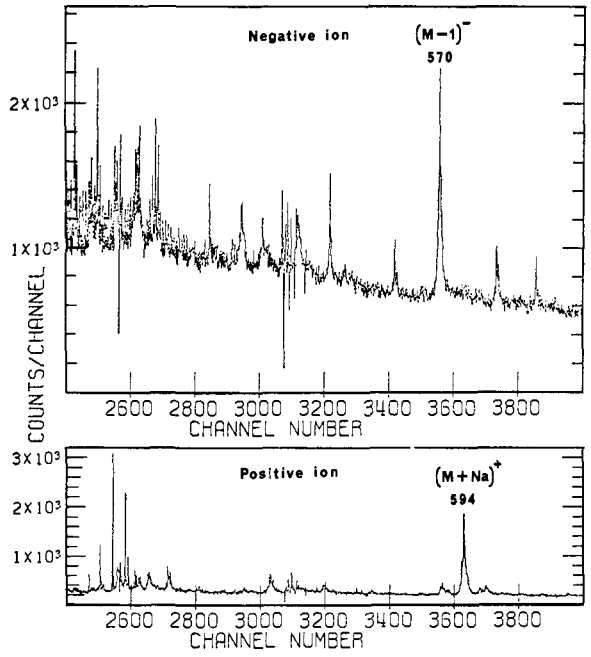


Figure 1. The positive and negative plasma desorption mass spectra (PDMS) of Q* nucleoside.

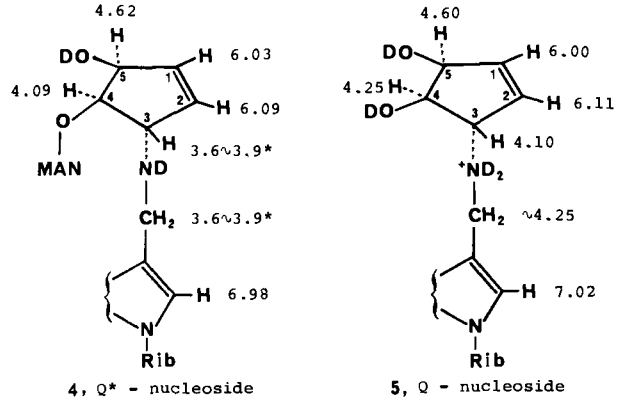


Figure 2. The 360-MHz ^{1}H NMR in D_2O, pD 7.5. The δ values shown in **4** are for the major isomer, the mannoside **2**: the shifts of 1-H, 2-H, 4-H, and 5-H for the minor galactoside **3** appeared at 0.05–0.10 ppm higher fields of corresponding protons in **2**. The asterisked chemical shifts in **4** could not be assigned with certainty due to overlap with hexose protons. Under conditions of measurements, pD 7.5, the amino group of Q nucleoside **5** (pK_a' 9) largely exists in the ammonium form.

The Q* nucleoside was isolated from rabbit liver tRNA as described previously.[4] The crude Q* nucleoside was separated from Q nucleoside by paper chromatography,[6] and further purified by high pressure liquid chromatography (HPLC)[7] to give ca. 0.6 mg of an amorphous material. The structural studies of Q* nucleoside are mostly based on spectral data due to scarcity of material. The uv spectra of Q* nucleoside **2** plus **3** under various pH's were identical with those of Q nucleoside **1**,[4] which had three pK_a values at 1.1, 7.7, and 10.4 as measured spectroscopically;[1] in addition, the side-chain amine function of Q nucleoside had another pK_a of ca. 9 (by NMR).[1] It was also deduced from MS studies[4] that the moiety below the dotted lines in structures **1–3** were identical in Q and Q*.

The ^{252}Cf plasma-desorption MS (PDMS)[8] of underivatized (**2** + **3**) directly gave a positive ion peak at 594 corresponding to 571 + 23 $(M + Na)^+$ and a negative ion peak at 570 $(M - H)^-$ (Figure 1), thus leading to a molecular weight of 571. This was corroborated by M^+ peak of *m/e* 767 for a side-chain N-acetylated permethyl (total of 11 methyls) derivative,[1] and of *m/e* 1291 for a trimethylsilyl (ten silyl groups) derivative[9] of Q* nucleoside. This 571 molecular weight corresponds to Q nucleoside having one additional hexose unit (in the upper half of the molecule) and is supported by the exact mass of the $M - CH_3CO$ ion in the mass spectrum of the *N*-acetyl, permethyl derivative: *m/e* 724.3696 (724.3770 required for $C_{34}H_{54}N_5O_{12}$).[10]

The 360-MHz ^{1}H NMR[11] spectrum of Q* nucleoside measured in D_2O, pD 7.5, DSS standard, surprisingly showed *three* anomeric protons at 5.89 (d, J = 6.5 Hz, 1′-H of ribose), 4.91 (d, $J < 1$ Hz), and 4.43 ppm (d, J = 7 Hz), roughly in the ratio of 1:0.75:0.25, the chemical shifts and coupling constants of the latter two peaks corresponding to the α-anomeric protons of β-mannosyl and β-galactosyl residues.[12,13] These results were confirmed by 1 N H_2SO_4 treatment of ca. 30 μg of Q* nucleoside at 100 °C for 4 h which gave a mixture of mannose (major) and galactose (minor) as identified by TLC.[14,15] The chemical shifts (360 MHz) of pertinent protons of Q* and Q nucleosides are shown in **4** and **5**, respectively (Figure 2). It is to be noted that although both spectra were measured in D_2O (pD 7.5), the 3-H, 4-H, and CH_2 peaks in **4** all absorb at higher fields than those in **5**. This difference could not be rationalized if the hexoses were attached to C-5. Namely, the amine function in **5** which has a pK_a' value of 9 should be mostly protonated at pD 7.5; the hexose linked to C-4 in **4**, on the other hand, would disfavor protonation of the amino group for steric reasons[16] and lead to a lowering of its pK_a, which is consistent with the ^{1}H NMR shifts. The similarity in the two 5-H proton signals in **4** (4.62 ppm) and **5** (4.60 ppm) fully supports this analysis. That the 4.62 ppm peak in **4** is due to 5-H and not to 4-H was established by the fact that the 5-H signal underwent a downfield shift of only 0.06 ppm upon acidification, i.e., 4.50 ppm in $Me_2SO + D_2O$ as compared to 4.56 ppm in $Me_2SO + D_2O + CH_3COOD$; if this were the 4-H signal, it should have shifted downfield by ca. 0.4 ppm as was the case for Q nucleoside **5**.[1]

As described before, the ^{14}C label is lost in the biosynthesis of Q nucleoside from [8-^{14}C]guanine.[17] Therefore, similar to the case of toyocamycin,[18] the 7-deaza ring is presumably ribose-derived. Studies to clarify the role played by the unique sugar residues in the first position of the anticodon, in particular in those of hepatoma tRNA's, are in progress.

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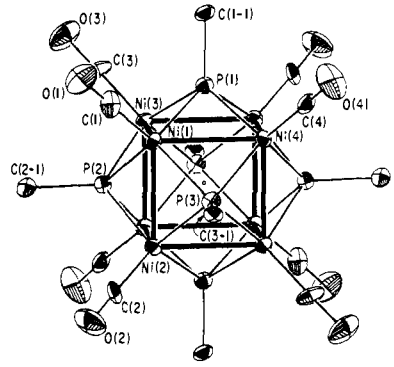
Synthesis and Structural Characterization of a New Type of Metal Cluster System, $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$, Containing a Completely Bonding Metal Cube. A Transition Metal Analogue of Cubane, C_8H_8

Sir:

We wish to report the preparation and structural analysis of $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$ which not only establishes the completely bonding metal cube as a basic structural unit in transition metal chemistry but also provides the first example of a $M_8L_8(\mu_4\text{-}X)_6$ type of metal cluster system. This work was a result of systematic studies designed to produce new transition metal cluster systems (with different MO electronic configurations) containing bridging main group ligands from the preformed metal cluster $[Ni_3(CO)_3(\mu_2\text{-}CO)_3]_n^{2-}$ (n = 2,[1] 3[2]) and $[Pt_3(CO)_3(\mu_2\text{-}CO)_3]_n^{2-}$ (n = 2, 3, 4, 5)[3] dianions.

The synthesis of $Ni_8(CO)_8(\mu_2\text{-}PC_6H_5)_6$ was accomplished by the addition of 0.28 g (1.6 mmol) of $C_6H_5PCl_2$ to a suspension of 0.85 g (1.0 mmol) of $[NMe_4]_2[Ni_3(CO)_3(\mu_2\text{-}CO)_3]_2$ in 40 ml of dry THF.[4] The mixture immediately changed from a bright orange-red to a deep purple color. After 1.5 h of stirring under N_2, the solvent was removed under vacuum and the residue washed three times with 15-ml portions of hexane. About 75 mg (ca. 20% yield based on P) of black plate-like crystals of $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$ were separated by the slow diffusion of heptane into a saturated toluene solution. Dark-red octahedral-shaped crystals of another compound also were isolated, but their unstable nature (as well as small yield) has prevented adequate characterization to date. Attempts to separate the compounds by elution from a silica gel column were unsuccessful due to extensive decomposition. The infrared spectrum for $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$ in CS_2 exhibited a very sharp carbonyl stretching frequency at 2020 cm^{-1}. Its proton NMR spectrum (JEOL MH-100) in CS_2 (with Me_4Si as internal standard) showed sharp resonances characteristic of a diamagnetic compound with overlapping phenyl resonances centered at δ 7.5.



$Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$

Figure 1. View of the $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$ fragment of the phenylphosphido-bridged molecule. All atoms are represented by 25% thermal ellipsoids. The phenyl rings have been omitted for clarity. The entire molecule possesses crystallographic $C_i - \bar{1}$ symmetry, while the fragment ideally conforms to cubic O_h symmetry.

An x-ray structural determination[5,6] revealed the existence of $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$ as a discrete molecule which with the neglect of the phenyl rings ideally conforms to cubic O_h symmetry, although an inversion center is the only crystallographically required molecular symmetry element. The molecular configuration (Figure 1) consists of a cube of nickel atoms with each square tetranickel face symmetrically capped by a phenylphosphido ligand. The additional coordination of one terminal carbonyl group, which is directed outward along one of the nickel cube's body-diagonals, results in a tetrahedral-like ligand environment of one carbonyl and three phosphorus atoms about each nickel atom. Each nickel atom then attains a noble-gas electronic configuration through a two-electron donation from the terminal carbonyl ligand, a one-electron donation from each of the three phenylphosphido ligands, and electron-pair Ni–Ni bonds with the three adjacent nickel atoms. This completely bonding (electron precise) homonuclear metal cube may be considered to be the first transition metal analogue of the C_8H_8 hydrocarbon[7] denoted as "cubane". Three octacopper cubane complexes, the $[Cu_8(i\text{-}MNT)_6]^{4-}$, $[Cu_8(DED)_6]^{4-}$, and $[Cu_8(DTS)_6]^{4-}$ tetraanions, have previously been found from structural analyses[8a-c] to possess a $[M_8(\mu_2 - X)_{12}]^{4-}$ type structure based upon a metal cube with edge-bridged X ligands. This geometry arises from the 12 sulfur atoms of the six bidentate sulfur ligands in each tetraanion being arranged in a distorted icosahedral array such that each Cu(1) is trigonally coordinated to three sulfur atoms from different ligands with a sulfur atom linked to two copper atoms along each of the 12 octacopper cube edges. Whereas a conformity to the EAN rule by each Cu(1) would effectively correspond to a two-thirds electron-pair Cu–Cu bond along each of the 12 cube edges, an alternative bonding model suggested from MO calculations[8d] involves no net Cu–Cu interactions.[9]

The Ni_8P_6 core in $Ni_8(CO)_8(\mu_4\text{-}PC_6H_5)_6$ may be viewed as the result of the interpenetration of a nonbonding P_6 octahedron by a bonding Ni_8 cube. The Ni atoms are ca. 2.6 Å apart, while the P···P distance along any of the three octahedral axes is 4.9 Å. The result is that each phosphorus atom lies approximately 1.1 Å out of its square Ni_4 face. This octametal-hexaligand architecture has a reciprocal structural relationship to the hexametal-octaligand $[Mo_6Cl_8]^{4+}$ type structure,[10] which may be viewed to evolve from a bonding