9 pulsed solenoid valve.[11] The reaction of La^{2+} with $Fe(CO)_5$ gives ~80% charge exchange product ions.[12] Carbonyl displacement reactions, however, are also observed (reaction 1). $La^{56}Fe(CO)_2^{2+}$ (~11% of the product ion intensity) was isolated by swept double resonance pulses and subjected to collision-induced

$$La^{2+} + Fe(CO)_5 \rightarrow LaFe(CO)_n^{2+} + (5 - n)CO \quad (n = 1, 2, 3) \quad (1)$$

dissociation (CID) with background argon at about 4×10^{-6} Torr.[13] As the collision energy is increased, first loss of one and then both carbonyls is observed, resulting in the formation of $La^{56}Fe^{2+}$. This procedure can result in some of the $LaFe^{2+}$ ions being nonthermal. $LaFe^{2+}$ accounts for ~30% of the CID fragment ions at a collision energy of 48 eV. This species was then isolated and its reactions with small hydrocarbons observed at extended trapping times. Reactions of Nb^{2+} and Ta^{2+} with $Fe(CO)_5$ give only charge exchange products.

There are two significant differences between our observation of $LaFe^{2+}$ and the earlier work on Mo_2^{2+}. First, the nominal mass of Mo_2^{2+} is the same as Mo^+, but its presence could be verified by its characteristic isotopic distribution which differs from that of Mo^+. In contrast $LaFe^{2+}$ is heteronuclear and, thus, there are clearly no isobaric interferences from either Fe^+ or La^+. Although, unfortunately, an exact mass measurement could not be obtained for $LaFe^{2+}$, it is generated in a reaction sequence starting with La^{2+} where the nominal masses are verified at each step. Sequential loss of CO from CID of $LaFe(CO)_2^{2+}$ rules out any significant contribution from a possible isobaric interference, $La(CO)_2^{2+}$. In addition, CID of $La(CO)_2^{2+}$ would also be expected to sequentially lose two carbonyls which is not observed when the proposed $LaFe^{2+}$ species undergoes CID, as discussed below. A second difference is that the lifetime of the Mo_2^{2+} species could only be given a lower limit of several times greater than 15 ns, the flight time of the ions in the instrument. Thus, it was not clear whether the ions were stable or metastable. In our system $LaFe^{2+}$ could be stored and observed on the order of seconds.

There are two possible explanations for the bonding in $LaFe^{2+}$. A charge separated species La^+-Fe^+ is possible if the inherent bonding of the species is large enough to overcome the electrostatic repulsion or, more likely, results in a kinetic barrier to the dissociation. Similar explanations have been applied to some nonmetallic polyatomic species.[14,15] Alternatively, the bonding may more closely resemble La^{2+}-Fe where the ion-induced dipole results in a strong attractive interaction. Interestingly, CID on $LaFe^{2+}$ yields La^+ and Fe^+ exclusively over the energy range studied (13-32 eV). Although suggestive, these results do not conclusively distinguish the charge separated model from the La^{2+}-Fe model, since in the latter case, a curve crossing is possible and even likely.

In light of the rich chemistry observed for small transition metal ionic species, the reactions of $LaFe^{2+}$ with a few small hydrocarbons were studied. No reaction is observed with methane,[16] while $LaFe^{2+}$ does react with ethane, predominantly (>75%) by reaction 2. $LaFe(C_2H_4)^{2+}$ from reaction 2 reacts further with

$$LaFe^{2+} + C_2H_6 \rightarrow LaFeC_2H_4^{2+} + H_2 \quad (2)$$

ethane to form $LaFe(C_2H_4)_2^{2+}$. The observation of $LaFeC_2H_4^{2+}$ and $LaFe(C_2H_4)_2^{2+}$ resembles the reaction of $LaFe^+$ with ethane.[17] However, La^{2+} is not observed to react with either methane or ethane.[16,18]

The reactions of $LaFe^{2+}$ with propane are shown in reactions 3-6. Only the reaction analogous to reaction 4 was observed for

$$LaFe^{2+} + C_3H_8 \rightarrow LaFeC_3H_4^{2+} + 2H_2\ 11\% \quad (3)$$

$$\rightarrow LaFeC_3H_6^{2+} + H_2\ 52\% \quad (4)$$

$$\rightarrow FeC_3H_6^+ + La^+ + H_2\ 10\% \quad (5)$$

$$\rightarrow LaC_3H_6^+ + Fe^+ + H_2\ 27\% \quad (6)$$

$LaFe^+$ and La^{2+}.[17,18] At longer trapping times, $LaFe(C_6H_{10})^{2+}$ and $LaFe(C_6H_{12})^{2+}$ were observed, arising from the secondary reactions of $LaFeC_3H_4^{2+}$ and/or $LaFeC_3H_6^{2+}$.

Ethylene reacts with $LaFe^{2+}$ predominantly (>65%) by splitting the cluster ion to form $LaC_2H_4^+$ and Fe^+. Some $FeC_2H_4^+$ and La^+, along with minor amounts of $LaFeC_2H_2^{2+}$ and $LaC_2H_2^+$, are also observed. Due to the difficulty of performing a double resonance experiment at this stage, it was not possible to determine whether the $LaC_2H_2^+$ arose from the reaction of the doubly charged cluster or from La^+ reacting with ethylene.

Finally, it is worth mentioning that $LaFe(CO)_2^{2+}$ reacts with ethane slowly, forming $LaFe(CO)_2(C_2H_4)^{2+}$ and $LaFe(CO)_2(C_2H_6)^{2+}$ in roughly a 1:3 ratio, and with propane to yield $LaFe(CO)(C_3H_6)^{2+}$ and $LaFe(CO)(C_3H_8)^{2+}$ in roughly a 3:1 ratio.

The in situ synthesis of a variety of stable doubly charged LaFe species has been demonstrated. Additional studies are underway to try to determine the bond energy and kinetic barrier associated with $LaFe^{2+}$ and its derivatives, as well as to extend these promising preliminary chemical studies. A detailed theoretical calculation on the bonding of this intriguing species is also underway.

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Studies on the Biosynthesis of the *m*-C_7N Unit in the Antibiotics Manumycin and Asukamycin

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The manumycin group of antibiotics, represented by manumycin (**1**),[1,2] asukamycin (**2**),[3,4] colabomycin,[5] U-62,162,[6] and U-56,407,[7]

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contains as a central structural element a multifunctional m-C_7N unit. This moiety, consisting of a six-membered ring carrying one carbon and one nitrogen atom in a meta disposition, is ubiquitous in nature in a variety of forms.[8,9] The m-C_7N unit is most commonly quininoid as exemplified by the rifamycins,[10] mitomycins,[11] and ansamitocins[12] or aromatic as in the case of pactamycin.[13] In these antibiotics, its biogenesis has been demonstrated to proceed by a branch of the shikimic acid pathway,[9] ultimately via 3-amino-5-hydroxybenzoic acid (AHBA) or 3-aminobenzoic acid (ABA). Other forms of the m-C_7N unit are found in the valienamine moiety of validamycin and acarbose and in the antibiotic kinamycin; in these cases it has been shown that the unit derives from intermediates of the pentose phosphate pathway[14,15] and from acetate, respectively.[16] The structural and stereochemical uniqueness observed in **1** (4*R*)[17] and **2** (4*S*)[4] led us to study the biosynthesis of these antibiotics in parallel. In this communication we present results confirming a new biochemical pathway leading to the m-C_7N unit.

Fermentation, isolation, and purification of **1** and **2** followed previously described procedures.[2,4,18] Isotopically labeled precursors were fed to *Streptomyces parvulus* Tü64 (**1**) or *Streptomyces nodosus* subsp. *asukaensis* ATCC 29757 (**2**) after 40 h in the production culture or, in pulse feeding experiments, after 40, 48, 56, and 64 h. Isotopic labeling patterns in the purified antibiotics (0.07 mmol/L **1**, 0.03 mmol/L **2**) were assessed by NMR spectroscopy in $CDCl_3$ at field strengths of 7.1T or 11.8T on IBM AF-300 or Bruker AM-500 instruments. Chemical shift assignments are based on chemical shift anisotropy and coupling patterns and results obtained from 2D-COSY, NOESY, and heteronuclear correlation experiments.

Following a prediction that the manumycin group antibiotics actually represent incomplete ansamycins,[19] Rickards et al.[20] hypothesized that their m-C_7N units originate from the shikimate pathway via either AHBA or ABA. To test this hypothesis, we synthesized[14,21] both potential precursors labeled with ^{13}C in the carboxy group (99 atom % ^{13}C). In feeding experiments with [7-^{13}C]AHBA (2.6 mmol/L) and [7-^{13}C]ABA (3.2 mmol/L) no incorporation of label into the m-C_7N units of **1** and **2** was observed. These results indicate that either the unit is not derived from the shikimate pathway or that neither precursor is able to penetrate to the site of biosynthesis within the cells. To provide further information, additional experiments were conducted with precursors corresponding to intermediates of primary metabolism.

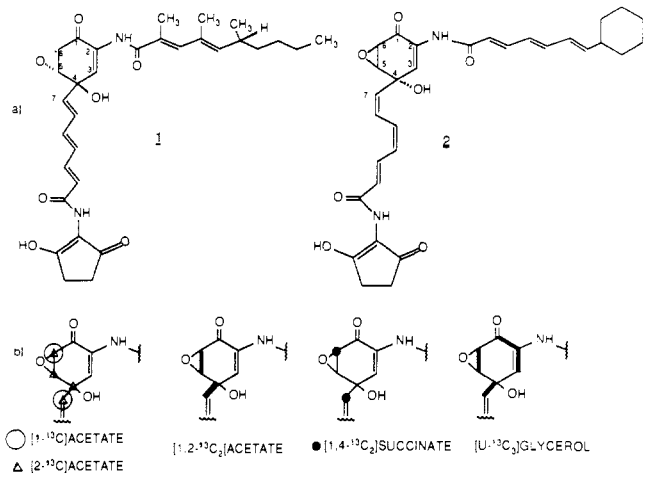

Figure 1. (a) Structures of manumycin (**1**) and asukamycin (**2**) and (b) summary of ^{13}C-labeling patterns in the m-C_7N unit from various precursors.

In both **1** and **2**, feeding of sodium [1-^{13}C]acetate (99 atom % ^{13}C, 10.8 mmol/L) clearly enriched positions C-6 and C-7 in the m-C_7N unit (Table I; Supplementary Material), suggestive of a "tail-to-tail" incorporation of acetate.[22,23] This arrangement was further confirmed by feeding sodium [2-^{13}C]acetate (10.8 mmol/L), which enriched C-4, C-5, C-6, and C-7 (Table 1, Supplementary Material). Enrichment in the four contiguous carbon atoms instead of only C-4 and C-5 is due to scrambling of the label from C-2 of acetate by the TCA cycle.[24] In both asukamycin and manumycin, profered sodium [1,2-$^{13}C_2$]acetate (10.8 mmol/L) gave rise to enrichment and spin coupling between C-4/C-7 and C-5/C-6 (Table I (Supplementary Material), Figure 1). All of these results suggest that the four-carbon segment extending from C-7 to C-6 derives from a TCA cycle intermediate (Figure 1). Confirmation of this was obtained from experiments with [1,4-$^{13}C_2$]succinic acid (1.6 mmol/L) which gave enrichments only at C-6 and C-7 in the m-C_7N units (Table I, Supplementary Material). These data prove that succinic acid or a metabolically related intermediate is involved in the biosynthesis of this moiety. The origin of carbon atoms 1, 2, and 3 was not ascertainable by the foregoing experiments.

[U-$^{13}C_3$]Glycerol[14] (99 atom % ^{13}C at all positions) was fed to the cultures at levels of 5.4 mmol/L. Metabolism of glycerol to acetate gave rise to labeling patterns in asukamycin and manumycin generally identical with those observed in the sodium [1,2-$^{13}C_2$]acetate experiments. Thus, C-4/C-7 and C-5/C-6 were found to be enriched and coupled (Table I, Supplementary Material). Examination of the proton-decoupled ^{13}C NMR spectrum revealed incorporation of glycerol into the C-1 to C-3 segment in both antibiotics **1** and **2**. Because the levels of incorporation of glycerol into these positions were not high, we tested for intact incorporation of the precursor by 1-D double quantum filtration, 2-D INADEQUATE,[25] and selective ^{13}C-homodecoupling experiments. Intact incorporation of glycerol into C-1 to C-3 of the m-C_7N unit was evident from the presence of a doubly coupled spin system in both antibiotics. The systems exhibited ABX patterns, in which coupling satellites for both the one- and two-bond ^{13}C–^{13}C couplings were observed (Table I).

The data reported herein demonstrate yet another biochemical origin of a m-C_7N unit, involving intermediates of the TCA cycle

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and the triose pool. Experiments to identify the actual substrates and the origins of the ring nitrogen and oxygen atoms are in progress.

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Registry No. 1, 52665-74-4; **2**, 61116-33-4; acetic acid, 64-19-7; succinic acid, 110-15-6; glycerol, 56-81-5.

Supplementary Material Available: Table listing data and details of the complete ^1H and ^{13}C NMR assignments of asukamycin and manumycin (1 page). Ordering information is given on any current masthead page.

Edge Inversion Barrier at a Four-Coordinate Main Group IV Center†

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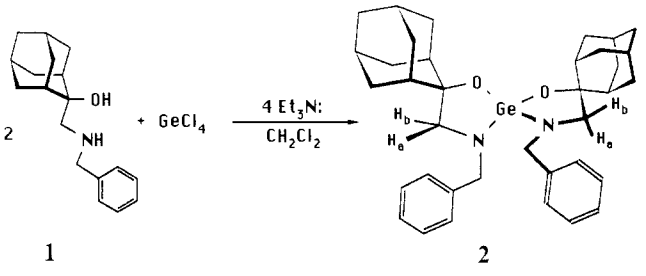
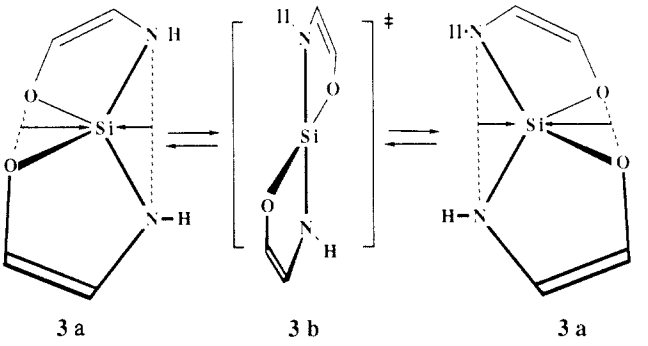
The barrier to edge inversion has been directly determined for a four-coordinate main group IV compound **2**. The experimentally determined barrier ($\Delta H^{\ddagger}$) for the germanium compound **2** is 22.2 (±2.5) kcal/mol (in agreement with theoretical expectations[1])



1 2 (1)

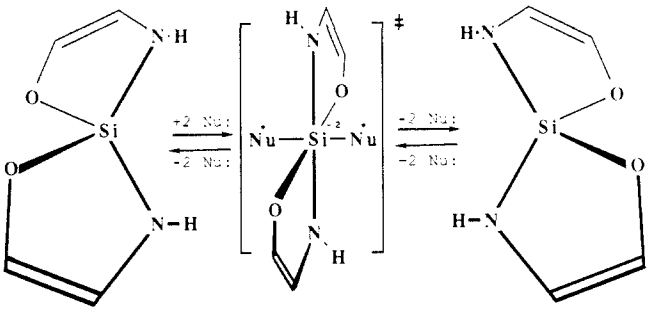
with an activation entropy ($\Delta S^{\ddagger}$) of 0.65 (±6.5) eu. These results substantiate the operation of an edge inversion process at main group IV centers analogous to the process recently predicted[2] for and subsequently measured[3] at pnictogen centers.

In previous reports[1a,c,d] we suggested that the edge inversion process (eq 2) should be considered in addition to the nucleophile assisted mechanism (eq 3) for configurational inversion of four-coordinate main group IV compounds (inversion may also be accomplished via a five-coordinate structure if accompanied by



3a 3b 3a

Edge Inversion Process (2)



3a Associative Inversion Process 3a (3)

pseudorotation). While the stereochemical outcome of these inversion mechanisms is the same, there is a difference in the molecularity of the three processes. The viability of the edge inversion process for such systems is supported by large basis set ab initio calculations of the barriers to edge inversion for the series of main group IV tetrafluorides.[1a] As with the pnictogen systems,[2,3] substitution of a main group IV center with electronegative groups (σ-acceptors) and π-donors can lead to a preference for the edge inversion process.

On the basis of our earlier studies on the pnictogen derived ADPnO[1e] systems[1c,d,4] high level ab initio molecular orbital calculations on **3** were performed. The geometries of the ground-state structure with a tetrahedral Si, **3a**, and that for the edge inversion transition state (C_{2h}) with a planar Si, **3b**, were gradient optimized[5] with the program GRADSCF[6] on a CRAY/1A computer. Force fields[7] and an MP-2 correlation correction were calculated at the optimum geometries.[8] The basis set is of the form (11s7p1d/9s5p1d/9s5p/4s)/[6s4p1d/3s2p1d/3s2p/2s] in the order Si/O,N/C/H.[9] The structure with a tetrahedral silicon, **3a**, is a minimum, while **3b** is a transition state. The single direction of negative curvature corresponds to the inversion mode (ν = 145i cm^{-1}). Structure **3b** is 24.6 kcal/mol above **3a** at the SCF level and is 17.6 kcal/mol above **3a** at the MP-2 level, suggesting that an experimental measurement on the barrier to the unimolecular edge inversion process for a compound like **3** is possible.

† Dedicated to Professor J. C. Martin on the occasion of his 60th birthday.

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