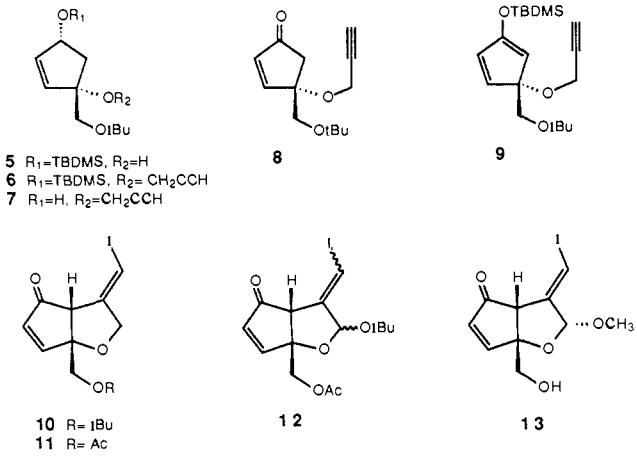
mation (*tert*-butyldimethylsilyl trifluoromethanesulfonate, triethylamine (Et_3N), CH_2Cl_2, 0 °C to 23 °C, 1 h)[10] coupled with a non-aqueous workup gave the cyclopentadiene **9**, which was submitted directly to mercuric chloride cyclization[2] (1.0 equiv of $HgCl_2$, 0.2 equiv of hexamethyldisilazane, CH_2Cl_2, 30 °C, 70 min). The cyclized vinyl mercurial was subjected without isolation to iodine mediated electrophilic substitution[4,11] (1.0 equiv of *N*-iodosuccinimide, 2 equiv of NaI, 0 to 23 °C, 4 h) to give, after workup and chromatography, the crystalline *E*-vinyl iodide **10**[12] (90.6% from **8**, mp 94-96 °C).



5 R_1=TBDMS, R_2=H
6 R_1=TBDMS, R_2= CH_2CCH
7 R_1=H, R_2=CH_2CCH

8 **9**

10 R= tBu
11 R= Ac

12 **13**

With the successful formation of **10**, three tasks remained to complete the synthesis: vinyl coupling, allylic oxidation, and unmasking of the primary alcohol. Treatment of **10** with acetic anhydride–ferric chloride[13] (0 °C, 1 h) provided acetate **11** (88.0%, mp 63 °C). Selenium dioxide oxidation[14] of **11** (0.7 equiv of SeO_2, 4.0 equiv of tBuOOH, 1,2-dichloroethane, 83 °C, 8 h) yielded an *E*,*Z* mixture of *tert*-butoxy acetal anomers **12** (81% combined yield, *E*:*Z* ca. 1:1), which, after methanolysis (catalytic TsOH, MeOH, 21-23 °C, 4 days) and chromatography, gave methoxy acetal **13** (24% yield from **11**) as the major *E*-vinyl iodide anomer. Coupling of **13** with tri-*n*-butylvinylstannane (catalytic $(Ph_3P)_2PdCl_2$, *N*,*N*-dimethylformamide, 23-24 °C, 18 h)[3] gave stereospecifically the (*E*)-diene **3** (72%, mp 127-128 °C, $[\alpha]_D^{21}$ +346.9° (*c* 0.179, $CHCl_3$)) which matched (^1H NMR, ^{13}C NMR, IR, UV, EIMS, TLC, mp, sign of specific rotation, and unit cell constants) acetal **3** derived from naturally occurring didemnenones A (**1**) and B (**2**). The acetal enriched in the opposite enantiomer[15] was obtained from (*S*)-**4**[8] by the same route. Hemiacetals **1** and **2** (1:1 mixture, $[\alpha]_D^{22}$ +514.8° (*c* 0.081, DMSO)) were obtained by hydrolysis of **3** (catalytic HCl, THF-H_2O, 2:1, 0-23 °C, 2.75 h, 69.9%) and were indistinguishable from the natural product mixture by ^1H NMR, IR, UV, EIMS, TLC, and sign of specific rotation.

The excess enantiomers of synthetic **1**, **2**, and (+)-**3** have the same absolute configurations as their naturally derived counterparts.[16] The absolute configurations at C2 and C6 are defined by the synthesis as 2*R*,6*R* for **1**, **2**, and (+)-**3**. On the basis of the structural correlations reported in our earlier work,[1] it follows that didemnenone C is 2*S*,6*S* and didemnenone D, most plausibly, is 2*S*,6*R*.

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Supplementary Material Available: Analytical data for **1-3** and **5-13** (5 pages). Ordering information is given on any current masthead page.

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(11) Riediker, M.; Schwartz, J. *J. Am. Chem. Soc.* **1982**, *104*, 5842-5844.
(12) While subsequent allylic oxidation yields an *E*,*Z* mixture of vinyl iodide isomers, assignment of the *E* configuration to **10** is supported by protonolysis of the vinyl mercurial and assignment of the ^1H NMR resonances of the resulting exocyclic methylene protons as described in ref 3, as well as by vinyl coupling to form the corresponding *E*-diene as for **13**.
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(15) (-)-**3**: $([\alpha]_D^{25}$ -245.9° (*c* 0.270 g/100 mL, $CHCl_3$, ca. 66% ee)).
(16) The specific rotation of the natural products **1** and **2** (1:1 mixture) is $[\alpha]_D$ +576.1° (*c* 0.49, DMSO), while that for naturally derived **3** is $[\alpha]_D$ +371.8° (*c* 0.86, $CHCl_3$).[1] The estimated optical purities of synthetic **1**, **2**, and (+)-**3** (ca. 89-93% ee) approximate that estimated for starting material (*R*)-**4** (ca. 94% ee).[8]

Origin of the Cyanamide Carbon of the Kinamycin Antibiotics†

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We have previously described the biosynthesis of the benz[*b*]carbazole skeleton of the kinamycin antibiotics **1**-**4**[2-5] from acetate via the benz[*a*]anthraquinone **5** (Scheme I).[6] The data suggested excision from **5** of two carbons, C-5 and C-6, originally derived from the same acetate unit. The only primary metabolic precursor of kinamycin remaining to be identified was that of the cyanamide unit. Efforts to identify the origin of this unusual moiety[7] were hampered by the apparent absence of its resonance signal from the ^{13}C NMR spectra of the kinamycins.[8] We now report experiments that uncovered the missing resonance as well as experiments that reveal the source of the cyano carbon.

Fermentation of *Streptomyces murayamaensis* in a defined medium[10] containing $(^{15}NH_4)_2SO_4$ as the sole nitrogen source afforded [^{15}N]kinamycin D, **4a**. The 40.5 MHz ^{15}N NMR spectrum (Figure 1) showed two doublets (J_{NCN} = 3.4 Hz)[11] at 344.5 and 241.6 ppm relative to $H^{15}NO_3$ at 362.0 ppm, for the two nitrogens. The 100.6 MHz ^{13}C NMR spectrum of **4a** (Figure 2) showed ^{15}N-coupled doublets for C-5′ (δ 131.7, J_{CN} = 2.9 Hz), C-6′ (δ 128.0, J_{CN} = 2.2 Hz), C-2 (δ 132.2, J_{CN} = 2.3 Hz), and C-3 (δ 128.4, J_{CN} = 2.8 Hz). To our surprise and delight a doublet of doublets was also observed at δ 78.5, J_{CN} = 21.2, 5.4 Hz. Examination of all previously obtained ^{13}C NMR spectra of **4** revealed the small singlet, almost overlapped by the $CDCl_3$ resonance, that had been ignored as an impurity. Although we cannot unequivocally explain the large upfield shift (ca. 30 ppm),[8] its observation has allowed us to determine the biosynthetic origin of the cyanamide carbon.

Feedings with typical one-carbon precursors labeled with ^{14}C led only to low incorporations ([$^{14}CH_3$]methionine, 0.08%; sodium [^{14}C]formate, 0.01%; sodium [^{14}C]cyanide, 0.01%; [^{14}C]urea, 0.01%; sodium [^{14}C]carbonate, 0.05%) except for [guanido-^{14}C]arginine, (0.61%),[12] [3-^{14}C]serine (0.49 and 1.90%[13]), and

† Dedicated to Professor Duilio Arigoni on the occasion of his 60th birthday.
(1) Career Development Awardee of the National Cancer Institute (Grant CA-00880), 1979-1984.
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(7) Only one other naturally occurring cyanamide has been reported: Bisset, N. G.; Choudhury, A. K.; Walker, M. D. *Phytochemistry* **1974**, *13*, 255-258.
(8) Ōmura[9] had first noted its absence and pointed out the chemical shifts for *N*,*N*-dimethylaminocyanamide (119.4 ppm) and *N*-cyano-*N*-methylaminoacetate (117.8 ppm). We have determined that the cyano resonance in *N*-cyanoaniline appears at 111.9 ppm.
(9) Ajisaka, K.; Takeshima, H.; Ōmura, S. *J. Chem. Soc., Chem. Commun.* **1976**, 571-572.
(10) The production medium—200 mL of 3% glycerol, 10 mM $(^{15}NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.04% $MgSO_4$, 0.01% $FeSO_4$ in a 1-L Erlenmeyer flask—was inoculated with 10 mL of a 48 h seed culture and incubated at 26-27 °C and 300 rpm for 36 h.

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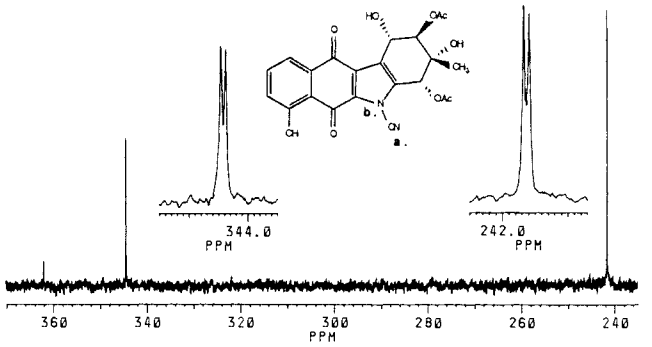


Figure 1. ^{15}N NMR of [$^{15}N_2$]kinamycin D; $CDCl_3$; 40.5 MHz; spectral width 10000 Hz; 64K data points, quadrature detection; 30° pulse; aquisition time 1.64 s; number of scans, 721.

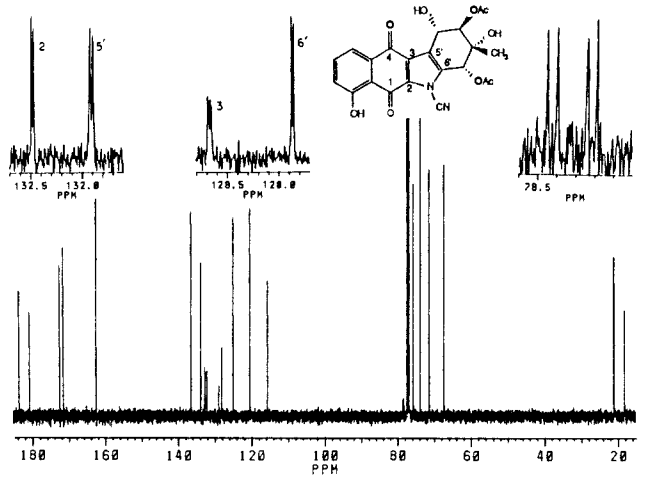
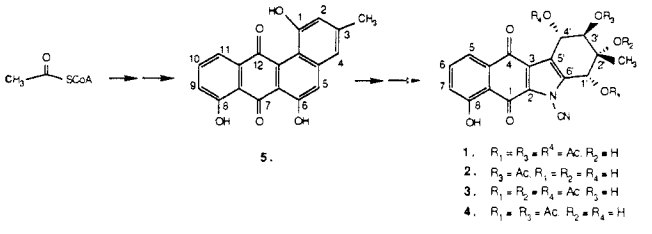
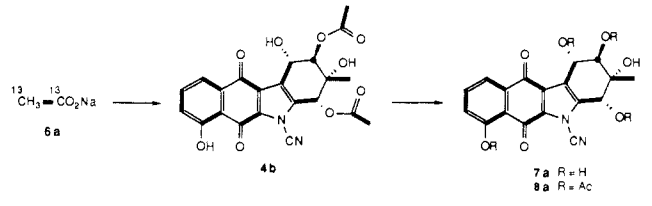


Figure 2. ^{13}C NMR of [$^{15}N_2$]kinamycin D; $CDCl_3$; 100 MHz; spectral width 18518.5 Hz; 128K data points; quadrature detection; 30° pulse; acquisition time 3.54 s; line broadening, −0.5 Hz; guassian broadening, 0.3 Hz; number of scans, 14364.

Scheme I



Scheme II



[2-^{14}C]glycine (0.50 and 4.94%[14]). However, examination of the ^{13}C NMR spectra of kinamycin D samples obtained from subsequent feedings with [3-^{13}C]serine and [2-^{13}C]glycine revealed that the former had been metabolized to [2-^{13}C]acetylCoA and the latter had been metabolized to [1,2-$^{13}C_2$]acetylCoA prior to incorporation. At this point, we appreciated that our original acetate feeding with sodium [1,2-$^{13}C_2$]acetate, **6a** (see Scheme II), had not provided an independent reference signal for measuring absolute enrichments. Careful hydrolysis (0.2 N NaOH, 0 °C, 15 min) of **4b**, derived from this latter experiment, provided a labeled desacetyl kinamycin **7a**[15a] that was then acetylated to give the tetraacetate **8a**,[15b] now containing methyl and carbonyl carbons at natural abundance for comparison. Examination of the ^{13}C NMR spectrum of this sample showed a 1.4% enrichment at the cyanamide carbon.

Separate feedings with sodium [1-^{13}C]acetate, **6b**, and with sodium [2-^{13}C]acetate, **6c**, provided kinamycin D samples **4c** and **4d**, respectively. Figure 3 shows relevant portions of the ^{13}C NMR spectra of **4** (Figure 3a, natural abundance), **4c** (Figure 3b), and **4d** (Figure 3c). By using the quinone carbonyls for reference (C-4

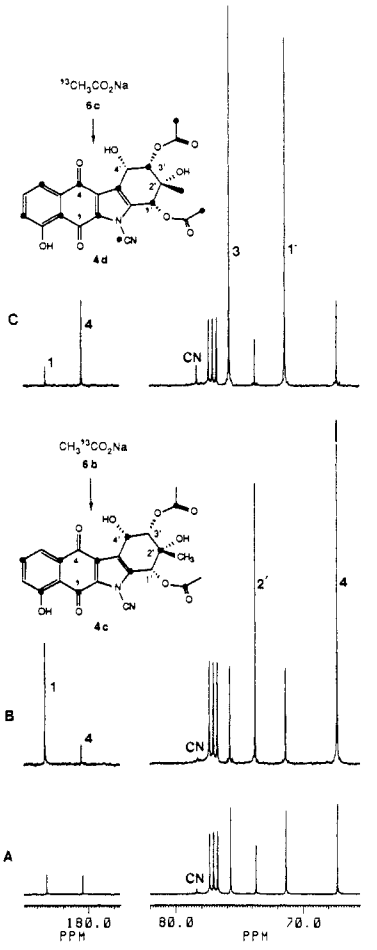
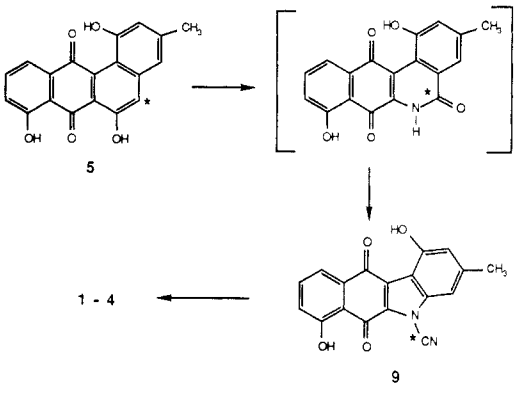


Figure 3. ^{13}C NMR of kinamycin D samples; $CDCl_3$; 100 MHz; spectral width 25000 Hz; 64K data points, quadrature detection; 30° pulse; aquisition time 1.64 s; line broadening, 1.0 Hz; A, natural abundance; number of scans, 6000; B, from incorporation of sodium [1-^{13}C]acetate; number of scans, 4000; C, from incorporation of sodium [2-^{13}C]acetate; number of scans, 4000.

Scheme III



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(12) When this was repeated with an additional 1.0 g/L of unlabeled arginine, the incorporation of ^{14}C was only 0.06%.
(13) Obtained when an additional 1.0 g/L of unlabeled serine was co-fed.
(14) Obtained when an additional 1.0 g/L of unlabeled glycine was co-fed.
(15) (a) Ōmura, S.; Nakagawa, A.; Yamada, H.; Hata, T.; Furusaki, A.; Watanabe, T. *Chem. Pharm. Bull.* **1973**, *21*, 931–940. (b) This was satisfactorily characterized spectroscopically.

for **4c** and C-1 for **4d**), it is clear from the cyanamide resonance that the cyanamide carbon is specifically enriched (2.7%) by **6c**, comparable to enrichments of the relevant ring carbons.

If the cyanamide carbon were biosynthetically added to a preformed benz[*b*]carbazole, any of the known independent pathways of acetate metabolism would have led through one of the compounds already tested. In view of the relatively uniform levels of enrichment from **6c**, we propose that the cyanamide carbon is derived from C-5 of **5** via oxidation, nitrogen insertion, and ring contraction/rearrangement as shown in Scheme III.[16] Additional, albeit circumstantial, support is provided by two additional benz[*b*]carbazole metabolites of *S. murayamaensis* that contain the cyanamide functionality; one of these is **9**.[17] No benz[*b*]carbazole metabolites have been found that lack the cyanamide unit. Efforts to synthesize **5** specifically labeled with ^{13}C at C-5 in order to test this hypothesis are in progress.

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(16) In order to test the proposed ring contraction and the intermediacy of **9**, we have attempted to prepare **5** and **9** sufficiently biosynthetically enriched in ^{13}C for subsequent feeding to *S. murayamaensis*. While we have obtained as much as 10% enrichment this has been insufficient—in part due to the insolubility of these compounds—to yield reliable enrichment in derived kinamycin D even with the known intermediate **5**.[6]

(17) These will be reported in a separate communication.

Crossed-Beam Study of HX Elimination Reactions

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Most studies of organic reactions are carried out in solution, where the role of the solvent is often complicated and very difficult to elucidate. Recently, there have been an increasing number of studies carried out in the gas phase or in beams where the states of the reactants and products can be better controlled and characterized. Studies of gas-phase acidities and basicities using ICR[1] have shown large deviations from the aqueous values. We have previously used crossed supersonic nozzle beams to study several aspects of three types of organic chemiionization reactions.[2]

We report here a preliminary study of the reactions of a strong organic base with an alkyl halide. The base abstracts a proton β to the halogen, and the halide negative ion is eliminated to give the protonated amine, the halide ion, and an olefin. In the present study we used all four isomers of butyl iodide reacting with tetrakis(dimethylamino)ethylene (TDMAE) $[(CH_3)_2N]_2C{=}C[N(CH_3)_2]_2$. Previous papers[2] have given detailed descriptions of the apparatus, the techniques of beam formation, and the data analysis. Briefly, each reactant beam is prepared by bubbling H_2 carrier gas through the liquid. The bubbler temperature determines the vapor pressure of the reagent in the beam. The mixture is passed through a Pyrex nozzle into the main vacuum chamber. The product ions are extracted by a grid, mass analyzed by a quadrupole mass analyzer, and detected on a continuous dynode electron multiplier. Each nozzle can be heated by passing a current through a coil of wire surrounding it. The kinetic energy of the beam can be calculated by using well-known formulas[3] and is varied by changing the temperature of the TDMAE nozzle. The temperature of the butyl iodide beam is kept at 26 °C to prevent decomposition.

Figure 1 shows the reactive cross section versus relative translational energy for the reaction of all four butyl iodides with TDMAE. The only ions that we observe are I^- and the protonated TDMAE. We cannot see the neutral product, but it would almost have to be some isomer of butene. The reactions of the four isomers have similar thresholds of 7.9 ± 1.0 eV. The cross sections were scaled in magnitude by a single multiplying factor but otherwise have a similar energy dependence. This is not surprising since the thermodynamic ΔH for each reaction is roughly the same. The heats of formation of the four butyl iodides and of the four butenes are close to each other. We cannot obtain the thermodynamic ΔH because the absolute proton affinity of TDMAE has never been measured. We have tentative evidence[4] that it is higher than the proton affinity of tri-*n*-butylamine. If this is so, then the thermodynamic threshold is less than 4.3 eV. The difference in threshold energies can be explained in several ways. There can be a barrier in the potential energy surface. The reaction may go at the thermodynamic threshold but requires that much of the energy be put into vibrational energy, or our extrapolation to obtain the threshold is in error. For example, the cross section might actually have a slowly rising exponential dependence at the threshold rather than the linear dependence which we assume.

One obvious difference between the four isomers is the number of β hydrogens. The tert isomer has nine, the sec five, the normal two, and the iso only one. While one would not expect that the cross section would be exactly proportional to the number of hydrogens, one would expect to see a trend, and this is exactly what is observed. The scaling factor for the cross sections plotted in the figure are *tert*-butyl 1.00, *sec*-butyl 0.44, *n*-butyl 0.31, and isobutyl 0.31. The relative cross section is given by the ordinate of the figure times the scaling factor. All four systems were run back to back while attempting to hold the experimental conditions constant. However, there is inevitably some drift in beam intensity and detector sensitivity. We estimate an error of 20% in the ratios.

Finally, we must exclude other possible processes which could give the same products. It is well-known that *tert*-butyl iodide decomposes at high temperatures to give isobutene and HI.[5] HI can then react with TDMAE to give the protonated amine and I^-. Indeed, we can clearly see this happening. As the temperature of the nozzle for butyl iodide is raised above 210 °C, the product intensity rises dramatically, the threshold energy drops from 7.9 to 3.9 eV, and we see cations produced by the elimination of $(CH_3)_2NH$ from the protonated amine. Evidently, the reaction of HI with TDMAE has a larger cross section than the reaction of butyl iodide, it has a lower threshold, and it produces a cation with appreciable vibrational energy. The behavior at high temperature is so different from that at low temperature that two very different reactions must be taking place. Furthermore, the other isomers of butyl iodide are very much more stable, yet they react readily with TDMAE at low temperatures.

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(5) Tsang, W. *J. Chem. Phys.* **1964**, *41*, 2487. Benson, S. W.; Amano, A. *J. Chem. Phys.* **1962**, *37*, 197. Bose, A. N.; Benson, S. W. *J. Chem. Phys.* **1963**, *38*, 878.

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