The Kinamycins are Diazofluorenes and Not Cyanocarbazoles

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Kinamycins A, B, C_1 and D, **1a–d**, isolated from *Streptomyces murayamaensis*, possess modest antitumor properties and antibiotic activity against Gram-positive organisms.[1,2] More recently, other structural variants, differing in the nature of the substituents R_1–R_4, have been reported.[3–5] The unique structural features assigned to these natural products such as the cyanamide group and the benzo[*b*]carbazole ring system have stimulated considerable interest in the biosynthesis[6,7] and the chemical synthesis[8–10] of the kinamycins. Biosynthetic studies have established that these natural products are derived from a polyketide precursor and that dehydrorabelomycin, **4**, is an intermediate. Phenanthroviridin aglycon, **5**, and prekinamycin, **7a**, have also been isolated from *S. murayamaensis* and have been proposed as intermediates between **4** and **1**. A mechanism consistent with the hypothetical biosynthetic pathway (**4** → **5** → **7a** → **1**) has been proposed to explain the formation of kinafluorenone, **6**, by a mutant strain blocked in kinamycin biosynthesis.[7] Disclosed herein is a regioselective synthesis of **7c** which incorporates the full ABCD ring system proposed for prekinamycin, **7a**, and evidence to prove that the kinamycins are derivatives of the 5-diazobenzo[*b*]fluorene ring system, **3**, rather than the 5-cyanobenzo[*b*]carbazole system, **1**.

Our synthesis of **7c**, based on previous model studies,[8,9] is shown in Scheme 1. That the key cycloaddition step (**17** → **18**) occurred with the desired regioselectivity was established by an X-ray diffraction study on the crystalline diasteromeric mixture of hydroquinones **19**.[11] The overall yield of **7c** from *o*-anisidine was 21%.

Spectroscopic analysis of **7c**,[12] however, clearly indicates that the compound prepared in this sequence is not a derivative of prekinamycin.[13] In the case of prekinamycin diacetate, a ^{13}C NMR signal at 83.7 ppm and an IR band at 2119 cm^{-1} were assigned to the cyanamide group and ^1H NMR signals at 6.84 and 6.95 ppm were assigned to the ring D protons of structure

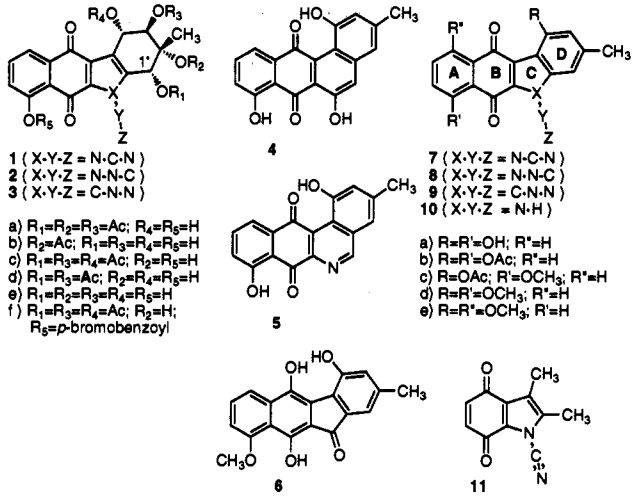
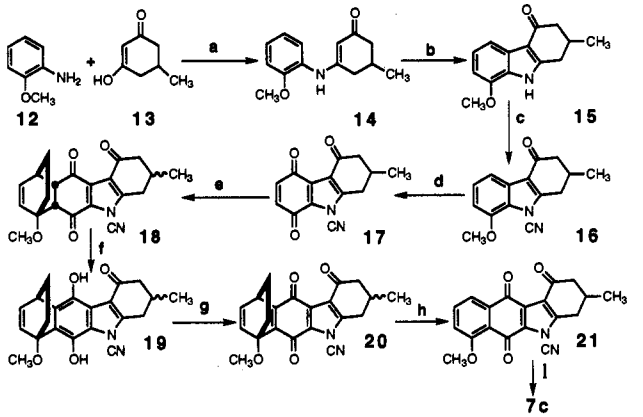


Figure 1.

Scheme 1[a]



[a] (a) *p*-TSA, $PhCH_3$, reflux, 6 h, 86%. (b) $Pd(OAc)_2$ (1.1 equiv), HOAc, reflux, 7 h, 64%. (c) Et_3N, PhOCN, DMSO, 23 °C, 12 h, 94%. (d) $Ce(NH_4)_2(NO_3)_6$, H_2O/CH_3CN, 23 °C, 12 h, 79%. (e) 1-methoxy-1,3-cyclohexadiene, DMF, 23 °C, 12 h, 89%. (f) $CDCl_3$ (acidic), CH_2Cl_2, 23 °C, 3 h, 100%. (g) DDQ, CH_3COCH_3/H_2O (20:1), −78 °C, 93%. (h) $PhCH_3$, reflux, 3.5 h, 94%. (i) DDQ, concentrated H_2SO_4, $H_2C{=}C(OAc)CH_3$, 23 °C, 14 h, 61%.

7b.[3] The cyano group of **7c**, on the other hand, gives rise to a ^{13}C NMR signal at 105.0 ppm and an IR band at 2253 cm^{-1}. The D ring ^1H NMR signals of **7c** appear at 7.03 and 7.47 ppm. In addition, comparison of the spectroscopic characteristics of all of the *N*-cyanoindole derivatives prepared in this laboratory with those of the kinamycins clearly reveals that the assignment of a cyanamide group to any of these natural products is untenable. For each of the 22 *N*-cyanoindole derivatives prepared unambiguously in this laboratory, the *N*-cyano group gives rise to an IR band in the 2237–2259-cm^{-1} range whereas the IR band assigned as a cyano stretch in each of the kinamycins is found in the 2119–2170-cm^{-1} range. Furthermore, each of our *N*-cyanoindole derivatives exhibits a ^{13}C NMR signal in the 104-109-ppm range whereas the ^{13}C NMR signal, previously assigned to the *N*-cyano carbon, has been reported at 78.5 ppm for kinamycin D[6] and 83.7 ppm for prekinamycin diacetate.[3]

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(11) For full details, see supplementary material.
(12) The connectivities in **11**[8] and **7c** was confirmed by $^1H/^{13}C$ (and, in the case of **11**, $^1H/^{15}N$) HMQC experiments (Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565).

(13) Echevarren *et al.*[10d] have recently reported that **7a**, prepared in a nonregioselective synthesis, was not identical to prekinamycin. It is our opinion, however, that the structural assignments in that report must be viewed with caution. The rearrangement reported to accompany the synthesis of the benzo[*b*]carbazole ring system is unprecedented. In addition, the unusual report that **10d** (but not the regioisomer **10e**) rearranges to an *o*-quinone upon exposure to nonaqueous base is at odds with out observation that **10c**, a byproduct of the aromatization of **21**, can be smoothly N-cyanated to give **7c** under basic conditions. Furthermore, the *N*-cyano ^{13}C NMR signals are reported[10d] to be in the 115–123-ppm range as compared with 104–109 ppm observed in our work for *N*-cyanoindoles with unambiguously established structures.

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Particularly convincing additional evidence to indicate that the kinamycins are not cyanamides is found in a comparison of the ^{15}N chemical shifts of the simple model **11**[12] with those reported for ^{15}N-enriched kinamycin D.[6] Whereas the ring and cyano nitrogens of **11** give rise to ^{15}N signals at 124 and 224 ppm, respectively, the signals assigned to the corresponding nitrogen atoms of kinamycin D are at 241.6 and 344.5 ppm.

The assignment of structures to prekinamycin[7] and to other newer members of the kinamycin family[3-5] has relied on spectroscopic comparisons with kinamycins A, B, C, and D for which the structures have been assumed to have been established unambiguously as **1a–d** by chemical and spectroscopic analysis[1] as well as by an X-ray diffraction study on the *p*-bromobenzoate of kinamycin C, **1f**.[2] Scrutiny of the original structural report,[2] however, reveals that the crystallographic data was employed to establish all of the connectivities shown in structure **1f** *except for the linear three-atom fragment X–Y–Z*. The quality of the data did not allow Furusaki *et al.*[2] to assign the final three atoms, two nitrogens and one carbon, unambiguously. The decision to assign a cyanamide group to the fragment X–Y–Z was based on chemical evidence. The fact that the hydrolysate obtained from **1e** gave a positive Nessler's reagent test for ammonia and a negative chromotropic acid test for formic acid was considered to be consistent with the assumption that the kinamycins were cyanamides **1** and not isocyanamides **2**.[1]

Our conclusion that the kinamycins are not cyanamides necessitates a reevaluation of the structural possibilities. Since long-range $^{1}H/^{13}C$ HETCOR experiments on kinamycin D[14] confirm the connectivities deduced on the basis of the crystallographic data for **1f**,[2] only the nature of the X–Y–Z group requires reconsideration. As a result, the only structural possibilities which remain are **2** and **3**. The fact that the ^{13}C NMR signal assignable to the X–Y–Z group (78.5–87.3 ppm) is well outside the range characteristic of isocyanide carbons (150–170 ppm)[15] excludes the isocyanamide possibility **2**. Thus the kinamycins must be represented by structure **3**, and prekinamycin must be **9a**.[16,17]

The validity of this proposal is confirmed by a comparison of the spectroscopic properties reported for the kinamycins with those of selected model compounds which incorporate a diazo group[18] (e.g., 9-diazofluorene, 1,4-benzoquinone 1-diazide, ethyl diazoacetate, diazocyclopentadiene, diazotetracyanocyclopentadiene, and the recently isolated natural diazo compound lagunamycin[19]). The ^{13}C NMR signals of the diazo carbon atoms of such compounds fall in a range (63–93 ppm) which encompasses the range observed for the kinamycins (78.5–83.7 ppm). The IR values for the kinamycins (2119–2159 cm^{-1}) fall in the range observed for the diazo bands of the same set of model compounds (2065–2200 cm^{-1}). Furthermore, while the ^{15}N NMR signals for kinamycin D are far removed from those observed for the cyanamide **11**, the signals at 241.6 and 344.6 ppm agree well with those expected for the internal (227–287 ppm) and terminal (332–441 ppm) nitrogens of a diazo compound.[20] In addition, the coupling constants observed for doubly ^{15}N labeled kinamycin D (J_{N-N} = 3.4 Hz, $^{1}J_{C-N}$ = 21.2 Hz, $^{2}J_{C-N}$ = 5.4 Hz) agree well with those observed for doubly ^{15}N labeled ethyl diazoacetate (J_{N-N} = 5.7 Hz, $^{1}J_{C-N}$ = 20.4 Hz, $^{2}J_{C-N}$ = 3.7 Hz).[20] Carbon–nitrogen bond lengths in diazo derivatives determined by X-ray crystallography are typically in the 1.31–1.37-Å range while those for the N–N bond are typically in the 1.10–1.13-Å range,[21] in good agreement with the values (1.323 and 1.082 Å, respectively) obtained in the crystallographic analysis of **1f**.[2] It is also worth noting that a major fragmentation pathway in the published mass spectrum of prekinamycin diacetate[3b] appears to involve the loss of 28 mass units as expected for a diazo compound.[18]

This structural revision raises important questions concerning the biosynthesis of the kinamycins from the established intermediate **4**. The suspected intermediacy of **5** is no longer plausible, and the recent isolation of kinafluorenone,[7] **6**, takes on added significance in this context. The novelty of the revised structures makes the kinamycins worthy subjects for renewed synthetic and biosynthetic studies as well as explorations of the mode of action[22] of these unusual antitumor antibiotics.

Acknowledgment. Professor S. J. Gould is thanked for very graciously delaying publication of his independent structural conclusions to allow for simultaneous disclosure of our results. Dr. S. Mooibroeck is thanked for her creative NMR experiments, and Professors D. Mackay and R. Rodrigo are thanked for helpful discussions. This work was supported by a University–Industry Collaborative Research and Development grant from the Natural Sciences and Engineering Research Council of Canada and Uniroyal Chemical Ltd. Research Laboratories, Guelph, Ontario.

Supplementary Material Available: Complete X-ray crystallographic data for **19** and complete experimental details for Scheme 1 (15 pages); listing of observed and calculated structure factors for **19** (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(16) We have found that the Nessler's reagent tests for dilute solutions of hydrazine or ammonia are qualitatively indistinguishable. Thus, the color test given by the hydrolysate from **3e**[1] may result from hydrazine generated from an azine byproduct commonly observed in solvolyses of diazo compounds.[18]

(17) Since submission of this manuscript we have been informed by Professor S. J. Gould that his group has independently established the diazofluorene structure for the kinamycins by an X-ray crystallographic study of the (*S*)-2-methylbutyric acid ester of kinamycin D: Gould, S. J.; Tamayo, N.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.*, preceding paper in this issue.

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(22) For a suggested mode of action for the kinamycins based on structures **1a–d**, see: Moore, H. W. *Science* **1977**, *197*, 527.