ORGANIC LETTERS

2008
Vol. 10, No. 3
381−384

A Biogenetically-Inspired Synthesis of a Ring-D Model of Kinamycin F: Insights into the Conformation of Ring D

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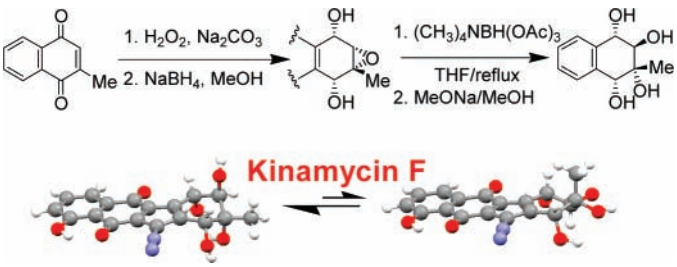
Received November 1, 2007

ABSTRACT



An efficient three-step construction of the highly oxygenated D-ring of the kinamycin antibiotics is reported for a simple model system. A comparison of the spectroscopic characteristics of the synthetic models with those of natural kinamycin F, which is suspected to be the bioactive form of the kinamycins, leads to the conclusion that the favored D-ring conformation of kinamycin F differs from that of the other partially or fully acylated variants.

The kinamycin antibiotics, first isolated from *Streptomyces murayamaensis*, were originally believed to be *N*-cyanobenzo[*b*]carbazole derivatives **1** (Figure 1).[1] Later crystallographic analysis by Gould and co-workers[2] and synthetic and spectroscopic studies in this laboratory[3] led to the discovery that these natural products were highly unusual diazobenzo[*b*]fluorene derivatives **2** and, in the particular case of isoprekinamycin, a diazobenzo[*a*]fluorene **3**.[4] More recently, He and co-workers have isolated a dimeric variant of the kinamycins in the form of lomaiviticin A (**5**) from *Micromonospora lomaivitiensis*[5] and have demonstrated very potent cytotoxicity against a broad range of tumor cell lines and antibacterial activity against Gram-positive bacteria. Interest in these natural products has been inspired by their structural novelty which has raised questions concerning

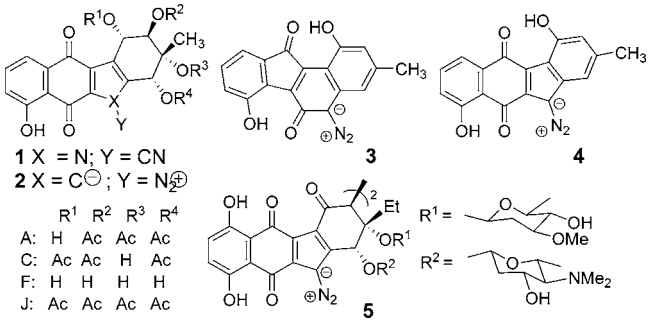


Figure 1. Structures of some kinamycins (**2**), isoprekinamycin (**3**), prekinamycin **4**, and lomaiviticin A **5**.

[†] Deceased November 2006.

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10.1021/ol702650u CCC: $40.75
Published on Web 01/10/2008

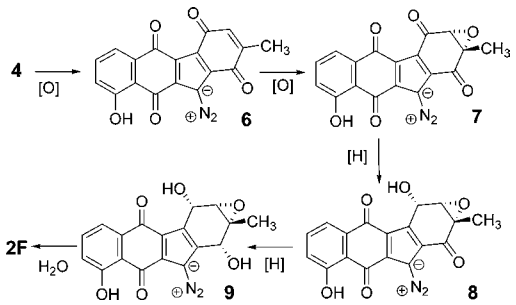
biosynthesis[6] and the possibility that the structural novelty may be indicative of a novel biological mode-of-action.[7−12]

Two syntheses of prekinamycin[13,14] have now been recorded, and our group has recently completed the first total synthesis of isoprekinamycin.[15] Lei and Porco[16] have recently reported an enantioselective and the first total synthesis of kinamycin C. The issue of constructing the highly oxygenated D-ring of the kinamycins has also been explored by Kumamoto and co-workers leading to an ingenious construction of racemic kinamycin C *O*-methyl ether[17] and, most recently, by Nicolaou and co-workers who have devised another asymmetric synthesis of kinamycin C.[18] Progress in model studies toward the synthesis of the lomaiviticins has also been reported.[19]

In this laboratory, we have been exploring the possibility that the problem of stereo- and regioselective construction of the kinamycin D-ring might be overcome in a relatively simple way by means of a synthesis that mimics, in part, the currently accepted biogenetic pathway. The isolation of prekinamycin **4**,[20] ketoanhydrokinamycin **8**,[21] and the *O*-acyl derivatives of the epoxy diol **9**[22] from *Streptomyces* species supports the biogenetic pathway shown below (Scheme 1).

We report a model study for stereoselective elaboration of the D-ring in three steps from an appropriate *p*-quinone intermediate analogous to the biogenetic intermediate **6** and spectroscopic and computational evidence for an unusual D-ring conformation for kinamycin F which possesses the most breadth of antibacterial activity of the kinamycin family[1] and which may be generated from the other kinamycins by esterase action in vivo to function as the bioactive growth inhibitory form of these anticancer agents.[11,12]

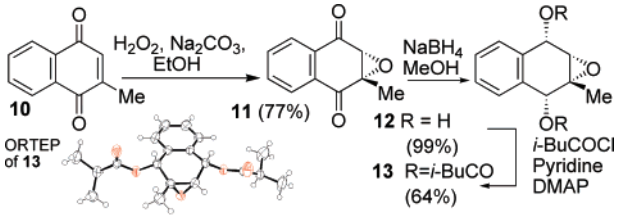
Scheme 1. Possible Biosynthetic Pathway to the D-Ring of the Kinamycin Antibiotics in *Streptomyces*[6]



Epoxidation of the simple model 2-methyl-1,4-naphthoquinone (**10**) with hydrogen peroxide under basic conditions afforded the corresponding epoxy diketone **11**. Although various strategies for reduction of the diketone **11** with the desired stereoselectivity were envisaged, in practice, direct reduction with $NaBH_4$ provided the epoxy diol **12** with very high diastereoselectivity.

The stereochemistry of the epoxy diol was established unambiguously by a single-crystal X-ray diffraction study on the bis-isobutyryl ester **13** (inset in Scheme 2). We believe

Scheme 2. Synthesis of a Model of the Epoxy Diol **12**



that solvation of the epoxide, by means of H-bonding interactions with the alcohol solvent, introduces a large steric barrier for approach of the nucleophilic reducing agent syn to the epoxide oxygen of **11** and provides the observed stereochemical outcome.

After numerous unsuccessful attempts at nucleophilic ring opening of the epoxy diol **12** to mimic the putative biosynthetic conversion of epoxide **9** to kinamycin F (**2F**), it was found that the desired stereo- and regioselective ring opening of the epoxide can be achieved in good yield by using $Me_4NBH(OAc)_3$ in refluxing THF (Scheme 3). Honda and Mizutani had previously used this reagent to induce ring opening of epoxy alcohols but not of epoxy diols.[23] In the case of 2,3-epoxycyclohexanols, it has been shown that this reaction succeeds only if the alcohol and epoxide are syn and that ring opening occurs by nucleophilic attack at C3. This has led to the suggestion that the epoxide ring-opening induced by $Me_4NBH(OAc)_3$ occurs by a mechanism analogous to that proposed by Caron and Sharpless for ring-

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(7) Kinamycin C (NCS138425) exhibits potent cytotoxicity against the 60 cancer cell line panel at the National Cancer Institute, and the spectrum of activity is unlike that of any known anticancer agents.

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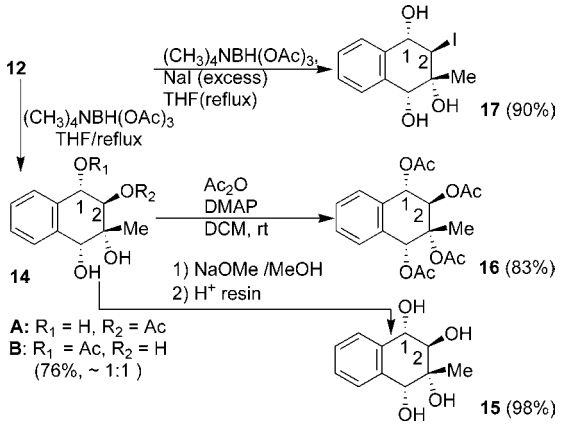
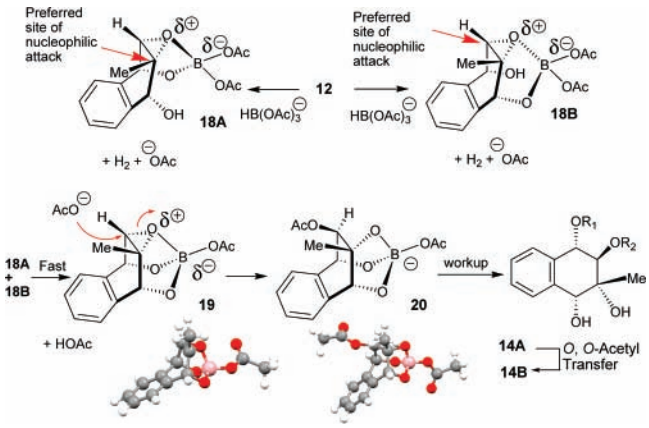
Scheme 3. Ring Opening of the Epoxy Diol **12**



opening of epoxy alcohols with Ti(O-*i*-Pr)$_4$.[24] Essentially, the Lewis acid is thought to react initially with the alcohol and then to provide a Lewis acid, tethered to the alcohol oxygen atom, to induce the epoxide ring opening.

In the case of our epoxy diol **12**, each alcohol group might react to form a distinct adduct with the reagent (Scheme 4).

Scheme 4. Likely Mechanism of Epoxide Ring Opening



The literature precedent would predict ring opening with rupture of the epoxide C−O bond at C3 for adduct **18A** but with rupture of the C−O bond at C2 for adduct **18B**. Since the accessibility of the reagent to the two alcohol groups in **12** is expected to be comparable, the high degree of regioselectivity observed in the present study is somewhat surprising.

We suspect that the initial adducts, **18A** and **18B**, do not undergo epoxide ring opening directly but, instead react further to form the same cyclic complex **19** in which the boron serves as a Lewis acid intramolecularly to assist with epoxide ring opening by the nucleophile by backside attack at the sterically less crowded site to give **20**. Aqueous workup then provides **14A**, initially, which equilibrates with **14B** (Scheme 4). Models of **19** and **20** generated by ab initio molecular orbital calculations (RHF 6-31G//6-31G) are shown below.[25,26]

The structures of the ring-opened products **14A** and **14B** were assigned on the basis of extensive NMR spectroscopic analysis (^{1}H, ^{13}C, HMQC, HMBC, difference NOE) analysis.[25] As expected, peracetylation of the mixture yielded a single tetra-acetate **16**, and deacetylation under Zemplen conditions[27] provided a single tetrol **15**.

It was also found that in the presence of excess NaI, the stereo- and regioselective ring opening of the epoxy diol **12**, assisted by $Me_4NBH(OAc)_3$ in refluxing THF, provided the iodohydrin **17** as the only product.[28,29]

The ^{1}H NMR spectra of the acetates **14A** and **14B** and the tetraacetate **16** reveal that the observed coupling constants ($J_{1,2}$ = 6.8−8.3 Hz in DMSO-d_6) are consistent with the predominance, in each case, of a half-chair conformation in which the C1 and C2 hydroxy or acetoxy groups are in pseudoequatorial orientations. The comparable magnitude of $J_{1,2}$ reported for kinamycins A, C, D, E, and J is likewise consistent with this conformational preference for the D-ring in these natural products.[30] X-ray crystallographic studies of the C7-*O*-*p*-bromobenzoate derivative of kinamycin C[31] and of the C1-O-*S*-2-methylbutyrate derivative of kinamycin D[2] suggest that this conformational preference persists in the solid state.

It was also observed that $J_{1,2}$ for the tetrol **15** (6.9 Hz in DMSO-d_6) was consistent with a similar conformational preference. It was surprising to find, however, that $J_{1,2}$ for the fully deacylated version of the kinamycins, kinamycin F, in DMSO-d_6 was just 2.7 Hz.[32] The conclusion that kinamycin F exhibits a different conformational preference for its D-ring as compared with the other known kinamycins is especially interesting given the recent suggestion that kinamcyin F may represent the bioactive form of the other kinamcyins and prompted us to probe this phenomenon in more detail. We have now found that the conclusion, based on the ^{1}H NMR analysis, that kinamycin F, unlike the model **15** and other kinamcyins, has a preference for a half-chair conformation **A** (Figure 2) where the C1-OH and C2-OH groups are in pseudoaxial orientations is supported by ab initio molecular orbital calculations.

In particular, ab initio molecular orbital calculations predict that the conformer **A** is favored by 0.4 kcal/mol in the gas

(24) Caron, M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1560−1563.

(25) See the Supporting Information for details.

(26) For examples of facile *O*,*O*-acyl migrations, see: Akira, K.; Taira, T.; Hasegawa, H.; Sakuma, C.; Shinohara, Y. *Drug Metab. Dispos.* **1998**, *26*, 457−464.

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(28) Since reagents exist for the dimerization of iodocyclohexanes, 2-iodocyclohexanones and related systems (ref 29), the possibility that related iodohydrins might be converted into dimeric analogues of the kinamycins related to the lomaiviticins is worthy of consideration.

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(30) For a compilation of coupling constants reported for all known kinamycins, see the Supporting Information.

(31) Furusaki, A.; Matsui, M.; Watanabe, T.; Omura, S.; Nakagawa, A.; Hata, T. *Isr. J. Chem.* **1972**, *10*, 173.

(32) The literature value is J = 3.4 Hz (ref 1).

Figure 2. Calculated energy minimum conformations of kinamycin F.

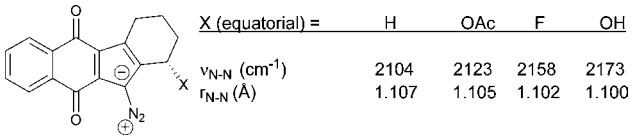

Figure 3. Calculated (RHF 6-31G//6-31G) diazo group stretching frequencies and N−N bond lengths.[25]

phase (Figure 2).[33] On the other hand, a similar computation for kinamycin E[25] reveals a substantial preference of the **B**-type conformation (favored by 4.8 kcal/mol) in parallel with the experimental data for this and other kinamycins but contrasting with the conformational preference described above for kinamycin F.

Furthermore, it appears that there is some influence of the conformational preferences for ring D of kinamycin F on the diazonium ion character of the diazo group. Experimentally, we have found that the diazo stretch in the solution IR spectrum is observed as two bands in DMSO solution at 2143 and 2165 cm^{-1} ($\Delta\nu = 22$ cm^{-1}), consistent with the existence of two conformers. Ab initio molecular orbital calculations overestimate the absolute values for the diazo stretching frequencies for the two conformers but the predicted $\Delta\nu =$ 24 cm^{-1}, with the higher stretching frequency being associated with conformer **A**, agrees well with the difference between the two solution IR bands observed for the diazo group of kinamycin F.

These observations suggest that some specific interaction of the diazo group, which is absent in the model tetrol **15**, might be responsible for the preference for the equatorial orientation of the C4-OH group in kinamycin F. Further ab initio molecular orbital calculations on simpler models (Figure 3) suggest that the effect on diazonium ion character may be a consequence of a favorable through-space interaction of the C−O bond dipole with the oppositely polarized C−N bond dipole of the diazo group which is enhanced when the bond dipoles are aligned.

In summary, this model study establishes an efficient method for constructing the highly oxygenated D-ring of the kinamycins in three steps from a *p*-quinone starting material. In addition, potential interactions of the C-4 substituent which might influence both the conformation of the D-ring and the reactivity of the diazo group have been identified and suggest that the electrophilicity and the electron affinity of the diazo group in the kinamycins might be fine-tuned by appropriate choices of C-4 substituent in synthetic analogues. Efforts to construct such kinamycin congeners employing extensions of the model reactions described above are in progress.[34]

Acknowledgment. This manuscript is dedicated to the memory of Dr. Nicholas Justin Taylor. The authors thank NSERC for support of this research by means of a Discovery Grant (to G.I.D.).

Supporting Information Available: Synthetic procedures, spectroscopic data, CIF for **13**, and computational experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

OL702650U

(33) Calculations employing the PCM solvation model indicate that the preference for this conformer increases substantially with increasing solvent polarity (3.4, 5.3, and 12.4 kcal/mol for $CHCl_3$, acetone, and DMSO respectively) (ref 25).

(34) Our previous studies of the reactivity of the kinamycins suggest that an intermediate such as **6** would not survive the redox chemistry developed herein for ring D elaboration (refs 4 and 8). The key to the success of this strategy to kinamycin synthesis is the design of a precursor which is compatible with the conditions for ring D elaboration while also being poised for conversion into the delicate diazoquinone functionality that characterizes these interesting natural products.