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COMMUNICATIONS

Published on Web 02/08/2002

Diazo Group Electrophilicity in Kinamycins and Lomaiviticin A: Potential Insights into the Molecular Mechanism of Antibacterial and Antitumor Activity

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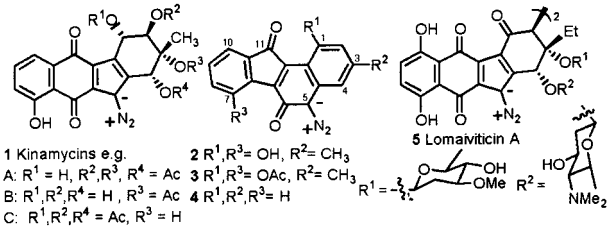
Received August 7, 2001

Microbial secondary metabolism has yielded a wealth of compounds with unusual structures and novel modes of action as antimicrobial and anticancer agents.[1] In this context, the structural novelty of the diazobenzo[*b*]fluorene ring system assigned to the kinamycins, **1**,[2] and the diazobenzo[*a*]fluorene structure assigned to isoprekinamycin, **2**,[3] is of considerable interest. This interest is heightened by the very recent report of the related lomaiviticin A, **5**, which exhibits very potent activity against a broad spectrum of cancer cell lines and against Gram positive bacteria.[4]

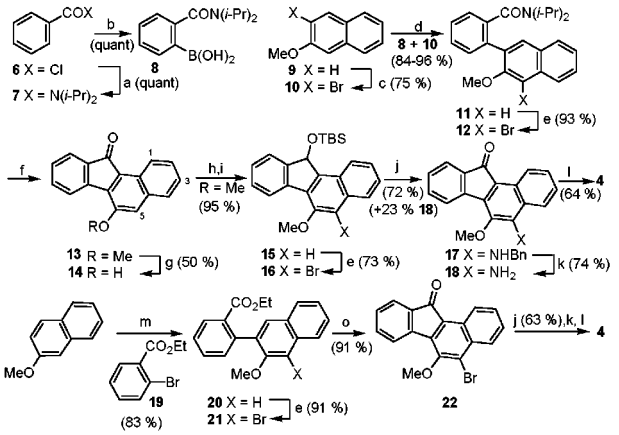


Although much is known about the biosynthesis of the kinamycins,[5] surprisingly little information exists concerning the chemical basis for their biological activity. It has been suggested that the diazo group in the kinamycins may serve as a precursor for carbene intermediates in vivo and experiments which demonstrate thermally[6] and photochemically[7] induced cleavage of DNA by 9-diazofluorenes, and heterocyclic analogues in the presence of metal ions (e.g. Cu^{2+}) in vitro have been presented as models for a possible mode-of-action for these antitumor antibiotics. More recently, DNA cleavage by **5** under reductive conditions has been observed but the details of these studies have yet to be disclosed.[4]

We report herein an enhanced reactivity of the diazo group in these natural products that may play a role in their antitumor and antibacterial activities. In particular, we disclose the synthesis of **4** and a comparison of its susceptibility to nucleophilic attack with that of isoprekinamycin, **2**, which indicates that this natural product is rendered more diazonium ion-like by virtue of the intramolecular H-bonding network present. Furthermore, we report the results of ab initio molecular orbital calculations which support not only the notion that **2** might act in vivo as a diazonium-like species but also that related and still greater diazonium ion-like character should be expected for the kinamycins, **1**, and lomaiviticin A, **5**.

Our synthetic strategy to the diazobenzo[*a*]fluorene **4**[8] was based on a Suzuki coupling.[9,10] Pd-catalyzed coupling of boronic acid **8** and aryl bromide **10** afforded the biaryl **11**. Anionic cyclization of **11** and demethylation gave **14**. Introduction of the diazo group proved to be problematic. Neither diazo transfer nor bromination could be effected at C-5 of **14**, presumably because of the electron-withdrawing effect of the keto group. Borohydride reduction of **13** followed by silylation yielded **15**, which was brominated smoothly at C-5 with NBS/DMF. Palladium-catalyzed amination of **16** with

Scheme 1 [a]



[a] (a) HN(*i*-Pr)$_2$, Et_2O, rt. (b) (1) *s*-BuLi, TMEDA, THF, −78 °C. (2) $B(OMe)_3$, −78 °C → rt. (c) (1) *n*-BuLi, THF, rt. (2) $BrCH_2CH_2Br$, −78 °C → rt. (d) $Pd(PPh_3)_4$, 2 M aq Na_2CO_3, DME, ⇵. (e) NBS, DMF, rt. (f) LDA, THF, 0 °C → rt (85% X = H or 49% X = Br). (g) 48% HBr, AcOH, ⇵. (h) $NaBH_4$, MeOH, MeONa, 0 °C → rt. (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C → rt. (j) $Pd_2(dba)_3$•$CHCl_3$, BINAP, PhMe, *t*-BuOK, $BnNH_2$, 80 °C. (k) 10% Pd−C, H_2 (1atm), AcOH, rt. (l) (1) $NaNO_2$, HCl, EtOH, 0−5 °C. (2) $NaHCO_3$. (m) (1) *n*-BuLi, THF, rt. (2) $B(OMe)_3$ −78 °C → rt. (3) **19** then step (d). (o) CH_3SO_3H, 65 °C.

benzylamine[11] also resulted in desilylation and oxidation to give ketone **17**. Hydrogenolysis gave **18**, which underwent diazotization and demethylation with HNO_2 to form **4**.

The problem arising from the poor nucleophilicity of the benzo[*a*]fluorenone ring was solved better by electrophilic substitution prior to cyclization. The amide **11** was brominated smoothly at C-5 (NBS/DMF), but the strong base used for anionic cyclization caused debromination of **12**. Thus, the Suzuki cross coupling strategy was modified to allow us to produce a brominated biaryl system suitable for cyclization under Friedel−Crafts rather than anionic conditions. Reaction of *o*-deprotonated 2-methoxynaphthalene with trimethyl borate gave the corresponding dimethyl aryl boronate, which reacted smoothly with ethyl 2-bromobenzoate in situ under Keay's modified Suzuki conditions[12] to give the biaryl **20**. Bromination with NBS followed by cyclization in methanesulfonic acid gave the brominated benzo[*a*]fluorenenone **22** in 91% yield. Amination produced **17**, which was converted into **4** as before.

With the model **4** in hand, we explored its properties in comparison with those of isoprekinamycin, **2**, in the hope that insights into the chemical properties of **2**, of importance to the mode-of-action of these diazo natural products, would emerge.

The available data concerning the biological activity of simpler *o*- and *p*-quinodiazides[13] and the related aryldiazonium ions[14,15] suggests that such compounds elicit some of their biological effects by chemical modification of nucleic acids by mechanisms involving aryl radicals. In the case of aryl diazonium ions, nucleophilic attack

10.1021/ja0167809 CCC: $22.00

at the terminal nitrogen of the diazonium group is an obligatory step prior to radical formation by loss of N_2.[16]

$$Ar{-}N_2^{+} + :Nu^{-} \rightleftarrows [Ar{-}N{=}N{-}Nu] \rightarrow Ar^{\bullet} + N_2 + {}^{\bullet}Nu$$

In the reactions of aryldiazonium ions with DNA, radical formation is preceded by nucleophilic attack by the C-2 amino group of guanine residues and by the C-6 amino group of adenine to form labile triazenes. Such intermediates, which are isolable in some cases,[17] decompose with loss of N_2 to form aryl radicals which combine with the purine ring radical to form C-8 aryl purines and also react in other ways with the initially modified DNA molecule to form depurinated sites as well as to cleave the phosphodiester backbone.

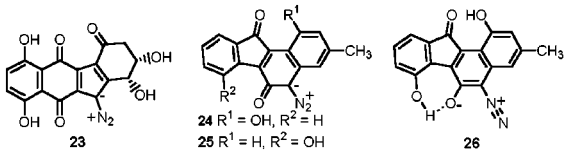


Table 1. Calculated C−N_2 Frequencies and N−N Bond Lengths

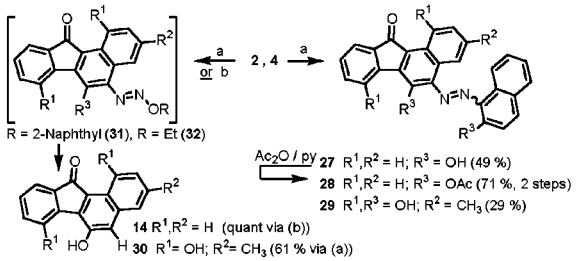
compound	calcd ν (cm^{-1})	calcd N–N (Å)
9-diazofluorene	1906	1.133
2,1-naphthoquinodiazide	2056	1.111
4	2087	1.108
24	2101	1.107
25	2125	1.105
2	2139	1.103
kinamycin B (**1**)	2188	1.099
23	2212	1.097
Ph-N≡N^{+} Cl^{-}	2212	1.100

In this context, we were intrigued by the IR stretching frequency assignable to the diazo group in the model compound **4** (2105 cm^{-1}), which was 57 cm^{-1} lower than that for isoprekinamycin (**2**) (2162 cm^{-1}). We have found that ab initio MO calculations reproduce this difference well (calculated $\Delta\nu = 52$ cm^{-1}) and reveal a shortening of the N−N bond in **2** relative to that in **4**.[18] Furthermore, the N−N bond lengths and stretching frequencies calculated for related systems indicate a trend of increasing diazonium ion character in the order 9-diazofluorene < 2,1-naphthoquinodiazide < **4** < **24** < **25** < **2** (Table 1). This trend indicates that the diazonium ion character of the diazo group in **2** is enhanced both by the keto group in ring B and by the intramolecular H-bonding network. As indicated by the higher computed C−N_2 frequency for **25** versus **24**, the diazonium ion character is influenced most strongly by the H-bond that stabilizes the partial negative charge on the quinodiazide oxygen atom (**26**). Similar calculations indicate even higher degrees of diazonium ion character in the N−N bond in the kinamycins and in the simplified model (**23**) of lomaiviticin A paralleling the trend in antitumor and antibiotic activities (**2** < **1** ≤ **5**).

Thus, we conclude that isoprekinamycin, the kinamycins, and the lomaiviticins should be activated toward attack by nucleophiles at the diazo group. To probe this predicted enhanced reactivity in the case of **2**, the model **4** and isoprekinamycin **2** were reacted with β-naphthol. At room temperature in the presence of Cs_2CO_3 as base, **4** gave the azo adduct **27** whereas **2** gave a 1:2 mixture of the adduct **29** and the hydrodediazonization product **30** (Scheme 2).[19,20]

At 0 °C, it was possible to test the relative electrophilicities of the diazo group of **2** and that of **4**. Under such conditions, complete conversion of isoprekinamycin to a mixture of **29** and **30** was observed in 9 h, whereas negligible reaction of **4** was observed after 17 h.[18,22] Since the mechanisms of both the hydrodediazoniation and azo coupling reactions involve nucleophilic attack on the diazo group, this observation is a clear indication of a much higher degree of electrophilic character for the diazo group of **2**.[23]

Scheme 2 [a,21]



[a] (a) β-naphthol, Cs_2CO_3, THF, 0 °C → rt. (b) H_2O (pH 9), EtOH, ⇵.

It is clear that much remains to be done before the modes-of-action of the kinamycins, isoprekinamycin, and lomaiviticin A are fully understood. We suggest, however, that recognition of the enhanced diazonium ion character of the diazo groups in these natural products may be a significant step toward an understanding of the molecular basis for their potent antitumor and antibacterial activity.

Acknowledgment. We thank V. Goodfellow and C. Beshara for isolating **2**, J. Venne for NMR studies, N. J. Taylor for X-ray crystallographic analysis, and NSERC for financial support.

Supporting Information Available: Synthetic procedures, and ab initio MO results (PDF) and crystallographic data for **28** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) See the Supporting Information.
(19) The structure of **27** was established by X-ray analysis of the diacetate **28**, and that of **29** by spectrometric comparison with **27** (ref 18).
(20) The greater tendency of **2** to undergo hydrodediazoniation is compatible with its higher diazonium character. Product **30** is expected to be formed via **31** (ref 16). The higher partial positive charge on the diazo group of **2** favors reaction with the harder site (O) in β-naphthoxide. Hydrodediazoniation of **4** does occur under more vigorous alkaline conditions (H_2O (pH 9), EtOH, ⇵), in the absence of β-naphthoxide, likely via **32** (ref 18).
(21) Yields for small scale reactions (2−5 mg) are only approximate (ref 18).
(22) Incubation of such solutions (48 h, rt) gave **27** (ref 18).
(23) H-Bonding also increases the electron affinities of **1**, **2**, and **5**, making them more prone to N_2 loss which might be induced by electron transfer under hypoxic conditions (ref 18).

JA0167809