

Total Synthesis of Isoprekinamycin: Structural Evidence for Enhanced Diazonium Ion Character and Growth Inhibitory Activity toward Cancer Cells

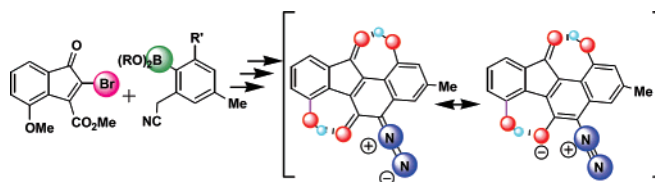
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ABSTRACT



The structurally novel diazobenzo[*a*]fluorene antibiotic isoprekinamycin (IPK) has been synthesized for the first time employing a Suzuki coupling of a brominated AB ring synthon with a boronate ester representing the D ring, followed by anionic cyclization and appropriate functional group manipulations. The first indication that the diazobenzo[*a*]fluorene system exhibits *in vitro* anticancer activity is provided and X-ray crystallographic evidence for enhancement of diazonium ion character as a consequence of intramolecular H-bonding is described.

The kinamycins, first believed to be *N*-cyanobenzo[*b*]-carbazoles **1**,¹ but now known to be diazobenzo[*b*]fluorenes **2**,^{2,3} are of current interest because of their antibacterial activity and an *in vitro* cytotoxicity profile against cancer cells suggestive of a mode of action different than that of anticancer agents in current clinical use.⁴ The discovery of the lomaiviticins **6**, which are dimeric analogues of the kinamycins possessing potent cytotoxicity against a range of cancer cell lines, has heightened interest in these unusual

natural products.⁵ A number of synthetic strategies have been disclosed,⁶ most notably a total synthesis of the biosynthetic precursor **7**,⁷ model studies toward a total synthesis of lomaiviticin B,⁸ and recent total syntheses of kinamycin C (**2C**)⁹ and its methyl ether.¹⁰

Isoprekinamycin (IPK), first assigned structure **3**,¹¹ is now recognized to be the diazobenzo[*a*]fluorene **4**.¹² The previ-

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(1) Omura, S.; Nakagawa, A.; Yamada, H.; Hata, T.; Furusaki, A. *Chem. Pharm. Bull.* **1973**, *21*, 931–940.

(2) Gould, S. J.; Tamayo, N.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 2207–2208.

(3) Mithani, S.; Weeratunga, G.; Taylor, N. J.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **1994**, *116*, 2209–2210.

(4) Hasinoff, B. B.; Wu, X.; Yalowich, J. C.; Goodfellow, V.; Laufer, R. S.; Adedayo, O.; Dmitrienko, G. I. *Anticancer Drugs* **2006**, *17*, 825–837.

(5) He, H.; Ding, W.-D.; Bernan, V. S.; Richardson, A. D.; Ireland, C. M.; Greenstein, M.; Ellestad, G. A.; Carter, G. T. *J. Am. Chem. Soc.* **2001**, *123*, 5362–5363.

(6) Gould, S. J. *Chem. Rev.* **1997**, *97*, 2499–2509.

(7) (a) Hauser, F.; Zhou, M. J. *J. Org. Chem.* **1996**, *61*, 5722–5723. (b) Birman, V. B.; Zhao, Z.; Guo, L. *Org. Lett.* **2007**, *9*, 1223–1225.

(8) Nicolaou, K. C.; Denton, R. M.; Lenzen, A.; Edmonds, D. J.; Li, A.; Milburn, R. R.; Harrison, S. T. *Angew. Chem., Int. Ed.* **2006**, *45*, 2076–2081.

(9) Lei, X.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2006**, *128*, 14790–14791.

(10) Kumamoto, T.; Kitani, Y.; Tsuchiya, H.; Yamaguchi, K.; Seki, H.; Ishikawa, T. *Tetrahedron* **2007**, *63*, 5189–5199.

(11) Seaton, P. J.; Gould, S. J. *J. Antibiot.* **1989**, *42*, 189–197.

(12) Proteau, P. J.; Li, J.; Williamson, T. R.; Gould, S. J.; Laufer, R. S.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **2000**, *122*, 8325–8326.

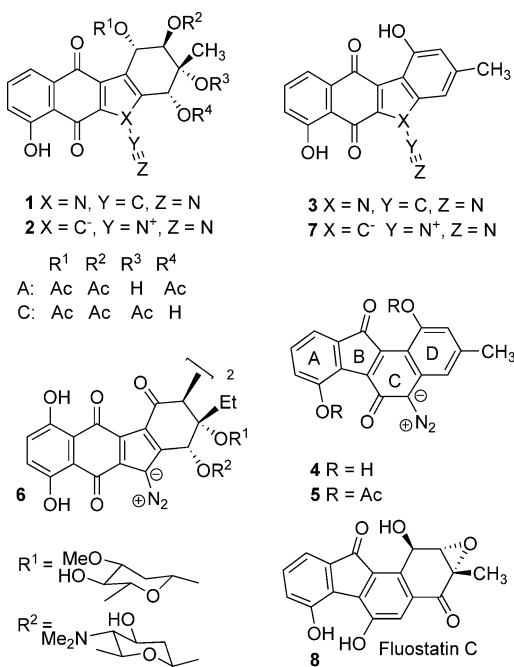
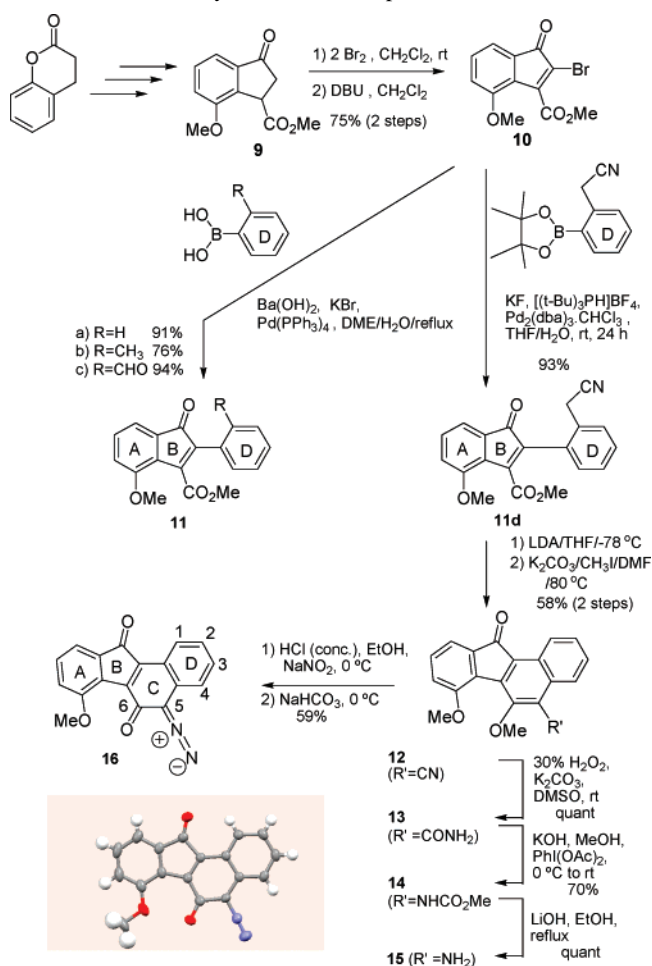


Figure 1. Structures of the kinamycins, lomaiviticin, isoprekinamycin, and fluostatins.

ously rare benzo[*a*]fluorene class of natural products now boasts several other members (e.g., **8**) with various degrees of oxygenation in ring D but lacking a diazo group.¹³ In this group, studies suggesting a substantial diazonium ion character in the diazo group of IPK, which might play a role in its bioactivity, have inspired us to design a practical synthesis of IPK.¹⁴ To date there have been no reports of the total synthesis of any of the benzo[*a*]fluorene natural products. Reported herein are the first total synthesis of IPK, structural data supporting the proposal that intramolecular H-bonding enhances its diazonium ion character, and the first indication that IPK is a potent cytotoxic agent, likely representing a variant of the same pharmacophore as the diazobenzo[*b*]-fluorenes.

Our synthetic strategy assumed that a precursor **11**, incorporating the AB and D rings of IPK, might be cyclized to the full benzo[*a*]fluorene system followed by selective functional group manipulations to provide the delicate diazo group. In practice, **10** was constructed from the commercially available dihydrocoumarin, via the known indanone **9**.¹⁵ Dibromination followed by DBU-induced dehydrobromination gave **10**.

Scheme 1. Synthesis of a Simplified Model of IPK



Although Suzuki coupling of **10** with arylboronic acids, under basic conditions with Pd(PPh₃)₄ as catalyst, gave the arylation products **11a–c**, nonbasic conditions, as developed by Fu and co-workers,¹⁶ with [(*t*-Bu)₃PH]BF₄, KF, and Pd₂(dba)₃·CHCl₃ were needed to generate **11d** in good yield by reaction of **10** with the appropriate commercially available pinacol boronate.

The hope that the potential competing intramolecular aldol reaction of deprotonated **11d** would be impeded by ring strain was realized and a good yield of the benzo[*a*]fluorene **12** was achieved via the desired Dieckman-like reaction. Cyano group hydrolysis to the amide **13** followed by a modified Hoffmann rearrangement in the presence of methanol¹⁷ provided the carbamate **14**. Saponification of **14** gave the aniline **15**, which was diazotized to provide the IPK model **16**, the structure of which was confirmed by a single-crystal X-ray diffraction study.

Adaptation of this approach to the preparation of IPK required access to an appropriate arylboronate, to serve as a D-ring synthon. The known *o*-iodobenzylic alcohol **17**^{15,18}

(13) (a) Akiyama, T.; Nakamura, K. T.; Takahashi, Y.; Naganawa, H.; Muraoka, Y.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1998**, *51*, 586–588. (b) Schneider, K.; Nicholson, G.; Ströbele, M.; Baur, S.; Niehaus, J.; Fiedler, H.-P.; Süßmuth, R. D. *J. Antibiot.* **2006**, *59*, 105–109. (c) Baur, S.; Niehaus, J.; Karagouni, A. D.; Katsifas, E. A.; Chalkou, K.; Meintanis, C.; Jones, A. L.; Goodfellow, M.; Ward, A. C.; Beil, W.; Schneider, K.; Süßmuth, R. D.; Fiedler, H.-P. *J. Antibiot.* **2006**, *59*, 293–297. (d) Fondja, C. B.; Schiebel, M.; Helmke, E.; Anke, H.; Laatsch, H. Z. *Naturforsch.* **2006**, *61b*, 320–325.

(14) Laufer, R. S.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **2002**, *124*, 1854–1855.

(15) See the Supporting Information for details.

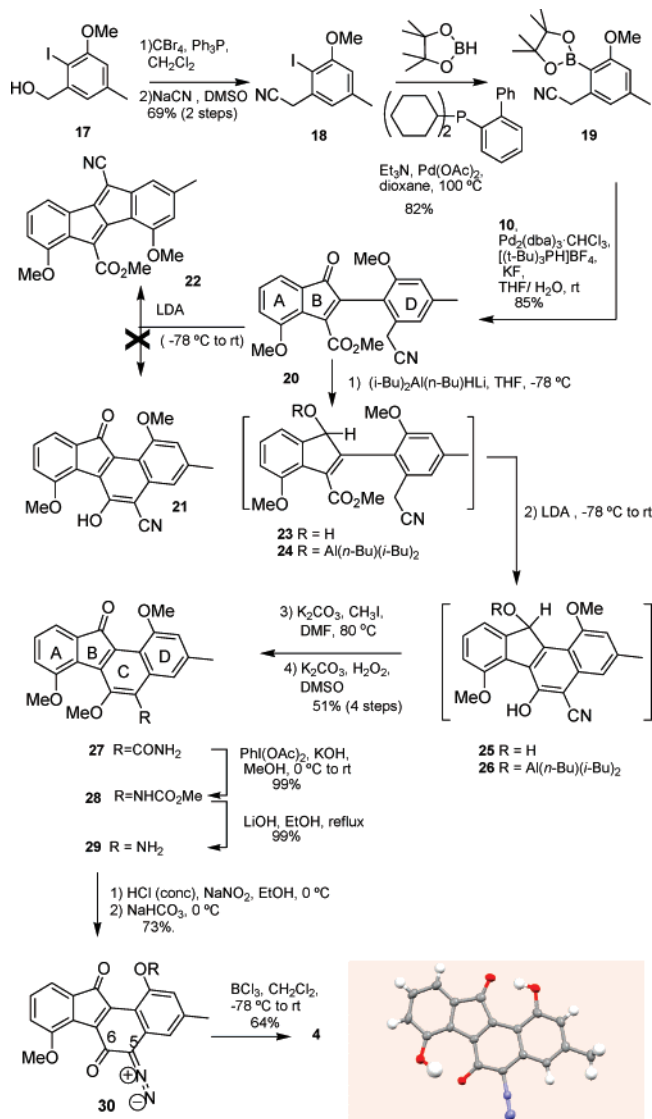
(16) Netherton, M. R.; Fu, G. C. *Org. Lett.* **2001**, *3*, 4295–4298.

(17) Moriarty, R. M.; Chany, C. J.; Vaid, R. K.; Prakash, O.; Tuldhar, S. M. *J. Org. Chem.* **1993**, *58*, 2478–2482.

(18) Qabaja, G.; Jones, G. B. *Tetrahedron Lett.* **2000**, *41*, 5317–5320.

was converted to the nitrile **18**, and then to the target boronate **19** via a palladium-induced coupling with pinacol borane.¹⁹ Suzuki coupling of **19** and **10** to give **20** proceeded well but, very disappointingly, attempted cyclization proceeded contrary to the favorable results of the model study and provided the aldol product **22** almost exclusively, clearly indicating the subtle energetics associated with the interaction of the D-ring methoxy group with the carbomethoxy group and with the keto group.

Scheme 2. Total Synthesis of Isoprekinamycin



To avoid the aldol reaction, **20** was reduced with borohydride but cyclization of **23** gave the desired Claisen product **21** and the aldol product **22** in low yields, likely because of regeneration of the keto group at the expense of degradation of the starting material.

Reduction of **20**, using a mixture of *n*-butyllithium and DIBAL at low temperature,^{20,21} gave the trialkylaluminum

24, which was treated with LDA in situ. Aqueous workup provided a mixture of the cyclized product **25** and the ketone **21**, formed by partial air oxidation. The most efficient route to amide **27** involved *O*-methylation of the mixture of **21** and **25** and hydrolysis of the cyano group to the amide with H₂O₂/K₂CO₃, which also led to oxidation to the ketone. The synthesis then paralleled that of **16** to give **30**, which upon demethylation with BCl₃ gave IPK.^{15,22}

Slow crystallization of IPK provided crystals for an X-ray diffraction study that confirms the assigned structure.¹² Comparison of structures of model **16** and IPK reveals increased diazonium ion character in IPK as evidenced by the alternating shorter N–N (1.104 versus 1.116 Å), longer C5–N (1.362 versus 1.334 Å), shorter C5–C6 (1.424 versus 1.457 Å), and longer C6–O (1.246 versus 1.227 Å) bond lengths which are entirely consistent with an increased diazonium ion contribution to the resonance hybrid representing the *o*-quinodiazide functionality in IPK relative to that **16**.¹⁴

The increased availability of IPK opens the door to more extensive biological studies. We have now found that IPK, previously shown to exhibit modest antibacterial activity,¹¹ significantly inhibits the growth of CHO (IC₅₀ = 5.8 μM) and K562 human leukemia cells (IC₅₀ = 6.4 μM).^{15,23}

Superimposition of the crystal structure or a computed model of **4** on a model of **7** reveals that the oxygen and

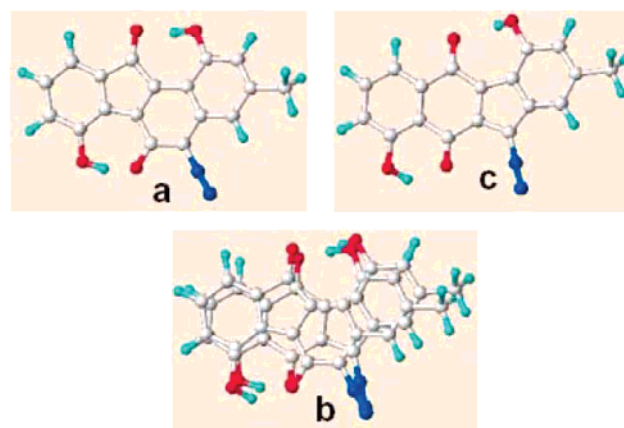


Figure 2. Structural comparison of the diazobenzo[*a*]fluorene and the diazobenzo[*b*]fluorene systems.

nitrogen atoms on the periphery align well despite the rearranged carbon skeleton, suggesting that the diazobenzo[*a*]- and diazobenzo[*b*]fluorenes are variants of the same

(20) Lithium trialkylaluminumhydrides are known to reduce ketones, but the concomitant use of the trialkylaluminum for protection of the alcohol, as in **26**, is new and offers advantages over trialkylsilyl protection when the alcohol is base-sensitive. Attempts at silylation of the alcohol **23** or its conjugate base led to oxidation and complex byproducts.

(21) Kim, S.; Ahn, K. H. *J. Org. Chem.* **1984**, *49*, 1717–1724.

(22) The synthesis of IPK is also a formal synthesis of prefluostatin (ref 13d) since we have generated it previously from natural IPK (ref 14).

(23) The clinically useful anticancer agent etoposide exhibits IC₅₀ = 1.4 and 3.4 μM, respectively, in the same assays.

(19) Baudoin, O.; Guénard, D.; Guéritte, F. *J. Org. Chem.* **2000**, *65*, 9268–9271.

pharmacophore. Consistent with this hypothesis is our finding that IPK-diacetate **5**, which is more soluble than IPK, inhibits the topoisomerase II α -catalyzed decatenation of kDNA (IC_{50} = 9.7 μ M) just as we observed recently for **2A** and **2C** (IC_{50} = 43 and 3.2 μ M, respectively).^{4,15,24}

The ease of synthesis of IPK relative to the kinamycins and lomaiviticins makes the diazobenzo[*a*]fluorene system attractive for generation of synthetic analogues. Efforts to create other benzo[*a*]fluorenes and congeners of IPK with improved solubility and drug-like properties are in progress.

Acknowledgment. Dedicated to the memory of Dr. Nicholas Justin Taylor. Taken, in part, from the M.Sc. thesis

(24) Unlike etoposide, **5** and the kinamycins inhibit topoisomerase II α but do not act as topoisomerase II poisons (ref 4).

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Supporting Information Available: Full experimental details for all reactions and bioassays and CIF files for IPK and **16**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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