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

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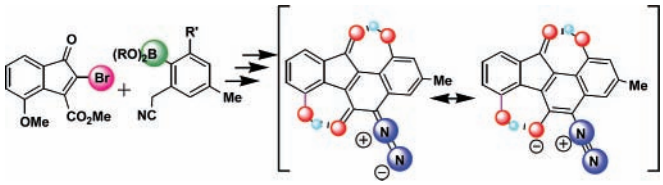
ABSTRACT



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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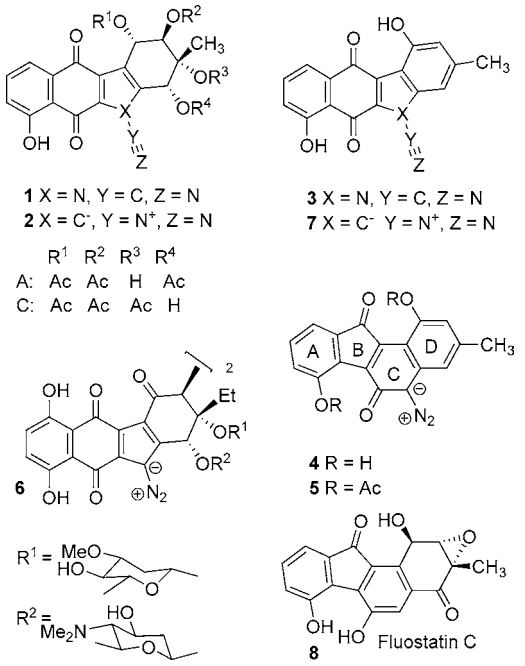
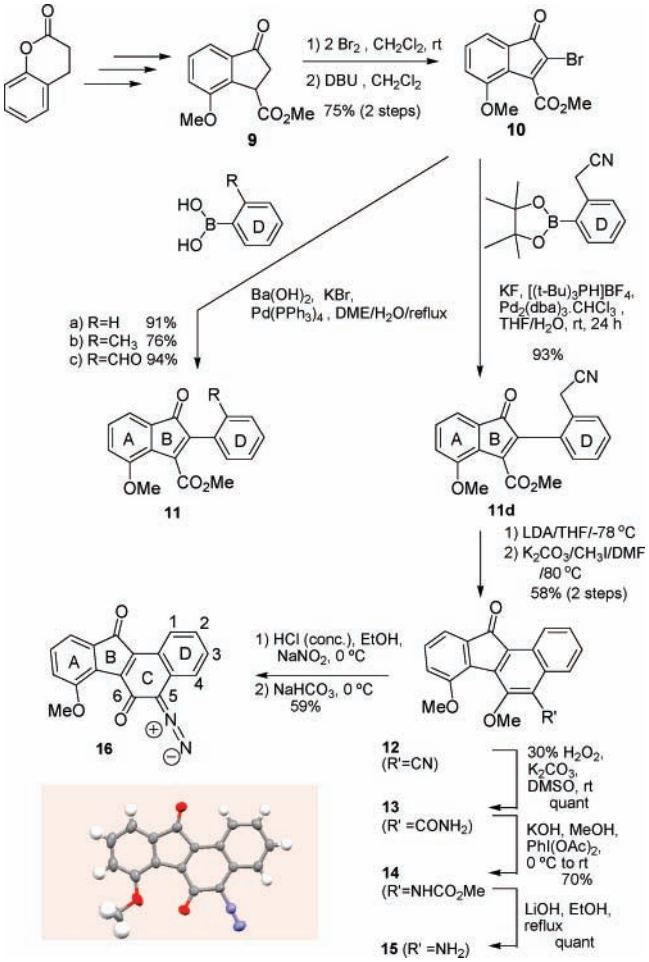


Figure 1. Structures of the kinamycins, lomaiviticin, isoprekinamycin, and fluostatins.

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 $Pd(PPh_3)_4$ as catalyst, gave the arylation products **11a**−**c**, nonbasic conditions, as developed by Fu and co-workers,[16] with $[(t\text{-}Bu)_3PH]BF_4$, KF, and $Pd_2(dba)_3{\cdot}CHCl_3$ 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 methanol[17] 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]

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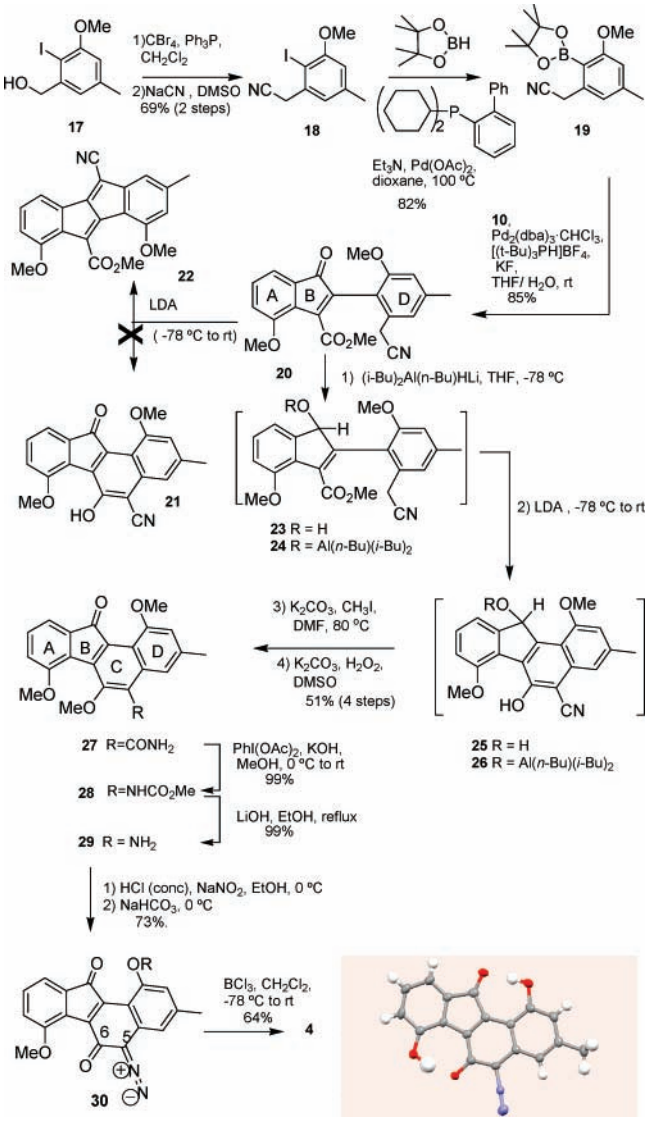
was converted to the nitrile **18**, and then to the target boronate **19** via a palladium-induced coupling with pinacol borane.[19] Suzuki coupling of **19** and **10** to give **20** proceeded well but, very disappointingly, attempted cyclization proceeded contrary to the favorable results of the 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 trialkylaluminate **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 H_2O_2/K_2CO_3, which also led to oxidation to the ketone. The synthesis then paralleled that of **16** to give **30**, which upon demethylation with BCl_3 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 (IC_{50} = 5.8 μM) and K562 human leukemia cells (IC_{50} = 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 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

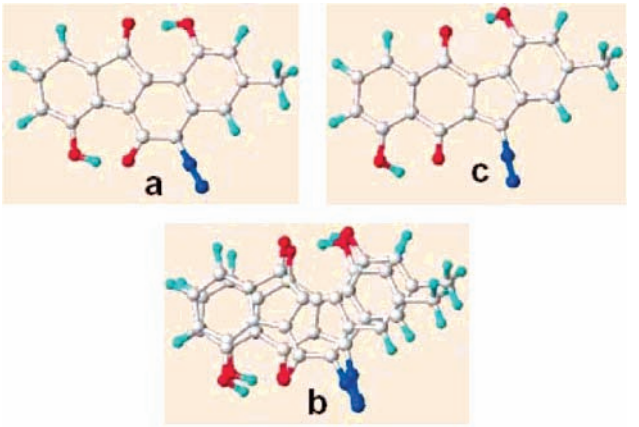


Figure 2. Structural comparison of the diazobenzo[*a*]fluorene and the diazobenzo[*b*]fluorene systems.

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

(20) Lithium trialkylaluminumhydrides are known to reduce ketones, but the concomitant use of the trialkylaluminate 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.

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(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 IC_{50} = 1.4 and 3.4 μM, respectively, in the same assays.

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

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