Syntheses of the Kinamycin Family
Insights into natural diazo containing benzo[b]fluorenes

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An Overview

• Isolation and characterization of the kinamycins, diazofluorene–containing natural products.

• Structural Reassignments

• Biological profile

• Total syntheses : 3 enantioselective and 1 racemic

• Efforts towards the synthesis of their dimer analogues
Meet the Kinamycins

- First isolated in 1970 by Omura et. al. from Streptomyces murayamaensis.
- Kinamycin family displays anti-tumor effects and is a potent Gram-positive antibiotic.
- Assigned based on derivatization studies, IR, NMR ($^1$H and $^{13}$C), X-Ray.
- Initially assigned as an N-nitrile (cyanamide).
- Cyanamide group postulated based on X-ray data on a derivative, ammonia liberation and IR absorption at $\sim$60 cm$^{-1}$.

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I
Kinamycin C
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“Prekinamycin”
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Omura et. al., J. Antibiot., 1970, 23, 315
Isolation

Streptomyces murayamaensis, isolated from soil samples near Murayama in Japan

An actinomycete, largest class of antibiotic producing bacteria

Spore former.

Omura et. al., J. Antibiot., 1970, 23, 315
Omura et. al., Chem. Pharm. Bull., 1973, 21
Gould et. al., J. Antibiot., 1989, 42, 189
Image from www.japannet.de/japan/japan3
Now with improved spectral data

- Gould and Dmitrienko independently reassigned structure.
- Anomalous shift of nitrile carbon at 78 ppm (expected at ~110 ppm)
- IR absorbance of synthetic relatives did not match the nitrile signal.
- Echavarren synthesized presumed prekinamycin, inconsistent with proposed structure (was later renamed isoprekinamycin)

Gould et. al., JACS, 1994, 116, 2207
Dmitrienko et. al., JACS, 1994, 116, 2209
Echavarren et. al. Tet. Lett., 1993, 34,
Hypothesized Biosynthesis

Annotated from the KEGG database

http://www.genome.jp/kegg-bin/show_pathway?rn01057+R06672

7,9,12-decaketide cyclization precursor

Annotated from the KEGG database

http://www.genome.jp/kegg-bin/show_pathway?rn01057+R06672
Hypothesized Biosynthesis

4, 8, O-acyl derivatives of 9 have been isolated.

Fairly extensive elaboration after diazotization.

Dmitrienko et. al., Org. Lett., 2008, 10, 381
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Structure and Biological Activity

- Weak binding to DNA ($K_{app} = 90 \, \mu M$)
- Unique activity against cancer cell line assay ($IC_{50} < 1\mu M$)
- Produces radicals under physiological conditions which cause DNA nicks.

Feldman et al., JACS, 2006, 128, 12562
H-bonding proposed as method to increase diazonium character in isoprekinamycin.

**Table 1.** Calculated C–N\textsubscript{2} Frequencies and N–N Bond Lengths

<table>
<thead>
<tr>
<th>compound</th>
<th>calcd $\nu$ (cm$^{-1}$)</th>
<th>calcd N–N (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-diazofluorene</td>
<td>1906</td>
<td>1.133</td>
</tr>
<tr>
<td>2,1-naphthoquinodiazide</td>
<td>2056</td>
<td>1.111</td>
</tr>
<tr>
<td>4</td>
<td>2087</td>
<td>1.108</td>
</tr>
<tr>
<td>24</td>
<td>2101</td>
<td>1.107</td>
</tr>
<tr>
<td>25</td>
<td>2125</td>
<td>1.105</td>
</tr>
<tr>
<td>2</td>
<td>2139</td>
<td>1.103</td>
</tr>
<tr>
<td>kinamycin B (1)</td>
<td>2188</td>
<td>1.099</td>
</tr>
<tr>
<td>23</td>
<td>2212</td>
<td>1.097</td>
</tr>
<tr>
<td>Ph-N≡N$^+$ Cl$^-$</td>
<td>2212</td>
<td>1.100</td>
</tr>
</tbody>
</table>

Dmitrienko et al., JACS, 2002, 124, 18
Feldman et. al., JACS, 2006, 128, 125€
Conformational Analyses

Dmitrienko et. al., Org. Lett., 2008, 10, 381

• Studies on Kinamycin derivatives by Dmitrienko.
• Favorable dipole interaction of C–N bond of the diazo group and the C–O ring bond postulated as possible reason for this conformational preference.
• Other kinamycins prefer conformation B
• Dmitrienko proposes that kinamycins get deacetylated to kinamycin F in vivo before displaying cytotoxic activity based on the superior activity of kinamycin F.

![Chemical structures and data]

Figure 2. Calculated energy minimum conformations of kinamycin F.

<table>
<thead>
<tr>
<th>X (equatorial)</th>
<th>H</th>
<th>OAc</th>
<th>F</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu_{N-N}$ (cm$^{-1}$)</td>
<td>2104</td>
<td>2123</td>
<td>2158</td>
<td>2173</td>
</tr>
<tr>
<td>$r_{N-N}$ (Å)</td>
<td>1.107</td>
<td>1.105</td>
<td>1.102</td>
<td>1.100</td>
</tr>
</tbody>
</table>
Feldman’s Mechanistic studies

Mechanism of Action of Prekinamycin

Scheme 5. Mechanistic Proposal for the Formation of Arene Adducts 36/39 from Diazoparaquinone 29

Feldman et. al., JACS, 2006, 128, 125
Feldman’s Mechanistic Studies

Feldman et. al., JACS, 2006, 128, 125
Synthetic Challenges

- 6–6–5–6 ring system
- Fluorene core
- Sensitive Diazo group
- Highly functionalized D ring.
- Tetrasubstituted quinone system
- Rich Ac substitution pattern on D ring.
- Dimer analog lomaiviticin A also has glycosylation pattern

\[ \begin{array}{cccc}
\text{R} & \text{R}_1 & \text{R}_2 & \text{R}_3 \\
\text{1a} \text{ kinamycin A} & H & Ac & Ac & Ac \\
\text{1b} \text{ kinamycin B} & H & H & Ac & H \\
\text{1c} \text{ kinamycin C} & Ac & Ac & H & Ac \\
\text{1d} \text{ kinamycin D} & H & Ac & H & Ac \\
\text{1e} \text{ kinamycin E} & H & H & H & Ac \\
\text{1f} \text{ kinamycin F} & H & H & H & H \\
\text{1g} \text{ kinamycin G} & Ac & Ac & COiPr & Ac \\
\text{1h} \text{ kinamycin H} & Ac & Ac & H & COiPr \\
\text{1i} \text{ kinamycin I} & Ac & COiPr & H & COiPr \\
\text{1j} \text{ kinamycin J} & Ac & Ac & Ac & Ac \\
\end{array} \]

Feldman et. al., JACS, 2006, 128, 125
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  – Porco
  – Nicolaou
  – Herzon
  – Ishikawa

• Efforts towards the synthesis of their dimer analogues and conclusions
First Total Synthesis

kinamycin A  $R_1 = H$  $R_2 = \text{Ac}$  $R_3 = \text{Ac}$  $R_4 = \text{Ac}$
kinamycin B  $R_1 = H$  $R_2 = \text{Ac}$  $R_3 = H$  $R_4 = H$
kinamycin C  $R_1 = \text{Ac}$  $R_2 = H$  $R_3 = \text{Ac}$  $R_4 = \text{Ac}$  \textbf{1}
kinamycin D  $R_1 = \text{Ac}$  $R_2 = H$  $R_3 = \text{Ac}$  $R_4 = H$

Porco et. al., JACS, 2006, 128, 14790
Synthesis of Fragment B

Porco et. al., JACS, 2006, 128, 14790
Synthesis of Fragment A

Absolute stereochemistry confirmed by X-Ray crystallography

Porco et al., JACS, 2006, 128, 14790
Combining the Fragments

Porco et al., JACS, 2006, 128, 14790
Last Touches

Features:
- Late Stage Diazo-introduction
- Oxidation of silylhydrazine
- Pd-based coupling, followed by Friedel–Crafts cyclization
- Enantioselective epoxidation as chirality source
- Selective acylation of secondary hydroxyls
- 1.1% overall yield, 23 step longest linear sequence

Porco et. al., JACS, 2006, 128, 14790
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Nicolaou Synthesis, Part A

1. BnBr, Ag₂O
2. Na₂S₂O₄, then NaH, Mel

1. tBuOK
2. OsO₄/NaIO₄

Nicolaou et al., JACS, 2007, 129, 103
Nicolaou Synthesis Part B

OTBS

MeMgBr, CuBr-Me₂S, TMSCl
then, Pd(OAc)₂, Air

90%

OsO₄, NMO

76%, >98% ee

OTBS

2-MeO-Propene

95%

LiHMDS, TMSCl
then Pd(OAc)₂, O₂

84%

I₂, pyr

92%

Nicolaou et. al., JACS, 2007, 129, 103
Joining the Halves

Nicolaou et. al., JACS, 2007, 129, 10356
Group Problem

Propose a reaction mechanism that leads to the given products and comment on the differing regioselectivities.

Nicolaou et. al., ACIE, 2009, 48, 5860
Labeling studies to determine the fate of the proposed samarium enolate

Peroxoide species 21 detected by NMR and MS

Nicolaou et. al., ACIE, 2009, 48, 5860
The Family Tree

Kinamycin C

95% yield from juglone

Features:
- Modular
- Hydrazine oxidation
- Benzoin-type condensation and Ullmann coupling conditions to synthesize the fluorene core

5.1% yield from juglone

- Late stage diazo introduction
- Enantioselective dihydroxylation to set stereochemistry
- 17 step longest linear sequence to shown late-stage product.

Kinamycin J

92%

Kinamycin F

80%

Nicolaou et al., JACS, 2007, 129, 103
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The Herzon Synthesis

Herzon et al., JACS, 2010, 132, 25
Kinamycin F

Features:
- Single step diazo introduction
- Late-stage construction of the D-ring
- Michael-reaction and Heck-type coupling to sew the pieces.
- Enantioselective dihydroxylation to set stereochemistry (poor yield, poor ee)
- No worries about acylation pattern
- 3.6% yield, 12 step longest linear sequence

Herzon et al., JACS, 2010, 132, 2540
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An Alternative Approach

Ring Elaboration

Ishikawa et. al., Tetrahedron, 2007, 63, 5189-5199

1. OsO₄
2. NaHSO₃

1. TMSOTf
2. mCPBA

3 d interconversion, then MeOH/H₂O

Ishikawa et. al., Tetrahedron, 2007, 63, 5189-5199
The Finish Line

Features:
Zn-catalyzed Diels-Alder
Racemic, although chiral DA catalysts known
Ring elaboration after DA

Late Stage Diazo-introduction
Hydrazine Oxidation
Selective Acylation
0.7% yield, 16 steps from Diels-Alder precursors

(±) O-methyl Kinamycin C

Ishikawa et al., Tetrahedron, 2007, 63, 5189-5199
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Whither Next?

Kinamycin family backbone

Dimer of kinamycin–like precursor

Herzon et. al., Org. Lett., 2009, 11,
Herzon’s Efforts

Herzon et al., Org. Lett., 2009, 11,
Nicolaou’s Efforts

Nicolaou et. al., ACIE, 2009, 48, 586C
To Be Solved for Lomaiviticin A

- Correct glycon monomer
- Efficient coupling reaction to make the dimer
- Derivative assays for activity.
- Biological profile: Mode of action, reason for superior activity, biosynthetic origins
- Relationship to the kinamycins both evolutionarily and enzymatically.
Conclusions

- Four different syntheses:
  - Porco: Stille Coupling, Friedel–Crafts reaction, epoxidation
  - Nicolaou: Ullmann Coupling, Benzoin–like condensation, desymmetrization of 4–OTBS–cyclohexanone
  - Herzon: Michael Reaction, Heck–type coupling, dihydroxylation
  - Kumamoto: Diels–Alder, racemic

- Two efforts towards lomaiviticin A, dimer of a kinamycin–like backbone

- Radical generation from diazo moiety indicated in bioactivity, derivative studies might help identify key structural features required for optimization.