Bergman Cyclization and Enediyne Antibiotics

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First reports of enediyne cyclizations

Studies by Bergman

enediyne 1a
40:60 cis:trans

300 °C, 30 sec

+ unaffected trans S.M.

1a and 1b must be interconverting through a symmetrical intermediate

Benzenediyl radical
2 is most well represented as a 1.4-diradical

optimization was carried out for the singlet and triplet electronic

to the prediction that butalene lies in a relative energy minimum,
35.9 kcal/mol above the singlet biradical. The transannular bond
surface of the biradical structure

difference found, a calculation performed at the equilibrium
geometry might lead to an inverted ordering of the electronic
calculations,
arbitrarily taken to be that of benzene. Wilhite and Whitten were
difference in the singlet and triplet biradical energies. The smallest
populated under the reaction conditions have yet to be charac-

In spite of the efforts of numerous investigators to generate and
Wilhite and Whitten4 reported a detailed ab initio study in

Abstract:

the C/E ratio leading from the radical pair to the reduced product, o-dipropylbenzene
hexachloroacetone solvent in a
(between 0.1 and 10 M) in the chlorobenzene reaction solution. This result indicates the presence of the singlet state of

Two approaches have been used to investigate the spin state@) of 1,4-dehydrobenzenes produced in the solution

Additional support for this analysis came from the reaction of

Other early studies

Table I. Calculated Energies of 1,4-Dehydrobenzene Structures

<table>
<thead>
<tr>
<th></th>
<th>relative energy of structures, kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>triplet</td>
</tr>
<tr>
<td>Wilhite and Whitten (1971)4</td>
<td>0</td>
</tr>
<tr>
<td>SCF–MO–CI</td>
<td></td>
</tr>
<tr>
<td>Dewar et al. (1974)5</td>
<td>+5</td>
</tr>
<tr>
<td>MINDO/3 (lim/CI)</td>
<td></td>
</tr>
<tr>
<td>Washburn et al. (1979)8</td>
<td>0 (+82)</td>
</tr>
<tr>
<td>ab initio 4-31G (no CI)</td>
<td></td>
</tr>
<tr>
<td>Mueller (1973)7</td>
<td>0 (+24)</td>
</tr>
<tr>
<td>modified MINDO/2 (no CI)</td>
<td></td>
</tr>
<tr>
<td>Noell and Newton (1979)6</td>
<td>+1.4</td>
</tr>
<tr>
<td>ab initio GVB (4-31G)</td>
<td></td>
</tr>
</tbody>
</table>

Chapman, 1976
Preliminary characterization attempts

Energy calculations were inconclusive

Understanding Cycloaromatizations

Bergman, Schreiner–Pascal, Myers–Saito, and Schmittel reaction of enediynes and enyne-allenes.

This need was enhanced by the discovery of natural enediynes in the 1980s. The natural enediynes calicheamicin (1987), es-paramicin (1987), and dynemicin (1990) were soon after their discovery in the years 1987 and 1990 considered as possible leads for antitumor antibiotics where this hope was based on their relationship to neocarzinostatin. Neocarzinostatin can be considered as an enediyne with an epoxidized double bond, and therefore its biological activity was related to a Bergman-type cyclization of an intermediate enyne-cumulene. Hence, the discovery of the natural enediynes and their chemistry established the importance of the cycloaromatization reaction discovered by Jones, Bergman, and Masamune in the early 1970s.

The Bergman reaction may be formally classified as an electrocyclic reaction. However, it has to be mentioned that for an electrocyclic reaction, the π system orthogonal to the symmetry plane of the intermediate should be directly involved in the formation of the new CC bond, which is not the case. Other authors have related the Bergman reaction to the Cope rearrangement and speak of a formal Cope rearrangement. Again, this can be questioned when considering just the Bergman reaction whereas the Bergman and retro-Bergman reaction seen together (i.e., considering both the formation of bond C1C6 to give and the cleavage of bond C3C4 in ) may formally see in this way (interaction of two in-plane ally radicals) although this would lead to the transition state of the Cope rearrangement being identical to the intermediate p-benzyne biradical. In this situation, it is appropriate to follow the suggestion of Alabugin and co-workers and to speak in the case of the Bergman and Myers–Saito rearrangements (Figure 1) of cycloaromatization reactions as a special class of ring-forming reactions.

The correct description of a singlet biradical such as requires a methodology, which was not available in the 1970s, however came into general use with the availability of coupled cluster methods and perturbation theory corrected complete active space methods in the late 1980s and early 1990s, respectively. A correct quantum chemical description of the Bergman cyclization implies more than just a reliable description of an organic biradical. It requires a balanced account of nondynamic (multireference) electron correlation effects (for the biradical) and dynamic electron correlation effects (especially including three-electron effects for the correct account of different types of electron delocalization in reactant and product). Even in the year 2013, this problem cannot be considered to be fully solved for the Bergman and the Bergman-related reactions (Figure 1) in the way that larger enediynes and enyne-allenes can be easily investigated.

In this situation, quantum chemists focused on the possibilities of density functional theory (DFT),
Understanding Cycloaromatizations

Radical cyclizations

Pericyclic reactions

Cycloaromatization reactions

break two $\pi$-bond form one $\sigma$-bond.

Two new radical centers are created from a non-radical reactant.

Understanding Cycloaromatizations

The enediyne antibiotics

Neocarzinostatin
- First isolated in 1965 as a 1:1 protein complex
- Structure of the chromophore component was determined in 1985.
- Crystal structure of the complete protein-chromophore complex determined in 1993.

Calicheamicins
- First isolated in 1986 as a series of related compounds from microbial fermentation products
- Active against both gram positive and gram negative bacteria. Extraordinary activity against murine tumours.

Esperamicins
- First isolated in 1985 from microbial fermentation products

Dynemecins
- Isolated and characterized in 1989

**The enediyne antibiotics**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Producing Strain</th>
<th><em>In Vitro</em> IC50 (nM)</th>
<th><em>In Vivo</em> ID50 (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neocarzinostatin</td>
<td><em>Streptomyces carzinostaticus</em></td>
<td>225~900</td>
<td>380</td>
</tr>
<tr>
<td>Lidamycin</td>
<td><em>Streptomyces globisporus C-1027</em></td>
<td>0.01~0.5</td>
<td>0.25~0.5</td>
</tr>
<tr>
<td>Kedarcidin</td>
<td><em>Actinomycete strain L585-6</em></td>
<td>1</td>
<td>2~3.3</td>
</tr>
<tr>
<td>Calicheamicins</td>
<td><em>Micromonospora echinospora ssp</em></td>
<td>6~9</td>
<td>0.5~1.5</td>
</tr>
<tr>
<td>Esperamicins</td>
<td><em>Actinomadura verrucospora</em></td>
<td>0.3~8.3</td>
<td>0.1~0.2</td>
</tr>
<tr>
<td>Dynemicins</td>
<td><em>Micromonospora chersina M956-1</em></td>
<td>0.9~10</td>
<td>30~60</td>
</tr>
</tbody>
</table>

IC50, half-inhibiting concentration; ID50, half-inhibiting dosage.

Below is an example of process for the isolation of calicheamicins from the fermentation broth of NRRL15839.

**Process for the isolation of calicheamicins**

**Fermentation broth (1,500 liters, ~0.1 μg/ml β¹Br)**

- **Mycelium and aqueous phase**
  - Extracted with 750 liters EtOAc

- **EtOAc phase (700 liters)**
  - Concentrated to a syrup stirred with excess hexane filtered through Celite

- **Hexane solution, containing fat**
  - Stirred with 20 liters EtOAc

- **Celite coated with calicheamicins**
  - Concentrated to 2.5 liters dried over sodium sulfate precipitated from diethyl ether - hexane

- **Celite**

- **Mother liquor**
  - **Crude calicheamicin complex (26.7 g)**
    - Silica Woelm column EtOAc saturated with 0.1m aq KH₂PO₄ to EtOAc-MeOH (95:5 to 90:10)

- **Crude calicheamicin β¹Br and γ¹ Br (2.0g)**
  - Sephadex LH-20 column MeOH-H₂O (90:10)

- **Partially purified calicheamicin complex (435mg, 10%β¹Br, γ¹ Br 4% )**
  - Silica gel 60 column EtOAc-MeOH (98:2)

- **Pure calicheamicin β¹Br (26mg, 80% pure)**
  - Sepanalyte C₁₈ column CH₃CN-0.2M NH₄OAC (45:55)

- **Calicheamicin β¹Br**
  - CPure calicheamicin /S-|Bromined with other γ¹Br samples of similar purity to give 18mg total
  - silica gel prep TLC

- **Calicheamicin γ¹ Br**
  - (4.5mg, 30% pure)

- **Pure calicheamicin γ¹ Br (4.3 mg)**

**Scheme 2. Schematic presentation of process for the isolation of calicheamicins pxBv and Ti31 from the fermentation of NRRL15839.**
Challenges in Chemistry and Biochemistry

• Early 1970s: The Bergman cyclization is discovered as a radical cycloaromatization involving a 1,4-diyl intermediate.

• Remains a chemical curiosity up to the mid 1980’s, and is underexplored in terms of reaction scope and synthetic utility

• Late 1980s: Characterization of unprecedented and highly potent enediyne antibiotics results in a massive renaissance in the chemistry of enediyynes. Numerous avenues for research investigations are suddenly apparent and many researchers are now involved.

Enediyynes as inspirations for mechanistic investigations into the BC.  

Biochemistry of enediynes  

Mode of action  

Synthesis of analogs and hybrids  

Total synthesis challenge  

Broadening synthetic utility
OBITUARY

Professor. Nakao Ishida (1923–2009)
Medical scientist, microbiologist, immunologist, entrepreneur, educator, and administrator

Our Japanese research communities recently lost a great mentor and friend, Professor Nakao Ishida on December 4, 2009. Late Professor Nakao Ishida, M.D. was born in 1923 at Nigata prefecture, Japan. He entered and graduated from Tohoku University Medical School, Sendai, Japan, in 1946 and pursued the academic track since then at Tohoku University. He was a Fulbright scholar at the Virus Institute of University of Michigan School of Public Health (1954–1956) under Professor Thomas Francis Jr. M.D. Professor Ishida became a full professor of the Department of Bacteriology, Tohoku University Medical School, Sendai in 1960 and served the professorship till 1985.

Professor Ishida discovered Sendai virus in 1951 from an epiatropic patient. This paramyxovirus not only became an indispensable tool in cell fusion, but also provided a basic concept of protease driven promotion of viral infectivity. The mechanism of proteolytic activation of the viral infectivity through cleaving F-protein was elegantly elucidated by Morio Homma of Ishida’s group in Sendai. This mechanism provided a clue to analyze the similar mechanism of HA cleavage of influenza virus, which confers the virus infectivity, as demonstrated by Hans-D. Klenk et al in Germany.

At the front of active viral research in his Department of Bacteriology, Professor Ishida as a chairman of the department, played a key role in the discovery of adenovirus type 11 as a causative agent of infantile acute hemorrhagic cystitis by Yoshio Numazaki et al, and also in the discovery of rotavirus, a causative agent of infantile gastroenteritis in the pediatric patients, by Tasuke Konno et al of Tohoku University. It was about the same time independently of the discovery of rotavirus by Ruth Bishop et al in Australia. Professor Ishida’s other research interest in virology include hepatitis virus B and C as well as influenza virus.

In the Ishida’s Department of Bacteriology, the research project on antibiotics was one of major efforts since 1943, the day of his predecessor Professor Chairman Masahiko Kuroya. Collaboration between the department and pharmaceutical companies was a routine scene, and screening program for antiviral and antitumor agents were undertaken in addition to antibacterial agents where many companies sent their scientists for training. Among numbers of newly discovered antibiotics, a proteinaceous antitumor agent named neocarzinostatin (NCS) was a successful case that became an approved drug used in clinics. This neocarzinostatin became the first prototype protein antitumor agents, among others such as actinoxanthin of Prof. A. Khovlove, Science Academy of Soviet Union, or macromomycin of Prof. Hamao Umezawa, University of Tokyo.

Extensive investigation of NCS was carried out under the leadership of Professor Nakao Ishida. Namely, antitumor activity was elucidated by Katsuo Kumagai, the mechanism of action in molecular biology using bacteria as DNA degradation and inhibition of DNA synthesis was established by Yasushi Ono, and subsequently, Kenzo Ohtsuki detailed molecular mechanism further. The chemistry, biochemistry, pharmacological properties, including amino acid sequence, in vivo half life, subcellular action were clarified by Hiroshi Maeda. The chemical structure of the unique endyene chromophore associated with NCS was established by Kiyoto Edo. The development for commercialization was undertaken by Kayaku Antibiotics Laboratories, Ltd, Tokyo, led by Yasuo Koyama, Shigehiro Matsumoto, Kazuso Toriyama et al. NCS was later conjugated with styrene-co-maleic acid polymer named SMANCS by Hiroshi Maeda, and it became the first polymeric drug approved by regulatory agency (1993), and opened up a new field of polymer therapeutics in cancer therapy.

Professor Ishida’s research interests were limitless, and immunology was another area. He was intrigued by that child-bearing mother who is immunologically tolerant to the baby, even though the baby is apparently non-self. Keiji Tamura pursued a plasma component that might...
The enediyne antibiotics: mechanism of action

Pioneering work:

- **1978-1981**: NCS binds to DNA and cleaves it. The transformation is accelerated drastically by added thiols and oxygen.

- **1983**: (Goldberg et. al.) In absence of DNA, NCS reacts irreversibly with nucleophiles to form a covalent adduct which binds but does not cleave DNA. An epoxide ring is opened. Spectroscopic data for the adduct.

- **1987**: (Myers) assignment of the adduct and cycloaromatization (Myers-Saito cyclization) proposal

The enediyne antibiotics: mechanism of action

Natural enediyne antitumor antibiotics: the 10-membered family

Calicheamicin γ1

Esperamicin A1

namenamicin

Shishijimicin A

Dynemicin A

Uncialamycin

10-membered enediynes
The enediyne antibiotics: mechanism of action

Natural enediyne antitumor antibiotics: the 9-membered family

- 9-membered enediynes as apoprotein complexes.
- N1999A2 is the only non-protein 9-membered cyclic enediyne found in nature.
The enediyne antibiotics: mechanism of action

<table>
<thead>
<tr>
<th>Structural Units</th>
<th>Structural Features/Functions</th>
<th>Pictorial presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warhead</td>
<td>The Enediyne.</td>
<td></td>
</tr>
<tr>
<td>Locking Device</td>
<td>Stabilizes the enediyne from undergoing rearrangement</td>
<td>![Diagram of Locking Device]</td>
</tr>
<tr>
<td>Triggering Device</td>
<td>It offers a mechanism by which locking is removed and enediynes become reactive</td>
<td>![Diagram of Triggering Device]</td>
</tr>
<tr>
<td>Binding Device</td>
<td>Gives Specificity</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Structural features of enediynes.

<table>
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<th>Pictorial presentation</th>
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<td>Warhead</td>
<td>The Enediyne.</td>
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</tr>
<tr>
<td>Binding Device</td>
<td>Gives Specificity</td>
<td></td>
</tr>
<tr>
<td>Trigger</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 9. Structural features of Calicheamicin.

calicheamicin γ₁
Calicheamicin: mechanism of action

![Chemical structures and reactions]

- **Stable warhead**
- **‘hot’ warhead**
- Strained enediyne due to pyramidalized bridgehead
- **Bergman cyclization**

These fundamental chain of events are more or less common to the mechanism of other enediyne antibiotics.

Dynemicin: mechanism of action

Bioreduction

BC, DNA damage

19
1. Calicheamicin + Glutathione + DNA gives a mixture of products:

Glutathione + DNA +

MeS-SH

+ G-SS

MeSSSS

GS-SSMe

end product
Calicheamicin: Intricacies in the mechanism of action

1. Calicheamicin + Glutathione + DNA gives a mixture of products:

G-S-SMe

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{HSS} \\
\text{H} \\
\text{OR} \\
\text{4}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{G-SS} \\
\text{H} \\
\text{OR} \\
\text{6}
\end{array}
\]

\[
\begin{array}{c}
\text{MeS-SH} \\
\text{+}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{MeSH} \\
\text{+}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{G-SSS} \\
\text{H} \\
\text{OR} \\
\text{5}
\end{array}
\]

\[
\begin{array}{c}
\text{MeS-SH} \\
\text{+}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{G-SSS} \\
\text{H} \\
\text{OR} \\
\text{5}
\end{array}
\]

\[
\begin{array}{c}
\text{MeSH} \\
\text{+}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{G-SSS} \\
\text{H} \\
\text{OR} \\
\text{5}
\end{array}
\]

4-6 converge to 3.
4 and 5 disappear faster than 6.

GS-SSMe

\[
\begin{array}{c}
\text{HO} \quad \text{NHCO}_2\text{Me} \\
\text{O} \\
\text{S} \\
\text{OR} \\
\text{3}
\end{array}
\]

end product

Calicheamicin: Intricacies in the mechanism of action

<table>
<thead>
<tr>
<th>Substrate</th>
<th>6</th>
<th>1</th>
<th>6</th>
<th>6</th>
<th>6</th>
<th>6</th>
<th>6</th>
<th>6</th>
<th>6</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>[GSH] (mM)</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>t (hrs)</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Rate of DNA cleavage by 6 is ~10 fold lower than that by 1 (calicheamicin)

MeS-SH + G-SS

Calicheamicin: Intricacies in the mechanism of action

<table>
<thead>
<tr>
<th>Substrate</th>
<th>1</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>[GSH] (mM)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>t (min)</td>
<td>60</td>
<td>3</td>
<td>10</td>
<td>20</td>
<td>60</td>
<td>120</td>
<td>300</td>
<td>1200</td>
<td>120</td>
<td>300</td>
<td>1200</td>
<td></td>
</tr>
</tbody>
</table>

Rate of DNA cleavage by 6 is ~10 fold lower than that by 1 (calicheamicin)

Rate of DNA cleavage by 4 lower than that by 1 but higher than 6.

MeS-SH

\[
\text{MeS-SH} + \text{G-SS} \rightarrow \text{Rate of DNA cleavage by 6 is ~10 fold lower than that by 1 (calicheamicin)}
\]

G-S-SMe

\[
\text{G-S-SMe} + \text{HSS} \rightarrow \text{Rate of DNA cleavage by 4 lower than that by 1 but higher than 6.}
\]

Calicheamicin: Intricacies in the mechanism of action

- Does the reaction occur as a ternary complex of 1, GSH, and DNA, or is 1 activated free in solution with subsequent binding of the reaction product(s) to DNA?

- What is the role of DNA in the primary and secondary activation steps?

Excess DNA inhibits cleavage by 6. The drug is partitioned between bound and free form where the latter reacts faster.
1. Reaction of 1 with GSH and DNA as a ternary complex produces 6 as the major product.
2. 6 must dissociate from the helix prior to reacting with GSH and produce 2.
3. 2 binds to DNA followed by cycloaromatization and DNA cleavage.
4. DNA cleavage will follow a bimodal kinetic profile where the initial cleavage event will occur with a half-life on the order of a few minutes and the second, major stage of the cleavage will occur with a half-life of several hours, depending critically upon the exact concentration of nuclear DNA.
### Contributing factors: the distance theory

- Nicolaou: In the absence of other factors, distance $d$ between the ends of the diyne system contributes to reactivity.

- Proposed that the critical range of $d$ values between 3.20 and 3.31 would be necessary for biologically relevant activities.

#### Table of Calculated $c,d$-distances and Stability of Conjugated Enediynes

<table>
<thead>
<tr>
<th>Entry</th>
<th>$(n = x)$</th>
<th>$c,d$-distance (Å)</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2.84</td>
<td>Unknown</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3.25</td>
<td>Cyclization at 25 °C</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3.61</td>
<td>Stable at 25 °C</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3.90</td>
<td>Stable at 25 °C</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4.14</td>
<td>Stable at 25 °C</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>4.15</td>
<td>Stable at 25 °C</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>4.33</td>
<td>Stable at 25 °C</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>4.20</td>
<td>Stable at 25 °C</td>
</tr>
</tbody>
</table>

Contributing factors: the distance theory

- Nicolaou: In the absence of other factors, distance \(d\) between the ends of the diyne system contributes to reactivity.

- Proposed that the critical range of \(d\) values between 3.20 and 3.31 would be necessary for biologically relevant activities.

![Diagram showing activation enthalpy vs distance for different structures A, B, C, and D.](image)
Attenuation of strain

- Magnus, Snyder: the cyclization rates of bicyclic enediynes are best interpreted as governed by strain-energy modulation in the pseudocyclic transition state.

\[
R = \text{TBDMS}
\]

\[
\begin{align*}
\text{A} \quad & \quad \text{B} \\
\text{C} \quad & \quad \text{D} \\
\text{E} \quad & \quad \text{F} \\
\text{G} \quad & \quad \text{H}
\end{align*}
\]

- A has lower inter alkylnyl distance but reacts slower.
- The cyclohexanone derivatives E, G enjoy a conformationally activated strain release mechanism from ground state to transition state.

\[
\begin{array}{|c|c|c|c|}
\hline
& \text{rel at } 85 \degree \text{C} & \Delta E^* \text{, kcal/mol} & \Delta E_{\text{ring}} \text{, kcal/mol} \\
\hline
\text{A} & 1 & 31.2 & 1.5 \\
\text{C} & 216 & 27.5 & -1.5 \\
\text{E} & 648 & 25.4 & -6 \\
\text{G} & \text{N.D, extremely rapid even at } 0 \degree \text{C} & 22 & -6.1 \\
\hline
\end{array}
\]

kcal (PRDDO). Secondly and more importantly, while the five-ring energy increases by 1.5 kcal as it moves from enediyne 10 to a biradicaloid transition state, the six-membered ring of 7 drops by 6.0 kcal (boat → chair) along the same route. At the

Activation and Triggers


**Activation and Triggers**

**[Diagram with chemical structures and reactions]**


Investigations to reveal the mechanisms of DNA cleavage by NCS were pioneered by Irving Goldberg at Harvard in the late 1980’s. Our understanding is primarily based on his series of elegant experiments. The major pathway involves single strand scission at thymine to produce a nucleoside aldehyde.

The initial NCS activation (diradical formation) is rapidly followed by uptake of 1 mol of O₂ per mole of chromophore. O₂ uptake is followed by the uptake of at least an additional sulfhydryl group. Oxygen label is selectively incorporated into the product nucleoside aldehyde.

Tritium labelling demonstrates that the chromophore selectively abstracts tritium only from the 5’-position and incorporates it into a stable, non-exchangeable form of the chromophore.

In the absence of oxygen, strand cleavage is poor and NCS-DNA adducts are seen.

Provide a reasonable mechanism that is consistent with these experimental observations.

Intermission
Total Synthesis highlights

Identified
Neocarzinostatin Chromophore
(Edo et al. 1985)

Total synthesis of Dynemicin A
(Myers et al. 1995)
(Danishefsky et al. 1995)

Total synthesis of N1999A2
(Kobayashi, Hirama et al. 2006)

Total synthesis of Maduropeptin Chromophore
(Sato, Hirama et al. 2009)


Total synthesis of Calicheamicin $\gamma^1$
(Nicolaou et al. 1992)
(Danishefsky et al. 1994)

Total synthesis of Neocarzinostatin Chromophore
(Myers et al. 1998)

Total synthesis of Kedarcidin Chromophore
(Myers et al. 2007)
Nicolaou (1988-1992)

Why not a direct cyclization from 2 to 6?
Nicolaou (1988-1992)

Nicolaou (1988-1992)

\[
\begin{align*}
\text{TBDMSO} & \quad \text{O} \\
\text{Me} & \quad \text{O} \\
\text{OH} & \quad \text{Ar} \\
\alpha: \beta & \quad \text{ca} \ 6:8:1 \\
11 & \\
\text{Ar} & = \text{m-Cl-phenyl}
\end{align*}
\]
includes NaCNBH₃ and DIBAL reductions, protecting group manipulations.

1. DIBAL, DCM, -78°C (91%)
2. Ph₃P, DEAD, AcSH, THF, 0°C (96%)

DCM, 0 to RT 57%
**Synthesis of C-1027 core (Inoue, 2008)**

![Chemical Structure]

Highly labile cores require novel assembly strategies.

**Scheme 1.** Total Synthesis of the C-1027 Chromophore Core: Extremely Facile

---

**Table 1. Condensed Results of 1,2-Elimination**

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>R²</th>
<th>t</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>Ms</td>
<td>2 h</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>b</td>
<td>Ms</td>
<td>0.7 h</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>Ms</td>
<td>7 h</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>d</td>
<td>Ms</td>
<td>0.5 h</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>e</td>
<td>Ms</td>
<td>5 min</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>f</td>
<td>Ms</td>
<td>1 min</td>
<td>93</td>
</tr>
</tbody>
</table>

Biosynthesis of C-1027

- Back to back reports in 2002 described the identification and cloning for the biosynthetic gene cluster of C-1027 and Calicheamicin.

- The enediyne core is assembled by a highly conserved PKS complex in both cases and a modular convergent overall biosynthesis.

Core biosynthesis is not well understood

- Studies with $^{13}$C labeled acetate, doubly labeled and mixed labeled.
- Core is likely derived from a linear precursor consisting of seven acetates assembled in a head to tail fashion.
- In the 9-membered system, the alkyne carbons are derived from the same acetate unit. This is not the case for the 10-membered systems.
- The exact identity of the precursor and its subsequent transformations to the core remain ambiguous.

Folding pattern for the 9-membered enediyne of neo-carzinostatin.

Folding patterns for the 10-membered enediyne of dynemicin A.
C-1027 is protected by the apoprotein

Table 4. Relationships in space between C6 or C3 of chromophore and hydrogens of the C-1027 apoprotein

<table>
<thead>
<tr>
<th></th>
<th>C6</th>
<th>Gly96 H21</th>
<th>Asn97 H2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (Å)</td>
<td>C6-H2</td>
<td>4.59(±0.62)</td>
<td>4.21(±0.28)</td>
</tr>
<tr>
<td></td>
<td>C3-C2</td>
<td>7.36(±0.45)</td>
<td>6.70(±0.27)</td>
</tr>
<tr>
<td>Angle (deg.)</td>
<td>C3-C6-H2</td>
<td>164.1(±6.1)</td>
<td>116.6(±6.6)</td>
</tr>
<tr>
<td></td>
<td>C6-H2-C2</td>
<td>93.5(±10.5)</td>
<td>109.2(±8.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C3</th>
<th>Pro76 Hβ1</th>
<th>Pro76 Hβ2</th>
<th>Pro76 Hγ2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (Å)</td>
<td>C3-Hβ (or C3-Hγ)</td>
<td>4.21(±0.43)</td>
<td>4.19(±0.75)</td>
<td>4.24(±0.47)</td>
</tr>
<tr>
<td></td>
<td>C6-Cβ (or C6-Cγ)</td>
<td>7.15(±0.61)</td>
<td>7.15(±0.61)</td>
<td>7.11(±0.59)</td>
</tr>
<tr>
<td>Angle (deg.)</td>
<td>C6-C3-Hβ (or C6-C3-Hγ)</td>
<td>151.1(±9.6)</td>
<td>160.8(±10.6)</td>
<td>128.0(±11.6)</td>
</tr>
<tr>
<td></td>
<td>C3-Hβ-Cβ (or C3-Hγ-Cγ)</td>
<td>103.2(±14.2)</td>
<td>104.4(±15.6)</td>
<td>118.9(±15.5)</td>
</tr>
</tbody>
</table>

EPR studies reveal that the chromophore is in equilibrium with the diradical and is presumably stable due to lack of suitable hydrogen donors nearby.
Sacrificial Proteins as instruments of self-preservation

- CalC confers resistance by binding and undergoing cleavage at Gly-113 after trigger activation.

Sacrificial Proteins as instruments of self-preservation

- CalC confers resistance by binding and undergoing cleavage at Gly-113 after trigger activation.
Therapeutic Prospects

- Without precise targeting, the extreme potency of enediyne antibiotics leads to undesired collateral damage.

**Structure of SMANCS**

![Structure of SMANCS](image)

**Styrene-Maleic acid copolymer-NCS conjugate**
Approved in 1993 and currently used in Japan

**Mylotarg – Antibody conjugated Calicheamicin**
FDA approved in 2000. Discontinued since 2010

A handful more antibody conjugates are undergoing clinical trials.

Single Molecule Visualization of Bergman Cyclization

Deposited on 2-monolayer thick NaCl island on a Cu-111 surface at 10K

9,10-dibromoanthracene (DBA)

Direct Observation of intermediates and products

Figure 2 | Structures and AFM imaging of the starting material, reaction intermediates and product. a–d, Chemical structures of the reaction products formed by successive STM-induced debromination of DBA (6) (a) and subsequent retro-Bergman cyclization: DBA, 9-dehydro-10-bromoanthracene (radical 7) (b), 9,10-didehydroanthracene (diradical 5) (c) and 3,4-benzocyclodeca-3,7,9-triene-1,5-diyne (diyne 4) (d). e–h, Corresponding constant-height AFM images of the molecules in a–d, respectively, on NaCl(2ML)/Cu(111) using a CO tip. Δf corresponds to the frequency shift of the oscillating cantilever.
Single Molecule Visualization of Bergman Cyclization

Figure 4 | Reversible Bergman cyclization. a–c, Laplace-filtered AFM images of diyne 4R (a), diradical 5 (b) and diyne 4L (c) on NaCl(2ML)/Cu(111). The molecule is adsorbed at a step edge of an NaCl(3ML)/Cu(111) island, seen in the lower part of the images. d, Current trace during a voltage pulse of $V = 1.64$ V at the position indicated by the white circle in b. The different current levels correspond to the molecular structures of the same colour shown in the inset.

e, Calculated energies of the Bergman cyclization using the distance between the carbons indicated by red circles ($d_{C-C}$) as the reaction coordinate.

Important Reviews:


*Other articles cited on each slide.*