Misassigned Natural Products and Modern Methods of Structure Determination

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Stoltz Group Literature Meeting
147 Noyes, 5pm
9/19/05

[α]D = +33 (c 0.03, CHCl3)

Outline

• Brief historical perspective (Quinine)
• Introduction to the basic modern methods of structure elucidation
  1. X-ray analysis
  2. Mass Spectrometry
  3. Optical Rotation
  4. Circular Dichroism
  5. IR
  6. NMR

  NOT included: 3D NMR, elemental analysis, UV-vis, and chemical degradation

• Case Studies of misassigned natural products
  1. Schizogamine and Ioschizogamine
  2. Batzelladine F
  3. Sporol and Neosporol
Mistaken Identity: Back in the Day
Case Study: Quinine

- 1854 Strecker determined molecular formula of quinine as C_{20}H_{21}N_{2}O_{2}
- 1849 Wilhelm Hofmann published idea that quinine could be synthesized from coal tar
- 1856 Sir William Henry Perkin (age 18) undertook Hofmann's project:

\[ 2 \text{MeC}_{10}H_{13}N + \frac{3}{2} \text{O}_2 \rightarrow \text{H}_2\text{O} \]

Summary in Nicolaou, K. C. and Snyder, S. A. Classics in Total Synthesis II.

Mistaken Identity: Back in the Day
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\[ 4 \text{MeC}_{10}H_{13}N_2 \overset{\text{K}_2\text{Cr}_2\text{O}_7}{\rightarrow} \text{H}_2\text{N}_2\text{N} \]

Summary in Nicolaou, K. C. and Snyder, S. A. Classics in Total Synthesis II.
**Mistaken Identity: Back in the Day**  
**Case Study: Quinine**

- 1854 Strecker determined molecular formula of quinine as $C_{30}H_{32}N_2O_2$  
- 1849 Wilhelm Hofmann published idea that quinine could be synthesized from coal tar  
- 1856 Sir William Henry Perkin (age 18) undertook Hofmann's project:

![Chemical reaction diagram](image)

- 1853 Pasteur performed degradation studies

![Chemical reaction diagram](image)

- 1908 Paul Rabe reported the correct molecular connectivities of quinine. Of course, no stereochemistry was known.

Summary in Nicolaou, K. C. and Snyder, S. A. *Classics in Total Synthesis II*. 
**Mistaken Identity: X-Ray Misassignments**

3D map of electron density + math = X-Ray Crystal Structure

- How can we determine the absolute stereochemistry of the small molecule?
  - stereocenters already known
  - heavier element present (heavier than Si)
  - anomalous dispersion occurs when the frequency of the X-ray is near the frequency of electrons within a cloud.
  - This is only large for heavy atoms.
  - With anomalous dispersion, additional calculations gives absolute stereochemistry

- How can we confuse atom identities?
  - experimental determination of van der Waals radius
  - electron density integration at a grid point gives
    - atomic number
    - nitrogen and oxygen have similar masses, and hydrogens are difficult to refine

Fenical, W.; Clardy, J. et al. JACS 1991, 113(6), 2303-2304,
Stout, Jensen X-Ray Structure Determination: A Practical Guide;
Giacovazzo, C. et al. Fundamentals of Crystallography

**Methods of Structure Determination**

**Mass Spectral Analysis**

- MS analysis is a destructive method of spectroscopy used to determine the molecular formula and identity (through characteristic fragmentations) of molecules of interest.

![](image)

- Analysis based on the absorption of energy, ionization, and identification of the m/z ratio of the resulting molecules/fragments.

  $M: \quad \text{--e}^- \quad M^* \quad \text{fragmentation rearrangement ?}$

- Upon ionization, loss of an electron and generation of a radical cation (molecular ion, $[M]^+$) yields a molecule nearly identical to itself in mass ($m_e = 5.49 \times 10^{-4}$ of $m_p$).

- Different methods of ionization allow the detection of the molecular ion and use on different substrates.

**Methods of Structure Determination**

*Ionization Methods*

**Electron Impact (EI):** Involves bombarding molecules in gas phase with a high-energy electron beam (50-100 eV). Ionization occurs by the interaction of the fields of both the electron and the molecule passing close to or through the molecule. Usually, a good deal of fragmentation occurs... "hard ionization"

**Chemical Ionization (CI):** A sample is ionized in the presence of a large excess of a "reagent" gas (CH₄, isobutane, or NH₃), which is ionized by EI. 1⁺ ions react to form 2⁺ ions that react with the sample. Generates a [M+1]⁺ ion (quasiautosomal ion) cleanly with little fragmentation... "soft ionization"

\[
\begin{align*}
\text{CH}_2^+ & \quad + \quad \text{M} \\ 
\text{C}_2\text{H}_5^+ & \quad + \quad \text{M} \\
\rightarrow & & \quad [\text{M+H}]^+ & \quad + \quad \text{CH}_4 \\
\rightarrow & & \quad [\text{M+H}]^+ & \quad + \quad \text{C}_2\text{H}_4
\end{align*}
\]

**Field Desorption (FD):** Method good for solid samples of low volatility or thermal instability. A sample is placed on the anode of a pair of electrodes, and subjected to an intense electric field. Generally see [M]⁺ and [M+1]⁺. Inferior to FAB procedures.

**Electrospray Ionization (ESI):** Ionization involves placing an ionizing voltage at atmospheric pressure across a nebulizer needle. As the mist of charged droplets evaporate, and Coulombic repulsions cause a molecular "explosion." Method can be used for molecules without any ionizable sites through formation of Na⁺, K⁺, or NH₄⁺ adducts. Generally obtain [M+nH]ⁿ⁺, and is widely used with HPLC.

**Fast-Atom Bombardment (FAB):** A sample is adsorbed on a matrix (usually glycerol, m-nitrobenzyl alcohol, or diethanolamine) and ionized by a high-energy Xe beam. [M+H]⁺ usually seen, as well as [M]⁺ and fragmentations. [M+Na]⁺ can also be seen with used of salt additives. FAB can achieved up to >10⁶ Da resolution, but beware of matrix background peaks.

**Matrix-Assisted Laser Desorption Ionization (MALDI):** The sample in a matrix (2,5-dihydrobenzoic acid or α-cyano-4-hydroxycinnamic acid, both m/z <400 and do not form adducts with proteins) is dispersed on a surface and is adsorbed and ionized by a high-energy laser beam (N₂ UV laser beam at 4 ns pulses). MALDI is excellent for large biomolecules and polymers, up to several hundred kDa.

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**Methods of Structure Determination**

*Elemental Abundance and Exact Mass*

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<thead>
<tr>
<th>Element</th>
<th>Atomic Weight</th>
<th>Nuclide</th>
<th>Mass</th>
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<tr>
<td></td>
<td></td>
<td>³⁷Cl</td>
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<td>Bromine</td>
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<td>⁷⁹Br</td>
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<tr>
<td></td>
<td></td>
<td>⁸¹Br</td>
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<tr>
<td>Iodine</td>
<td>126.9045</td>
<td>¹²₇I</td>
<td>126.9045</td>
<td>100</td>
</tr>
</tbody>
</table>

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• Higher resolution spectrometers can distinguish very similar molecular formulas.

\[
R = \frac{M_s - M_n}{M_n - M_n} = \frac{250.9133}{250.1933 - 250.1807} \approx 20,000
\]

\[
M_s = \text{higher mass number of two adjacent peaks} \\
M_n = \text{lower mass number}
\]

• Index of hydrogen deficiency (degree of unsaturation) helps predict the number of "pairs" of hydrogens to remove from the corresponding "saturated" formula to produce the molecular formula.

\[
\omega = \frac{2C - H + 2 - X + N}{2}
\]

\[
\text{C}_6\text{H}_6\text{O} \\
\omega = 6
\]
**Methods of Structure Determination**

*Fragmentation and Rearrangement*

- **Nitrogen Rule:** A [M]** of even weight will contain either no or an even number of nitrogens. A [M]** of odd weight will contain an odd number of nitrogens.

- **Odd/Even Fragmentation Rule:** An odd numbered [M]** will create even-numbered ion fragments. An even numbered [M]** will create odd-numbered ion fragments.

- Fragmentation occurs in either a homolytic or heterolytic manner from [M]**; lower lying orbitals (p* or n) typically absorb ionizing radiation, initiating fragmentation.

- Fragmentations eliminating very stable, neutral molecules (H₂O, NH₃, CO) are highly favorable, as well as cleavage of C-C bonds near heterocatoms.

- Rearrangements are not a simply the cleavage of bonds but an intramolecular rearrangement (typically H-atom migration). Characterized by an observation of a fragment that differs by 1 from the odd/even fragmentation rule, indicating the retention of H-atom in the rearrangement.

**Mistaken Identity: MS Analysis**

*Halipeptins*

\[\text{R= Me, Halipeptin A} \quad \text{R= H, Halipeptin B (Proposed)}\]

Potent anti-inflammatory agent isolated from marine sponge *Haliclona* sp.

**Key Characterization Data:**

- **NMR:** ¹H, ¹³C, ¹⁵N, DEPT, COSY, TOCSY, HMQC, HMBC, ROESY
- ¹⁵N shows peak from δ ~260 to ~270 ppm (similar to N-O bond)
- **MS:** HRFAB m/z 627.4073 [M+H]**
  - Also determined using chemical degradation (MeO⁻) and MS analysis, as well as FAB and ESIMS/MS fragmentations.
  - Subtracting other characterized motifs leaves C₄H₄NO₂, which predicts 2 degrees of unsaturation.
  - Data seems to fit oxazetidine motif...

**Mistaken Identity: MS Analysis**

**Halipeptins**

- MS data for recently isolated Halipeptin C suggested a sulfur atom instead of 2 oxygens.
- MS re-evaluation of Halipeptin A and B reveal same trend.
- Molecular formula left with C₄H₂NS, requiring 2 degrees of unsaturation.

"...our first HRMS measurements... were more in agreement with C₃H₁₀N₂O₈ than with C₃H₁₅N₂O₇, leading to misinterpretation of the NMR data... new data were obtained on superior instrumentation..."

- ¹H and ¹³C suggest thiazoline moiety, as well as HMBC correlatins between quaternary Me and α-CH₂.
- Reassignment is also supported by NMR calculations of related oxazetidines and thiazolines, as well as ¹³C comparisons to a synthetic analog.


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**Methods of Structure Determination**

**Chirotopical Properties: Optical Activity**

**Chirotopical properties:** Properties of chiral substances arising from their nondestructive interaction with anisotropic radiation, allowing the differentiation between two enantiomers.

**Light Polarization:** To confine vibrations of the electric vector of light waves to one direction, usually by reflection or transmission through certain substances; "plane polarized light"

**Optical Activity/ Rotation:** The change of rotation in the plane of polarization that occurs when polarized light is passed through an optically active substance.

**Polarizability:** The measure of the response of a molecule to an external electric field; when placed in a field, the displacement of electric charge induces a dipole in the molecule.

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**Technique** | **Principle** | **Chromophore Necessary?**
--- | --- | ---
Optical Rotation | Refraction | No
Circular Dichroism | Absorption | Yes

optical activity occurs when \( n_\parallel \neq n_\perp \)  
\((n = \text{index of refraction})\)

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Methods of Structure Determination

Chirotopical Properties: Optical Activity

- Optical rotation data obtained using polarimeter, and the data is analyzed utilizing Biot's Law:

\[ \alpha = |\alpha| \cdot c \cdot l \]

\[ |\alpha| \cdot c = \frac{\alpha}{l \cdot (\text{dm}) \cdot c(g/mL)} = \frac{100\alpha}{l \cdot (\text{dm}) \cdot c'(g/100\ mL)} \]

\(\alpha\) = observed angle of rotation of the plane of polarization

|\(\alpha|\) = specific rotation (proportionality constant), dependent upon temperature (T) and wavelength (\(\lambda\)), usually Sodium D line (589.3 nm); also dependent on solvent

l = length of cell, usually in dm

c = concentration of sample, in units of g/mL for neat liquids, but more commonly g/100 mL (wt/V%) for solutions (see second equation)

- Molecules characterized by the direction of rotation:
  (+) = dextrorotatory
  (−) = laevorotatory

- Traditional notation:
  \(d = (+), l = (−)\), and \(dl = (\pm)\)

  \(\pm\) = right handed enantiomer, related to \(\pm\)-Glyceraldehyde

  \(\pm\) = left handed enantiomer, related to \(\pm\)-Glyceraldehyde

- The direction of which light is rotated is not dependent on whether the stereogenic center is \(R\) or \(S\); therefore, \(\pm\) can be (+) or (−). Also, absolute stereochemistry can not be determined by rotation alone.

Mistaken Identity: Optical Rotation

Terpestacin


- Both reports support same optical rotation values.

- "New" isolate from a different species reported in 2001 identical to Terpestacin isolated in 1993 except for the optical rotation; therefore, assigned as its enantiomer.

- Meyers asymmetric synthesis of Terpestacin in 2002 supports the latter optical rotation data...?

What’s going on?

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific Rotation ((\lbrack \alpha \rbrack_d))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993 Isolation</td>
<td>+26 (c 0.5, CHCl₃)</td>
</tr>
<tr>
<td>1998 Synthesis</td>
<td>+27 (c 0.22, CHCl₃)</td>
</tr>
<tr>
<td>2001 Isolation</td>
<td>−16.5 (c 0.29, CDCl₃)</td>
</tr>
<tr>
<td></td>
<td>−21.5 (c 0.32, MeOH)</td>
</tr>
<tr>
<td>2002 Synthesis</td>
<td>−17 (c 0.58, CDCl₃)</td>
</tr>
<tr>
<td>(Meyers)</td>
<td>−18 (c 0.06, MeOH)</td>
</tr>
</tbody>
</table>

**Circular Dichroism**

- CD arises from unequal absorption of right- and left-handed circularly polarized light (cpl) by chiral chromophores.

\[
[\alpha]D = +33 \ (c \ 0.03, \ CHCl_3)
\]

- Depending on the lot and age of CHCl₃, varying quantities of Cl-containing product formed.
- CHCl₃ stored over K₂CO₃ generates small quantities of Cl₂!
- CHCl₃ stored over MS4Å did not test positive for Cl₂ and gave stable solutions and reproducible rotations.

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</table>


**Useful for identifying protein structures**

![CD Spectrum of Subtilisin](image)
Circular Dichroism
The Octant Rule for Carbonyl Compounds

- The Octant Rule can be used to predict the sign of the observed CD based on the absolute structure, or vice versa.

\[(R)-3\text{-methylcyclohexanone} \quad \text{observed CE: } \Delta \alpha = +0.56\]

To apply the octant rule, first superimpose three-dimensional axes over the structure such that the carbonyl bond resides along an axis and the carbonyl carbon resides at the origin. Next, rotate so that you view down the carbonyl bond, with the O atom in front, to produce a 2D picture. The visible octants are labeled as shown. The sign of the octant containing the most polarizable group determines the sign of the CD.

Elie, "Stereochemistry of Organic Compounds" Ch. 13
Computational predictions (VCD & DFT):

IR Spectroscopy
Reference Data

**Overview of Advanced NMR Experiments**

\(^{13}\text{C} \text{ DEPT (Distortionless Enhancement by Polarization Transfer)}\)

- Three experiments which differentiate C, CH, CH\(_2\), CH\(_3\)
- Variable proton pulses at 45\(^\circ\), 90\(^\circ\), and 135\(^\circ\) affect the carbon types differently

**References for NMR experiments:**
- Silverstein & Webster, "Spectrometric Identification of Organic Compounds"
- Roberts, "ABCs of FT-NMR"
- Claridge, "High-Resolution NMR Techniques in Organic Chemistry"

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Overview of Advanced NMR Experiments

1D Difference NOE (Nuclear Overhauser Effect)

- NOEs arise from dipole-dipole interactions of atoms in close proximity to one another (up to 5 Å). NOE interactions can also be used to determine internuclear separation.
- By irradiating a particular resonance, atoms within range will be affected by either an enhancement or depression of the signal. Subtracting the reference spectrum from the enhanced spectrum reveals the resonances experiencing NOEs.
- 1D NOE detects steady-state NOEs and therefore are only applicable to MW < 1000
- NOEs also lead to a signal enhancement in ¹H decoupled ¹³C

Spectrum A - ¹H NMR

Spectrum B - Difference NMR from irradiation of protons 1. Proton 2 shows through space signal enhancement.

Spectrum C - Difference NMR from irradiation of protons 3. Protons 2 and 4 show through space signal enhancement.

Overview of Advanced NMR Experiments

2D NOESY (Nuclear Overhauser Effect Spectroscopy)

- Observes the same phenomena as the 1D NOE but transient effects. These are dependent on the kinetic rate that these effects develop. As a result, determining internuclear distances from these spectra are not as reliable as in the 1D NOE experiment.
- Transient NOEs are observable for small molecules (MW < 1000) and large molecules (MW > 2000)
- For medium sized molecules a different observation technique is used to detect the same effects (ROESY). ROESY requires more scans and therefore longer acquisition.

2D NOESY of Andrographolide peaks in red indicate positive NOE effects
Overview of Advanced NMR Experiments

2D COSY (COrelation Spectroscopy)

- 2D COSY reveals scalar, through-bond couplings between protons.
- One $^1$H spectrum is shown on each axis.
- Start from cross-peaks on the diagonal and trace either vertical or horizontal. Off-diagonal cross-peaks indicate coupled protons.
- The number of protons in a particular spin system is readily determined.

Overview of Advanced NMR Experiments

2D DQF-COSY (Double-Quantum Filtered COrelation Spectroscopy)

- Modified 2D-COSY technique
- An additional pulse to the pulse sequence, single quantum transitions are suppressed, leaving double quantum and higher transitions. As a result, non-coupled resonances like methyl singlets, will be greatly reduced. This allows better resolution of coupled cross-peaks.
- The spectrum on the whole appears "cleaner"
Overview of Advanced NMR Experiments

TOCSY (TOtal Correlation SpectroscopY)

- TOCSY shows correlations between protons in the same spin system even if they aren't directly coupled. DQFCOSY only shows protons directly coupled.

- 1D TOCSY shows correlations between protons in the same spin system even if they aren't directly coupled.
- Mixing time is critical, but longer mixing times cause loss of resolution and signal.
Overview of Advanced NMR Experiments

2D TOCSY (TOtal Correlation Spectroscopy)

- 2D TOCSY shows correlations between protons in the same spin system even if they aren't directly coupled.
- Mixing time is critical, but longer mixing times cause loss of resolution and signal.
- TOCSY shows that anomeric protons 1 and 2 are in separate spin systems. Furthermore, the complex multiplet between 3.5 and 4.0 are separated into the two component ring resonances.

β-lactose

Overview of Advanced NMR Experiments

2D HETCOR (HETeronuclear chemical shift CORrelation)

- HETCOR is the $^1$H-$^{13}$C COSY. $^{13}$C is the detected nucleus.
- Shows couplings between carbons and the attached protons.
- Unlike COSY, there is no symmetry to the 2D spectrum. Tracing vertically down from each carbon resonance indicates the number and identity of attached protons.
- A related experiment is HMOC (Heteronuclear Multiple Quantum Coherence) detects $^1$H instead of $^{13}$C. This increases sensitivity, but resolution suffers. This variant is sometimes called inverse-detected HETCOR.

ipsenol
Overview of Advanced NMR Experiments

2D HMBC (Heteronuclear Multiple Bond Coherence)

- HMBC shows long-range (2-3 bond) ¹H-¹³C correlations. Couplings to attached carbons are not observed. ¹H is the detected nucleus.
- Two-bond correlations are almost always found, but three-bond correlations are sometimes absent.
- Artifacts from ¹³C satellites occasionally appear, especially with methyl carbons. These are easily identified since the apparent cross-peak does not match a resonance in the 1D spectrum.

![HMBC Spectrum]

ipsenol

Overview of Advanced NMR Experiments

INADEQUATE (Incredible Natural Abundance Double QUantum Transfer Experiment)

- Similar to COSY, but targeting ¹³C instead of ¹H. Shows ¹³C-¹³C couplings.
- Readily reveals C-C connectivity; a cross-peak in the INADEQUATE spectrum indicates the carbons are connected to one another.
- At high resolution, each cross-peak appears as a doublet.

- Downside: requires large quantities of material (~250 mg) due to the low natural abundance of ¹³C
Schizogane Alkaloids
Modern NMR Analysis to the Rescue!

\[\text{Schizogamine} \quad \text{Isoschizogamine}\]

IR = 6.07 μ (1647 cm\(^{-1}\))
\(\lambda_{\text{max}} = 264 \text{ (log } \varepsilon = 4.09) , 302 \text{ (log } \varepsilon = 4.00)\)
\([\alpha]_D = -7.9^\circ \text{ (CHCl}_3 , c = 1 , 25 ^\circC)\)
MP 123-125 °C

IR = 5.93 μ (1686 cm\(^{-1}\))
\(\lambda_{\text{max}} = 259 \text{ (log } \varepsilon = 4.12) , 290 \text{ (log } \varepsilon = 3.87)\)
\([\alpha]_D = -239^\circ \text{ (CHCl}_3 , c = 1 , 25 ^\circC)\)
MP 184-185 °C

Typical IR stretching frequency for tertiary amide carbonyls: 5.99-6.14 μ (1670-1630 cm\(^{-1}\))
Typical IR stretching frequency for δ-lactam carbonyls: -5.95 μ (~1680 cm\(^{-1}\))
Typical IR stretching frequency for γ-lactam carbonyls: -5.88 μ (~1700 cm\(^{-1}\))

Isolation:
Renner, Experientia 1963, 19, 244-246.
Renner, Lloydia 1964, 27, 267.

Structure Revision:

Total Synthesis:

Other approaches:

Isoschizogamine
Problems with NMR Data

\[\text{Isoschizogamine} \quad \text{proposed structure}\]

\[\text{Expected } ^{13}\text{C NMR resonances:}\]

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<th>δ (ppm)</th>
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<td>30</td>
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<td>21</td>
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<td>3°</td>
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\[\text{Observed } ^{13}\text{C NMR resonances:}\]

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<td>84.4</td>
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<td>3°</td>
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<td>37.66</td>
<td>3°</td>
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\[\text{Expected } ^{1}\text{H NMR features:}\]

C-H singlet from C21
4 H spin system from C16 & C17
5 H spin system from C5, C6 & C7

\[\text{Observed } ^{1}\text{H NMR resonances:}\]

No C-H singlet
10 H spin system (\(^{1}\text{H}-^{1}\text{H} 2D-COSY) of the form
N-CH\(_2\)-CH\(_2\)-CH-CH\(_2\)-CH\(_2\)

Something is obviously wrong!
Data are okay for Schizogamine, however.
**Isoschizogamine - A Biosynthetic Structure Revision**

Accounts for observed NMR features:
- C18 is a γ-lactam
- C21 is an aminal
- 10 H spin system from C5, C6, C7, C2, C16, C17

Isoschizogamine - A Biosynthetic Structure Revision

NOE data also agree with this structure assignment.
Isoschizogamine
Total Synthesis

\[
\text{KHMDS; \ Bu_2BOTf; \ H_2, Pd/C; PhMe, } \Delta \quad (49\% \ yield)
\]

\[
95:5 \text{ dr}
\]

\[
\text{Isoschizogamine Total Synthesis; Bu_2BOTf; H_2, Pd/C, -40 °C; PhMe, Δ.}
\]

\[
88:12 \text{ dr}
\]

(74\% yield, 2 steps)

Isoschizogamine
Total Synthesis

\[
[\text{Ph(CF}_3\text{)}_2\text{CO]}_2\text{SPh}_2, \text{CH}_2\text{Cl}_2, \text{rt} \quad (74\% \ yield)
\]

\[
\text{single diastereomer}
\]

(74\% yield)

1) \ NaBH}_4, \text{Cu(acac)}_2, \text{EtOH, THF}

2) \ LiAIH}_4, \text{THF}

(29\% yield, 4 steps)

Isoschizogamine
8 total steps, 7\% overall yield
Case Study: Batzelladine F

Introduction


- Relative stereochemistry of C1-C9 based on nOe data, however, no information or analysis was provided in the isolation paper.

- Lengths of connecting chain (C10-C16) and nonyl chain at C25 assigned based on MS fragmentation data, although this was also not presented or discussed in the isolation article!

- Relative or absolute configuration at C16 could not be determined.

Case Study: Batzelladine F

Batzelladine D Revises Guanidinium Stereochemistry

- Snider and Chen were the first to prepare analogues and reassign stereochemistry based on $^1$H and $^{13}$C NMR, as well as chemical degradation (Snider, B. B. et al. Tetrahedron Lett. 1996, 37, 6977-6980).

- Overman followed with proving the absolute and relative stereochemistries based on Biginelli condensation.

**Case Study: Batzelladine F**

*Determining the Relative Stereochemistry of the Left-Hand Fragment*

Comparison of synthetic analogues to determine relative configuration of batzelladine F left-hand fragment.

<table>
<thead>
<tr>
<th>carbon #</th>
<th>A</th>
<th>natural batz. F</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.7</td>
<td>20.7</td>
<td>20.7</td>
</tr>
<tr>
<td>2</td>
<td>48.9</td>
<td>47.2</td>
<td>47.3</td>
</tr>
<tr>
<td>4</td>
<td>56.5</td>
<td>57.5</td>
<td>57.5</td>
</tr>
<tr>
<td>7</td>
<td>56.4</td>
<td>57.4</td>
<td>57.5</td>
</tr>
<tr>
<td>9</td>
<td>53.1</td>
<td>51.6</td>
<td>51.6</td>
</tr>
</tbody>
</table>


---

**Case Study: Batzelladine F**

*Possible Diastereomers for the Left-Hand Fragment*

No information on absolute stereochemistry, therefore, 4 possibilities for left-hand fragment.
Case Study: Batzelladine F
Retrosynthetic Analysis of the Proposed Structure

\[ \text{Batzelladine F} \]
(proposed)

\[ \text{Batzelladine F} \]


Case Study: Batzelladine F
Synthesis of the Four Proposed Diastereomers

\[ \text{(S)-BINAP•RuCl}_2\text{NEt}_3 \]
H$_2$Cl, MeOH, 40 °C
(90%)

\[ \text{I} \]
8 Steps
24% overall yield

\[ \text{Batzelladine F} \]

**Case Study: Batzelladine F**

*Synthesis of the Four Proposed Diastereomers*

![Chemical structures and reactions](image)

1. aq. NaBF₄
2. MeCl, Bu₄N, CH₂Cl₂, 0 °C
3. Et₃N, CHCl₃, 70 °C (55% over 3 steps)

64% + <10% of the syn isomer at C20 and C23

- All four diastereomers prepared in same manner, however **NONE** matched the HPLC trace of natural batzelladine F (co-injection).
- What is in error? NMR is not useful due to essentially identical spectra, but what about MS?

**Case Study: Batzelladine F**

*MS Analysis of Prepared Diastereomers vs. Natural*

- Synthesized Diastereomers
  - m/z 625 [M–H]^+

- m/z 276
  + m/z 350

- MS analysis for synthetic material yields two major peaks, m/z = 276 and 350.
- MS analysis of natural material yields two different major peaks, m/z = 304 and 322.


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**Case Study: Batzelladine F**

*MS Analysis of Prepared Diastereomers vs. Natural*

- batzelladine F (authentic) → m/z = 322
  + m/z = 304

- batzelladine F (authentic)
  - NaOMe, MeOH
  - 110 °C
  - Exact Mass: 336.26651
  - $C_{15}H_{20}N_3O_2$
  + Exact Mass: 322.2858
  - $C_{14}H_{16}N_2O$

**Fragmentation of Synthetic material gave fragments that are ± 28 amu than expected.**

**Not Consistent with 2D NMR Data!**
Case Study: Batzelladine F
MS Analysis of Prepared Diastereomers vs. Natural

What could account for an exchange of 28 amu on either side, yet still match 2D NMR data?

−CH₂CH₂− = 28

The previous synthetic routes are still applicable, however, there are still four diastereomers possible for left-hand fragment.


Case Study: Batzelladine F
Synthesis of Revised Structure and Diastereomers

All four possible diastereomers prepared for comparison.

Case Study: Batzelladine F
HPLC Analysis of Four Diastereomers of Revised Structure

1 co-elute with 2 and authentic batzelladine F
2 co-elute with 1 and authentic batzelladine F

NMR and Optical Rotation Not Useful...

3 NOT batzelladine F by HPLC
4 NOT batzelladine F by HPLC


Case Study: Batzelladine F
Circular Dichroism Saves the Day!

1 HPLC co-elute with 2 and authentic batzelladine F
2 HPLC co-elute with 1 and authentic batzelladine F

Case Study: Sporol and Neosporol

NMR Comparisons

- 1 methine (next to ether)
- 7 methylenes
- 1 next to alcohol
- 2 methyls


Case Study: Sporol and Neosporol

Retroynthetic Analysis

**Case Study: Sporol**

**Claisen Rearrangement: Enol Ethers with Controlled Geometry**


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**Case Study: Sporol**

*Preparation of the Dioxane*

**Case Study: Sporol**

*End Game*

![Chemical structure]

Ph$_3$PCH$_2$Br, tBuOK
THF, reflux (82% yield)

![Chemical structure]

twice repeated:
DIBAL-H

Hexanes (37% yield)

---

**Mistaken Identity: Organic Synthesis**

*Assigned Patchouli Structure*

![Chemical structure]

CH$_3$CO$_2$H, NaOAc, 1 - 4 °C

![Chemical structure]

Ac$_2$O, pyridine reflux

Is this patchouli?

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**Mistaken Identity: Organic Synthesis**

*Assigned Patchouli Structure*

Büchi, G. et al. *JACS* 1964, 86, 4438-4444

**Büchi, G. et al. *JACS* 1964, 86, 4438-4444**

**Biginelli Condensation**

*Defining Syn and Anti Stereochemistries*