# Structure Determination of Macrocyclic Compounds with Micro ED

**Literature Seminar**
**2024. 04. 20**

**M2 Manaka Matsumoto**

# Contents

# Contents

# beyond Rule of 5 (bRo5) Chemical Space

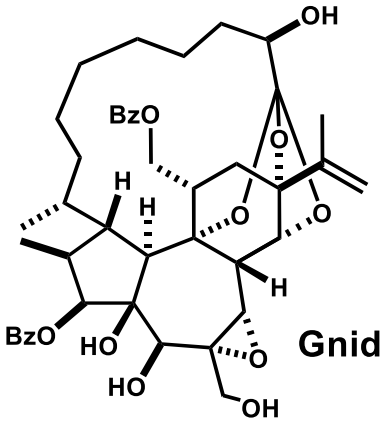


**Lipinski's rule of 5**

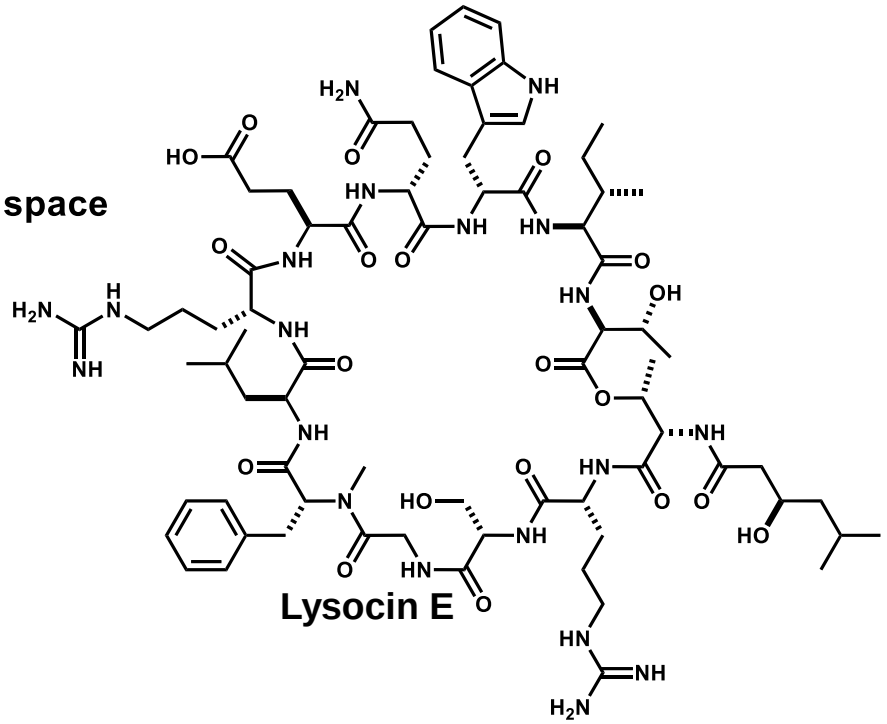
1. MW ≤ 500 Da
2. clogP ≤ 5
3. hydrogen bond acceptors (HBAs) ≤ 10
4. hydrogen bond donors (HBDs) ≤ 5

**new modalities in 'beyond rule of 5' chemical space**

**· macrocyclic natural products**

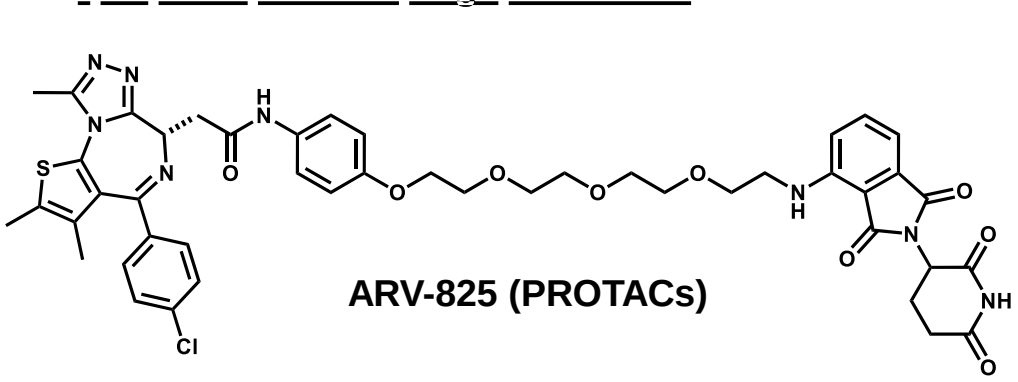


Gnidimacrin

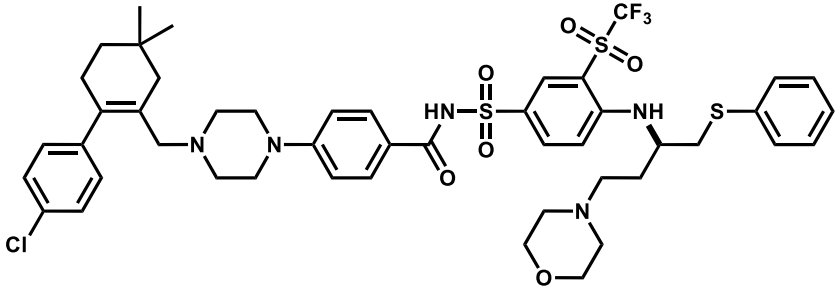


Lysocin E

**· De novo desined large molecules**



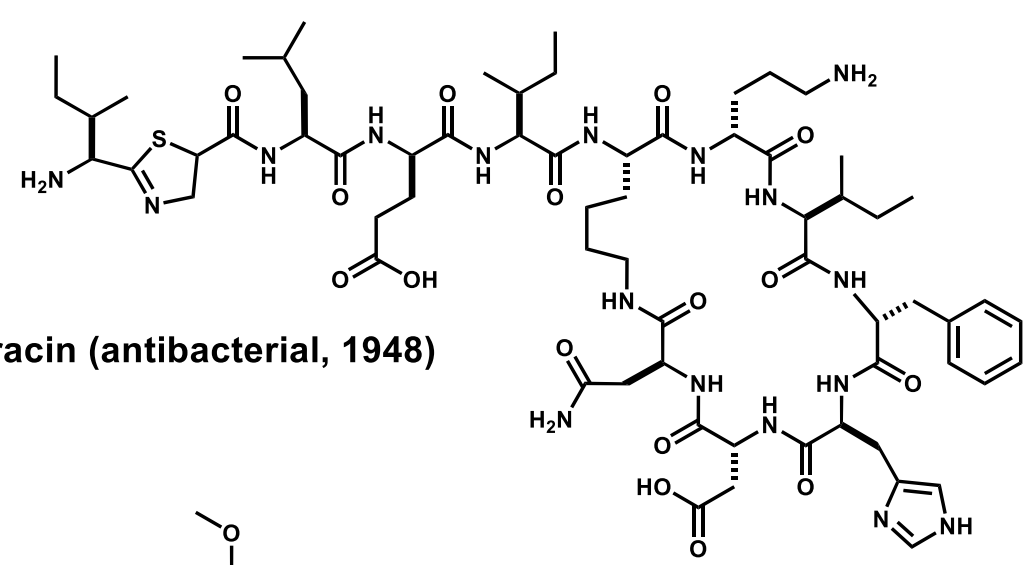
ARV-825 (PROTACs)



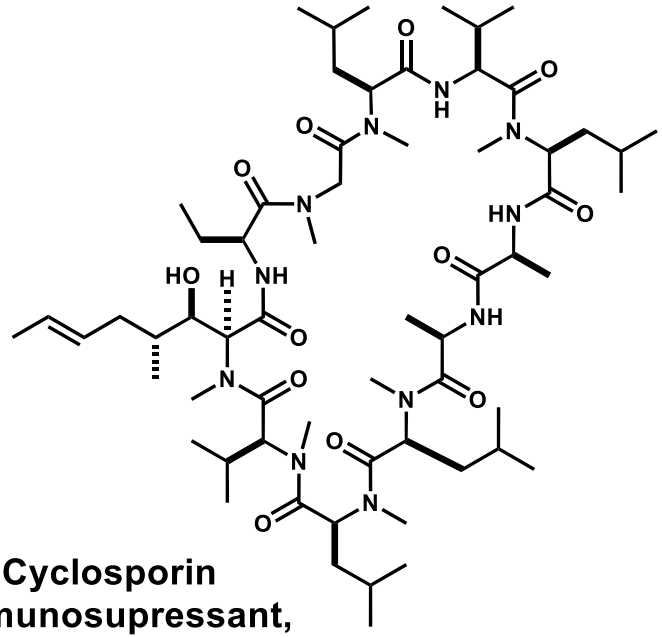
Navitoclax (apotosis regulator)

# Macrocyclic Compounds as Drug Leads

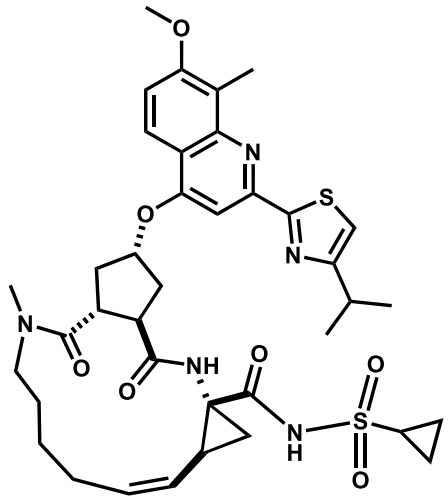




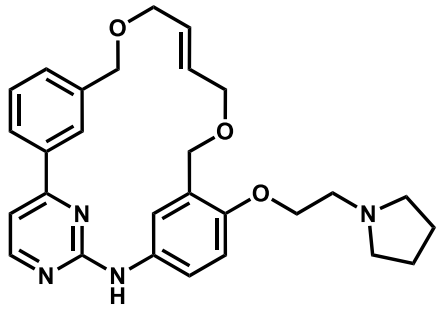
Bacitracin (antibacterial, 1948)



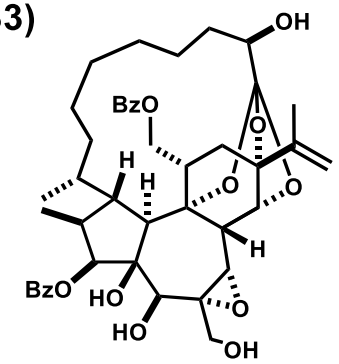
Cyclosporin (immunosupressant, 1983)



Simeprevir (antiviral, 2013)



Pacritinib (anticancer, 2022)



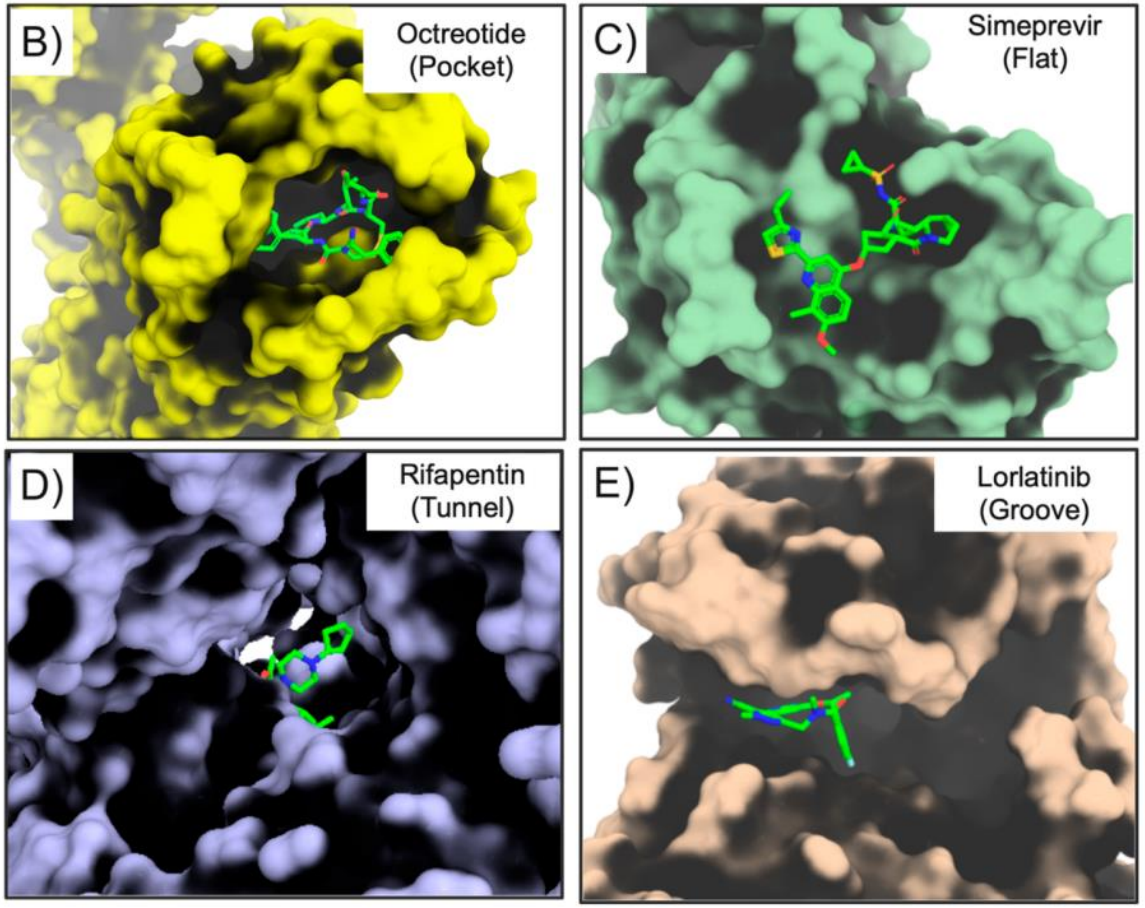
Gnidimacrin (anti-HIV, future)

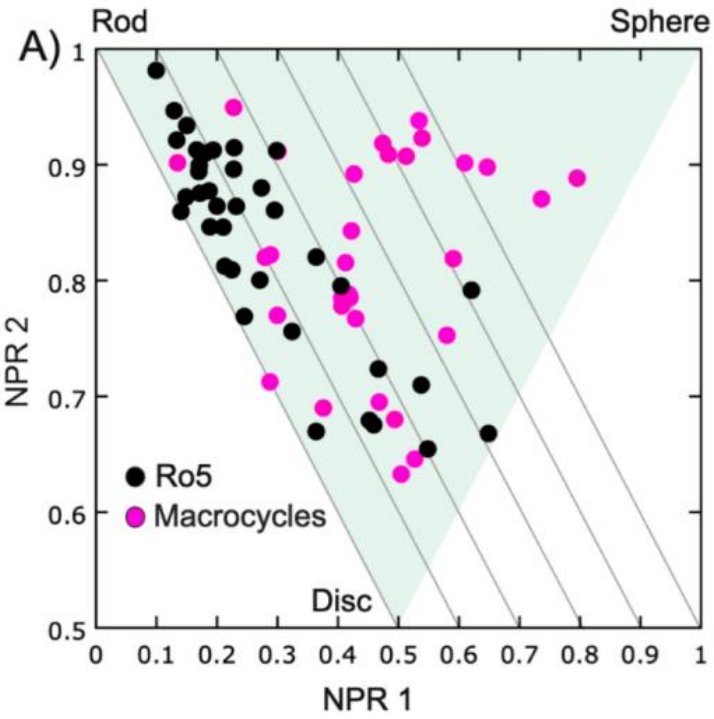
# Affinity and Selectivity



**pre-organized but flexible structure**
**→ access to 'undruggable' binding sites**
**i.e. flat, tunnel, groove**

**likeliness to adopt desc- and sphere-like conformtaions**

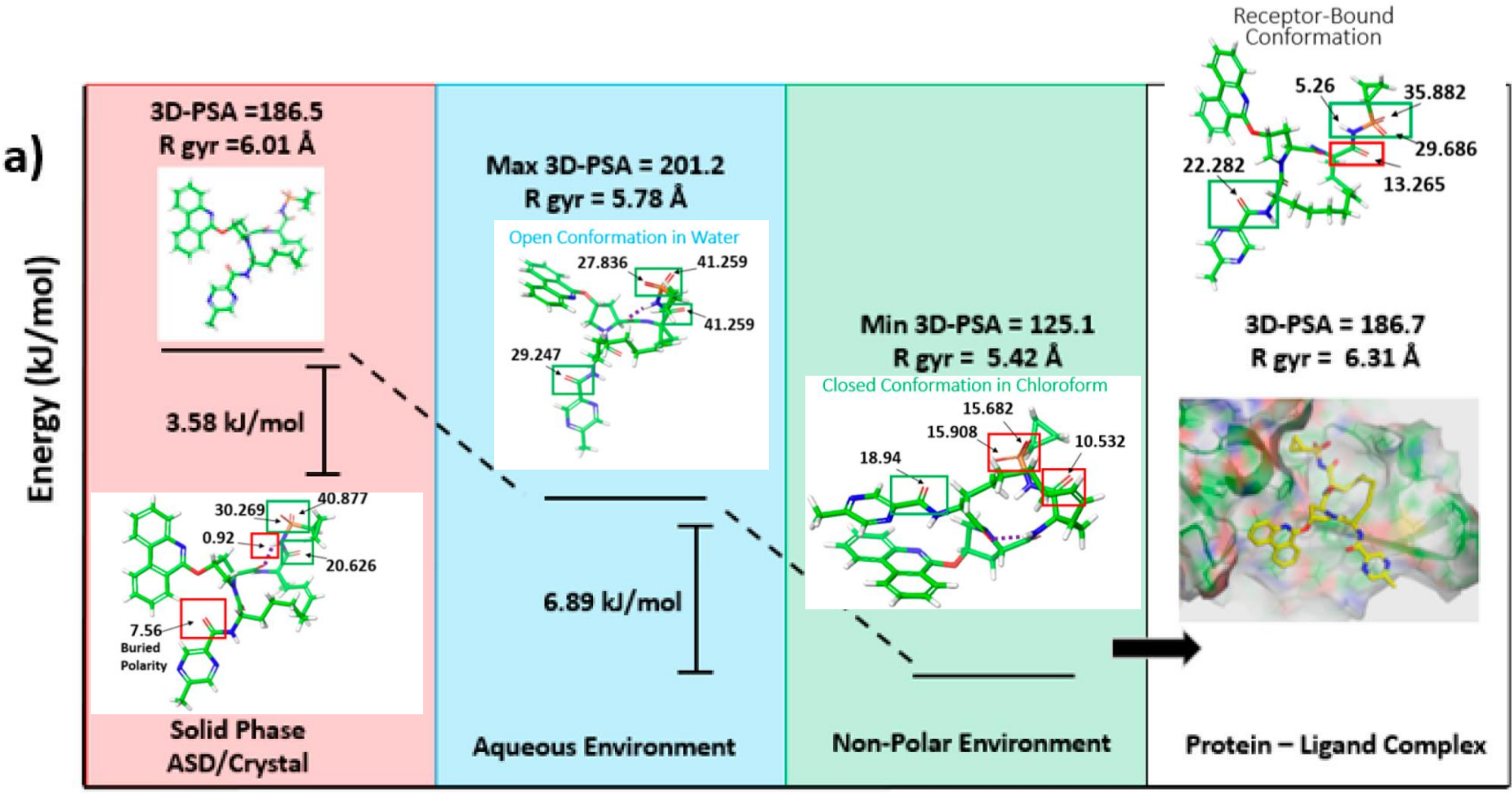



**some act as molecular glue for two proteins that form the groove**

1) Garcia Jimenez, D.; Poongavanam, V.; Kihlberg, J. . *J. Med. Chem.* **2023**, *66*, 5377

# Molecular Chameleons




**Flexible macrocycles can change their conformation responding to the external environment. This chameleonic behavior is important for uptake and permeability.**

1) Sheikh, A. Y.; Mattei, A.; Bhardwaj, R. M*J. Am. Chem. Soc.* **2021**, *143* , 17479

# Introduction of Prof. Gonen

**Prof. Tamir Gonen**

**1998 Bachelor of Science @ The University of Auckland, New Zealand**
**2002 Ph.D. @ The University of Auckland (Prof. Edward N. Baker)**
**2002-2005 Postdoctoral fellow @ Harvard Medical School (Prof. Thomas Walz)**
**2005-2011 Assistant Professor @ University of Washington**
**2011 Associate Professor @ University of Washington**
**2011-2017 Group leader @ Howard Hughes Medical Institute Janelia Research Campus**
**2017- Professor @ University of California Los Angeles**

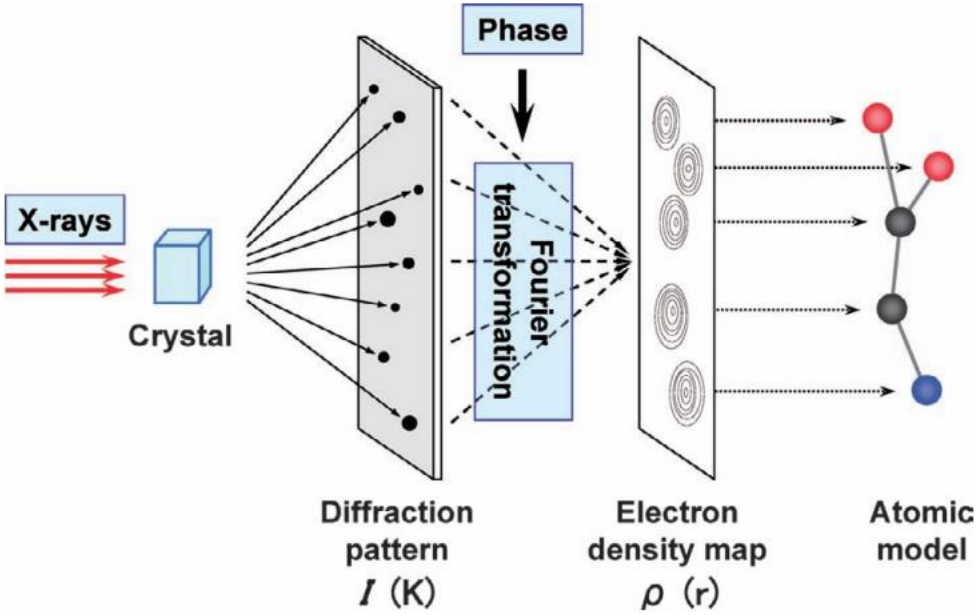
**Research topic: membrane biophysics, crystallography and cryo-EM**

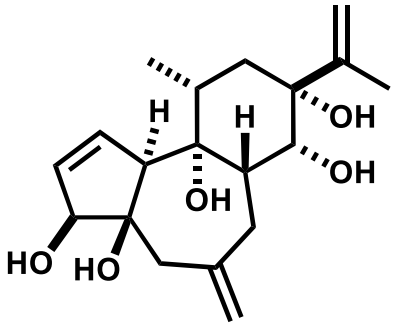
1) https://cryoem.ucla.edu/tamir

# Contents

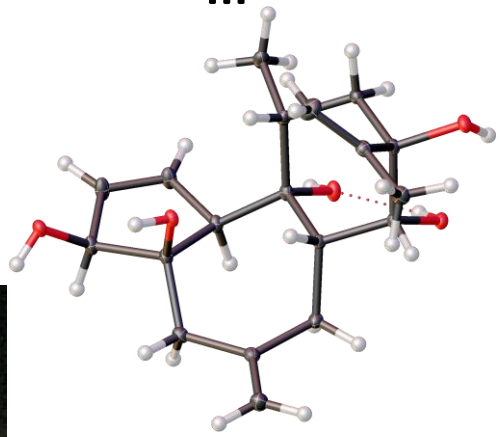
# X-Ray Crystallography

**X-ray crystallography: definitive tool to get unequivocal 3D structural information**

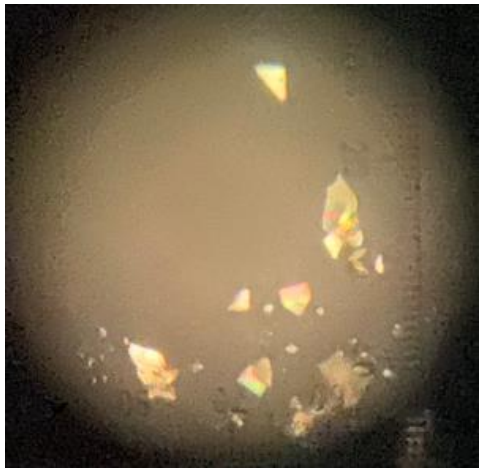



**III**



**bottleneck: requirement of high-quality and large single crystal**

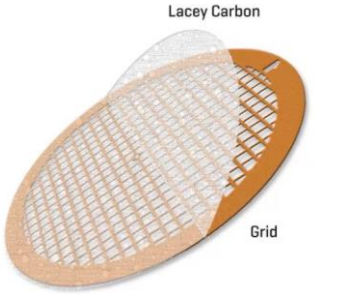


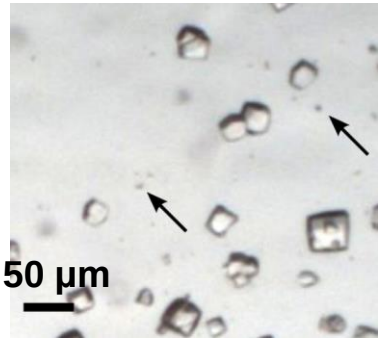
# MicroED

**electron crystallography:**
- **use electron beam instead of X-ray photon**
- **electron beam interacts with nucleus and electrons (strong)**
  **X-ray photons interacts with valence electron (weak)**
  **→ obtain large amount of diffraction data from much smaller crystals**

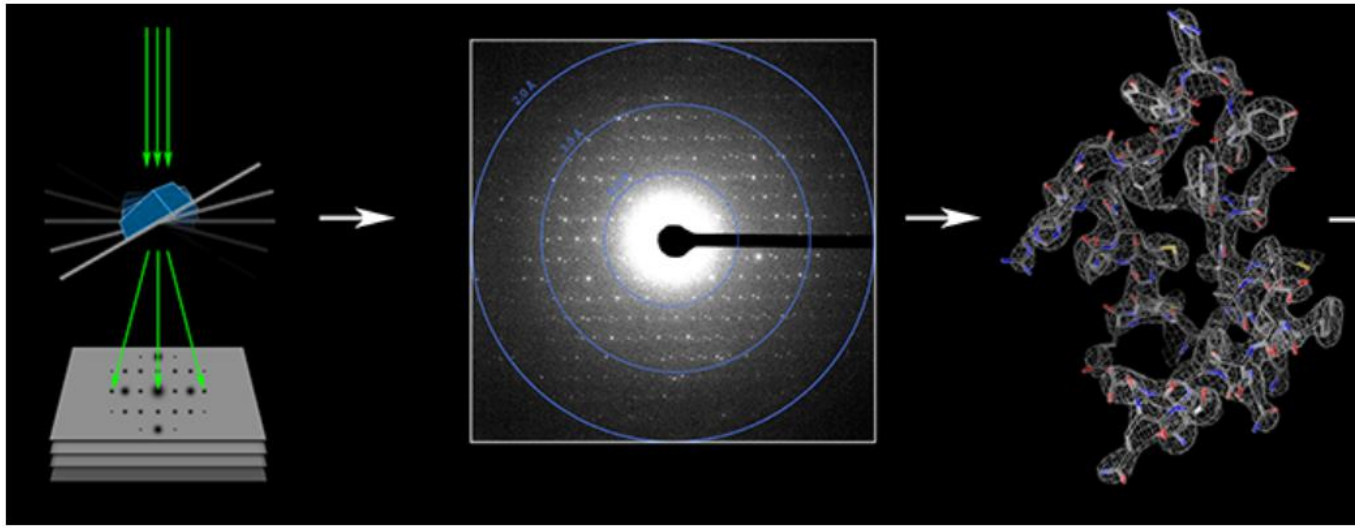
**limitation:**
**one diffraction pattern from each crystal due to beam induced damage**

**Micro ED: developed by Gonen's group in 2013**
- **prevention of the damage by reducing the electron dose to 1/200**
- **data collection using high-resolution camera**




**microcrystals**



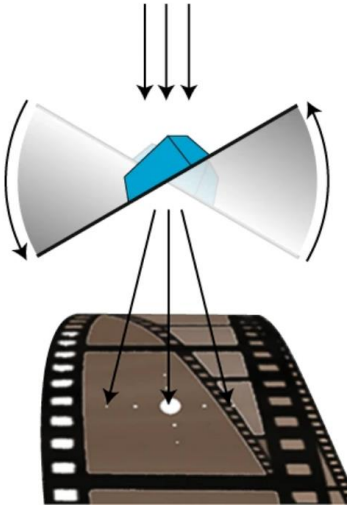
**Cryo Transmission Electron Microscopy (TEM)**

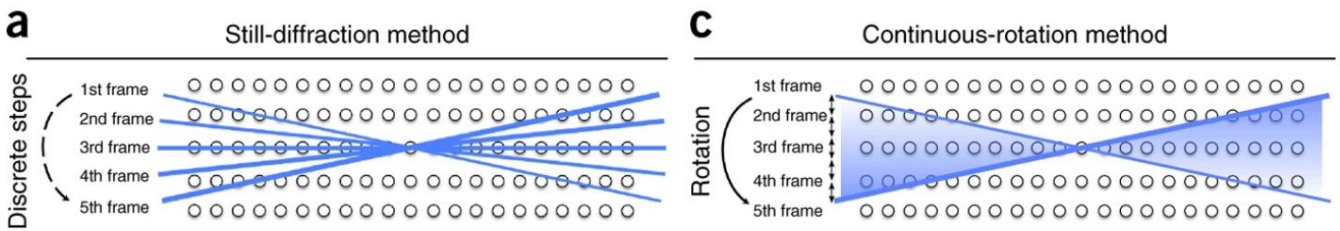
**Lysozyme (2.9 Å)**



1) Shi, D.; Nannenga, B. L.; Iadanza, M. G.; Gonen, T. *eLife,* **2013**, *2*, e01345.

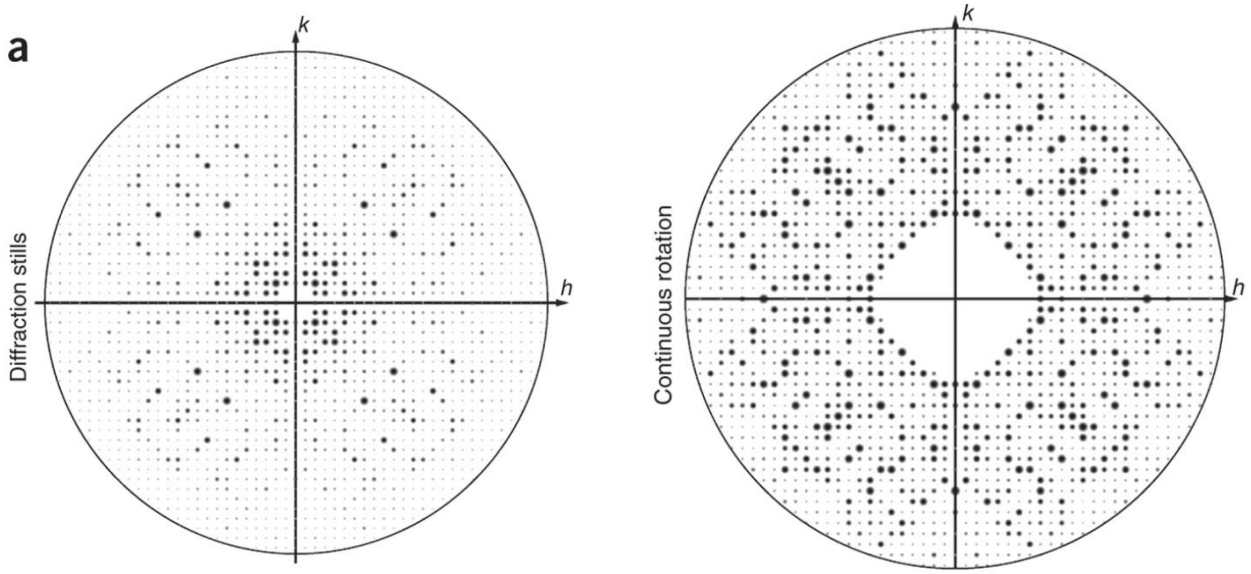
# Continuous Rotation






**- coutinuously rotating the sample**
**- record the diffraction by movie**

**Quality of MicroED data was dramatically improved.**

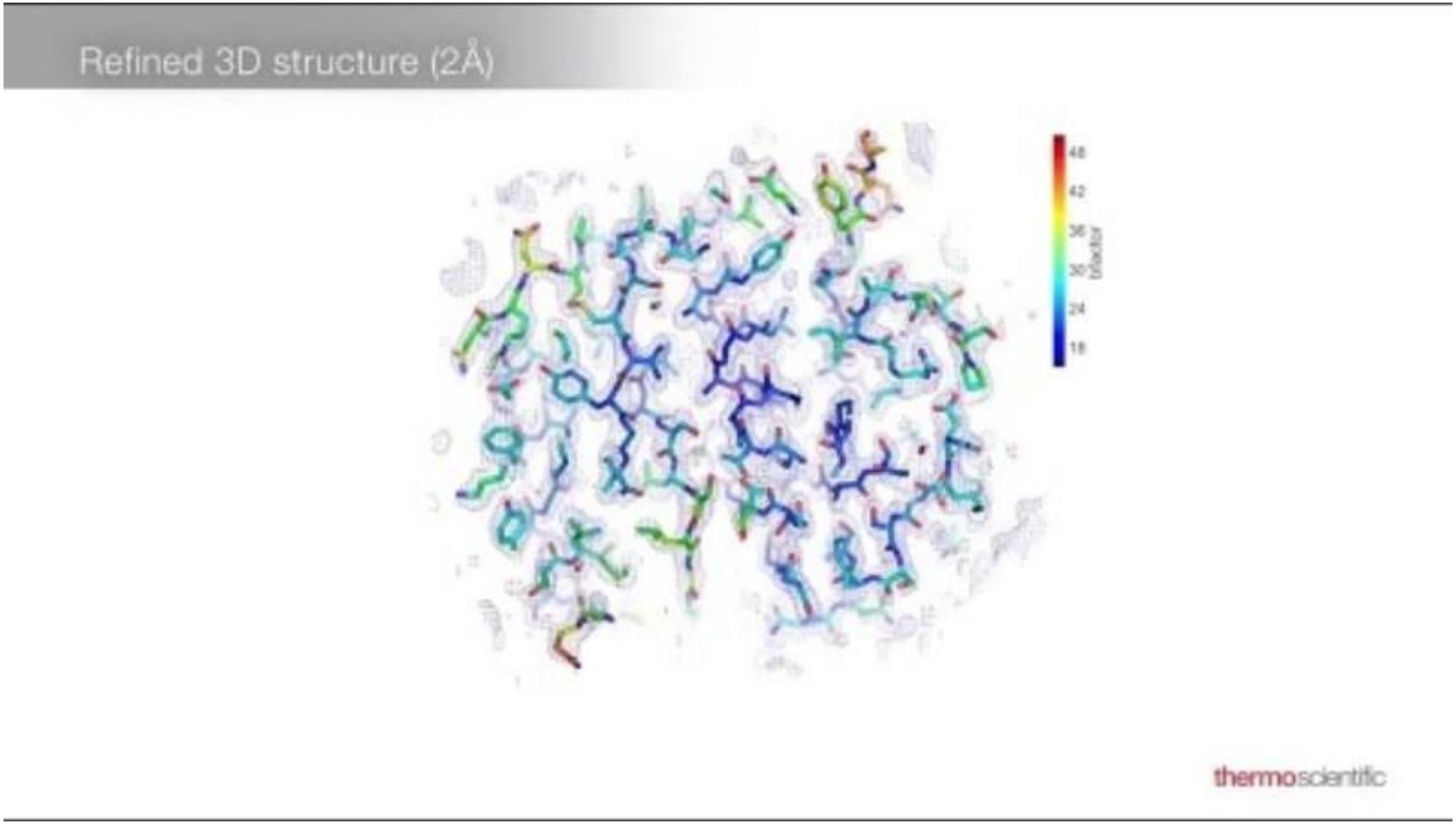





1) Nannenga, B.; Shi, D.; Leslie, A.; Gonen, T. *Nat Methods* **2014**, *11*, 927.

# Methods of MicroED (Movie)

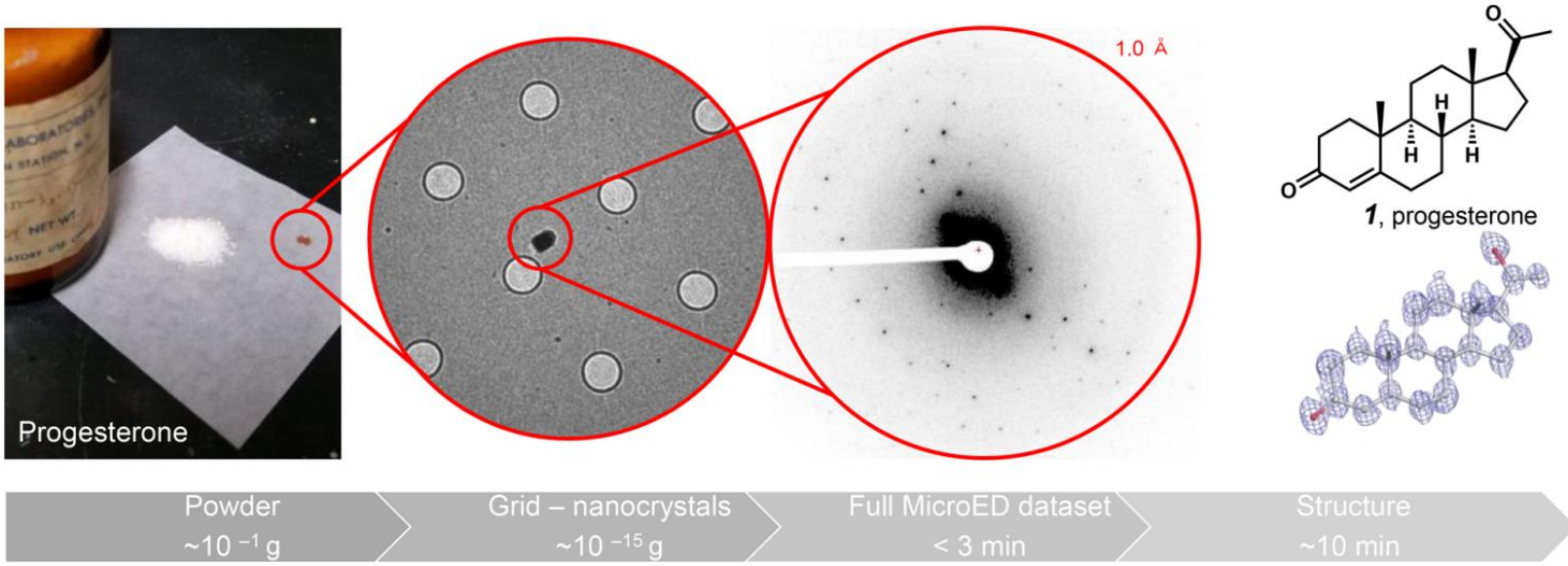





1) https://youtu.be/RxOp9NP3uho?si=EFw9YU9EQWjYUTA1

# Application to Small Molecule

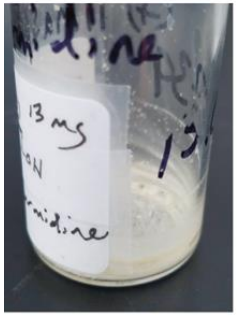


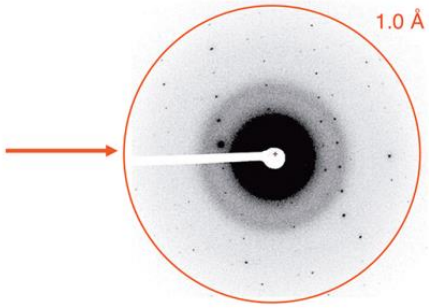



1) Jones, C. G.; Martynowycz, M. W.; Hattne, J.; Fulton, T. J.; Stoltz, B. M.; Rodriguez, J. A.; Nelson, H. M.; Gonen, T. *ACS Cent. Sci.* **2018**, *4*, 1587.

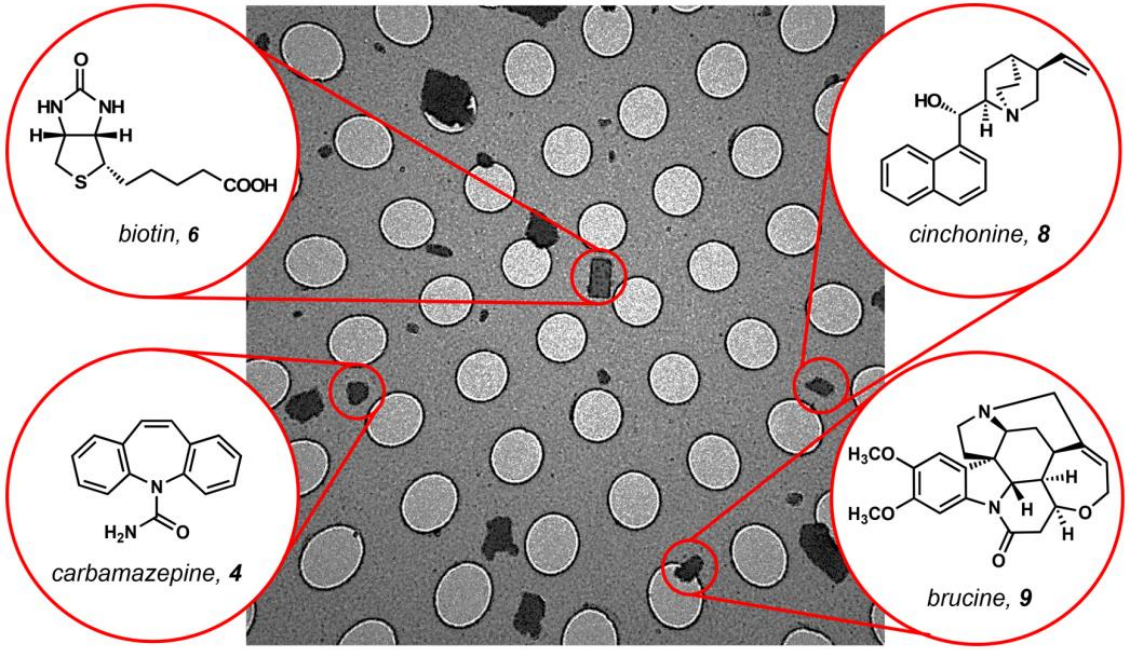
# Advantages of MicroED



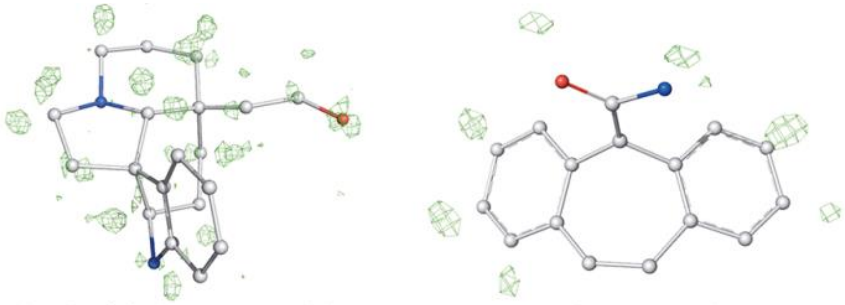





**high resolution data from small amount of microcrystal (apparent amorphous tolerated)**




**mixture of several compounds**



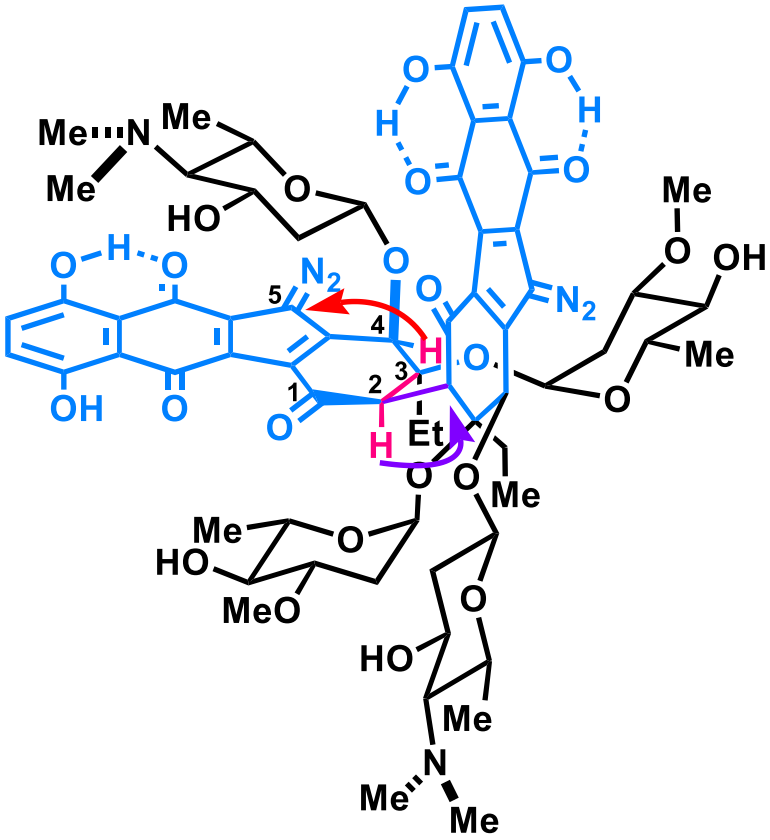
*synthetic (+)-limaspermidine, **10*** carbamazepine, ***4***

**H atom can be detected**

1) Jones, C. G.; Martynowycz, M. W.; Hattne, J.; Fulton, T. J.; Stoltz, B. M.; Rodriguez, J. A.; Nelson, H. M.; Gonen, T. *ACS Cent. Sci.* **2018**, *4*, 1587.

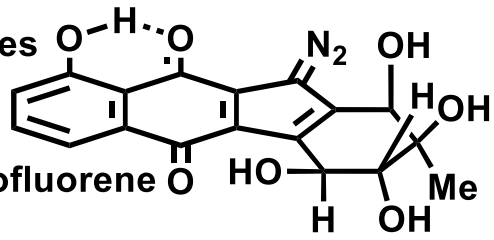
# Structural Revision of the Lomaiviticins (1)





(-)-lomaiviticin A
(2001 structure assignment
by He and coworkers)

relative stereochemistry:
$^{3}J_{H,H}$ coupling constants
comparision to natural glycosides



presense of diazofluorene:
inference from monomeric diazofluorene

only 6/19 carbons on aglycon are proton-attached
complicated assignment of core structure

H2 and H4 seemed singlet (500 Hz $^{1}$H NMR)
but shown COSY correlation
→ W-plane coupling
H2 and H4 are syn, separated by 4 bonds

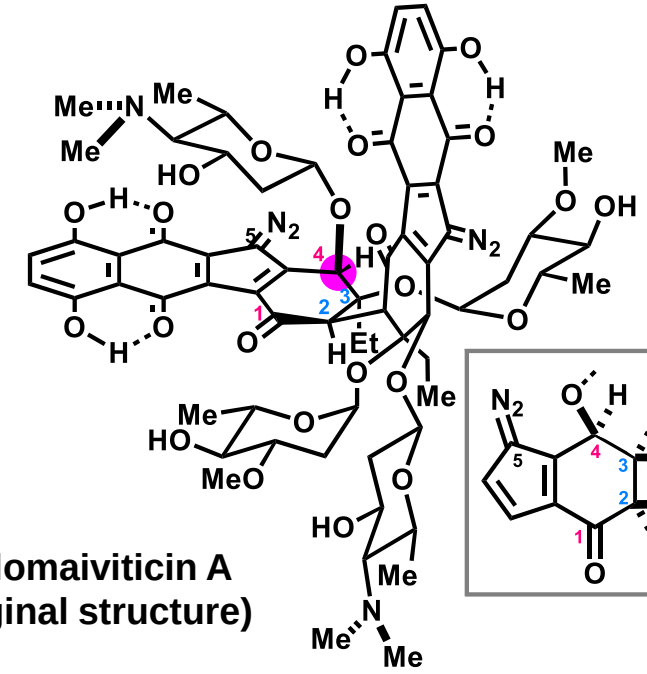
HMBC correlation between H4 and C5
→ $^{3}J_{C,H}$ coupling to locate aminosugar
with respect to the diazocyslooctadiene

HMQC and HMBC correration between H2 and C2
→ location of bridging C-C bond

1) Kim, L. J.; Xue, M.; Li, X.; Xu, Z.; Paulson, E.; Mercado, B.; Nelson, H. M.; Herzon, S. B. *J. Am. Chem. Soc.* **2021**, *143,* 6578.

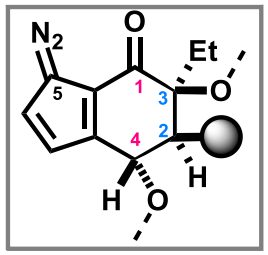
# Structural Revision of the Lomaiviticins (2)

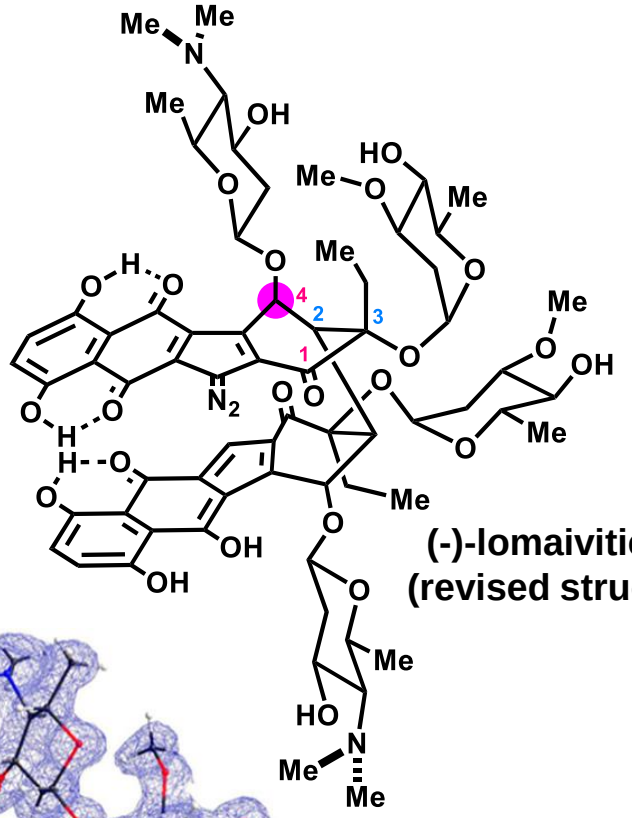




(-)-lomaiviticin A
(original structure)

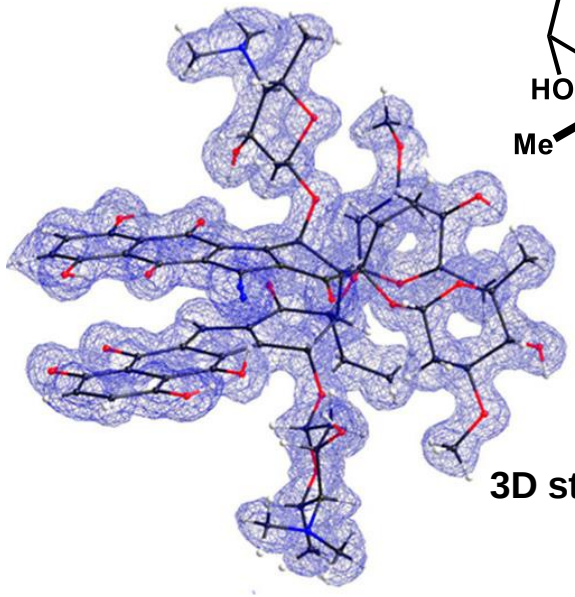






(-)-lomaiviticin A
(revised structure)

- H2 and H4 seemed singlet (500 MHz $^{1}$H NMR)
$^{2}J_{H,H}$ coupling constant 1.1 Hz (800 MHz 1H NMR)
- HMBC correlation between H4 and C5
: not $^{3}J_{C,H}$ but $^{4}J_{C,H}$

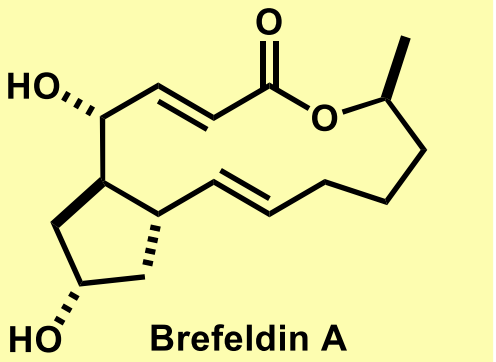


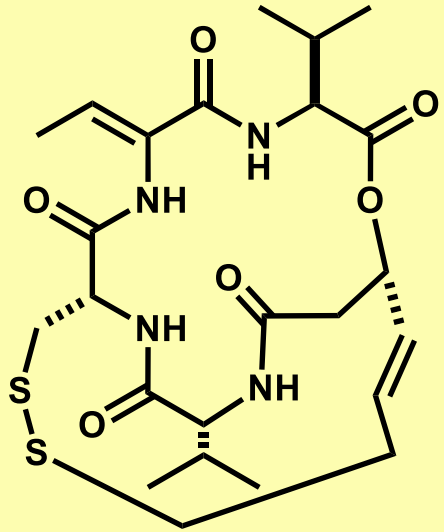
3D structure determined
with Micro ED

1) Kim, L. J.; Xue, M.; Li, X.; Xu, Z.; Paulson, E.; Mercado, B.; Nelson, H. M.; Herzon, S. B. *J. Am. Chem. Soc.* **2021**, *143,* 6578.

# Outline of Main Paper

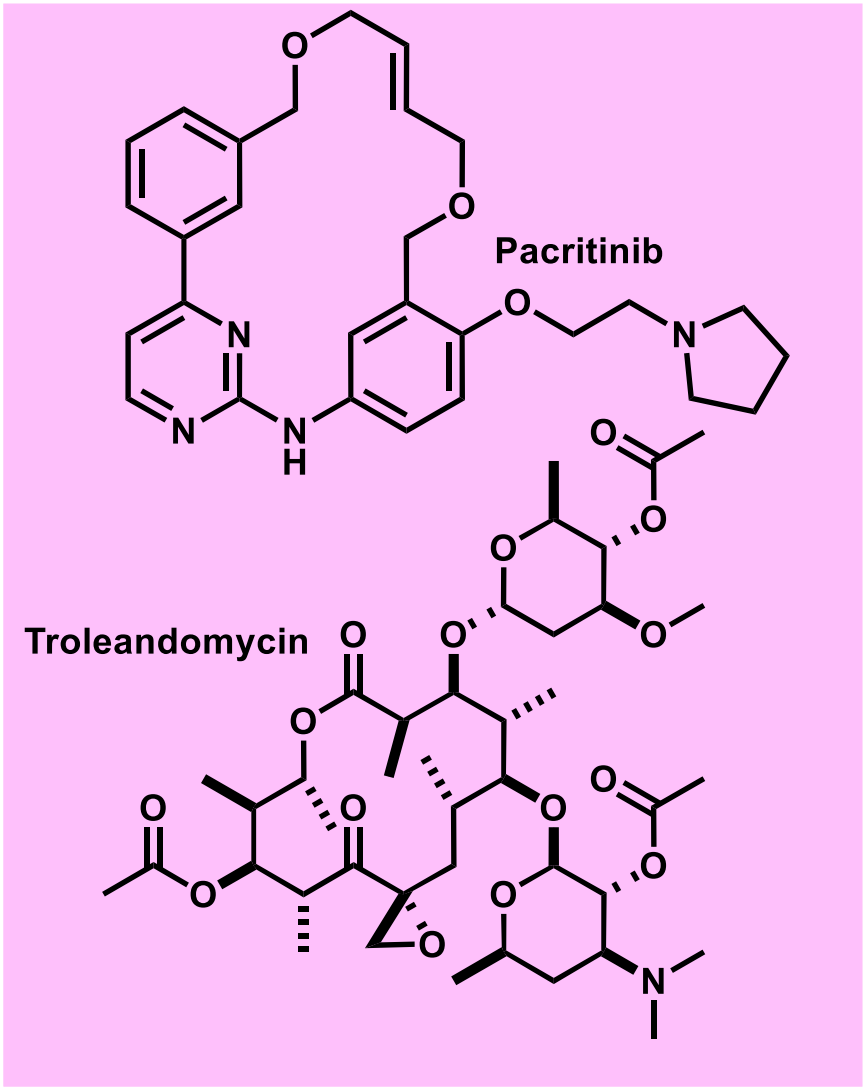
proof of concept




small, rigid macrocycles
XRD 3D structure available

application




larger, more flexible macrocycles

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.

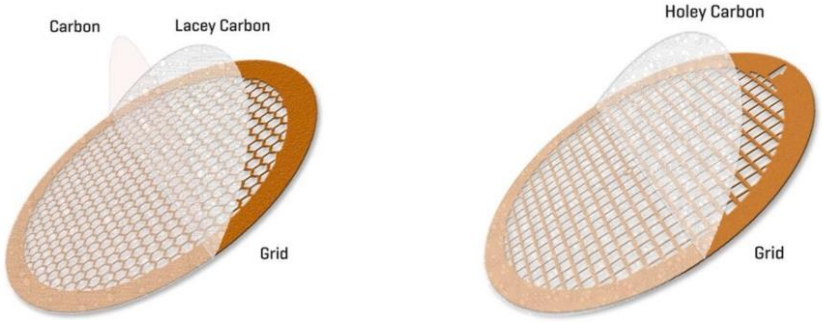
# Grid Preparation and Diffraction Screening



**<u>Procedure A</u>: General**

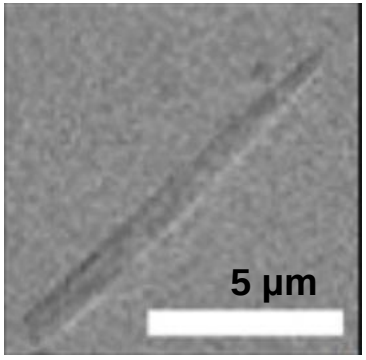
1. **grind the powder between two coverslips and apply it to the EM grid**
2. **freeze the grid and load to the TEM**
3. **evaluate the quality of grid preparation by low magnification TEM images**

**Needle microcrystals appeared slightly bent**
**→ continuous carbon grids (more rigid and flat) were used instead of holey grids**




**<u>Procedure B</u>: For complex macrocycles**

1. **dissolve powders into minimal amounts of MeOH**
2. **let the solvent evaporate at rt for about 20 h and get thin needle microcrystals**



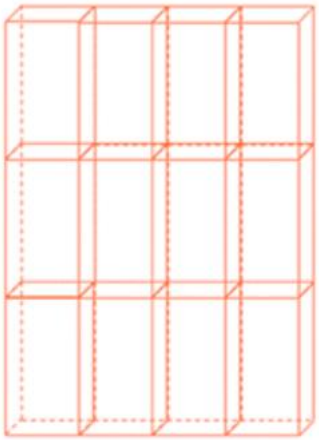

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.

# Data Collection

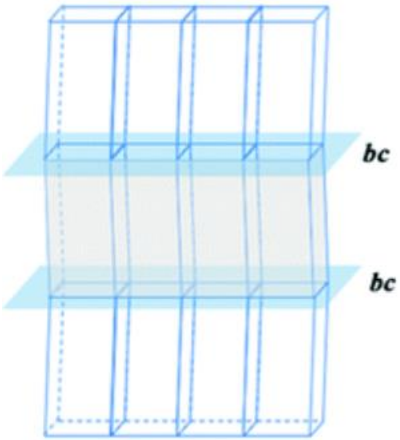
**Procedure A: General**
**exposure: 2 s/frame**
**continuous rotating : 0.6 deg/s**
**electron dose rate: 0.01 e-/Å$^2$/s**
**stage range: -70 deg to +70 deg**

**Procedure B: For radiation sensitive macrocycles (disulfide bonds, ester group)**
**exposure: 0.5 s/frame**
**continuous rotating : 2 deg/s**

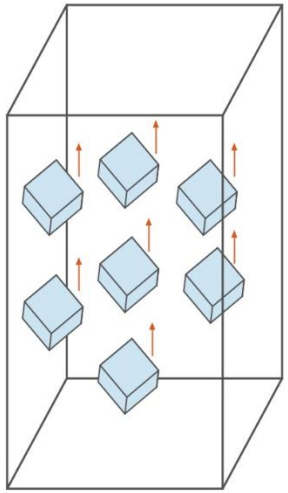
**Procedure C: For macrocycles from which complete data collection is difficult**
**SerialEM-based high-throughput autonomous data collection was employed**
**→ hundreds of MicroED data sets from each sample were automatically generated by using detector overnight**



**single crystal**




**twinned crystal**

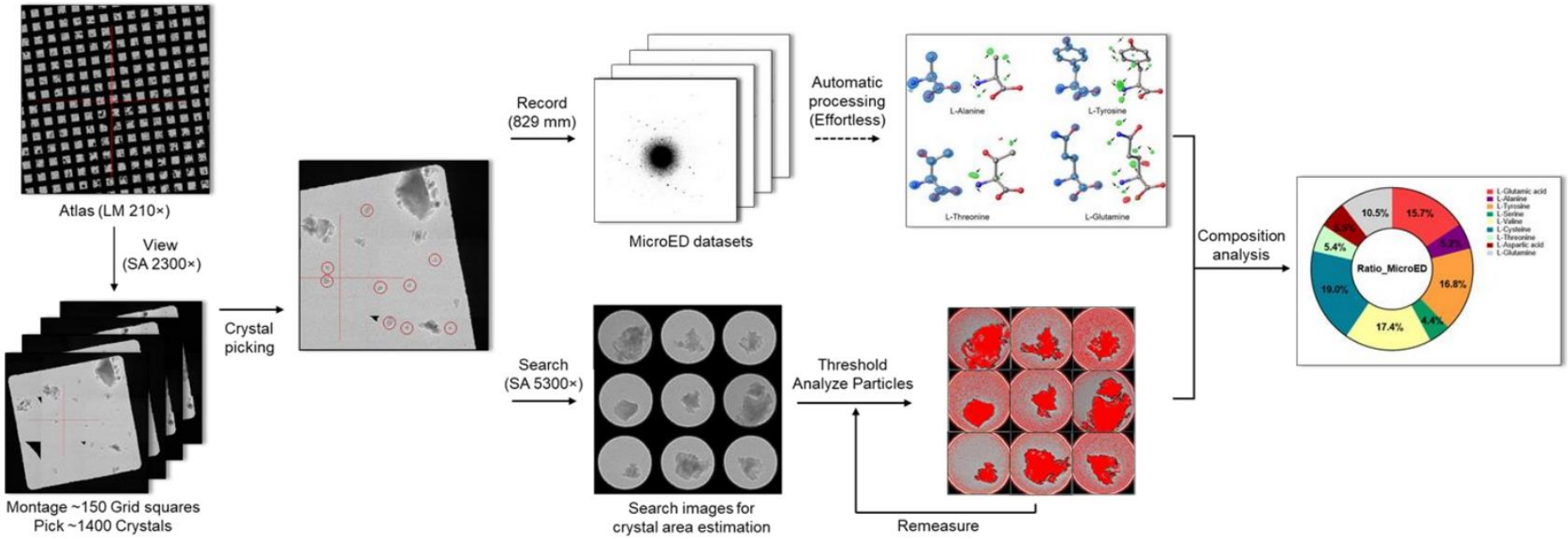


**preferred orientation**

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.

# Data Collection with Serial EM

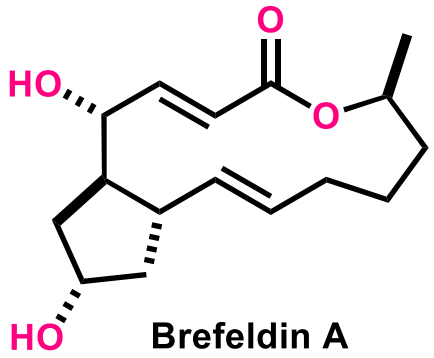





**Data collection and processing are automatically done and provide hundreds to thousands of MicroED datasets when the measurement is run overnight.**
**This enables collection of sufficient amount of data from small amount of single crystal.**

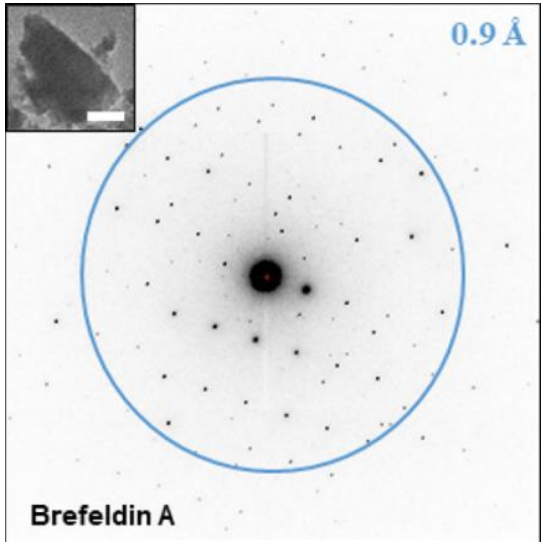

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.
2) Unge, J.; Lin, J., Weaver, S. J.; Sae Her, A.; Gonen, T. *ChemRxiv.* **2023**

# Proof of Concept -Brefeldin A- (1)




Brefeldin A

**isolation**:
from the toxic fungus *Penicillium brefeldianum*
**bioactivity**:
antiviral; a lead compound for cancer chemotherapy
**target**:
the guanine nucleotide exchange factor GBF1
**structural features**: small macrocyclic lactone
**structural study**:
4 single crystal XRD structures in the CCDC,
2 target bound structures in the pdb

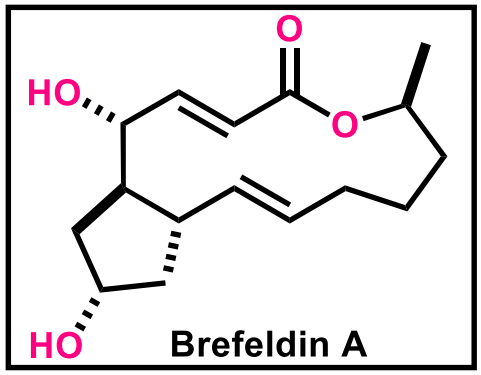



microcrystal image and
electron diffraction data

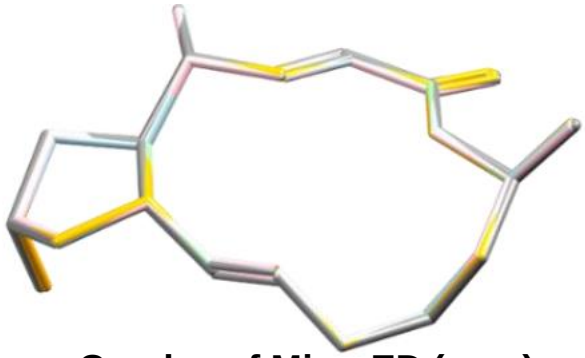

MicroED structure

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.

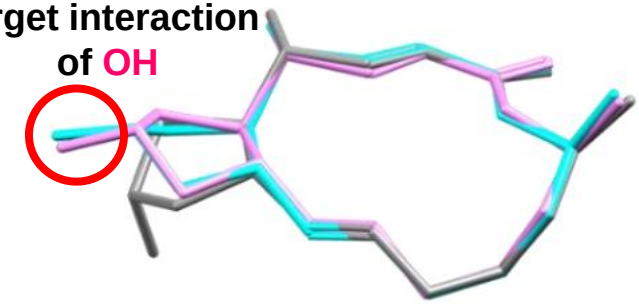
# Brefeldin A (2)

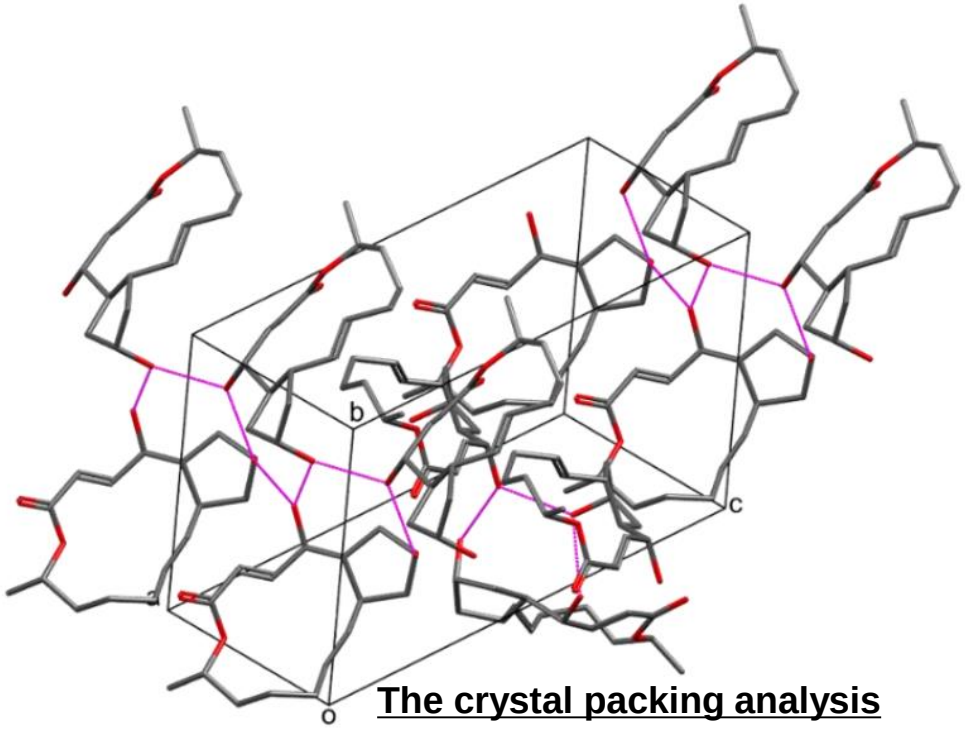


Brefeldin A


Overlay of MicroED (gray) and SCXRD (yellow)

target interaction of OH


Overlay of MicroED (gray) and target bound (cyan and pink)




**The crystal packing analysis**

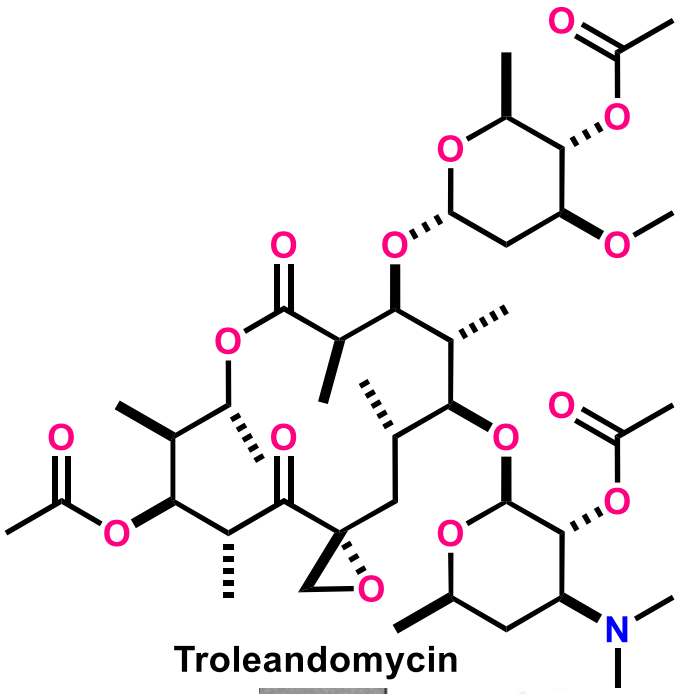
Each OH forms strong intermolecular hydrogen bonds (pink)

**Note:**
**The covalent bond between H and O was longer (av. 0.251 Å) in MicroED structure than in XRD**

**H atom can be more accurately located in electrostatic potential map generated by electron diffraction**

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.

# Troleandomycin (1)



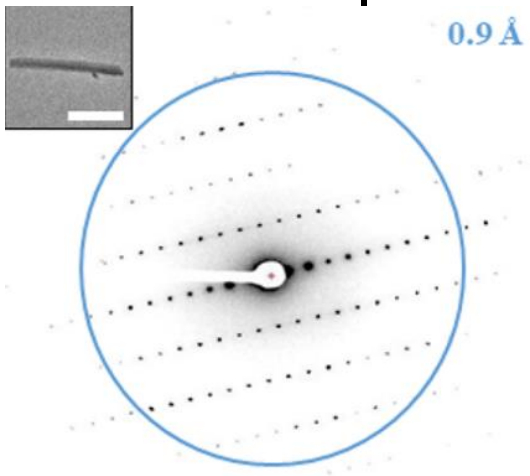
Troleandomycin

**semisynthetic**

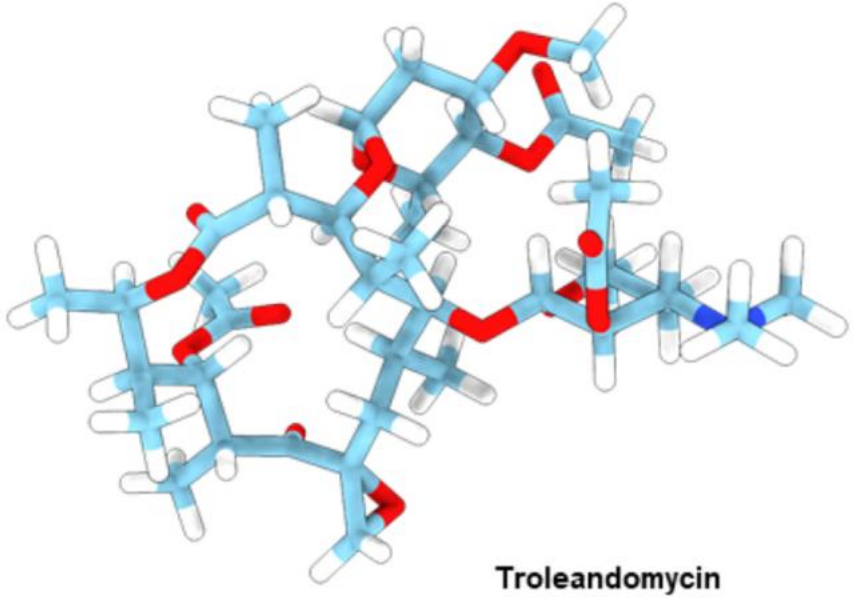
**structural features**:
macrocyclic lactone ring with two flexible sugar substituents

**bioactivity**: antibiotic (1969 FDA approved)

**structural studies**:
1 bound structure to a ribosomal subunit




microcrystal image and electron diffraction data

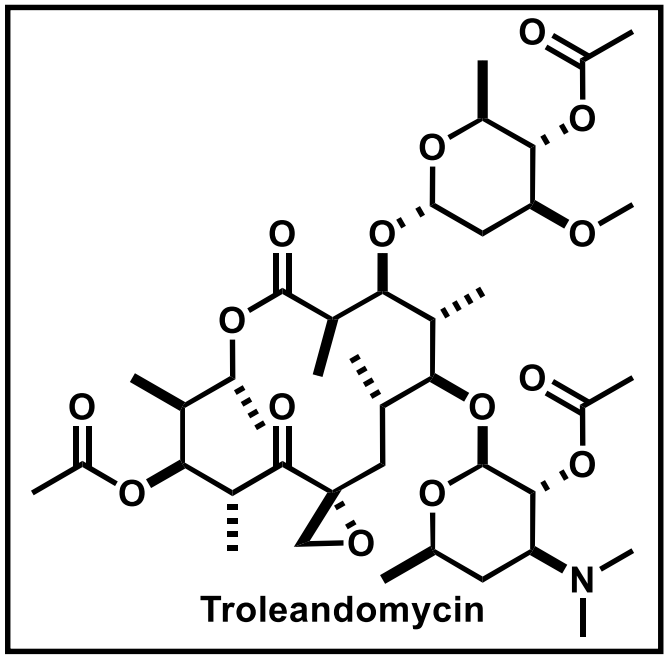


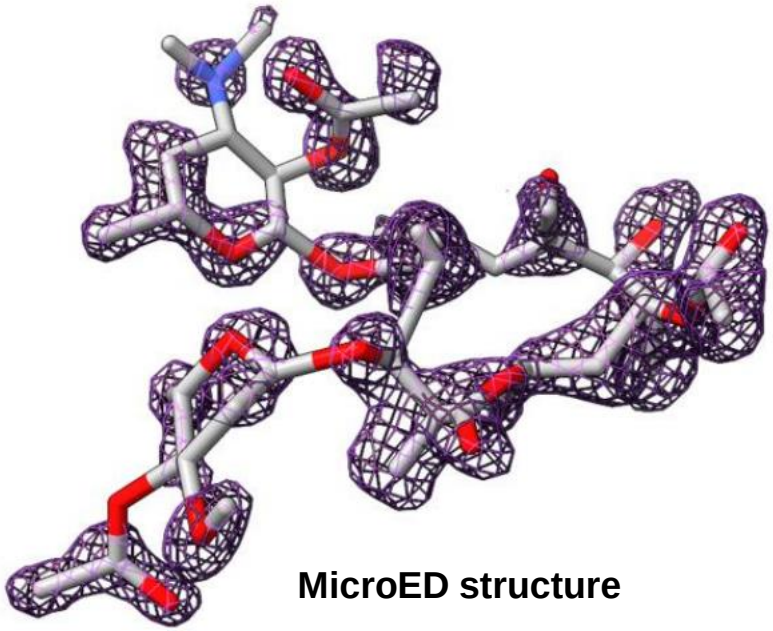

MicroED structure

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol*. **2023,** *18,* 2582.

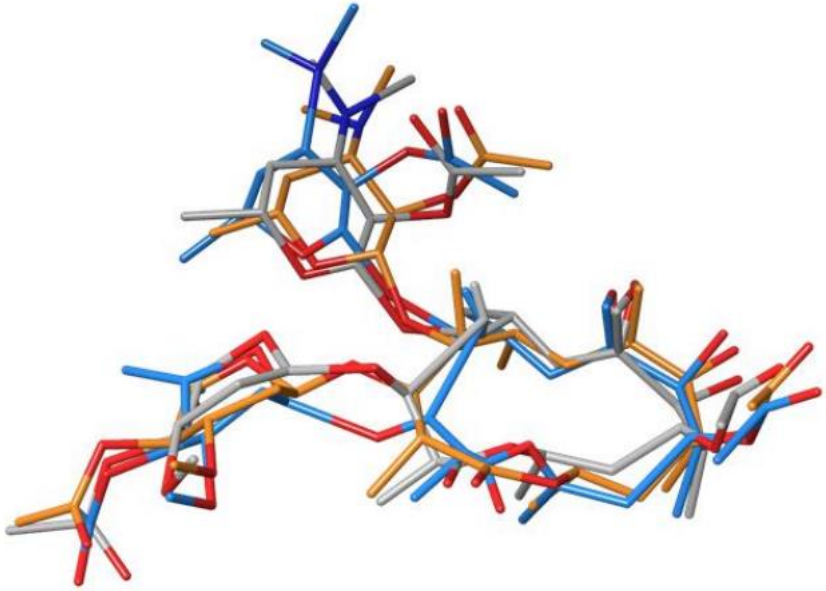
# Troleandomycin (2)





MicroED structure

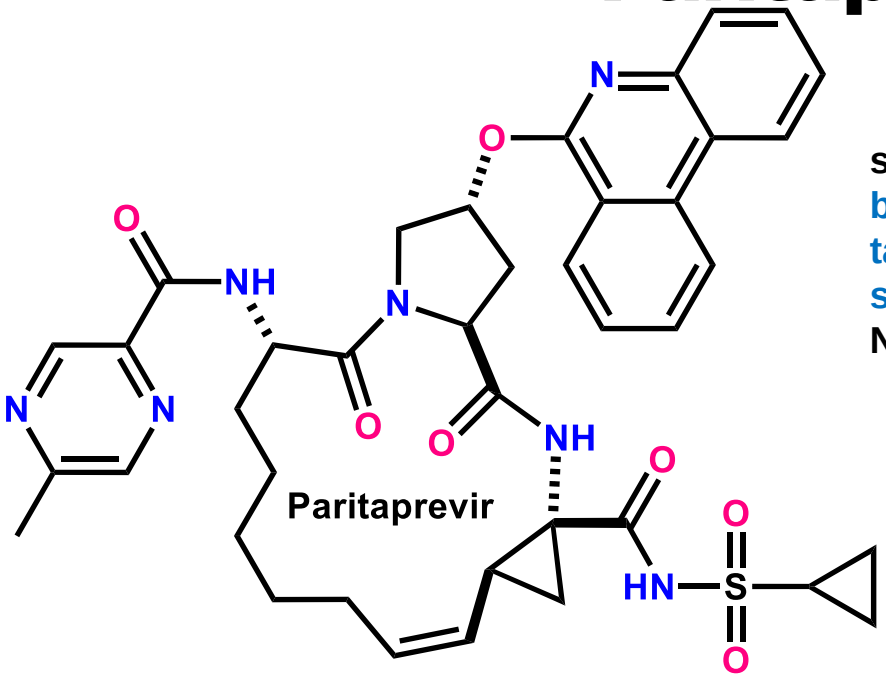


**2 conformations from MicroED (gray, blue) and target-bound structure (orange)**

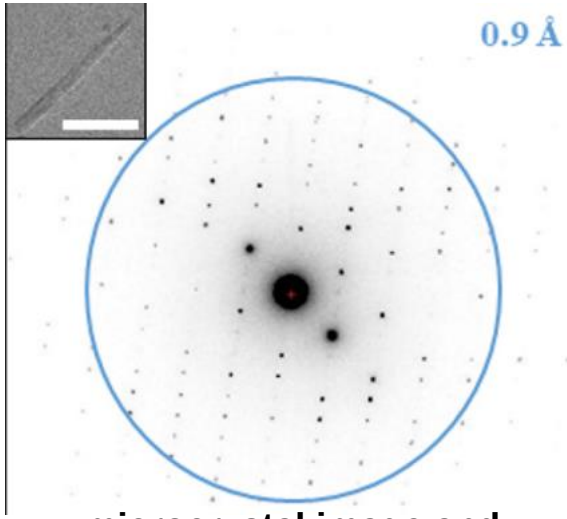
**All three conformations "open and flat" which imply the high-affinity to the target protein**

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol*. **2023,** *18,* 2582.

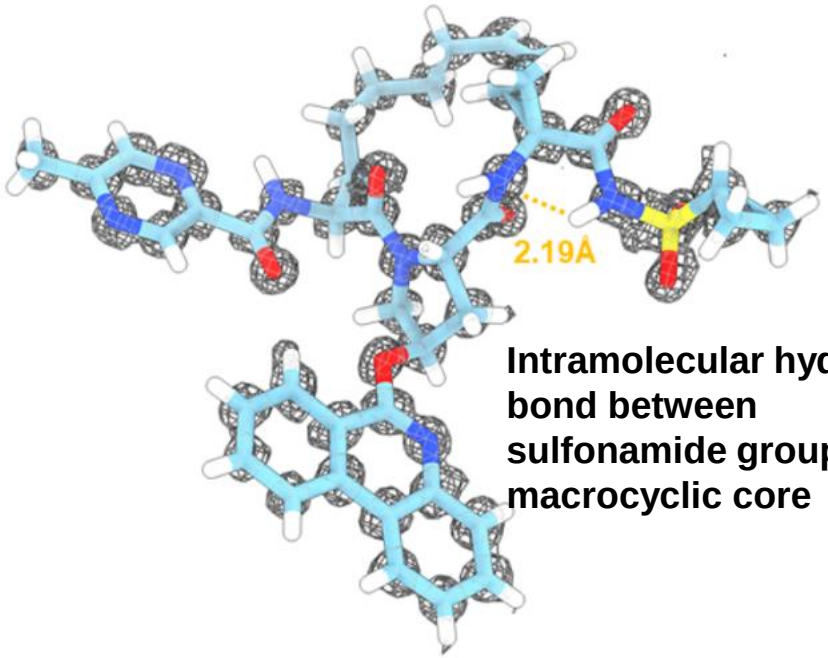
# Paritaprevir (1)




**synthetic**
**bioactivity: anti hepatitis C virus**
**target: serine NS3/4a protease inhibitor**
**structural studies:**
**No crystal data available**




**microcrystal image and electron diffraction data**

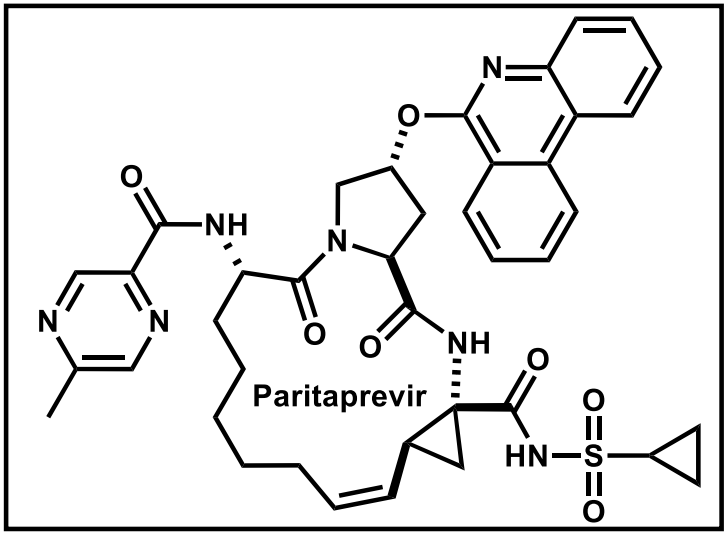


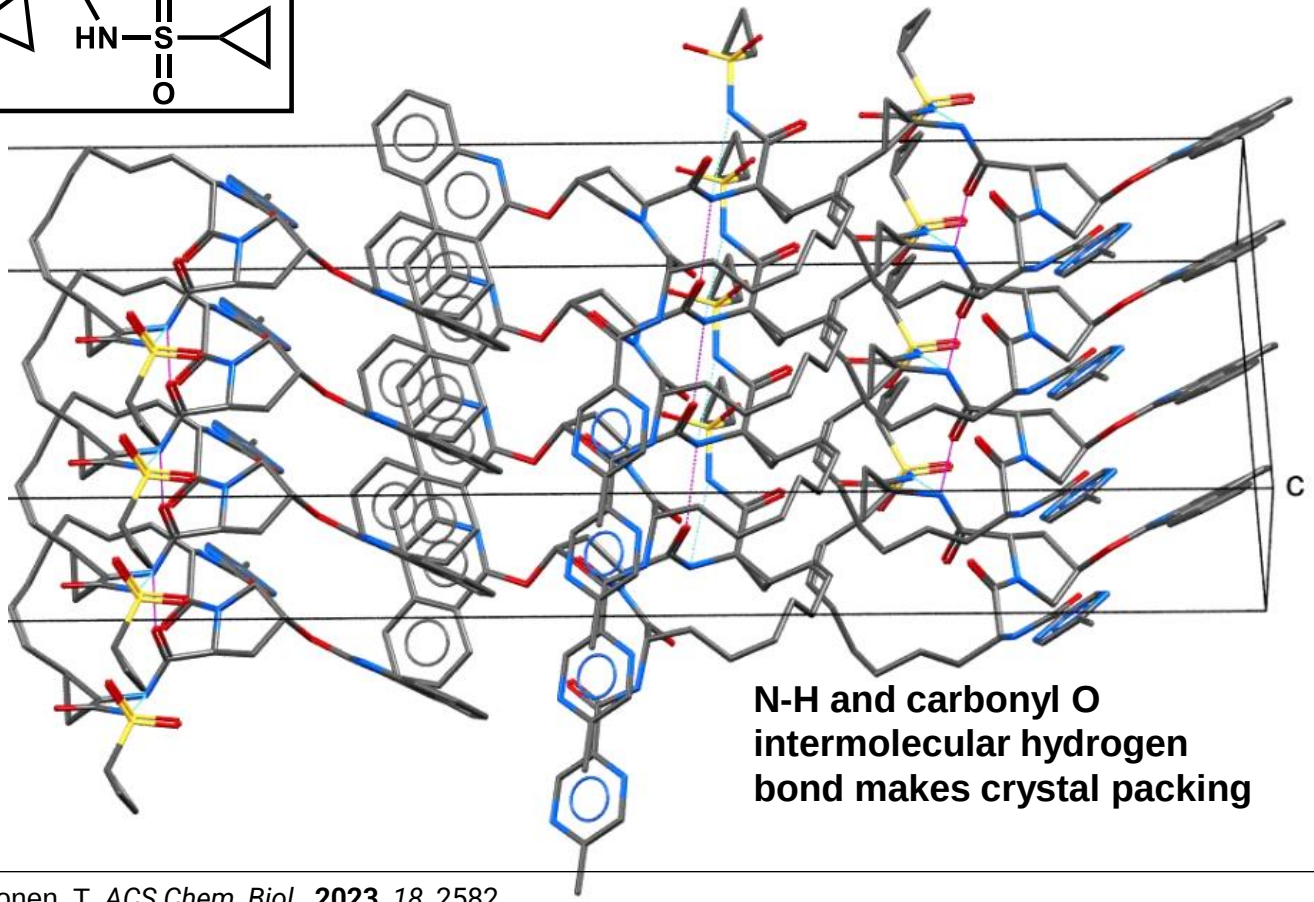

**Intramolecular hydrogen bond between sulfonamide group and macrocyclic core**

**MicroED structure**

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol.* **2023,** *18,* 2582.

# Paritaprevir (2)

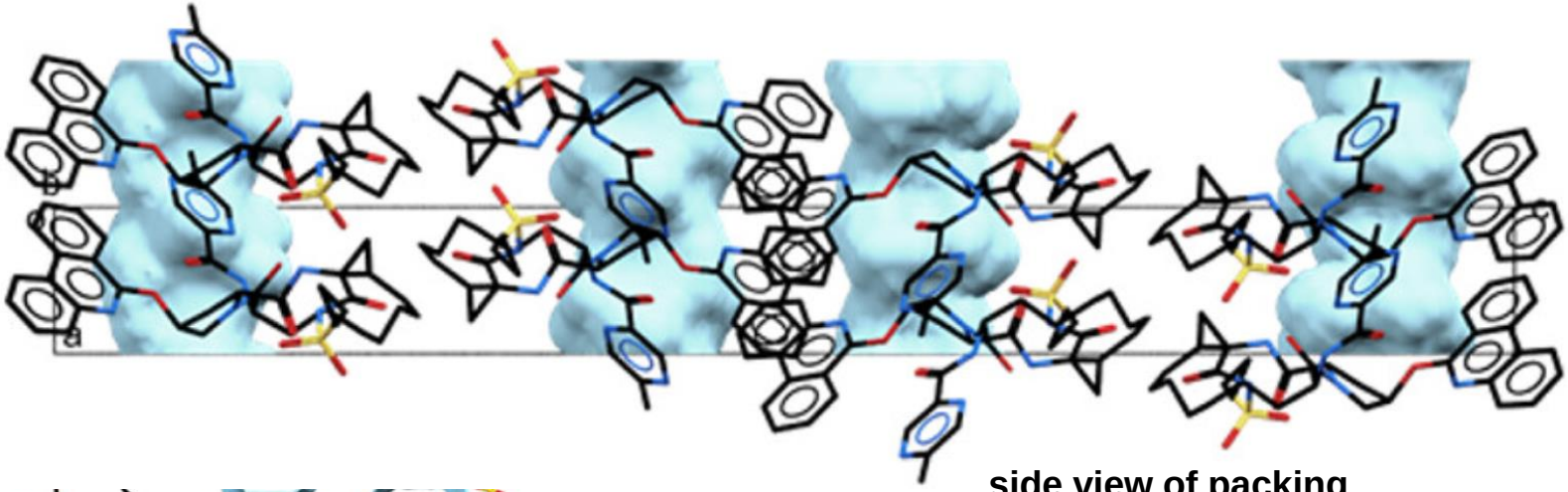


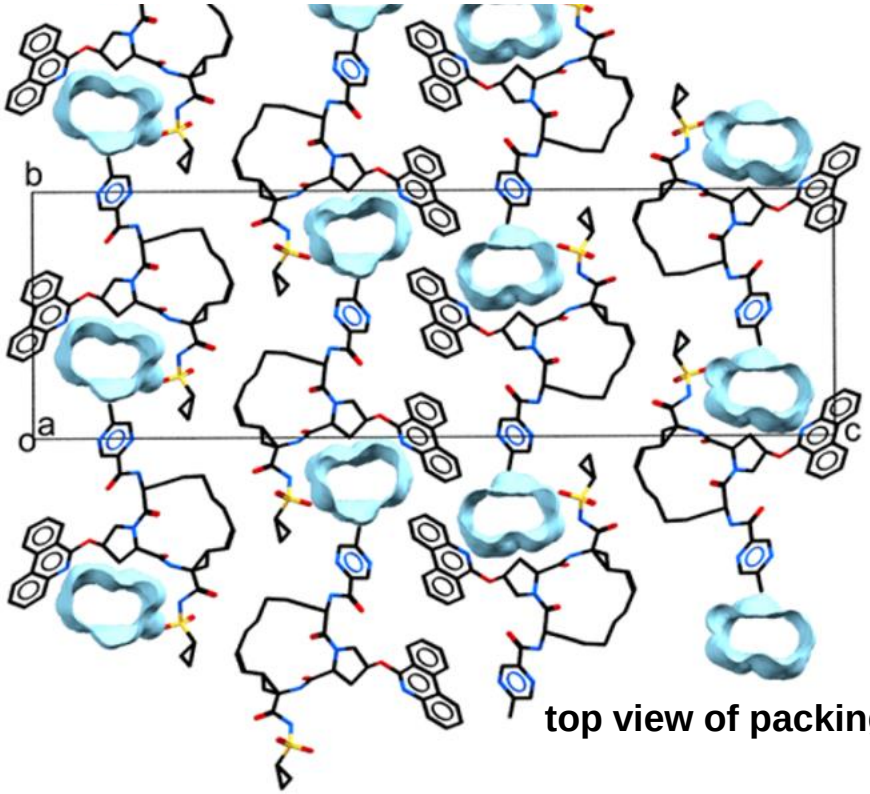

**N-H and carbonyl O intermolecular hydrogen bond makes crystal packing**

1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol*. **2023,** *18,* 2582.

# Paritaprevir (3)




side view of packing




top view of packing

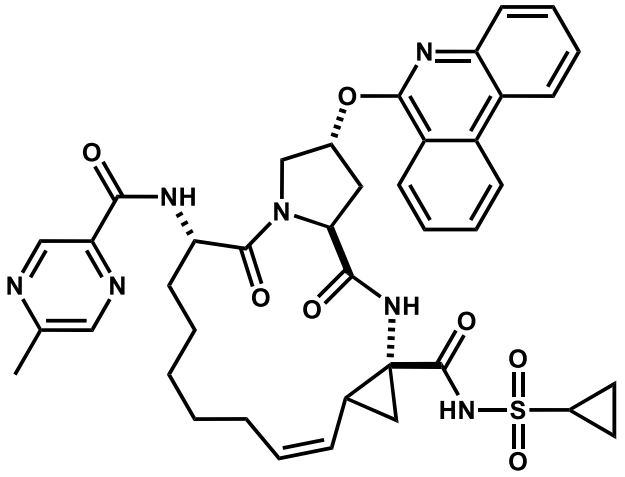
**large voids were detected along the crystallographic axis**
**= solvent accessible channel**

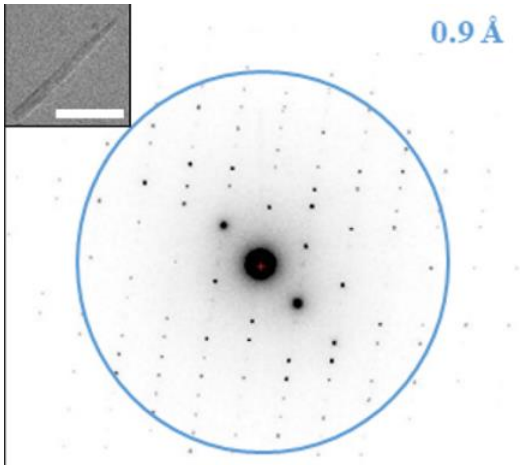
**Voids can accommodate a significant amount of water, which can be crucial for the solubility, adsorption, and bioavailability.**

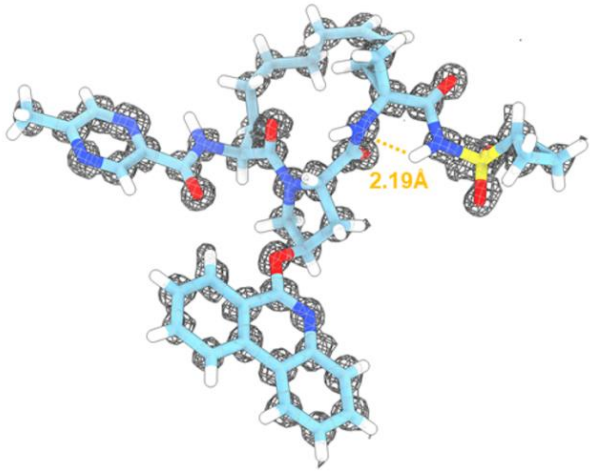
1) Danelius, E.; Bu, G.;, Wieske, L.; Gonen, T. *ACS Chem. Biol*. **2023,** *18,* 2582.

# Summary

**Development of MicroED method**




- hardware to get high-resolution data
- software to automate data collection and analysis

3D structure determination of flexible macrocyclic molecules is now possible.

**Application to drug discovery**

- structure determination of large, complex and flexible natural products which are difficult to crystallize
- prediction of solubility and permeability
- optimization of structure for efficient target binding