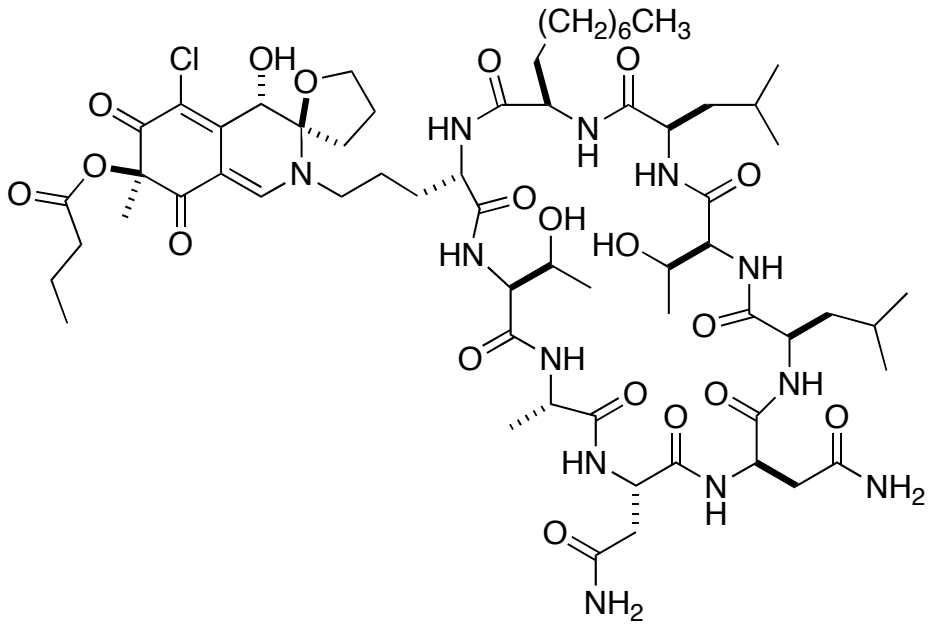
Total Synthesis, Stereochemical Reassignment, and Absolute Configuration of Chlorofusin



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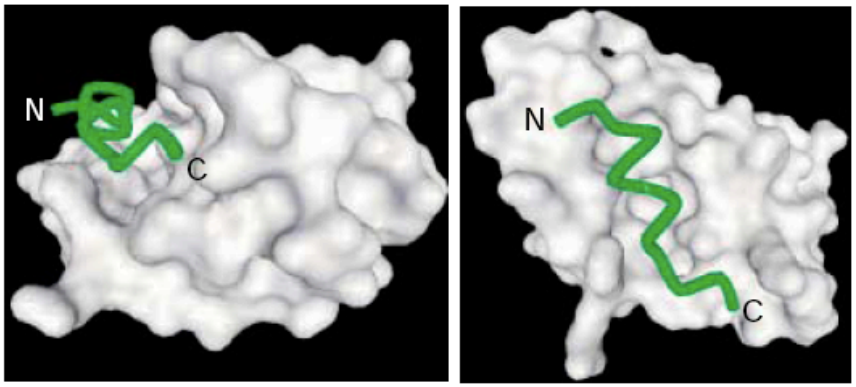
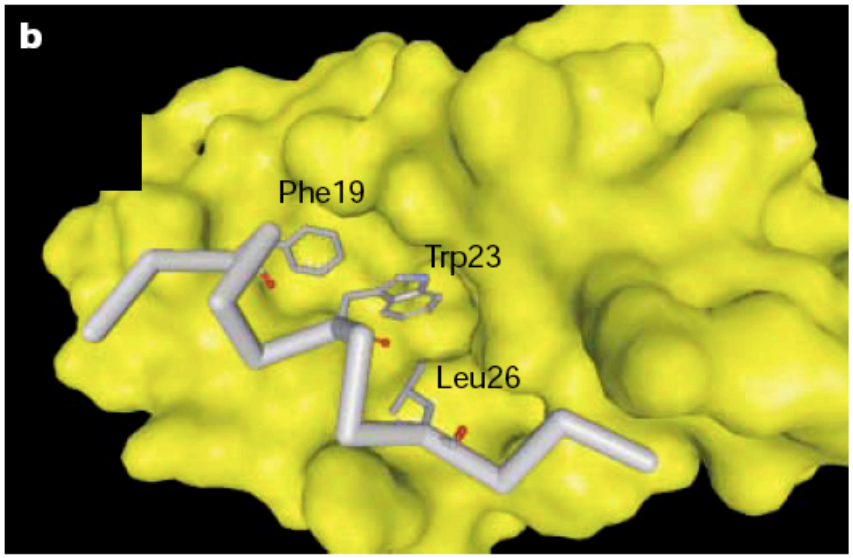
Scripps Research Institute
JACS ASAP

Joshua Pierce
Wipf Group Current Literature
July 28, 2007

Isolation and Biological Activity

Novel peptide derived from fermentation of a microfungus assigned to the genus *Fusarium*.
Discovered during a screen of 53,000 fermentation cultures.





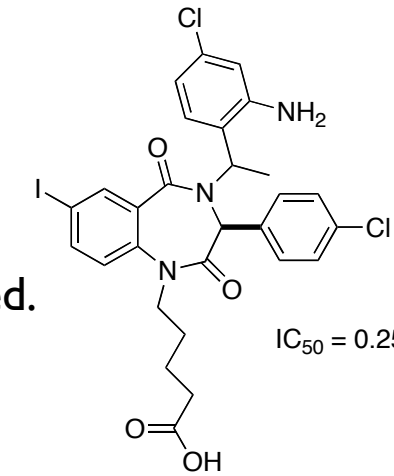
MDM2 (surface) binding to key residues of p53

Initial IC_{50} of 4.6 μM (disrupts MDM2-p53 binding)

The compound showed no cytotoxic effects.

At a test concentration of 7.3 μM, chlorofusin did not exhibit any antimicrobial activity against a variety of NCI strains.

Small molecules with ability to disrupt this protein-protein interaction have been developed.

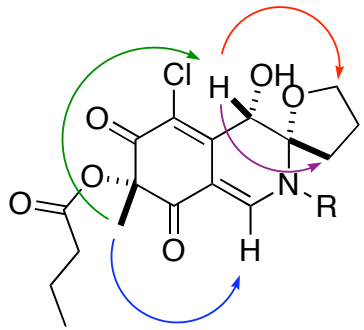


IC_{50} = 0.25 μM

JACS **2001**, *123*, 554.
Bioorg. Med. Chem. Lett. **2006**, *16*, 3463.

Determination of Structure

NOE Analysis: Relative Stereochemistry



50 sec mixing

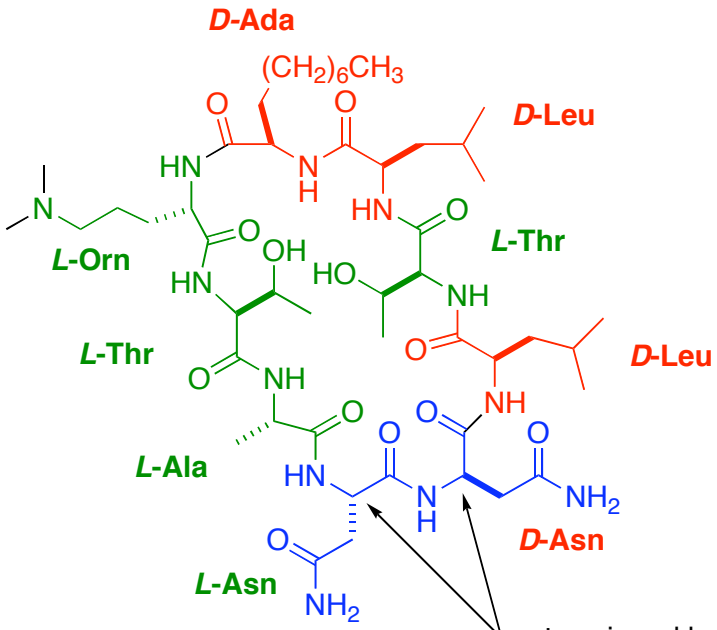
25 and 50 sec mixing

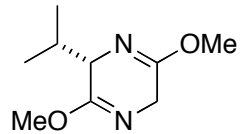
200 sec mixing

500 sec mixing

JACS **2001**, *123*, 554.

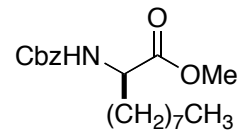
Cyclic Peptide



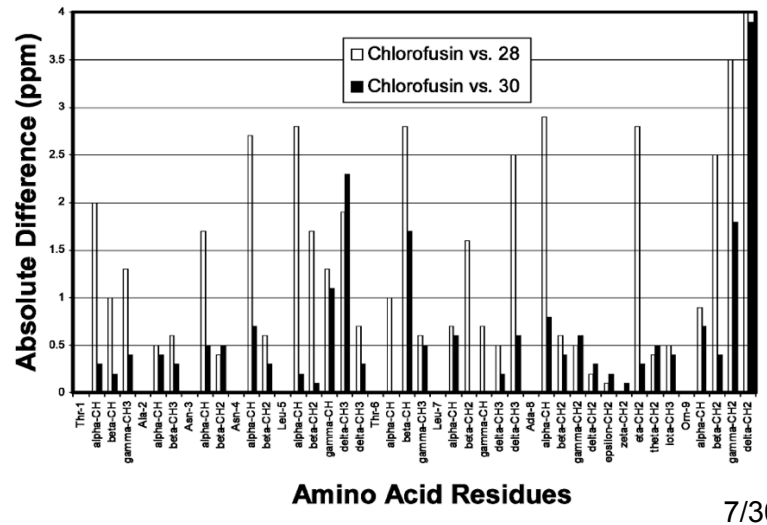


1. *n*-BuLi, $C_8H_{17}I$ (94%)

2. 0.5 M HCl (quant)
3. CbzCl (95%)



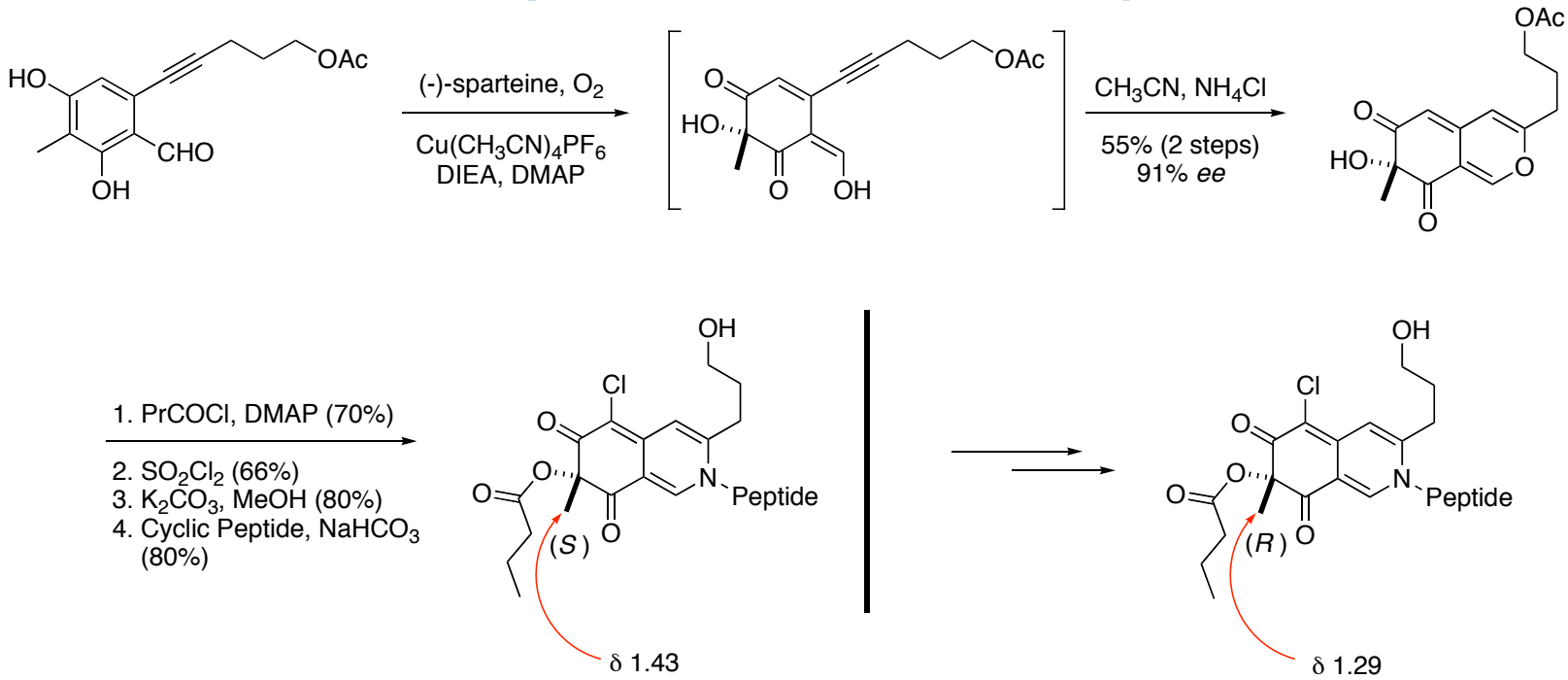
> 96% *ee*
D-2-aminodecanoic acid (*D*-Ada)



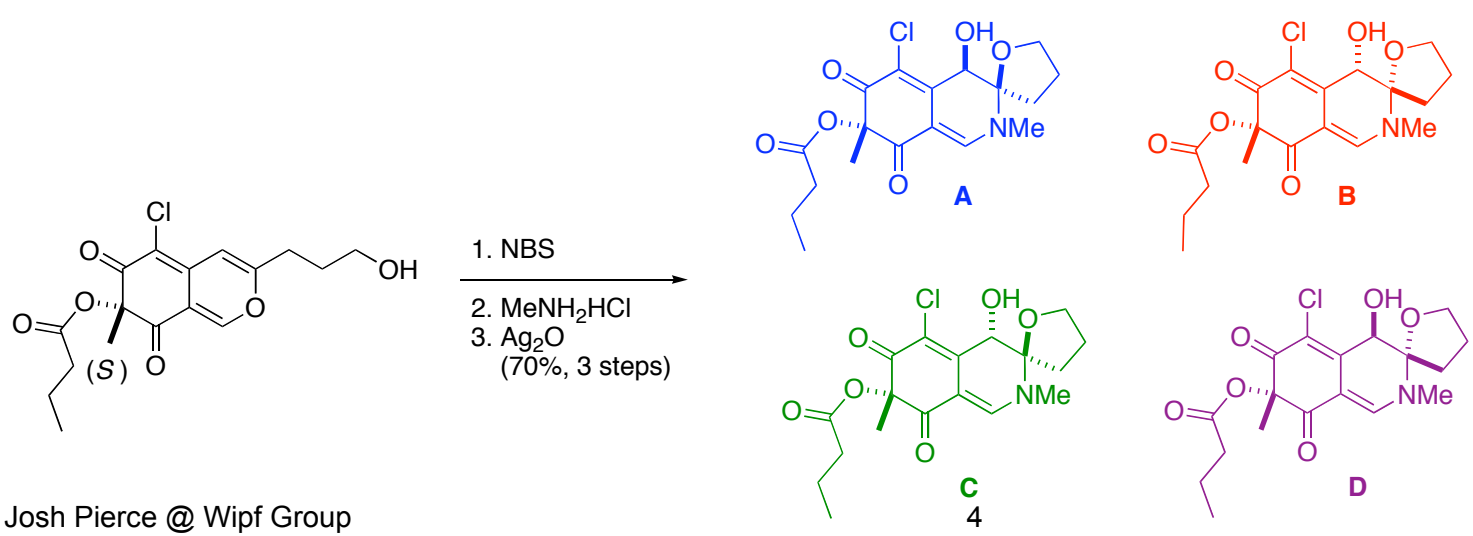
Synthesis of *D,L* (28) and *L,D* (30) diastereomers allowed for determination of the cyclic peptide structure.

OL **2003**, *5*, 5047.

Initial Synthetic Studies by Yao



Natural chlorofusin has methyl group at δ 1.41, therefore configuration was assigned as (*S*)!



4

A:B:C:D = 1:1:2.5:4

Structures of all 4 diastereomers confirmed through X-ray analysis.

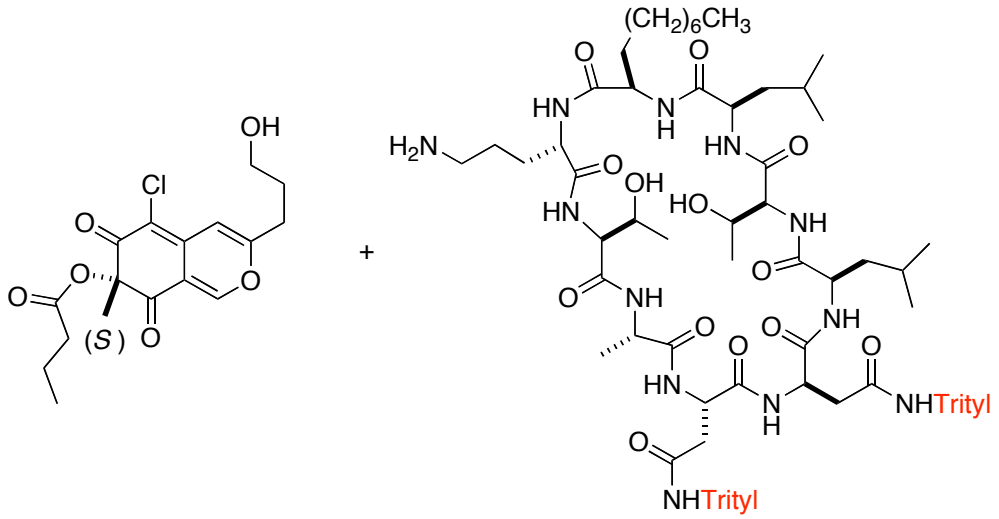
D found to match closest to natural chlorofusin.

Josh Pierce @ Wipf Group

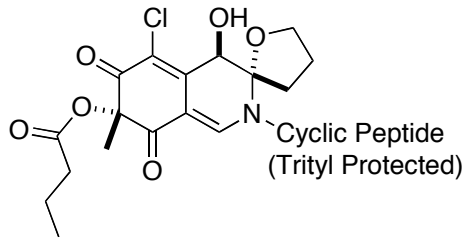
7/30/2007

JACS **2007**, *129*, 6400.

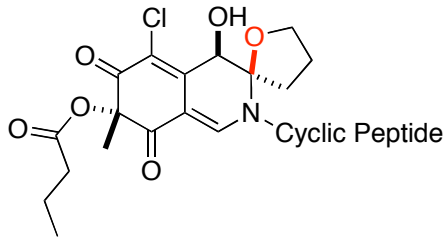
Yao's Completion of Chlorofusin



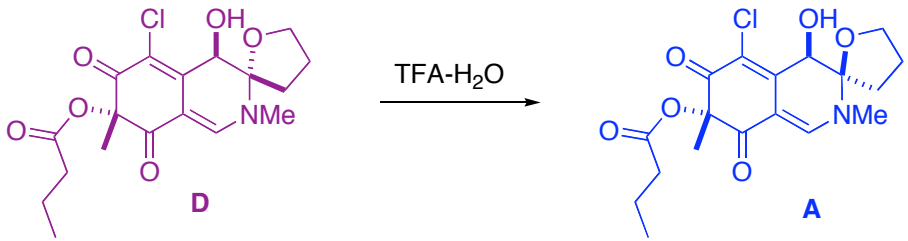
1. NBS
2. Amine, NaHCO3
3. Ag_2O
(23%, 3 steps)

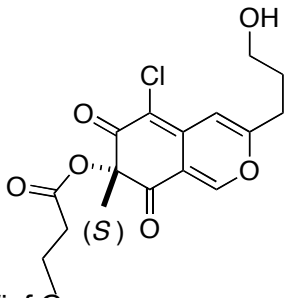


TFA-H_2O (10:1)
i-Pr_3SiH (2 eq.)
80%



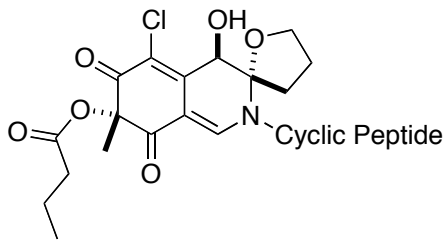
Confirmation of unexpected stereoisomer formation:





+ Free Cyclic Peptide

1. NBS
2. Amine, NaHCO3
3. Ag_2O
(25%, 3 steps)

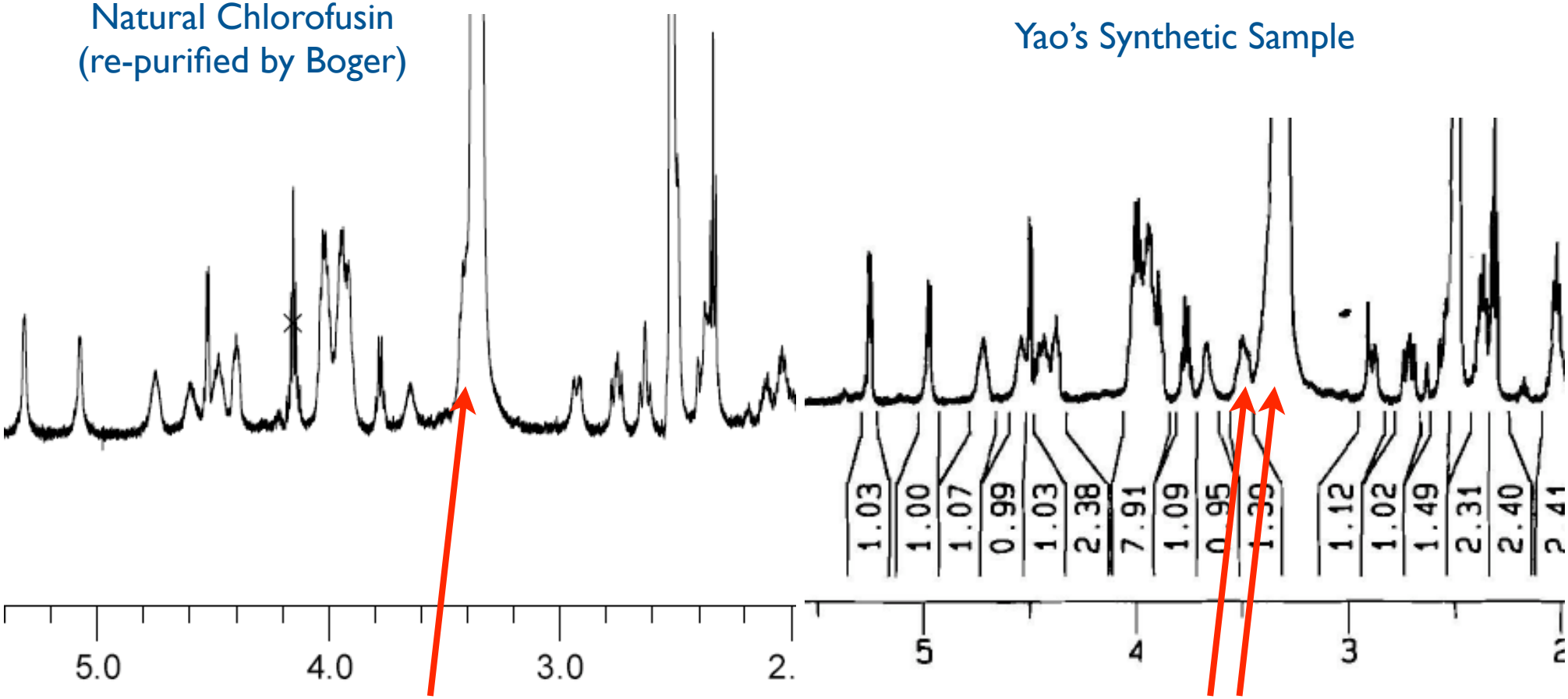


NMR match with natural chlorofusin!

NMR Data that Concluded "NMR Match"

Natural Chlorofusin
(re-purified by Boger)

Yao's Synthetic Sample

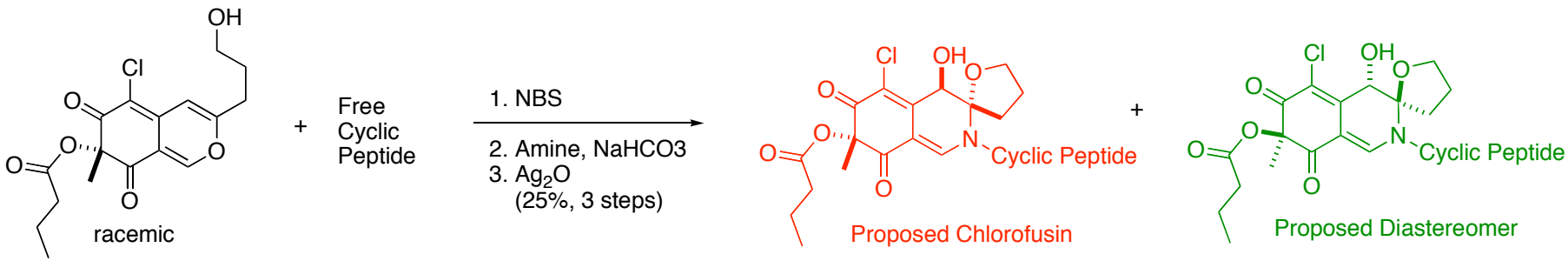


Data Reported by Williams: Yao:

Orn-9		
NH	6.67	6.70
α-CH	4.57	4.54
β-CH_2	1.73	1.73
γ-CH_2	1.53	1.53
γ-CH_2	1.73	1.73
δ-CH_2	3.40	3.42 and 3.50[b]

2-D NMR studies allowed for assignment

Additional Confirmation of Structure?



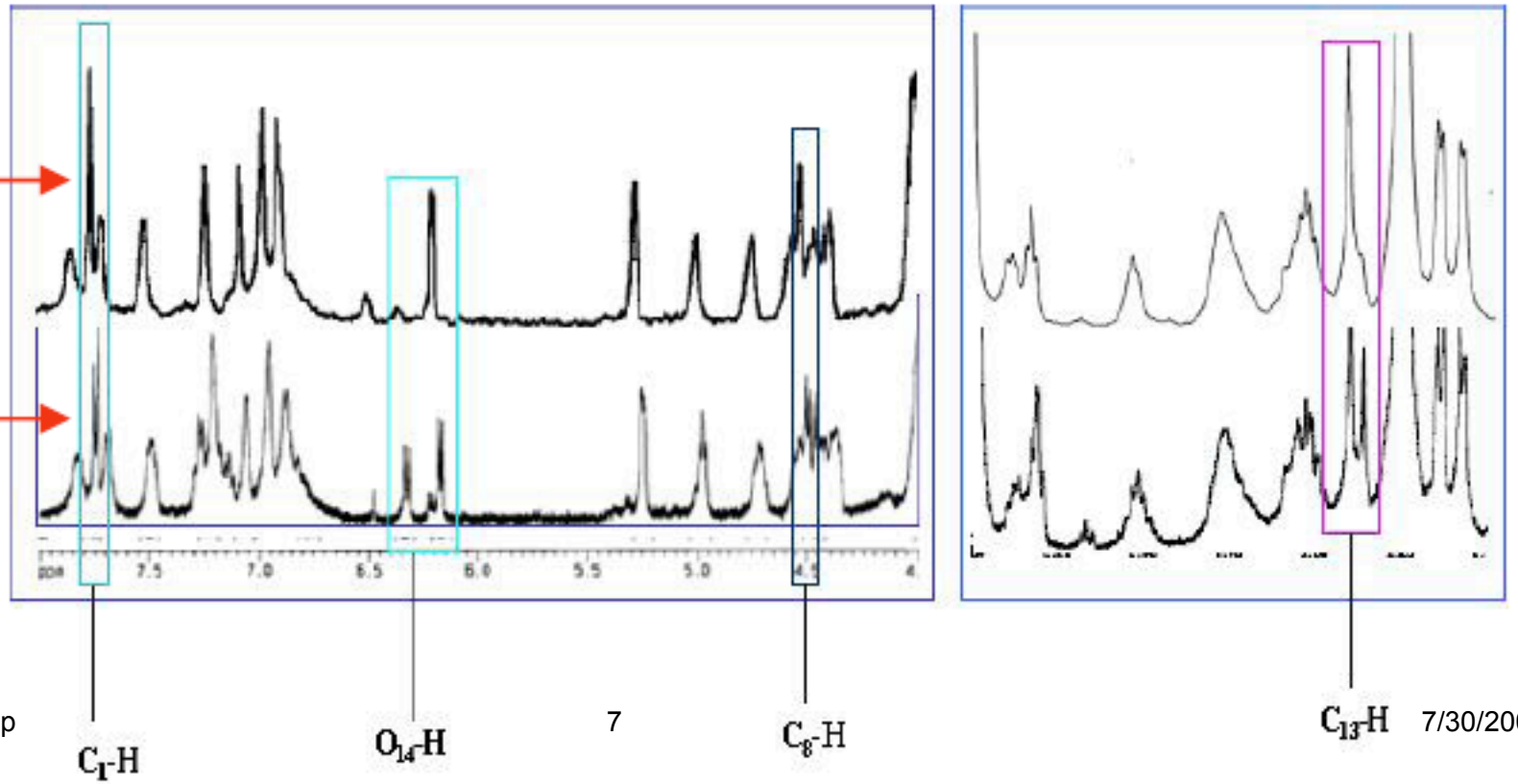
II-8b. ^{1}H NMR comparisons after HPLC purification.

Proposed Chlorofusin:

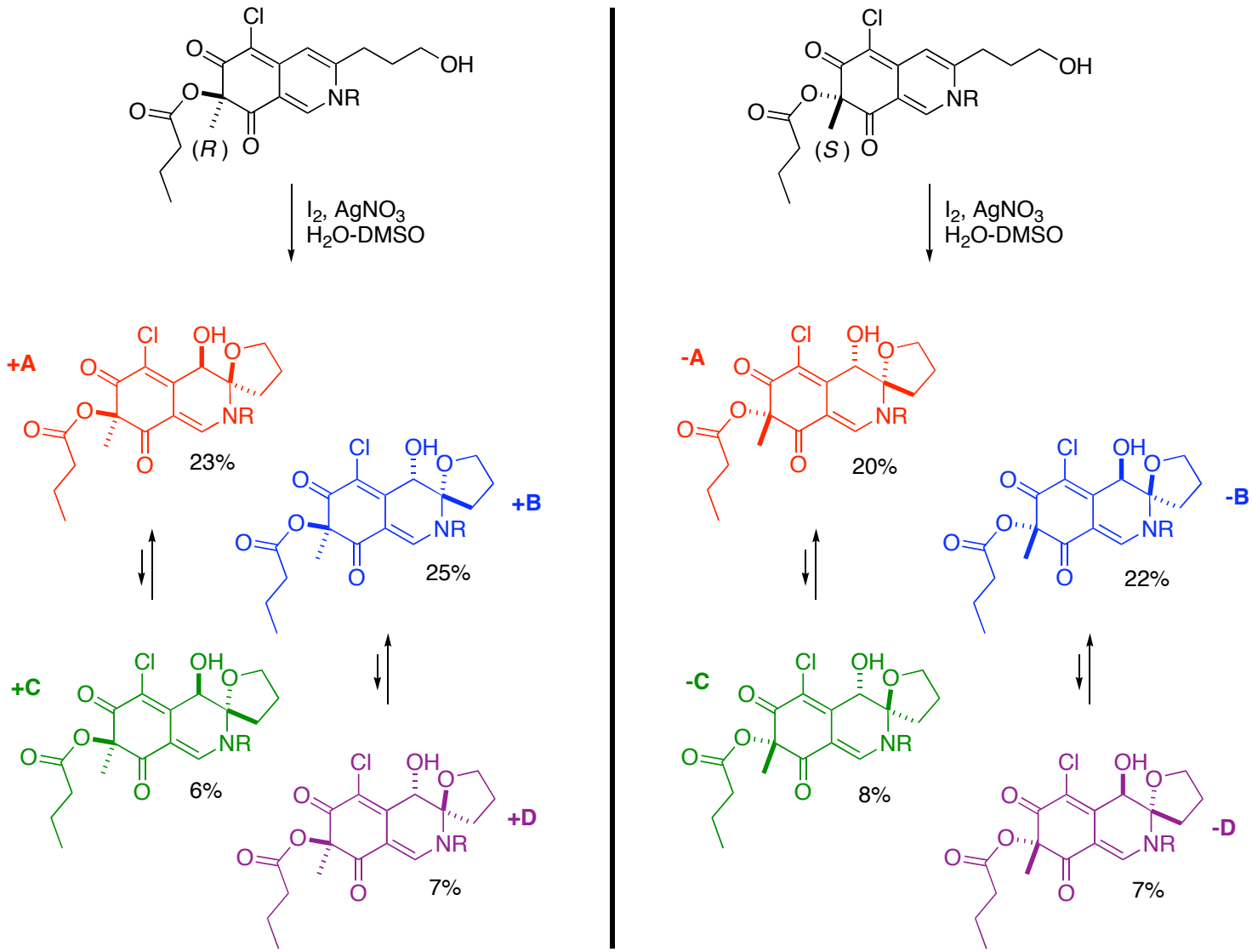
1a

1a & 14

Proposed Chlorofusin + Diastereomer

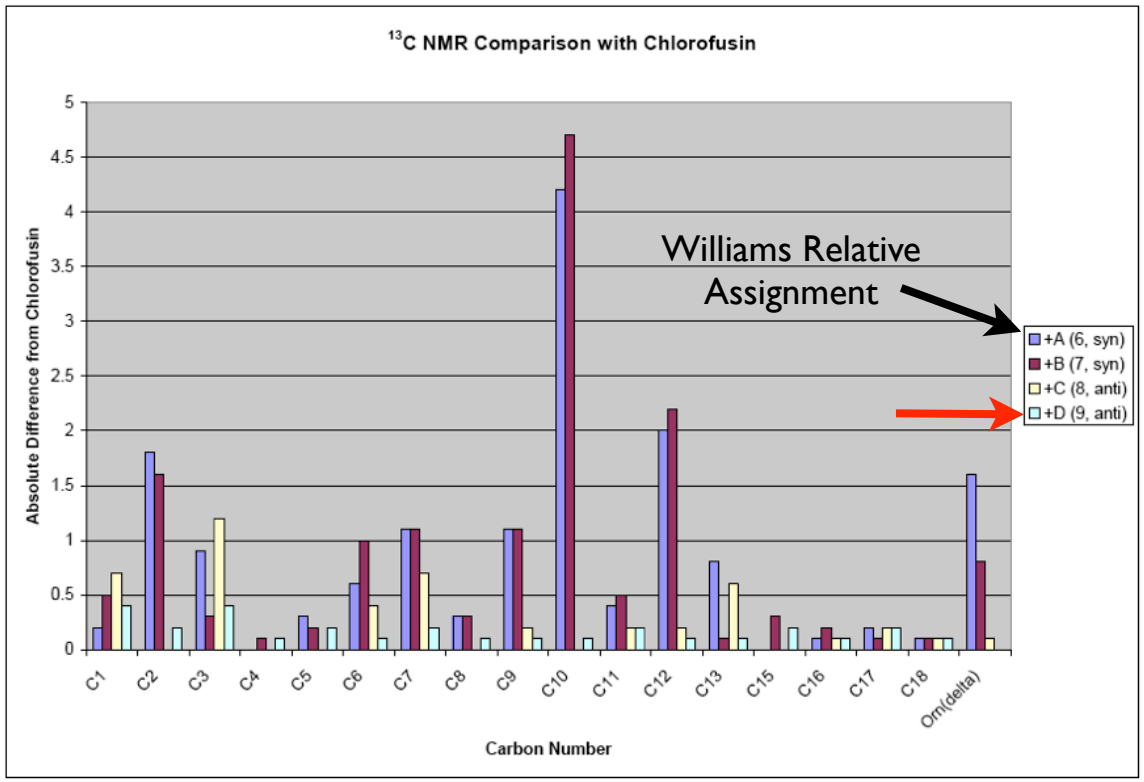


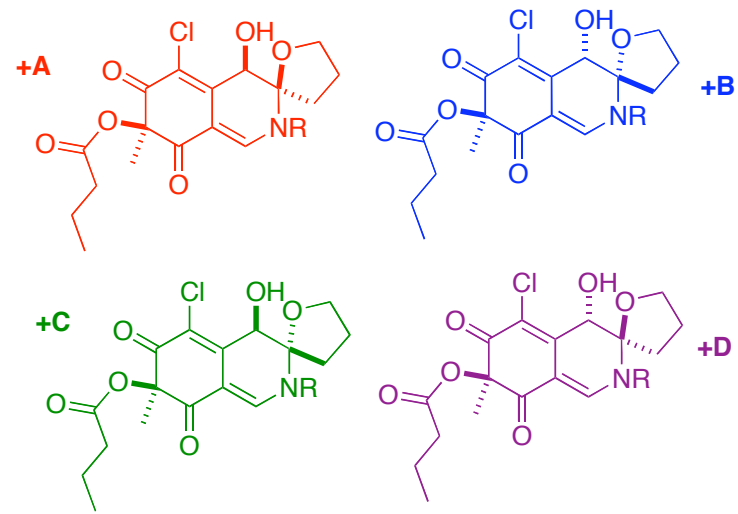
Boger: Synthesis of All Possible Diastereomers



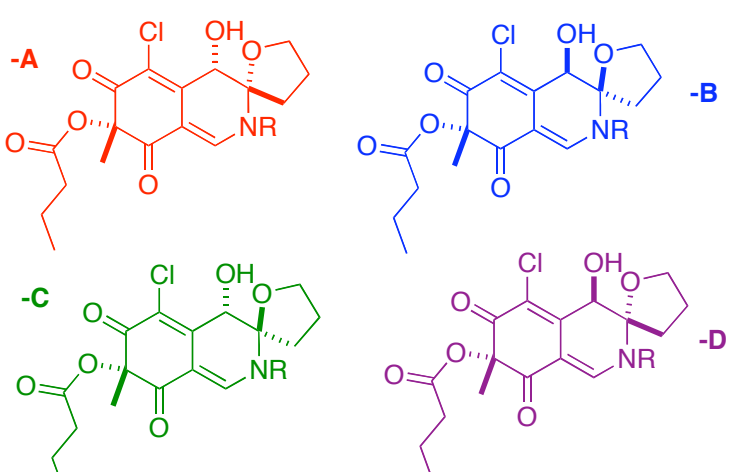
R = Fmoc-L-Orn-L-Thr-OBn

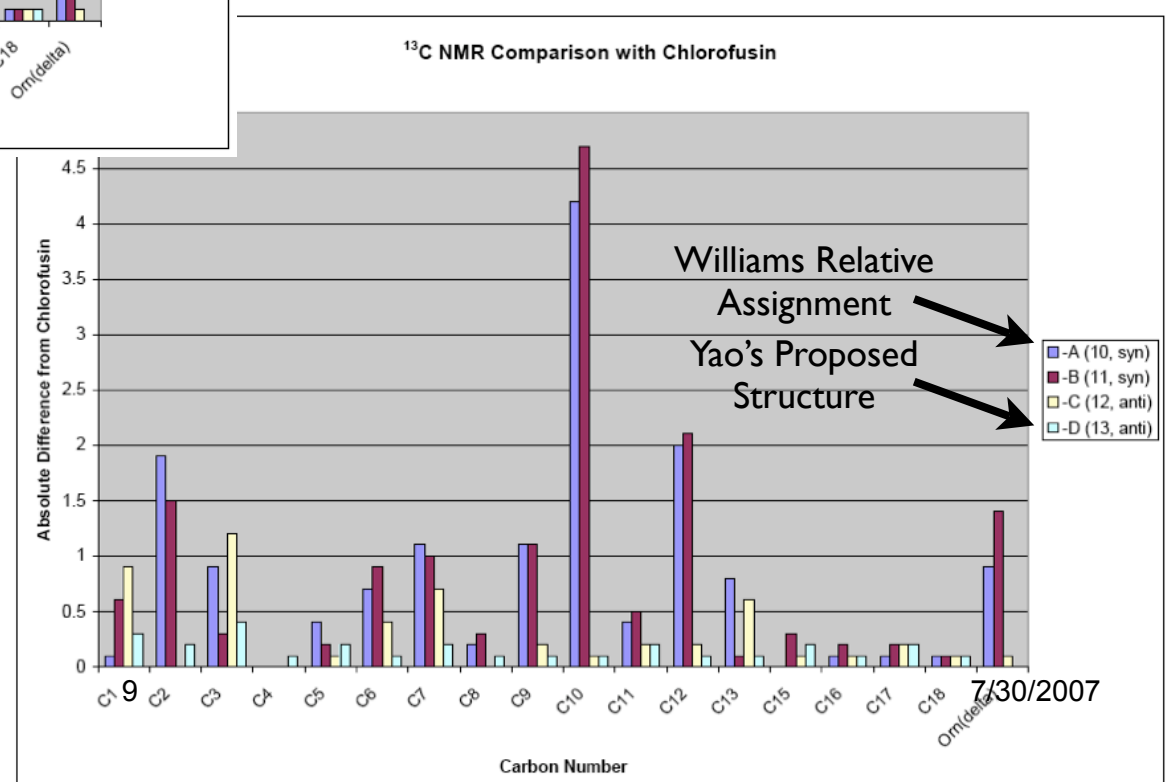
Comparison of Carbon NMR Data



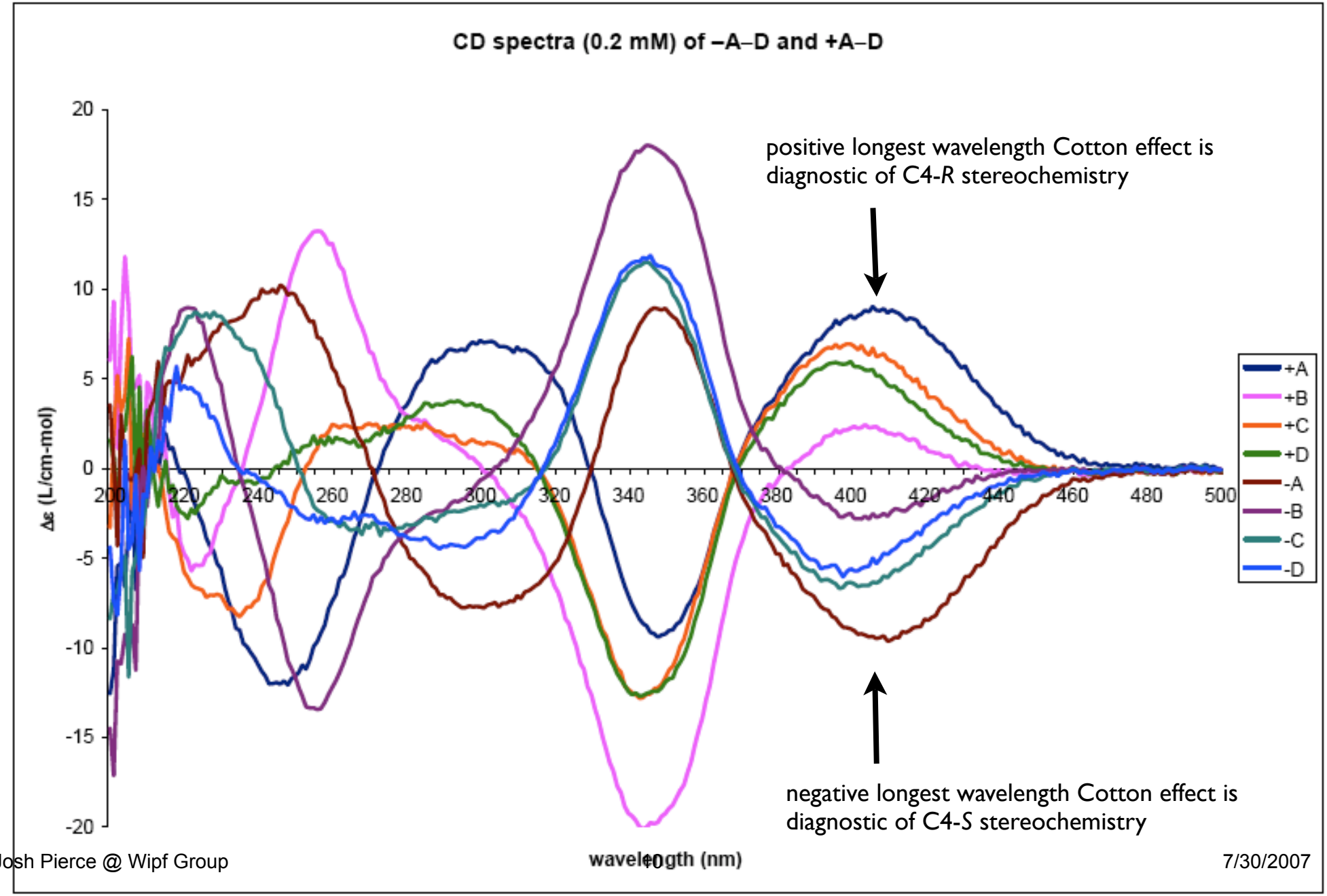


Closest match of NMR data

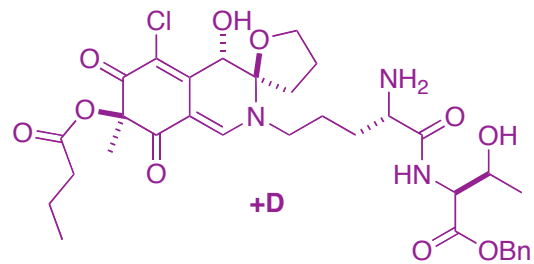




Comparison of CD Data



A Reassignment of a Reassignment of Stereochemistry



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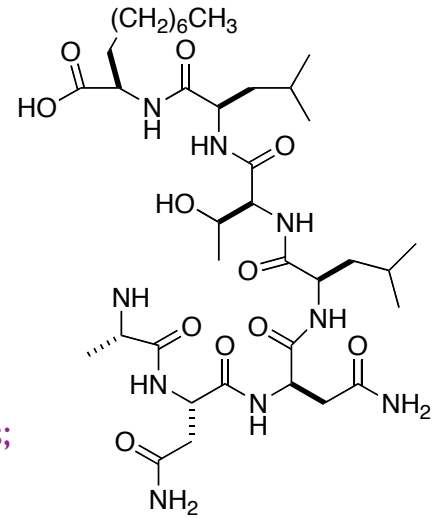


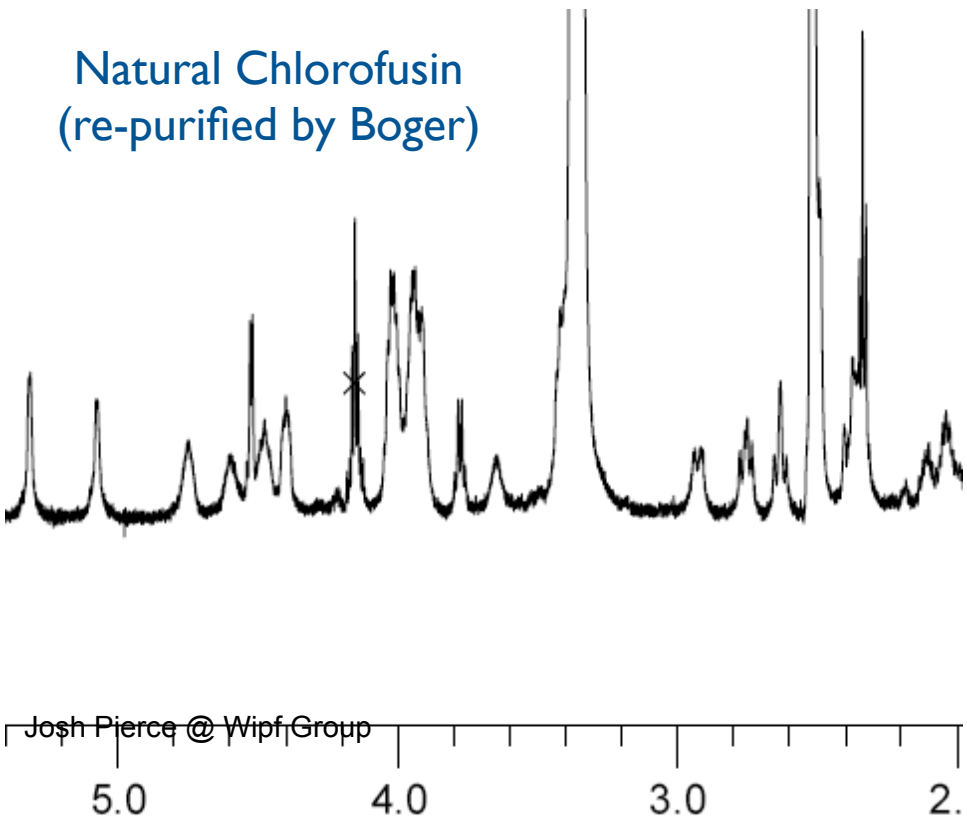
1. EDCI, HOAt (55%)
2. H_2, Pd/C
3. EDCI, HOAt (60%)

→ Chlorofusin

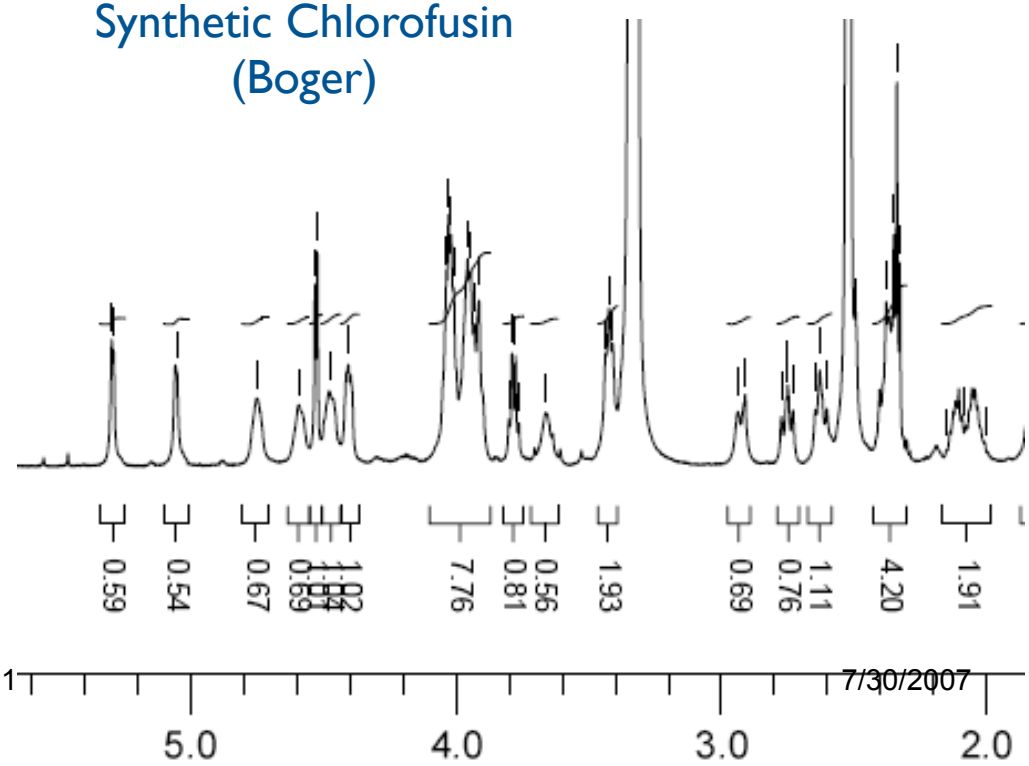
Opposite relative stereochemistry than Williams;
Opposite absolute stereochemistry than Yao.

Boger also makes all possible diastereomers of chlorofusin and compares NMR and CD data.

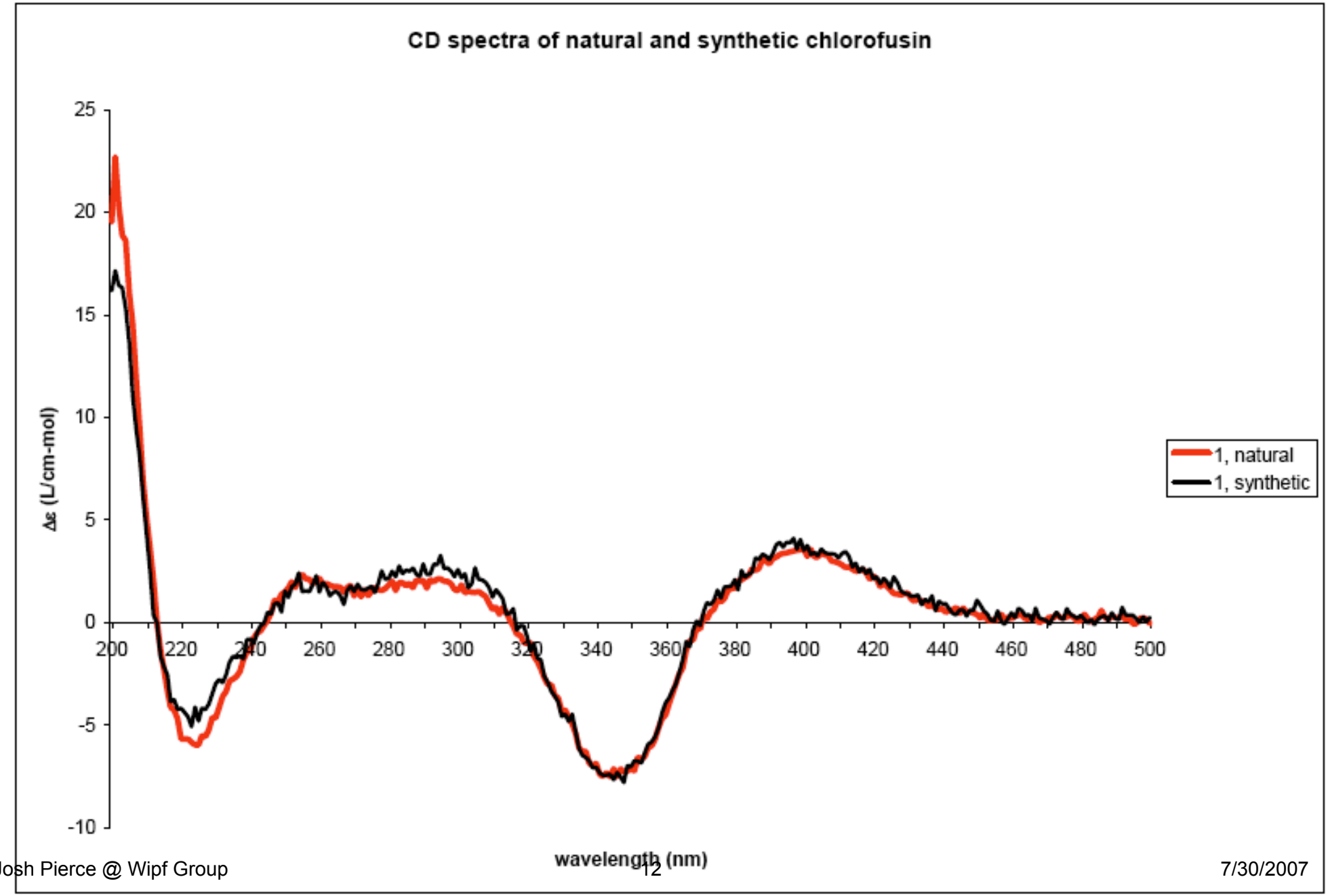
Natural Chlorofusin
(re-purified by Boger)



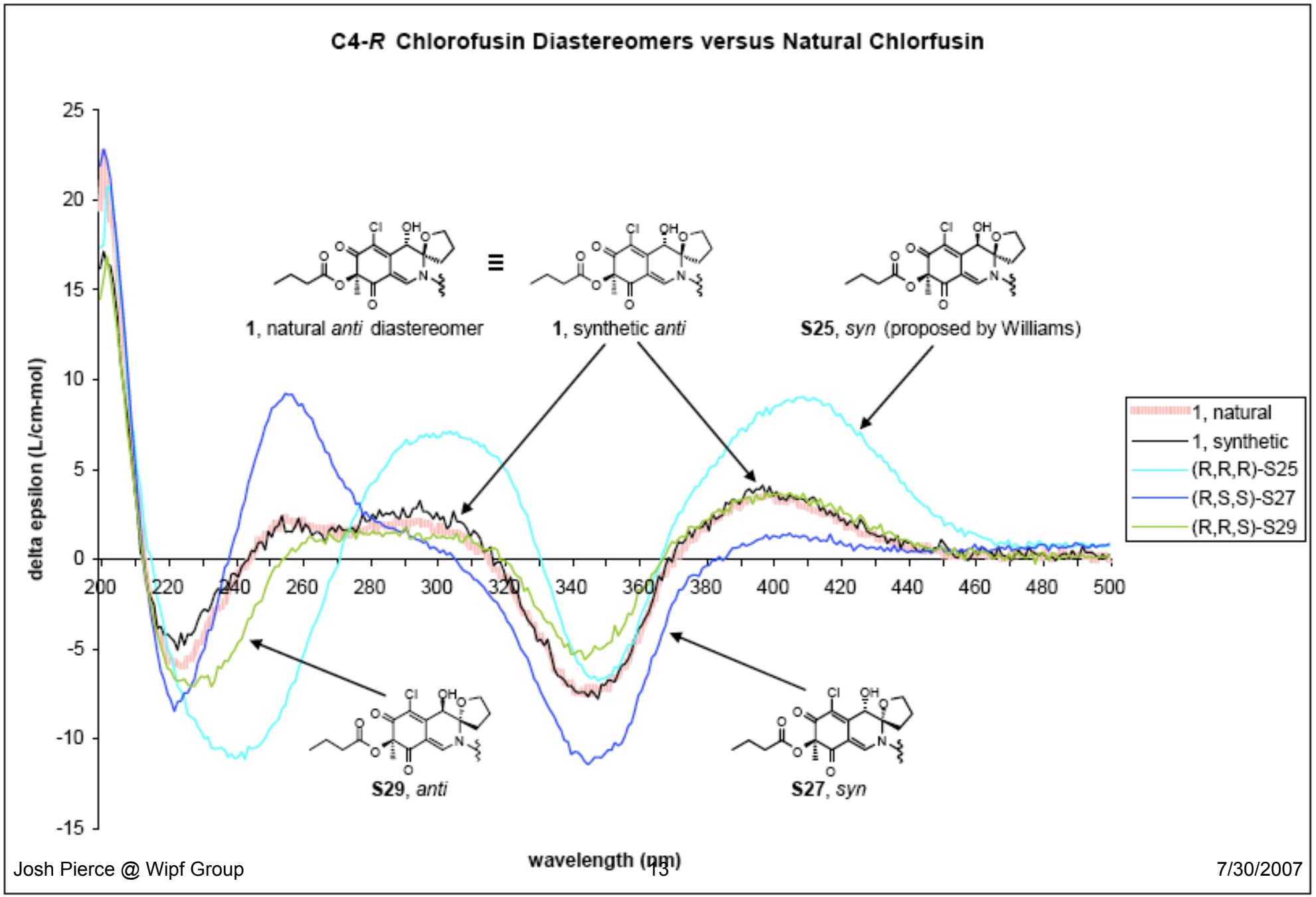
Synthetic Chlorofusin
(Boger)



Comparison of CD Data: A Match!



Additional Confirmation via CD



Conclusions and Lessons Learned

Chlorofusin was isolated by Williams in 2001; however, no stereochemistry was assigned for 2 amino acid residues and only relative stereochemistry designated for the chromophore.

Boger synthesized the cyclic peptide domain and assigned the 2 unknown amino acid side chains as well as confirmed the overall structure of the peptide domain.

Yao, claiming the first total synthesis of chlorofusin in 2007, correctly reassigned the relative stereochemistry based on NMR correlation. An absolute stereochemical assignment was put forth, however has since been challenged.

Recently, Boger has completed the synthesis of chlorofusin and through a systematic study of all possible diastereomers has uncovered the correct absolute stereochemisty. Additionally, an array of studies point to where other groups had gone wrong in their analysis.

Complex problems of this type remind us never to force data to fit a hypothesis and not to assume the factuality of literature without thorough analysis. In addition, while one could argue that the review process should have questioned Yao's assignment (and possibly did), it is reassuring that the chemical community was quick to challenge this work and propose an alternative assignment.