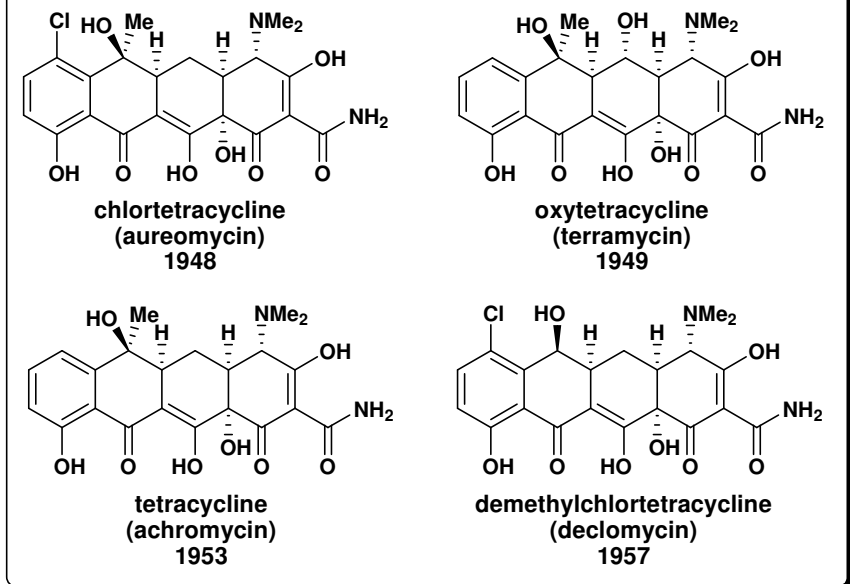


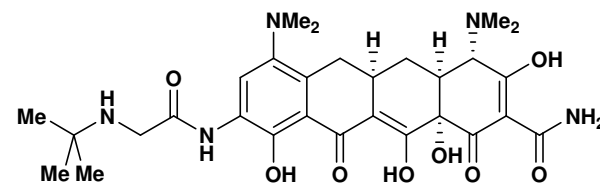
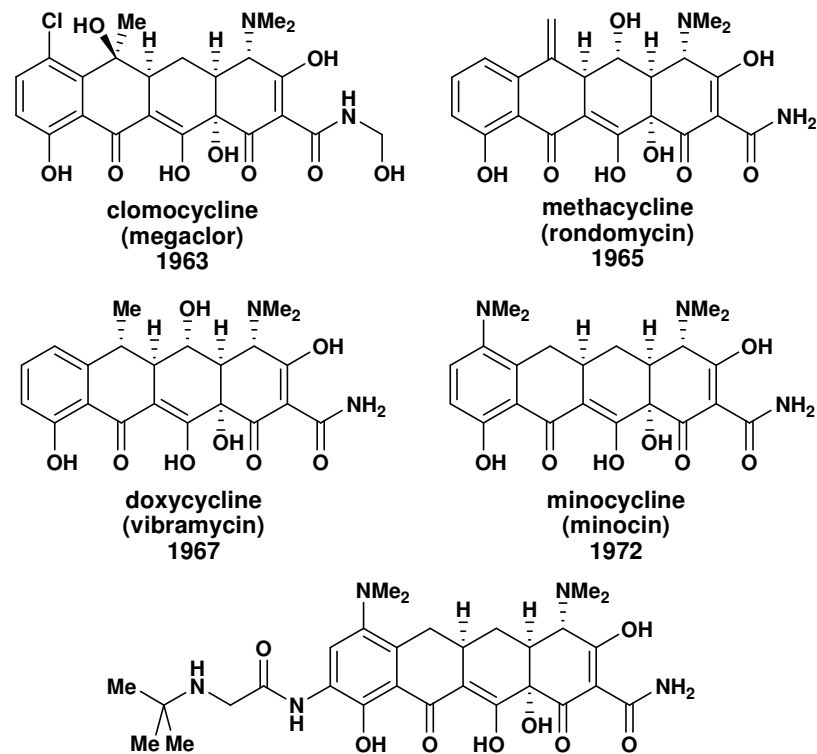
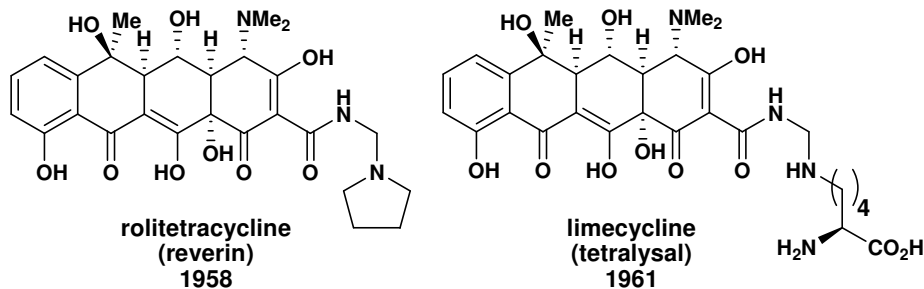
The Tetracyclines

The tetracycline family of antibiotics

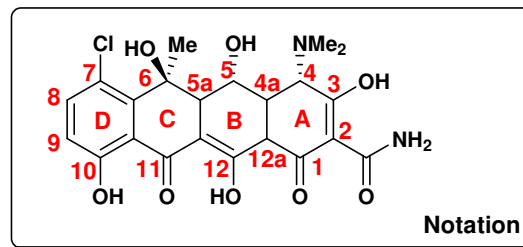
The natural products



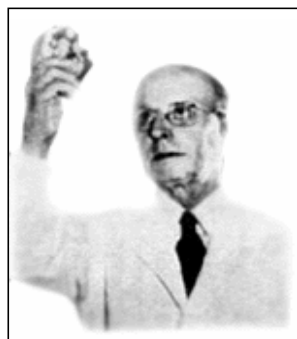
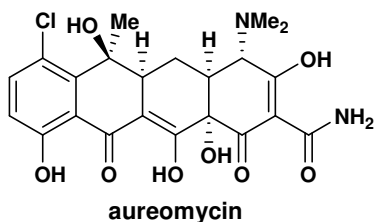
Semisynthetic derivatives on the market



1993
(Phase III clinical trials in progress)



Discovery and The Dawn of Semisynthetic Antibiotics



Bayer Pharmaceuticals

Benjamin Duggar
University of Missouri

The first tetracycline antibiotic discovered, aureomycin was isolated in 1948 from a Missouri soil sample by Lederle Laboratories. The Lederle team was led by Benjamin Duggar - a consultant who was a 73-year-old emeritus professor of botany who had recently retired from the University of Missouri! As Jie Jack Li cracks, "Your greatest discovery could happen in your retirement."

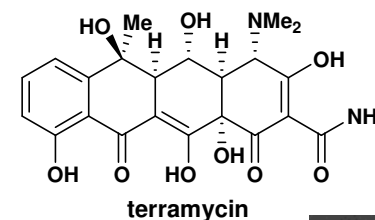
About Lederle Labs:

Lederle Labs was founded in 1902 in an old farmhouse on the Pearl River in New York. Aureomycin was one of many lifesaving products developed by Lederle, including vaccines for polio and smallpox. It is now a part of Wyeth Pharmaceuticals.



Ebay

Ad for aureomycin as additive in cattle feed



The Nobel Prize Committee
R. B. Woodward

About the same time as the Lederle discovery of aureomycin, Pfizer was scouring the globe for new antibiotics. Soil samples were collected from jungles, deserts, mountaintops, and oceans. But ultimately terramycin was isolated in 1949... from a soil sample collected on the grounds of a factory in Terre Haute, Indiana, owned by Pfizer!

From the beginning, terramycin was a molecule enveloped in controversy. It was the subject of the first mass-marketing campaign by a modern pharmaceutical company. Pfizer advertised the drug heavily in medical journals, eventually spending twice as much on marketing as it did to discover and develop terramycin. Still, it turned Pfizer - then a small company - into a pharmaceutical giant.

Pfizer and R.B. Woodward collaborated to determine the structure of terramycin, succeeding for the most part in 1952 (*JACS* **1953**, 75, 5455). The stereochemistry at C_{4a} was revised after X-ray crystallography and NMR studies in the 1960's (*JACS* **1965**, 87, 134; *JACS* **1963**, 85, 851).

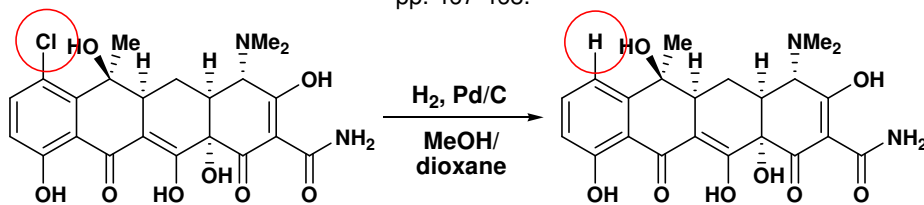
Big Pharma Behaving Badly: In 1955, Conover discovered that hydrogenolysis of aureomycin gives a deschloro product that is just as active as the original product. This proved for the first time that chemically-modified antibiotics could have biological activity. Within a few years, a number of semisynthetic tetracyclines had entered the market, and now most antibiotic discoveries are of novel active derivatives of older compounds.

Conover's discovery, however, provoked further controversy for tetracycline. Pfizer became embroiled in a patent dispute with American Cyanamid, which owned the rights to aureomycin (the starting material for Conover's procedure to make tetracycline). Pfizer and American Cyanamid eventually settled the dispute out of court when they realized that neither company held truly exclusive rights to the drug, and agreed to cooperate on selling the drug in order to drive off competitors trying to enter the tetracycline market. At one point, Pfizer employed a private detective to tap the phones of Bristol-Meyers, a competitor seeking to enter the tetracycline market! Bristol-Meyers agreed to overlook this brazen act in exchange for a share of the tetracycline market. Eventually five companies colluded in order to maintain artificially high prices for tetracycline. However, the Federal Trade Commission stepped in after several years, finding Pfizer and company guilty of patent fraud and anti-trust violations, and broke up the monopoly.

Legal issues aside, for this discovery Lloyd Conover is now in the American Inventors' Hall of Fame, alongside Thomas Edison and the Wright brothers.

U.S. Federal Trade Commission, "Anticipating the 21st Century: Competition Policy in the New High-Tech, Global Marketplace".

M. Mintz. "Golden Ox of Antitrust." *The Nation* 14 April 1969, Vol. 208, Issue 15. pp. 467-468.



Conover, L.H. 1955. U.S. Patent No. 2,699,054.



About.com

Lloyd Conover
Pfizer

Now, Back to Actual Science

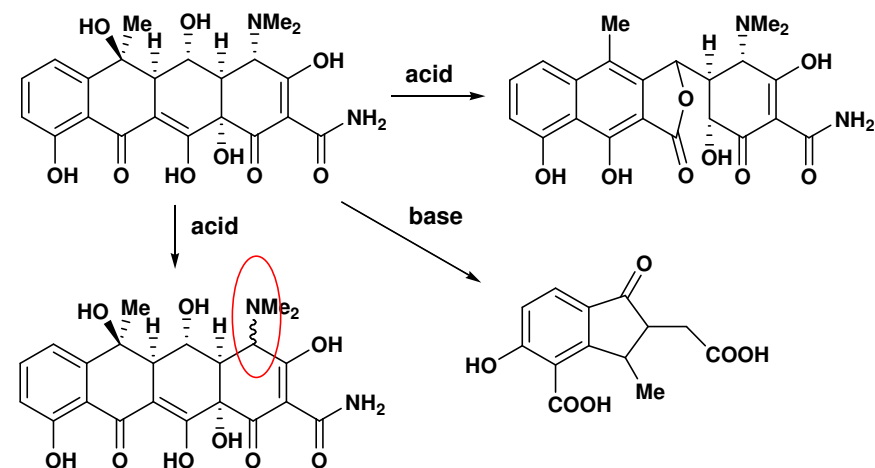
Biosynthesis and Biological Activity: Tetracyclines are polyketide antibiotics, biosynthesized in a fashion similar to that of fatty acids, erythromycin and a host of other antibiotics. Tetracyclines are produced naturally by *Streptomyces aureofaciens* (T. Nakano, et al. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1345.).

Tetracyclines bind to the bacterial ribosome, preventing the binding of aminoacyl-tRNA to the ribosomal A site. This prevents bacterial protein translation (I. Chopra, M. Roberts. *Microbiol. Mol Biol. Rev.* **2001**, *65*, 232.).

The Challenge to Synthetic Chemists: Muxfeldt and colleagues outline the basic obstacles to achieving a total synthesis of any of the natural tetracyclines:

Stereochemical Complexity. There are up to five contiguous asymmetric centers (tetracycline) which must be established.

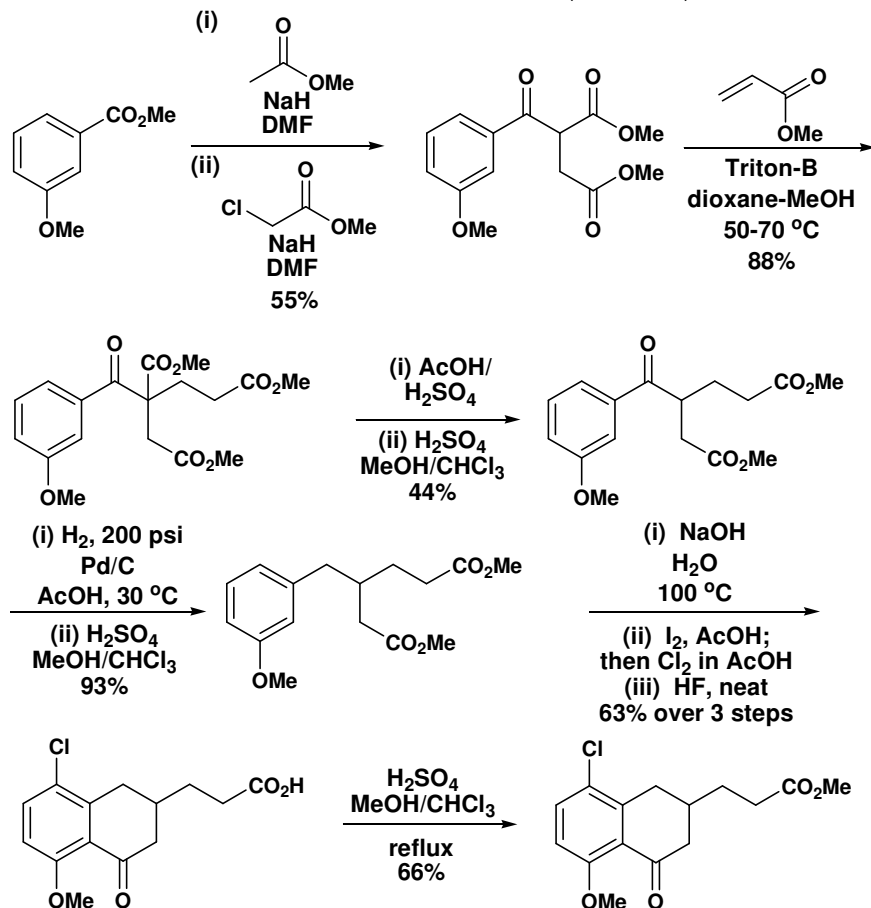
Chemical Sensitivity. For the 6-methyl-6-hydroxy tetracyclines, mild acid rapidly catalyzes dehydration, ketalization and a retro-aldol to produce the lactone below. Mildly basic conditions results in deprotonation of the C5 and C6 hydroxyls, initiating a cascade of events which leads to decomposition of the molecule. Finally, the C4 stereocenter is readily epimerized upon exposure to acetic acid or aqueous buffers.



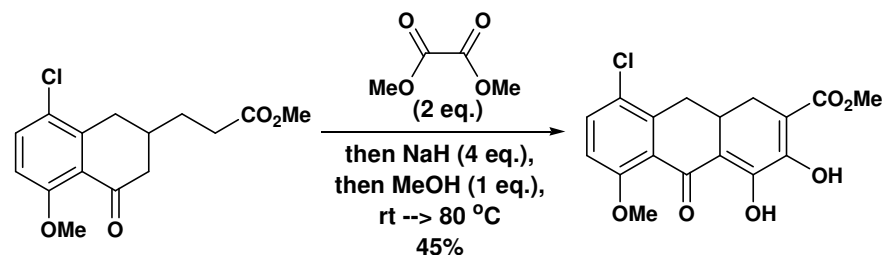
H. Muxfeldt, et al. *J. Am. Chem. Soc.* **1979**, *101*, 689.

Woodward's First Total Synthesis of a Biologically-Active Tetracycline, 6-Demethyl-6-deoxytetracycline.

L.H. Conover, et al. *J. Am. Chem. Soc.* **1962**, *84*, 3222. (Initial communication)
 R.B. Woodward. *Pure Appl. Chem.* **1963**, *6*, 561. (A personal account)
 J.J. Korst, et al. *J. Am. Chem. Soc.* **1968**, *90*, 439. (Full article)

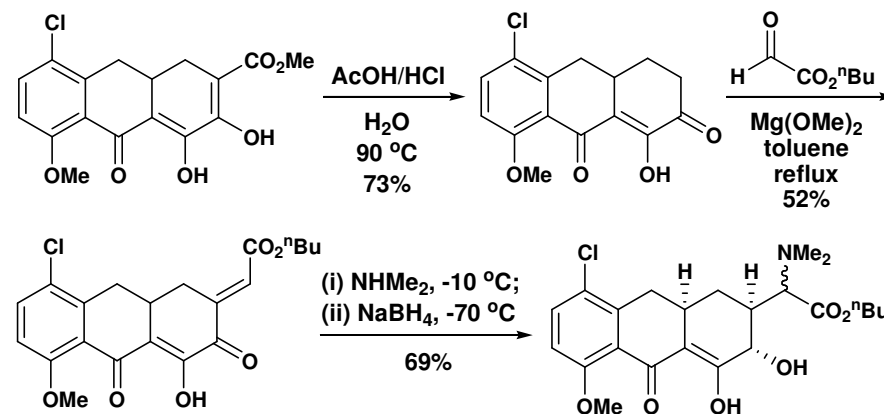
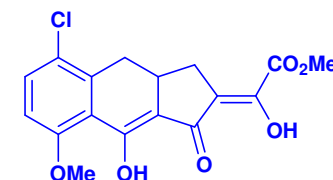


Chlorination blocks the *para* position, forcing condensation onto the more hindered *ortho* position.



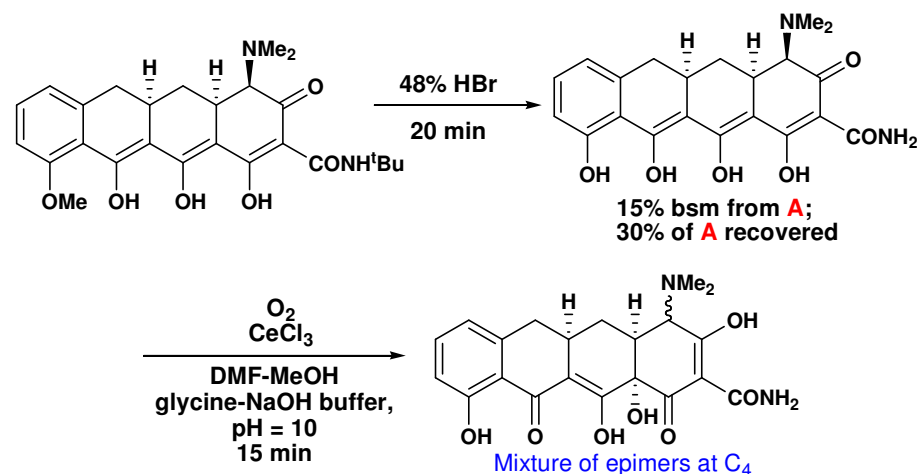
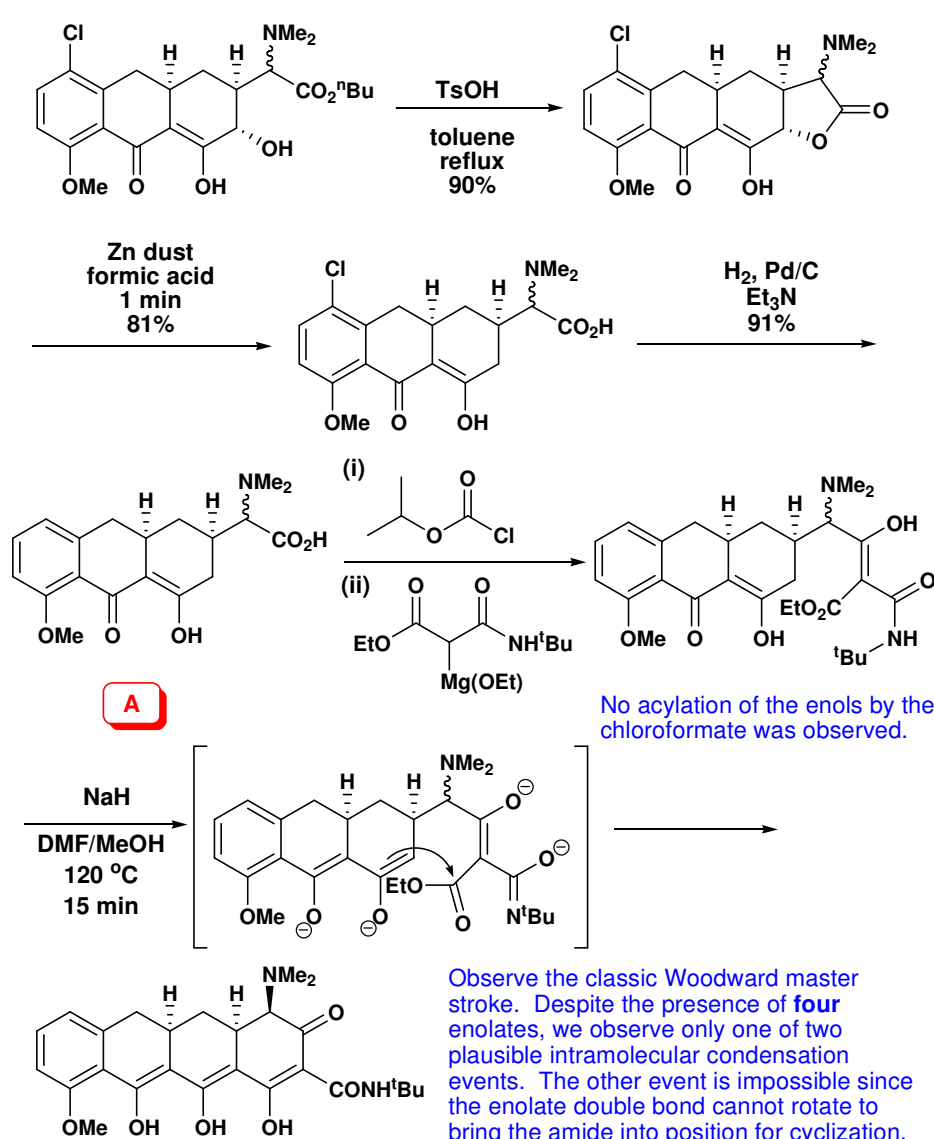
intermolecular condensation outcompetes intramolecular!

MeOH is essential to suppressing the kinetically favorable intramolecular condensation and permitting the intermolecular condensation with the oxalate prior to formation of the desired tricycle. In the absence of MeOH, Woodward observed formation of the intramolecular product first, followed by condensation onto the oxalate to form the five-membered ring shown:

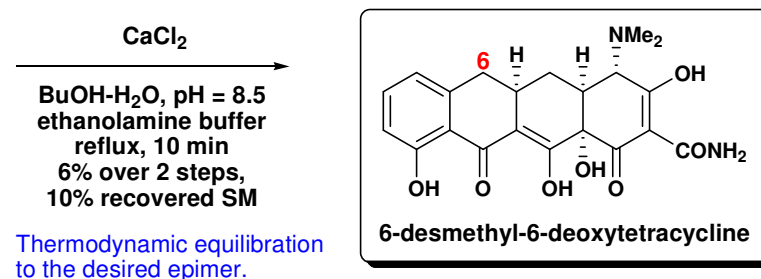


The thermodynamically more favorable diastereomer is formed exclusively in this step, with the carboxyamino substituent assuming an equatorial position and thus establishing the *cis* relationship of the bridgehead hydrogens. Ketone reduction is also stereoselective.

The Tetracyclines



This was a difficult step to optimize - Woodward himself noted dryly that "the case at hand was by no means the smoothest we had encountered." Competitive hydroxylation at C_{11a} was also observed, as well as rapid decomposition of the product under prolonged reaction conditions, forcing Woodward and colleagues to halt the reaction prematurely.

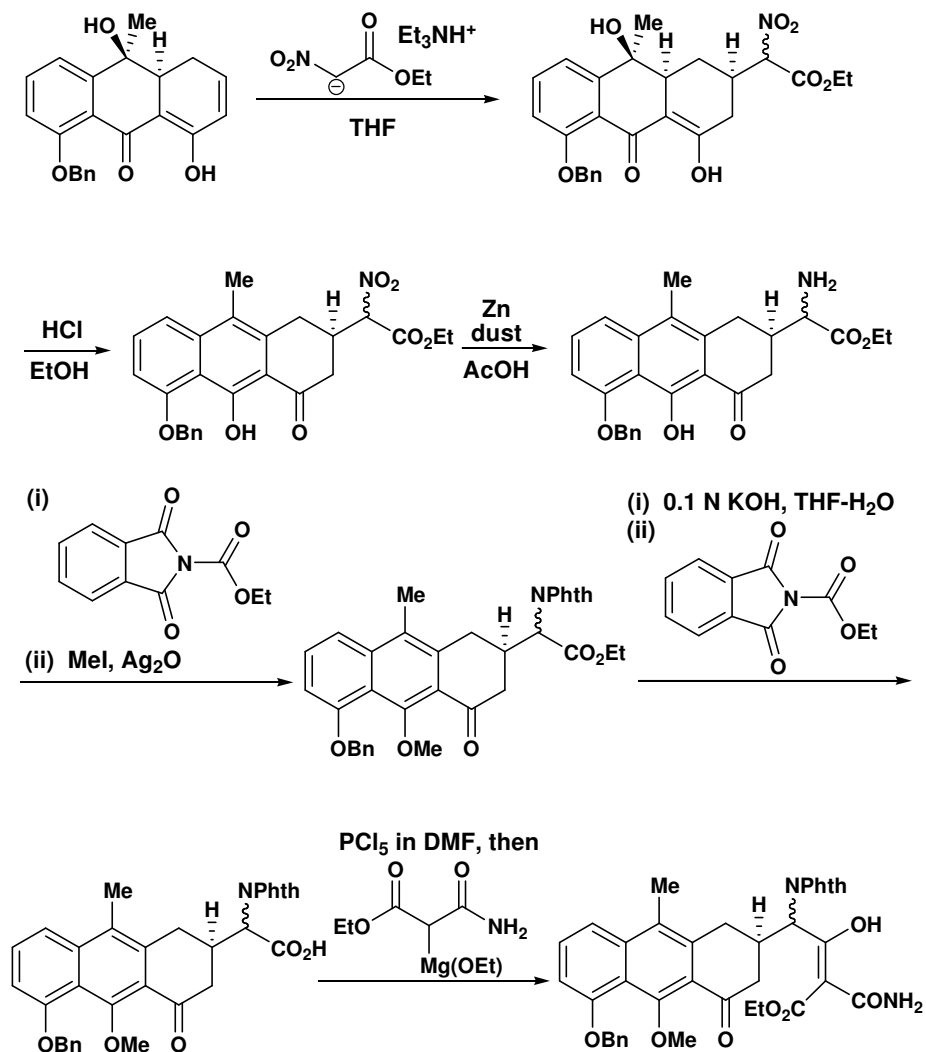
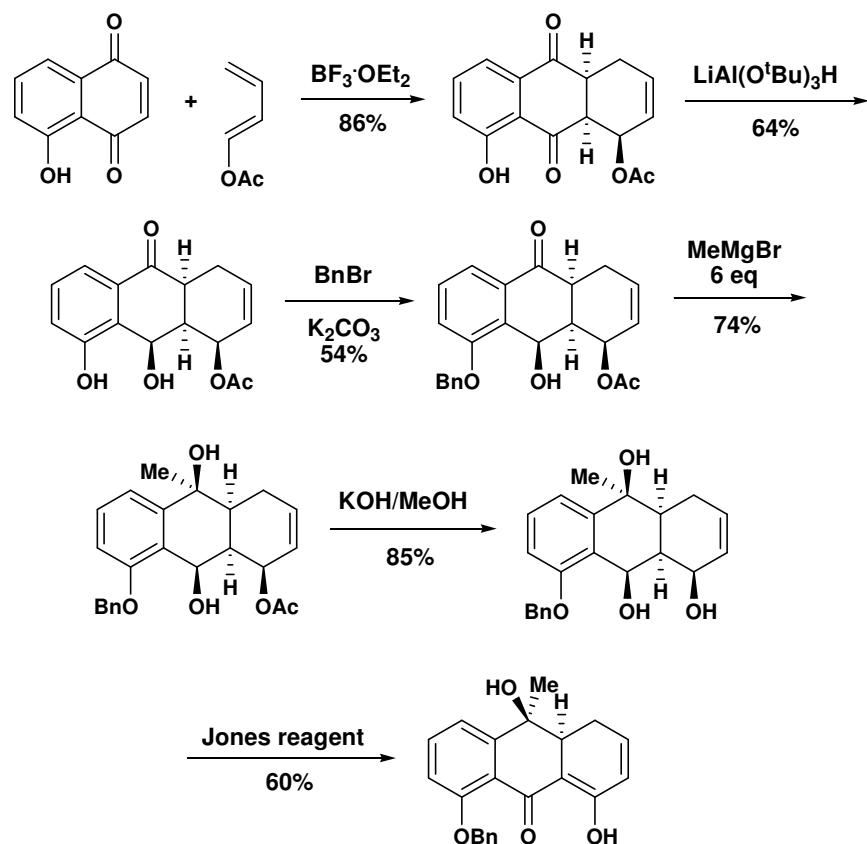


This was the first total synthesis of a tetracycline with all the requisite functionality for full antibiotic activity. Note, however, that this is not the total synthesis of a tetracycline natural product. Substituents at the C_6 position are missing.

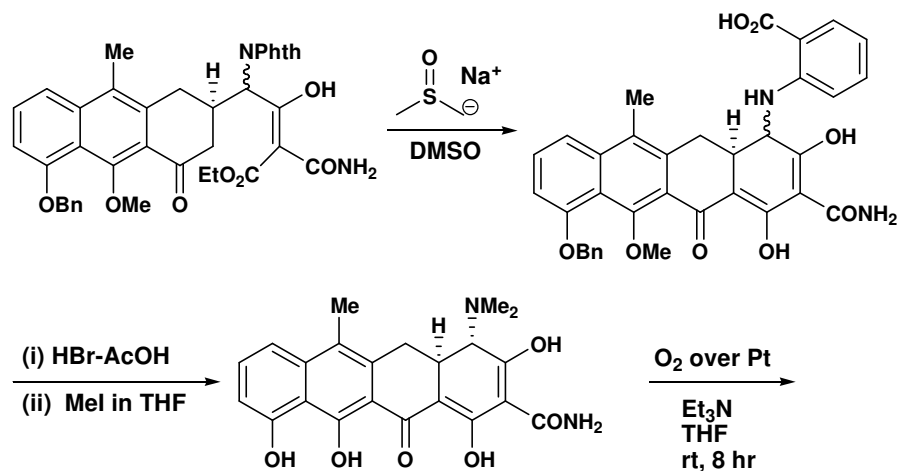
Shemyakin: The First Total Synthesis of a Tetracycline Natural Product

A.I. Gurevich, et al. *Tet. Lett.* **1967**, *8*, 132.M.N. Kolosov, S.A. Popravko, M.M. Shemyakin. *Lieb. Ann.* **1963**, *668*, 86.B.-M.G. Gaveby, J.C. Huffmann, P. Magus. *J. Org. Chem.* **1982**, *47*, 3779.

Note that the *Lieb. Ann.* reference cites a number of obscure Russian journals. The JOC reference, however, illustrates Shemyakin's approach to the tricyclic precursor produced below.

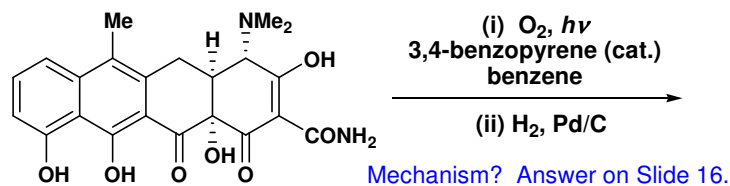


Notice Shemyakin adopting the Woodward approach to ring A.

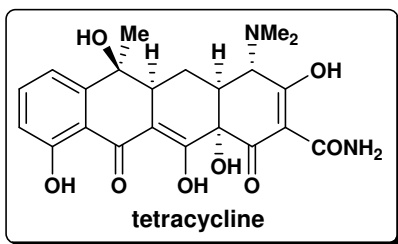


This intercepts a degradation product which had previously been elaborated into tetracycline.

A.I. Gurevich, M.G. Karapetyan, M.N. Kolosov. *Khim. Prirodn. Soedin., Akad. Nauk Uz.SSR* 1966, 141.

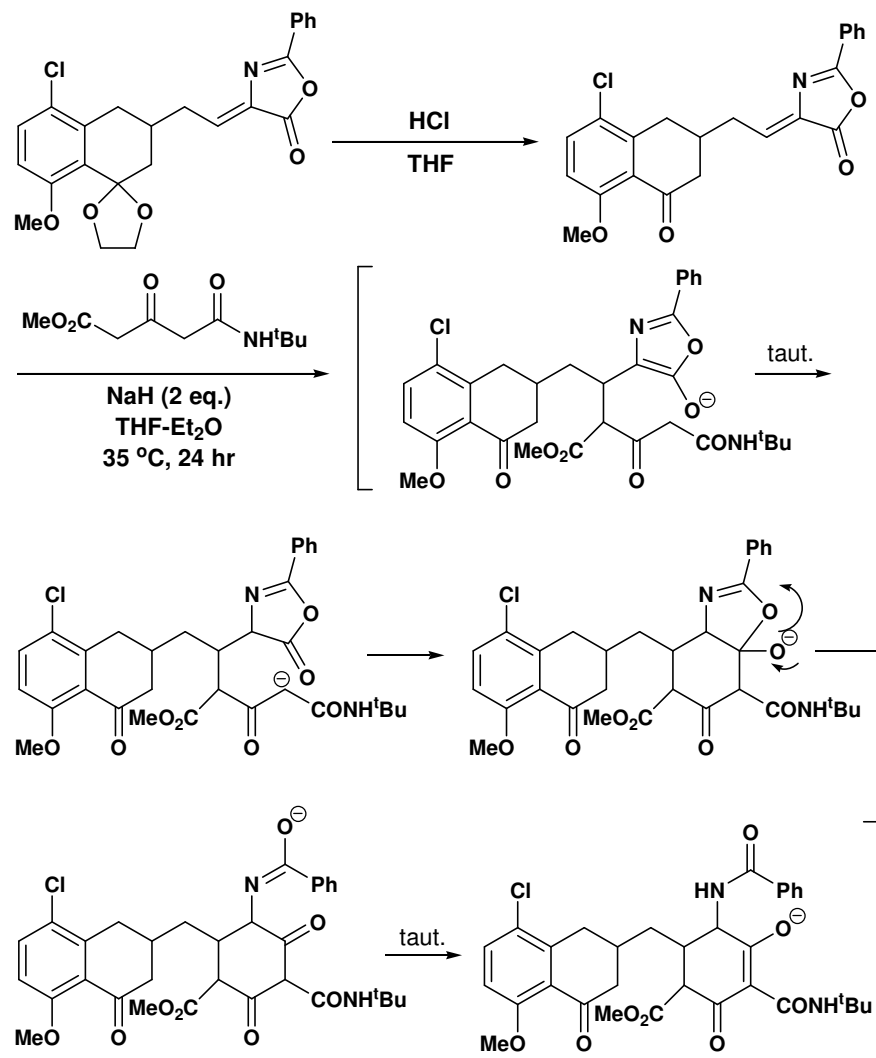
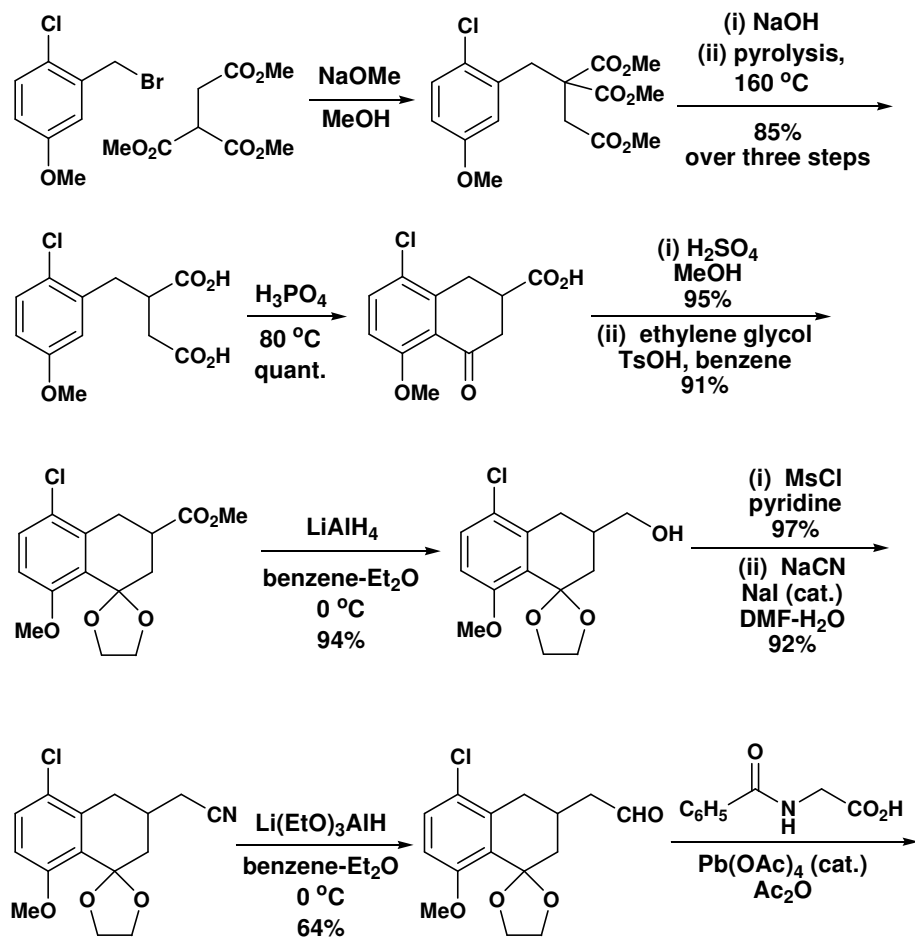


M. Schach von Wittenau. *J. Org. Chem.* 1964, 29, 2746.

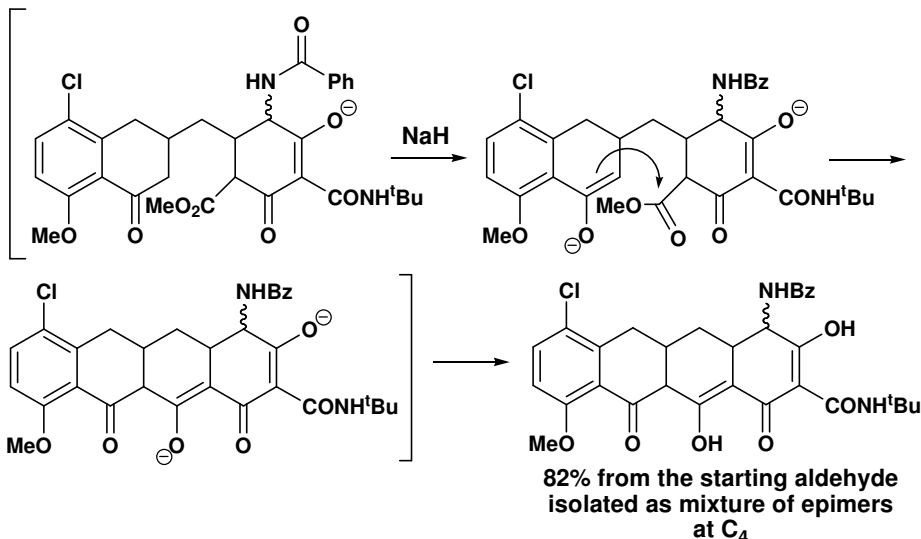


Muxfeldt's Total Synthesis of 6-Desmethyl-6-deoxytetracycline

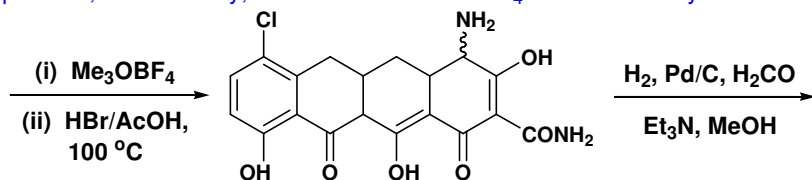
H. Muxfeldt, W. Rogalski. *J. Am. Chem. Soc.* **1965**, *87*, 933. (Communication)
 H. Muxfeldt, E. Jacobs, K. Uhlig. *Chem. Ber.* **1962**, *95*, 2901. (Prep of precursors)



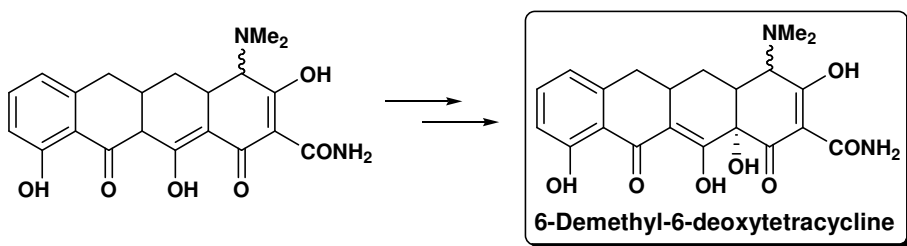
Now the stage is set for the second cyclization in this magnificent transformation. Only one equivalent of NaH used so far!



Muxfeldt thus effects the construction of the A and B rings in a single step! The only problem, unfortunately, is the failure to control C₄ stereochemistry.



(i) deprotects the benzoyl amide; (ii) deprotects the remaining functional groups.



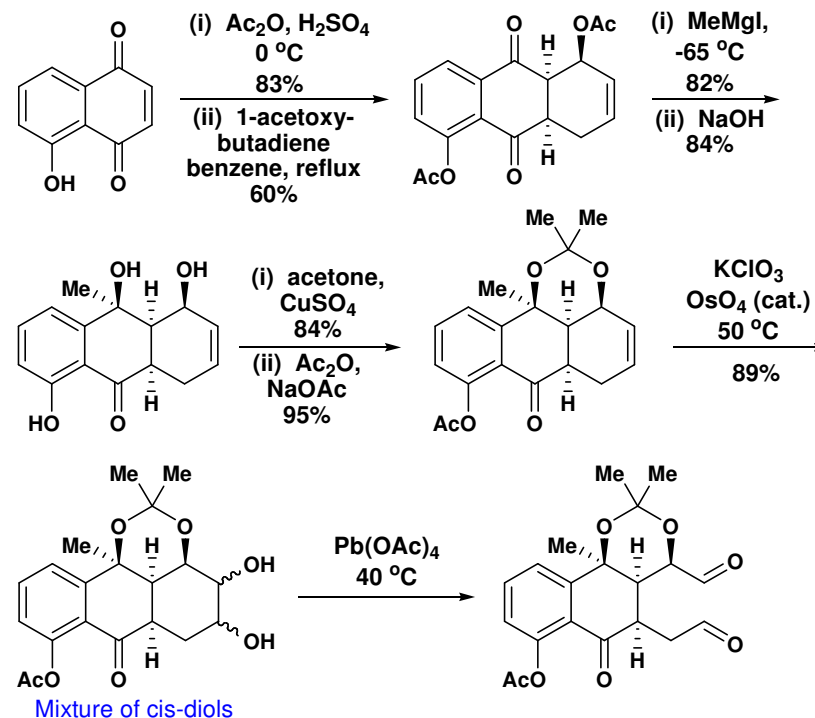
Here they intercept an intermediate from the Woodward synthesis. They also report hydroxylation with O₂ over platinum (*Angew. Chem. Intl. Ed. Eng.* **1962**, 1, 157).

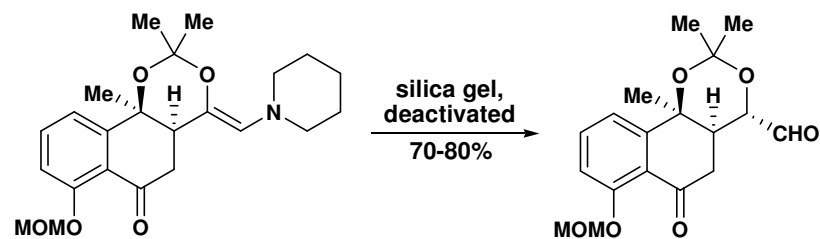
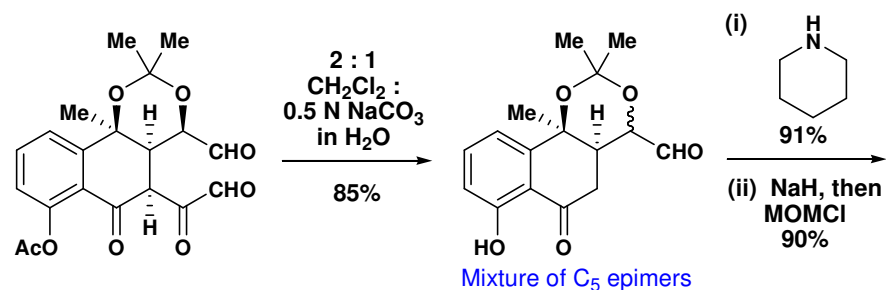
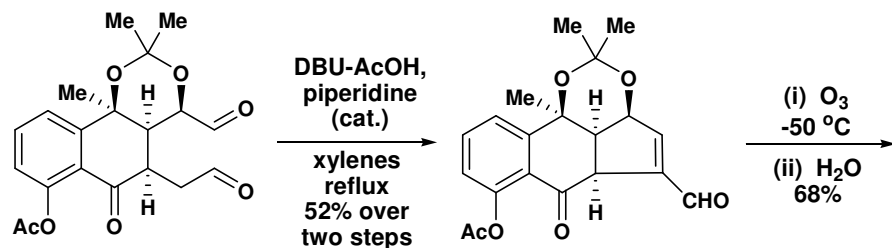
Muxfeldt's Last Hurrah: Total Synthesis of Terramycin

H. Muxfeldt, et al. *J. Am. Chem. Soc.* **1968**, 90, 6534.

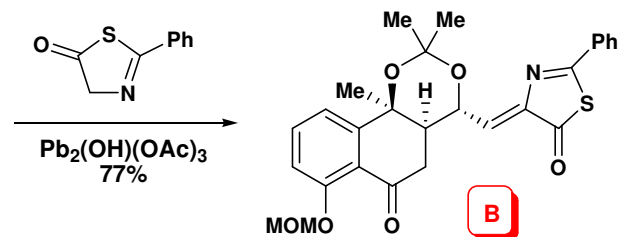
H. Muxfeldt, et al. *J. Am. Chem. Soc.* **1979**, 101, 689.

Terramycin is a much more difficult target than the prototypical tetracyclines discussed previously - Woodward and Muxfeldt avoided many of the problems outlined earlier with by targeting a structure without the troublesome C₅ and C₆ substituents, while Shemyakin targeted a tetracycline which did not have the C₆ hydroxyl. Here Muxfeldt and colleagues (including a young Edwin Vedejs!) tackle those problems head-on! Sadly, this is reported in a posthumous communication from the Muxfeldt laboratories.

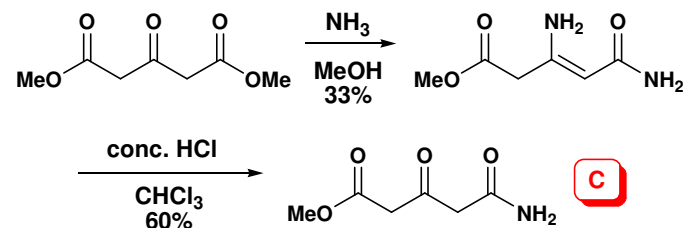




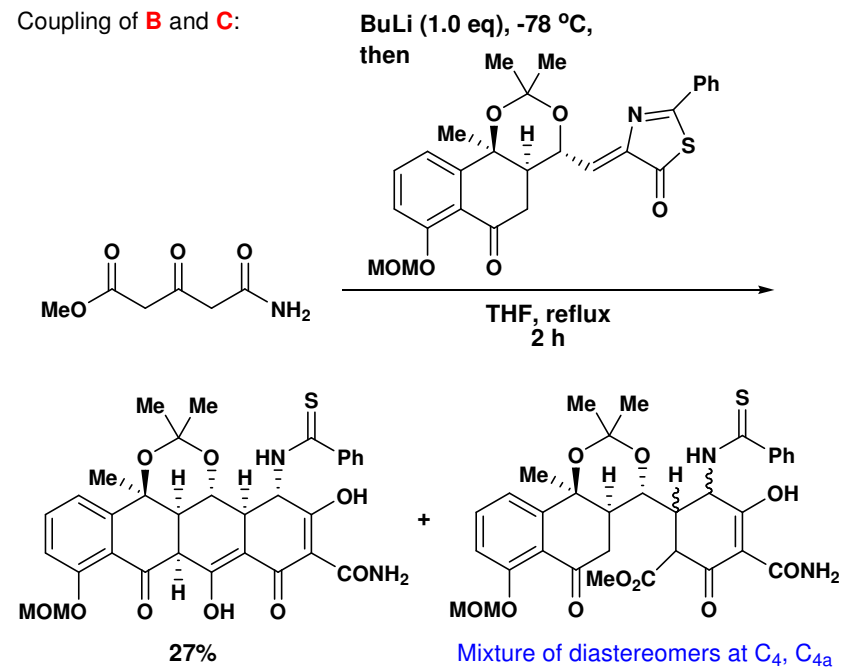
The thermodynamically more favorable epimer is obtained exclusively.



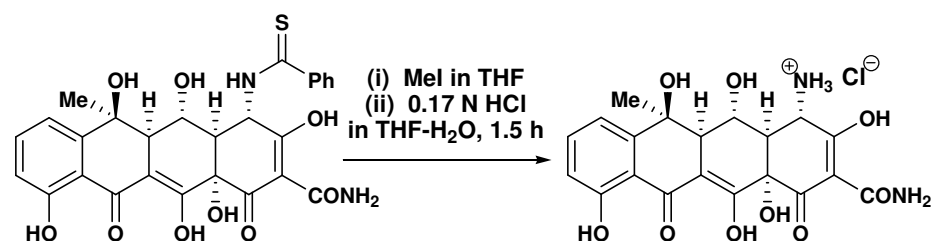
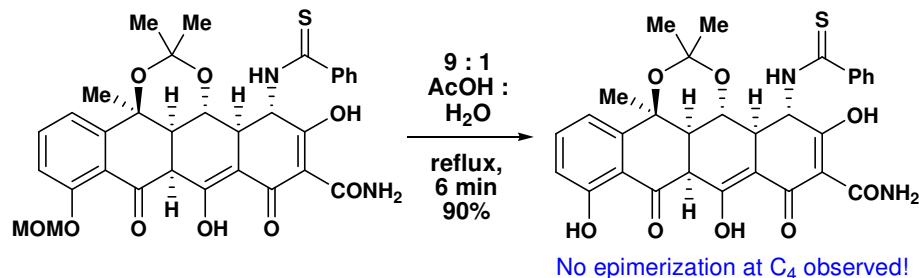
Preparation of **C**:



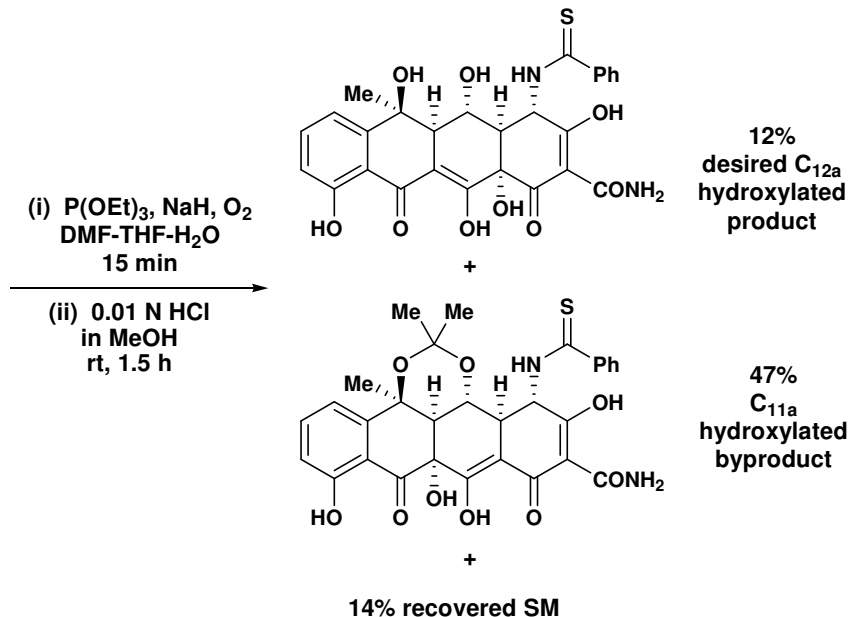
Coupling of **B** and **C**:



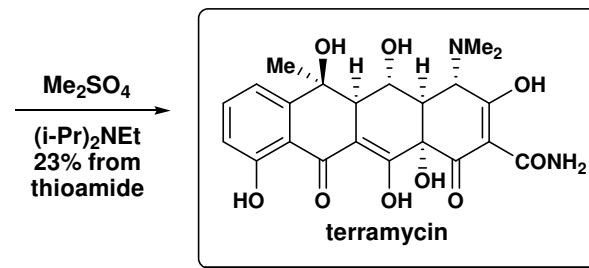
Once again, Muxfeldt employs his beautiful method for forming the A and B rings in a single step. And once again, there is little stereocontrol - all four possible epimers at C₄ and C_{4a} are formed in solution. Fortunately, the desired diastereomer readily crystallizes. The reason for employing the thiazolanone rather than the oxazolanone employed before will become clear shortly...



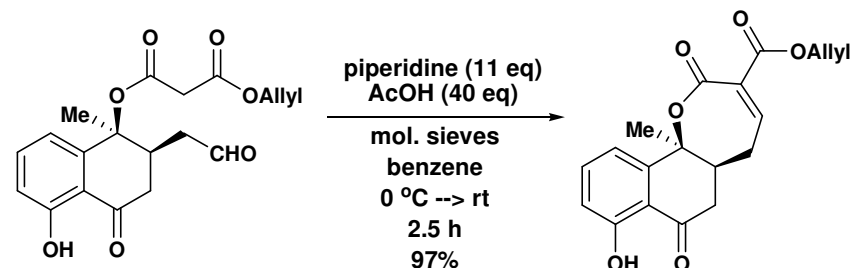
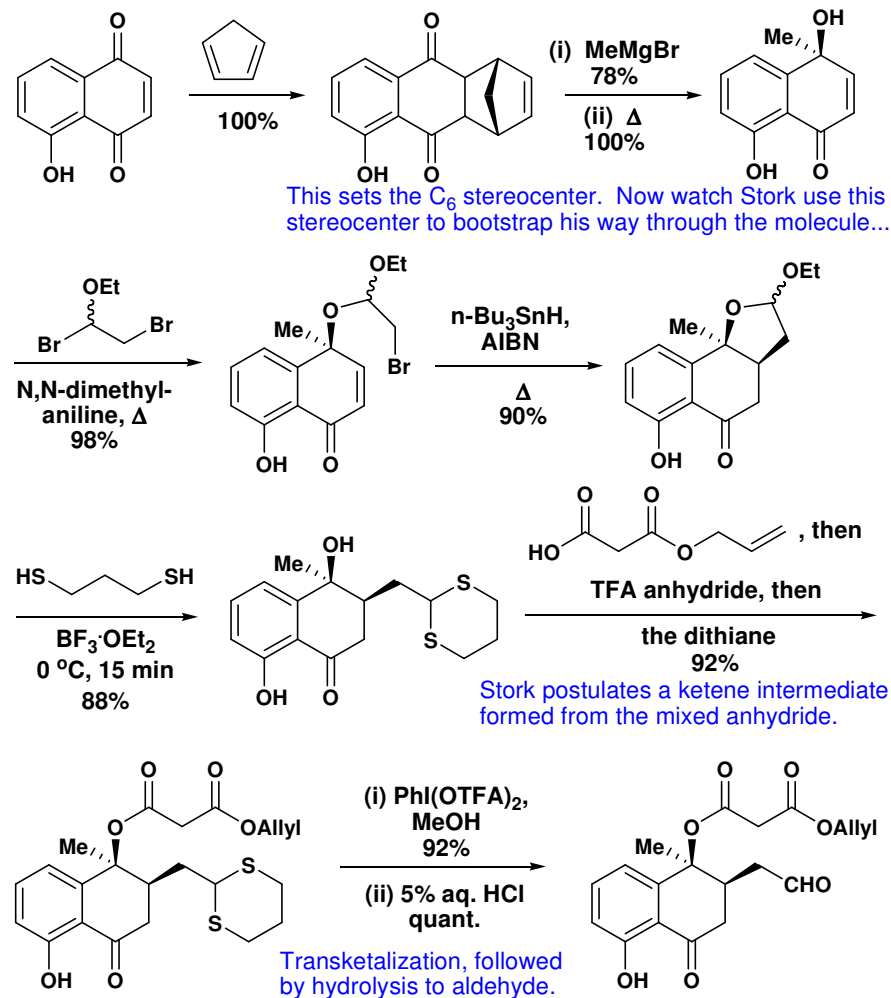
While the oxazolone substrate could also be carried to this step, the resulting benzoyl amide could not be deprotected at this stage, nor could any other amide devised, without decomposition. By contrast, deprotection conditions for the thioamide proved to be sufficiently gentle.



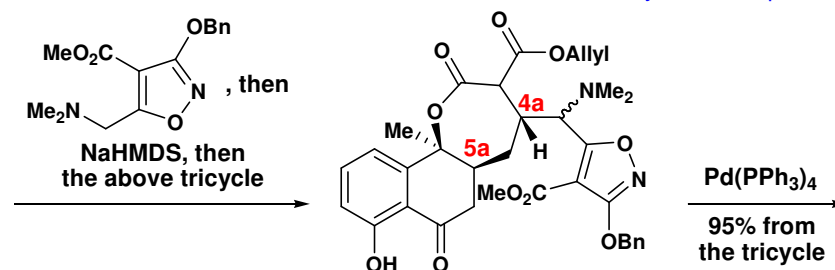
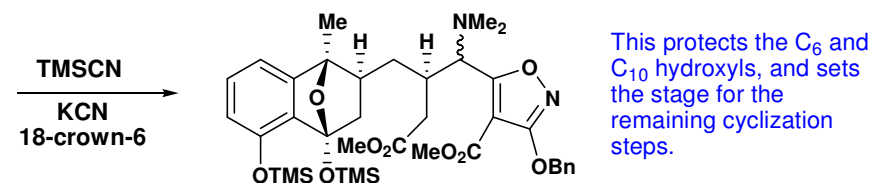
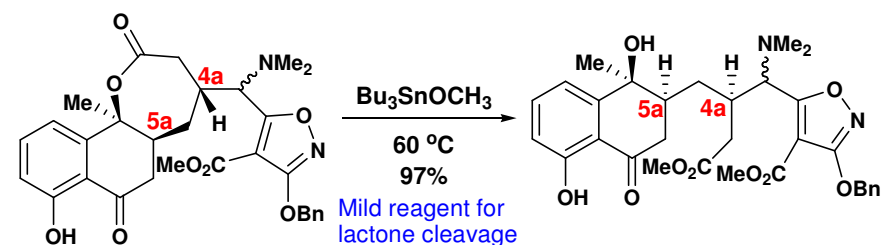
(i) hydroxylates the molecule; (ii) cleaves the acetonide. Unfortunately, hydroxylation occurs principally at C_{11a}. In a fortunate accident, however, it turned out that the acetonide could not be cleaved unless the C_{12a} hydroxyl was present. Thus separation of the desired deprotected product from the undesired major product was quite facile by polyamide chromatography.

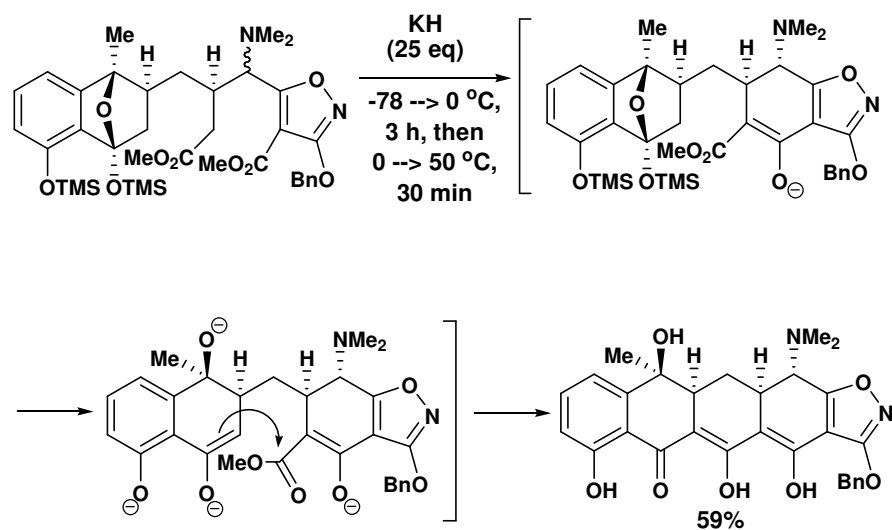


This concludes an elegant synthesis which assembles the A and B rings in a single step. Unfortunately, Muxfeldt and colleagues never satisfactorily address the issues of controlling the C₄ and C_{4a} stereocenters, nor do they improve upon Woodward's solution to the C_{12a} hydroxylation problem.

Stork: Controlling the C₄, C_{4a} StereocentersG. Stork, et al. *J. Am. Chem. Soc.* 1996, 118, 5304.Here Gilbert Stork and colleagues take a completely different approach in order to achieve stereocontrol at the C₄ and C_{4a} centers.

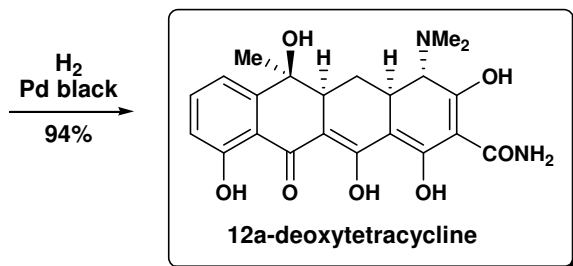
45% overall yield from start of the synthetic sequence!

Here Stork exploits the stereochemistry of the tricycle to direct conjugate addition to the more accessible face. Observe that the C_{5a} and C_{4a} stereocenters are now set.



A Note on C_{12a} Hydroxylation: This intercepts an intermediate which has been hydroxylated at the C_{12a} position according to literature reports, completing in principle the formal synthesis of tetracycline. However, Stork and colleagues were unable to successfully apply any of the C_{12a} hydroxylation methods previously reported. The presence of the C₄ dimethylamino substituent seems to interfere with the hydroxylation. Clearly a satisfactory solution to the C_{12a} hydroxylation problem is still needed...

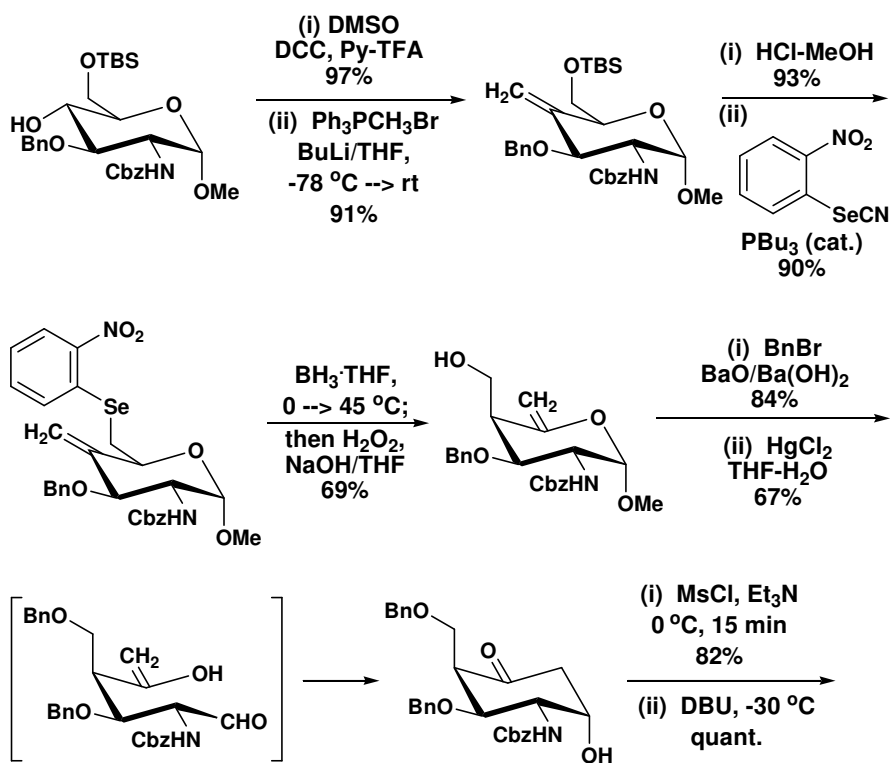
The protecting group scheme permits formation of the A ring first, followed by in situ deprotection and cyclization of the B ring to complete the basic tetracycline framework. Previous studies had indicated that failure to protect the C₁₁ ketone resulted in formation of a BCD tricycle for which conditions to complete A ring cyclization could not be found.



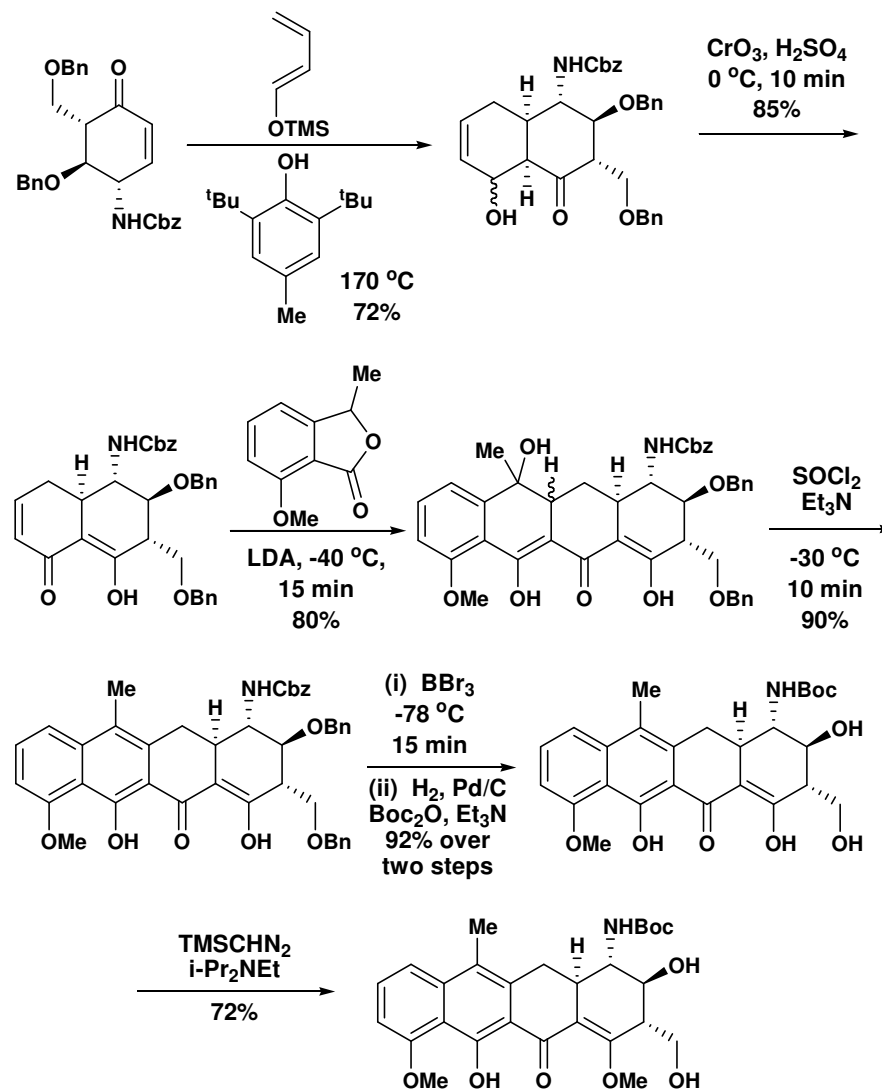
Tatsuta: Asymmetric Total Synthesis of Natural (-)-Tetracycline

K. Tatsuta, et al. *Chem. Lett.* 2000, 647.

Here Tatsuta and colleagues not only produce an asymmetric total synthesis, but they also take a very different approach to the synthetic problem, constructing the A and B rings first and exploiting the carbohydrate chiral pool for starting materials. And as a bonus, they solve the C_{12a} hydroxylation problem as well!

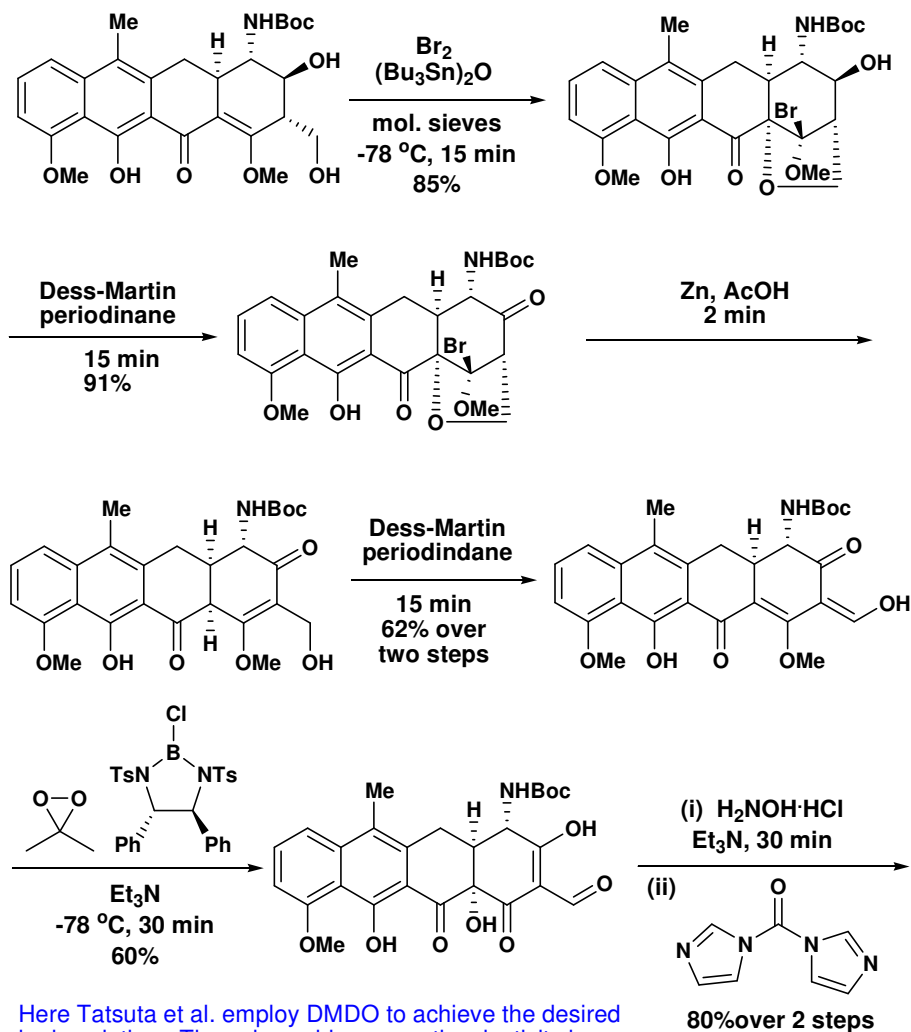


In addition to eliminating to the enone, (ii) also epimerizes to the thermodynamic diastereomer.



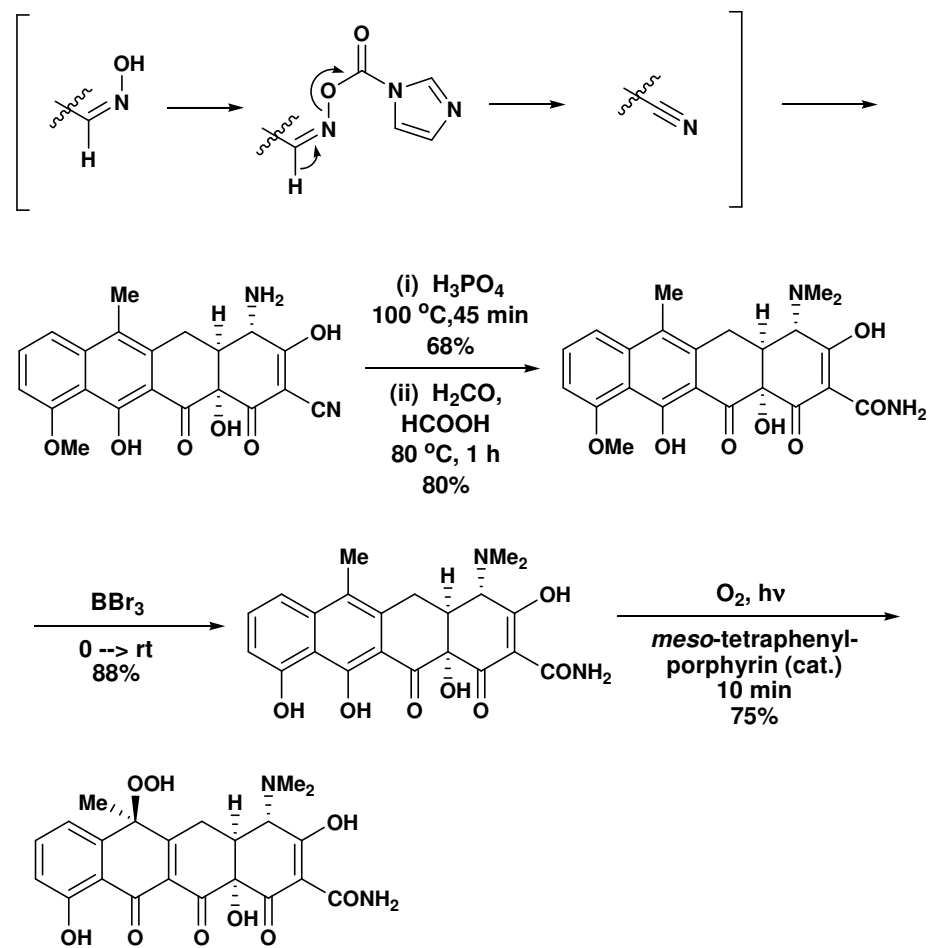
Attempts to directly oxidize this 1,3-diol to the 1,3-dicarbonyl failed, requiring the following detour of sequential alcohol oxidations.

The Tetracyclines



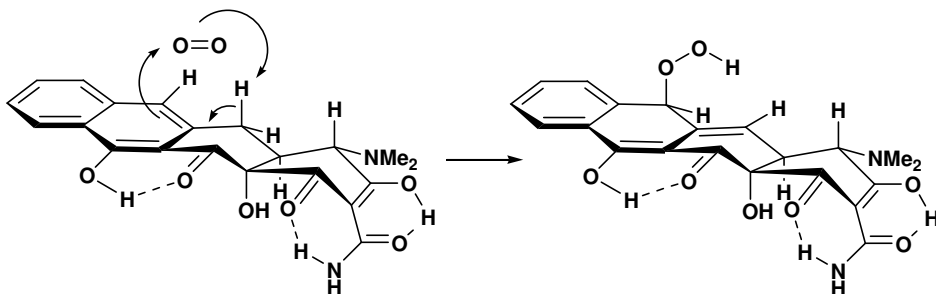
Here Tatsuta et al. employ DMDO to achieve the desired hydroxylation. They also achieve enantioselectivity by exploiting the chiral boron catalyst which Corey developed for enantioselective aldols and Diels-Alder reactions. Note the fantastic yield!

Mechanism?

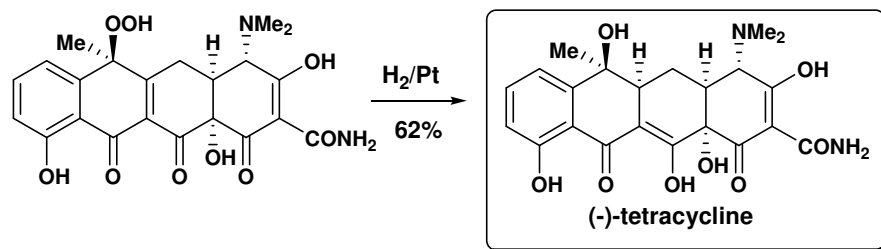


Here Tatstuta et al apply a protocol developed by Wassermann, Lu and Scott for hydroxylating anhydrotetracyclines. Provide a mechanism for this reaction, and rationalize the stereospecific nature of this reaction.

H. Wassermann, T.-J. Lu, A.I. Scott. *J. Am. Chem. Soc.* **1986**, *108*, 4237.



Wassermann, Lu and Scott invoke a formal ene reaction. The orbital alignment requirements dictate that only the axial hydrogen can participate in the reaction, inducing hydroperoxidation on the upper face of the molecule and thus ensuring the proper stereochemistry at C₆.

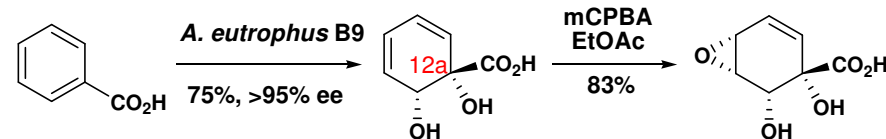


There are many elegant features to this synthesis. Tatsuta and colleagues mimic Stork's Diels-Alder approach to establishing stereochemistry, but employ it to define the troublesome C_{4a} stereocenter immediately. They construct the central tetracycline scaffolding in just three steps from simple precursors. And they solve the C_{12a} hydroxylation problem with a very mild oxidant in the presence of a chiral catalyst, and introduce the C₆ hydroxyl stereospecifically at a very late stage of the synthesis.

Myers' Rapid Asymmetric Access to Analogs of Tetracycline

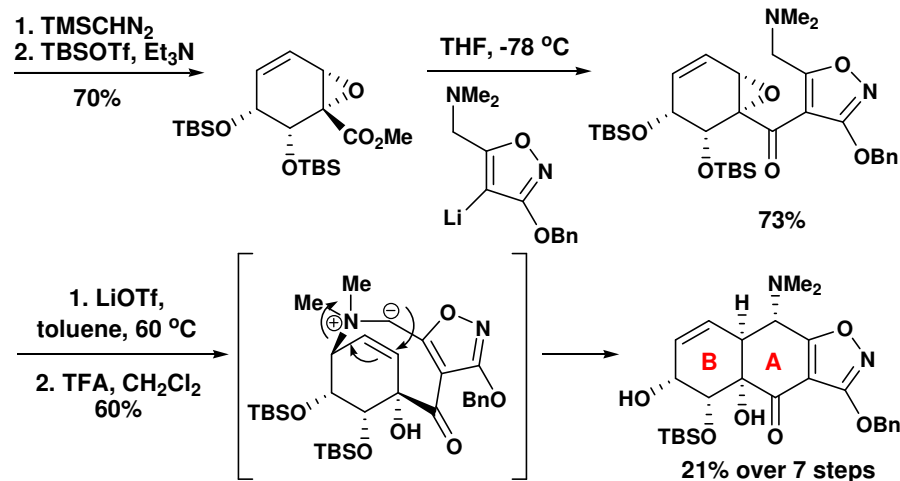
M.G. Charest, C.D. Lerner, J.D. Brubaker, D.R. Siegel, A.G. Myers. *Science* **2005**, *308*, 395.

In an extraordinary report, Myers and colleagues present a highly efficient and enantioselective method for accessing the tetracyclines.



This bacterial-catalyzed reaction can be run on a 90 g scale!

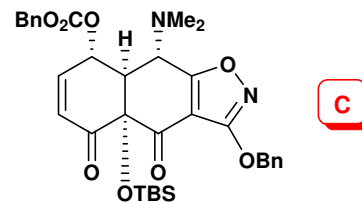
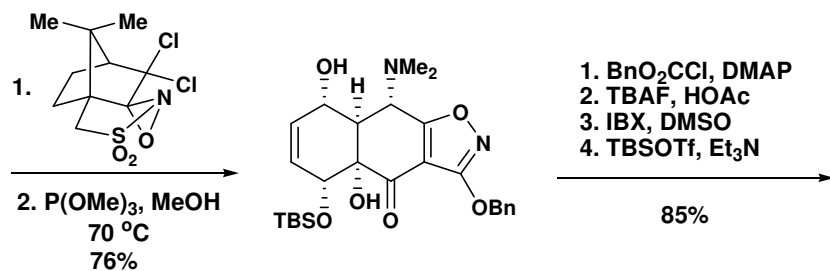
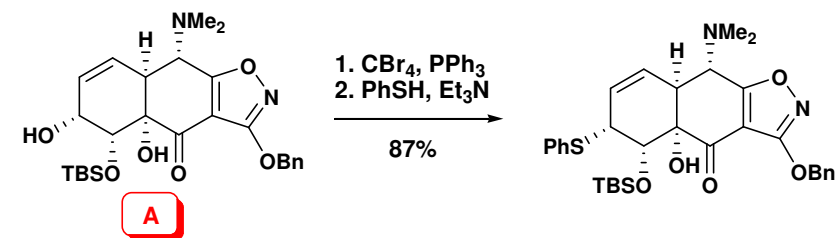
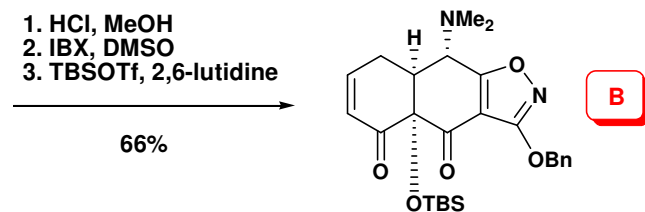
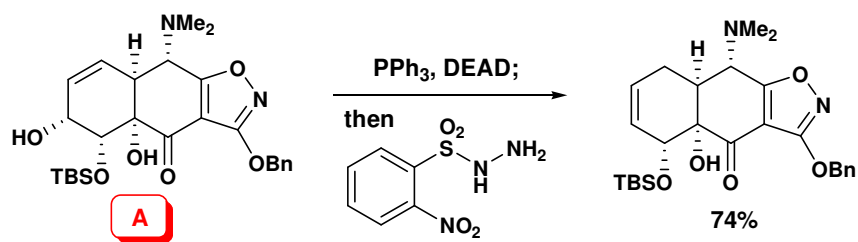
Notice how Myers begins with installation of the troublesome C_{12a} hydroxyl group, and then proceeds to build the molecule around it!



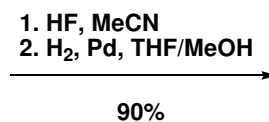
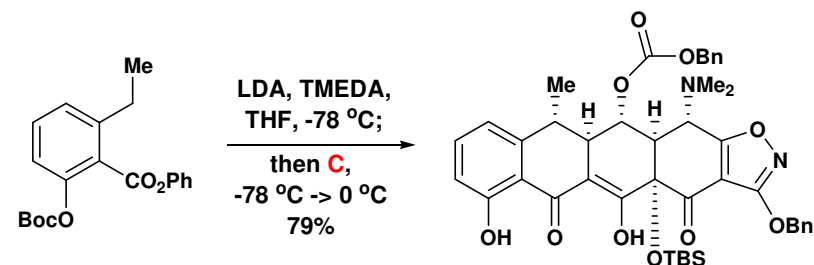
Here Myers closes the ring and sets the C₄ amine stereochemistry. Myers compares this key ring-closing step to a Sommelet-Hauser rearrangement, where the amine initially undergoes an intramolecular S_N-prime epoxide ring opening, followed by ylide formation and finally a [2,3] sigmatropic rearrangement. TFA selectively deprotects the allylic alcohol. Notice the remarkable yield so far!

The Tetracyclines

Now Myers takes his key intermediate **A** and converts it into two fragments: **B**, which will go on to form C₆-deoxy analogs of tetracycline, and **C**, which will go on to form analogs with the normal C₆-oxygenation.



With these fragments in hand, Myers now can install the C and D rings, and he proceeds to do so in a fashion that allows for analogs of tetracycline with deep-seated structural modifications to the D ring.

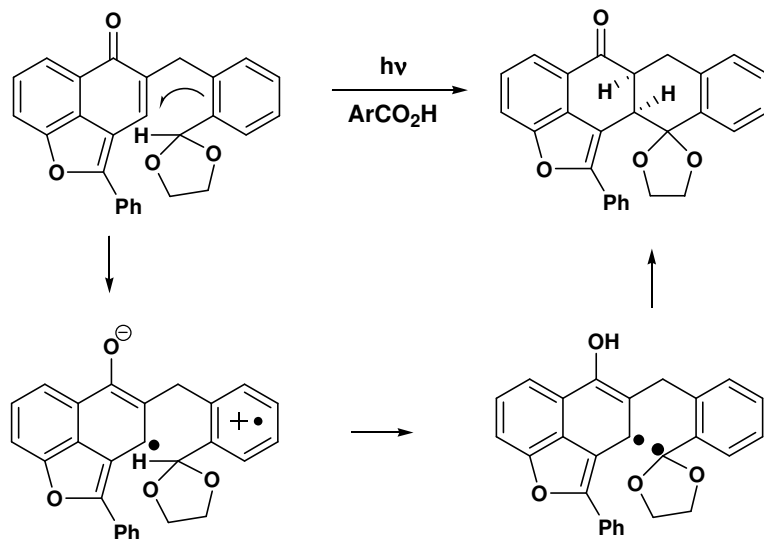


(-)-doxycycline
18 steps, 8.3%

Addendum: Tetracycline Tidbits

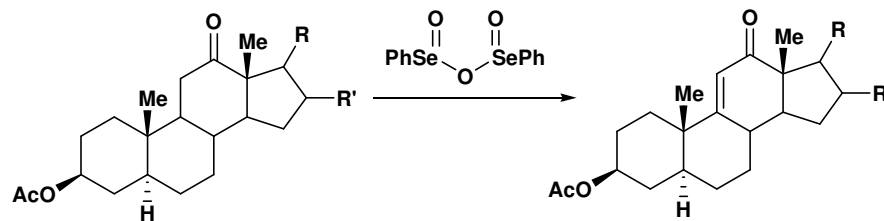
D.H.R. Barton spent over a decade tinkering with tetracycline, but never completed a total synthesis of the molecule. Over the course of this work, however, he discovered some interesting chemistry (naturally).

Photocyclizations of acetals onto enones:



D.H.R. Barton, et al. *J. Chem. Soc. Perkin Trans. I* **1976**, 503.

Barton and colleagues (including a young Steven Ley!) also discovered the utility of phenylseleninic anhydride for the deprotection of dithianes. This led to their applying this reagent in a variety of transformations:



D.H.R. Barton, D.J. Lester, S.V. Ley. *J. Chem. Soc. Perkin Trans. I* **1980**, 2209.

In his book *Reason and Imagination*, Barton concludes his chapter on the tetracyclines with the following perspective on the role of academic research in synthetic chemistry today:

"Just as the studies on the bitter principles [a class of natural products] convinced me that X-ray crystallography was a superior procedure for structure determination, the major effort on tetracycline synthesis convinced me that this sort of work should be left to Industrial friends who have the money and the resources to finish any multi-step synthesis, if it is economically justified. So it is the originality in the reactions and the reagents and any new principles that finally justify academic effort in synthesis. We are far away from the Woodwardian dogma of completely planned synthesis."