Synthetic Strategies Toward the Synthesis of Tetracyclines

A “diabolical concatenation of atoms”
R. B. Woodward, 1956

John Heemstra Jr.
June 21, 2005
- First so called “broad-spectrum” antibiotics
- Isolated by fermentation of various strains of *Streptomyces*
- Yellow, odorless, bitter, light sensitive, crystalline compounds
- Effective against both gram negative and gram positive bacteria
Target and Biochemical Action of the Tetracyclines in Bacteria

Bacterial resistance to Tetracyclines is not acquired by mutation in ribosomal RNA or proteins.
Tetracycline are very sensitive to acidic, alkaline and reducing agents, and are most stable between pH range 2-8.

6-deoxytetracyclines are considerable more stable than their 6-hydroxy counterparts.

Also, they show equal or greater potencies in antibacterial assays.

Chemically Modified 6-Deoxytetracyclines

7-chloro-6-demethyltetracycline (naturally occurring)

Tigecycline

Minocycline

- Currently in Phase 3 clinical trials.
- Reported to be the most promising new antibacterial agent in line for approval.

- Discovered in the 1970’s
- Until recently, was effective against bacteria resistant to other tetracyclines


Chemical properties of the Tetracyclines

1. Hydration of the C ring in anhydrotetracyclines to create tetracycline

X = Cl 7-chloroanhydrotetracycline
X = H anhydrotetracycline

method 1 (1962): O₂, hv, 5 days
X = Cl 70% yield
X = H no reaction

method 2. (1986): O₂, hv, TPP, CHCl₃, 10 min
X = H 49% yield

Correctly sets the stereochemistry at C6 and C5a

Scott, A. I. et. al. JACS, 1962, 84, 2271
Chemical properties of the Tetracyclines

2. Stereospecific hydroxylation of 12a-Deoxygenytracyclines to create tetracycline

![Chemical structure diagram]

\[ \text{catalysts:} \]

- Mn(OAc)_2, AgNO_3, CeCl_3, CoCl_2, CdCl_2, SnCl_2, PtCl_4, CaCl_2, BaCl_2, SrCl_2, CrCl_3
- Ru/C, Ir/C, Pd/C, Ag/C, Pt/C, Pd/ZnO, Pd/CaCO_3

Little to no experimental detail
Yields not given

3. Epimerization of the C4 amino group

![Chemical structure diagram]

reversible 1st order reaction influenced by multivalent cations

Total Synthesis of (±)-6-Demethyl-6-deoxytetraycycline (Sancycline)

Woodward/Pfizer 1962

epimerization
autoxidation
mostly Claisen condensations
methyl m-methoxybenzoate
condensation
condensation
condensation
conjugate addition

Woodward/Pfizer 1962
Elaboration of Methyl $m$-Methoxybenzoate

NaH, DMF

2 steps 55% yield

NaH, DMF

29% yield

diemethyl succinate

NaH, DMF

88% yield

Triton B, dioxane

1) H$_2$SO$_4$, reflux 98% yield

2) CH$_3$OH, H$_2$SO$_4$, reflux 44% yield

acetic acid 200 psi, 10% Pd/C 98% yield

Woodward, R. B. et al. JACS, 1968, 90, 439
Construction of the C and D Rings

1) MeOH, H₂SO₄
   69% yield

2) NaOH, H₂O
   97% yield

Acetic acid, chlorine
97% yield

NaH (2 equiv), A (1 equiv), DMF, rt
60% yield

H₃CO-CH₃

NaH (4 equiv), A (2 equiv), CH₃OH (1 equiv), DMF, rt to 80 ºC
45% yield

Intra than inter

Inter than intra

isolated in "low yield"

Woodward, R. B. et al. JACS, 1968, 90, 439
Establishing the Structure of the Hydroanthracene Triketone

Woodward, R. B. et. al. *JACS*, 1968, 90, 439
Establishing the Relative Stereochemistry at C4a and C5a

bulky a-dimethyl amino ester occupies a equatorial position

Woodward, R. B. et. al. *JACS*, 1968, 90, 439
Closure of the A Ring

Woodward, R. B. et. al. JACS, 1968, 90, 439
Introduction of the C12a Hydroxyl Group

Summary 6-Deoxy-6-Demethyltetracycline (Sancycline)

- First total synthesis of a fully biologically active tetracycline antibiotic.
- First example that late-stage introduction of the C12a hydroxyl is not practical.

25 steps
~0.002% yield

- provides access to substitution at C6
- resolution would permit non-racemic synthesis

conjugate addition of HNMe₂
epimerization by way of Ca complexes

"stereospecific" oxidation of a metal chelate

Key intermediate
Total Synthesis of (+)-Oxytetracycline (Terramycin)

Hans Muxfeldt, 1968

"the crazy German who thinks he can synthesize Terramycin."
Double Ring Closure in a One-Pot Reaction

3 new C-C bonds formed in a one-pot reaction

Reaction is not stereospecific, all eight possible stereoisomers are isolated

Muxfeldt, H. et al. JACS, 1979, 101, 689
Synthesis of the Terramycin Precursor Aldehyde

walnuts → 1) steps → juglone acetate + \( \text{OAc} \) → benzene reflux → 60% → exclusive regioselectivity → MeMgl, \( \text{Et}_2\text{O} \) 85%

resonance donation to C4 renders C1 most electron withdrawing carbonyl group

DBU, acetic acid piperidine → 4 steps 44%

DBU =  \[
\begin{array}{c}
\text{N} \\
\text{N}
\end{array}
\]

1) Acetic anhydride
2) cat. OsO4, HIO4, THF, 50 °C
3) Pb(OAc)4, 40 °C

Muxfeldt, H. et. al. JACS, 1979, 101, 689
Synthesis of the Terramycin Precursor Aldehyde

Muxfeldt, H. et al. JACS, 1979, 101, 689
Closure of the A and B Rings

Muxfeldt, H. et. al. JACS, 1979, 101, 689
dimethyl 3-oxoglutarate

H<sub>3</sub>CO\(\text{-}\text{CH}_3\)

MeOH, NH<sub>3</sub>

33% → H<sub>3</sub>CO\(\text{-}\text{NH}_2\)

methyl 3-oxoglutaramate

H<sub>3</sub>CO\(\text{-}\text{NH}_2\)

HCl aq.

60% → H<sub>3</sub>CO\(\text{-}\text{NH}_2\)

thiobenzoylglycine

HO\(\text{-}\text{N}\text{-}\text{S}\)

PBr<sub>3</sub>
dioxane

BrHN\(\text{-}\text{C}_6\text{H}_5\)

2N NaOAc

2-Phenyl-3-thiazolin-5-one
Introduction of the C12a Hydroxyl Group

1) CH₃I, THF
2) aq. HCl
3) dimethyl sulfate i-Pr₂NEt

1) NaH, Et₃P, O₂
THF, DMF,
cat. H₂O
2) acidic workup

3 steps
26%

52%
C12a hydroxylation

14%
C11a hydroxylation

separated by chromatography on polyamide and crystallization

Muxfeldt, H. *et. al. JACS, 1979, 101, 689
Summary (±)-5-Oxytetracycline (Terramycine)

• One pot procedure affects a double ring closure (B and A) by creating 3 new C-C bonds.

• However, reaction is not stereospecific and requires separation of 4 diastereomers (27% yield of desired diastereomer).

• C12a hydroxylation proceeded in a 52% yield (6% in Woodward’s synthesis)

• This method has been used for the synthesis of several natural and non-natural tetracyclines by varying the substituents on the aldehyde portion.
Total Synthesis of (±)-12α-deoxytetracycline

Gilbert Stork, 1996
Conjugate Addition of the Benzyloxyisoxazole

"Shemyakin ketone"

Stork, G.; et. al. *JACS*, 1996, 118, 5304

Stork, G.; Hagedorn III, A. *JACS*, 1978, 100, 3611
Setting the Relative Stereochemistry at C6 and C5a

Stork, G.; et al. *JACS*, 1996, 118, 5304
Synthesis of the Unsaturated Lactone

Stork, G.; et. al. JACS, 1996, 118, 5304
Conjugate Addition of the Benzyloxyisoxazole

Conjugate Addition of the Benzyloxyisoxazole

"intense efforts" failed to achieve closure of A ring

Stork, G.; et. al. JACS, 1996, 118, 5304
Synthesis of the Benzyloxyisoxazole

\[
\begin{align*}
\text{H}_3\text{CO}_2\text{OCH}_3 + \text{LiOCH}_3 & \rightarrow \text{H}_3\text{CO}_2\text{OCH}_3 \quad \text{LiOCH}_3 \\
& \text{100 °C} \\
& \text{1 week} \\
& \text{36%} \\
\text{CH}_3\text{C(OCH}_3)_3 & \rightarrow \text{H}_3\text{CO}_2\text{OCH}_3 \quad \text{HCl H}_2\text{NOH} \\
& \text{CH}_3\text{ONa, CH}_3\text{OH} \\
& \text{66%} \\
\text{BnBr} & \rightarrow \text{K}_2\text{CO}_3, \text{acetone reflux, 14h} \\
\text{NaBH}_4, \text{CH}_3\text{OH, -20 °C} & \rightarrow \text{H}_3\text{CO}_2\text{OCH}_3 \quad \text{O}_3, \text{CH}_3\text{OH} \\
& \text{S(CH}_3)_2 \\
& \text{2 steps} \\
& \text{72%} \\
\text{H}_3\text{CO}_2\text{OCH}_3 \rightarrow \text{H}_3\text{CO}_2\text{OCH}_3 \quad \text{LiHMDS, -78 °C} \\
& \text{2) PhCHO, -78 °C to rt} \\
\text{H}_3\text{CO}_2\text{OCH}_3 & \rightarrow \text{H}_3\text{CO}_2\text{OCH}_3 \quad \text{1) MsCl, Et}_3\text{N} \\
& \text{2) 40% (CH}_3)_2\text{NH in water} \\
& \text{93%} \\
\text{MsCl} &= \text{H}_3\text{C-S-Cl}
\end{align*}
\]

Closure of the A and B Rings

Stork, G.; et al. *JACS*, 1996, 118, 5304

[Diagram of the chemical process involving the closure of the A and B rings of a tetracycline derivative, showing the reaction with Bu$_3$SnOCH$_3$ in toluene at 60 °C, leading to a 97% yield. Also shown is the use of TMSCN (8 equiv), KCN (0.5 equiv), and 18-crown-6 (0.1 equiv) for further reactions.]

- TMSCN (8 equiv) KCl (0.5 equiv) 18-crown-6 (0.1 equiv)
- KH, THF -78 °C to rt then rt to 50 °C
- Pd black H$_2$ (1 atm) THF, MeOH 2 steps 59%

Mixture of 12 products, none of which are tetracycline

12a-deoxytetracycline

Presumptive A ring closes first
Summary (±)-12a-deoxytetracycline

16 steps 18 to 25% yield

“Methods have been claimed for the stereospecific 12a-oxidation of A. We are not aware, however, that a reproducible method for the oxidation of A to tetracycline is available.”

• Efficient and high-yielding synthesis; However,

• Absence of the C12a hydroxyl is associated with greatly reduced antimicrobial activity.

• Enantioselective formation of the tertiary hydroxyl could lead to non-racemic synthesis.

• Development of the Benzyloxyisoxazole as a masked vinylogous carbamic acid function will be used by in future syntheses by other researchers.
Total Synthesis of (-)-Tetracycline,
Andrew Myers, 2005

photooxygenation and hydrogenation

oxidative elimination

Diels-Alder cycloaddition

?-tetraacycline
Construction of the A and B Rings

C12a hydroxyl introduced in first step!

Provide a mechanism for this transformation

1) 5 mol% LiOTf, PhCH₃, 60 °C

2) TFA, CH₂Cl₂

Sₜₜ-prime

62% yield

[2,3] Sigmatropic rearrangement
Preparation of the Enone for the Diels-Alder Cycloaddition

**Chemical Structures and Reactions:***

1. **Preparation of Enone:**
   - Reaction of a precursor with PPh₃, DEAD, and PhCH₃ at -30°C.
   - Products include aryl sulfoxides.

2. **Diels-Alder Reaction:***
   - Treatment with 1) HCl, MeOH followed by 2) IBX, DMSO.
   - Product is isolated in 66% yield.

3. **Elimination Reaction:***
   - Sigmatropic elimination of N₂ results in a product.

**Note:**
Construction of the C Ring

(10 steps, 11% from benzoic acid)

1) HPy Br₃, CH₂Cl₂
2) PhSH, DBU, DMF

66%

1) Et₃N 3HF, THF, rt
2) IBX, DMSO

76%

77%

Meyrs, A. G.; et. al. *JACS*, **2005**, *127*, 8292
Synthesis of the Trimethylsilyloxybenzocyclobutene

3-benzyloxy benzyl alcoho

\[
\text{OH} \quad \text{CH}_3
\]

1) nBuLi, THF, -78 °C
2) MgBr\(_2\), -78 °C to rt

67% (+7% cis)

\[
\text{OH} \quad \text{CH}_3
\]

1) CH\(_3\)MgBr, THF, -5 °C
2) TEMPO, NaBr, NaOCl, NaHCO\(_3\), 0°C

94%

TEMPO = \[
\begin{array}{c}
\text{H}_3\text{C} \\
\text{N} \\
\text{CH}_3 \\
\text{H}_3\text{C} \\
\text{H}_3\text{C} \\
\end{array}
\]
Stereoselective Autoxidation

TFA, m-CPBA

CH$_2$Cl$_2$, -78 °C to rt

TFA prevents oxidation of amine

H$_2$ (1 atm) Pd black
dioxane

42% from A

(-)-tetracycline

Meyrs, A. G.; et al. JACS, 2005, 127, 8292
Synthesis of 6-deoxytetracycline Antibiotics

Convergent Assembly of Structurally Diverse Tetracyclines

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<thead>
<tr>
<th>Bacterial Strains Tested</th>
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<tbody>
<tr>
<td>Gram-Positive Organisms</td>
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<tr>
<td>S. aureus ATCC 29213</td>
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<tr>
<td>S. epidermidis ACH-0016</td>
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<tr>
<td>S. haemolyticus ACH-0013</td>
</tr>
<tr>
<td>E. faecalis ATCC 700802</td>
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<td>S. aureus ATCC 700699</td>
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<th>Gram-Negative Organisms</th>
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<tr>
<td>P. aeruginosa ATCC 27853</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 13883</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
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<tr>
<td>E. coli ACH-0095</td>
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<tr>
<td>E. coli pBR322</td>
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<thead>
<tr>
<th>D-Ring Precursor</th>
<th>Conditions</th>
<th>Tetracycline Analog</th>
<th>MIC (µg/mL)</th>
<th>Testing Control</th>
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[Chemical structures and reaction conditions]

MIC (µg/mL)

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<th>MIC (µg/mL)</th>
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<td>16</td>
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<td>8</td>
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<td>64</td>
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Testing Control:

Chemical structures and reaction conditions

Michael-Dieckman reaction sequence
Summary (-)-Tetracycline

- Second total synthesis of tetracycline (1st synthesis 34-steps, 0.002% yield).
- Versatile synthetic route allowing for the synthesis of tetracycline analogs.
- C12a hydroxyl introduced in first step(!) and used to set all of the remaining stereogenic centers in the molecule.
Summary

Tetracycline derivatives are still being developed as antibacterial agents for the treatment of multidrug-resistant (MDR) pathogens.

- The polar functionality shown in red is required for the binding of tetracyclines to the bacterial ribosomes. However, wide structural variation on or near the D ring has been cited as a means to overcome bacterial resistance.

- The diverse range of compounds accessible in sufficient quantities by Myers route may lead to the development of antibiotics with even greater potencies than tigecycline.

- However, the most promising route for the development of new tetracyclines remains the chemical modification of compounds readily accessible by fermentation.
1) CBr₄, PPh₃
2) PhSH, Et₃N
87% yield

1) H₂C₃CH₃
2) PhSH, Et₃N
76%

1) TBAF, HOAc
2) IBX, DMSO
3) TBSOTf, Et₃N