Total Synthesis of the Naturally-Occurring GRP78 Modulators
(+)-Prunustatin A and (+)-Brefeldin A, and Immunosuppressant (+)-SW-163A

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Research Interests of the Hale Group

- Total Synthesis of Pharmacologically-Active, Complex, Natural Products of Potential Value to Human Medicine
- Chemical Biology and Medicinal Chemistry
- New Reaction Development
- Physical Organic Chemistry, and the Elucidation of Complex Reaction Mechanisms Through the Use of Novel Small Molecule Reporter Probes
- Methods for Linking Small Molecules to Proteins
Research Interests of the Hale Group

Total Synthesis of Pharmacologically-Active, Complex, Natural Products of Value to Human Medicine

Some of the Complex Bioactive Natural Products Synthesised By The Hale Group Over The Past Decade

- **(+)-A83586C** (Antitumour)
  - Low nM β-Catenin/TCF4 Disrupter
  - (E2F Inhibitor)
  - (Osteopontin Inhibitor)

- **(-)-Agelastatin A**
  - (Antitumour)
  - (Nanomolar β-Catenin Downregulator)
  - (Osteopontin Inhibitor)

- **(+)-Eremantholide A**
  - (Antitumour)

- **(+)-Pumiliotoxin B**
  - (Cardiotonic)

- **(-)-Echinosporin**
  - (Antitumour)
  - Formal Total Synthesis

- **(+)-Monanchorin**
  - (Mast Cell Inhibitor)

- **(+)-Brefeldin A**
  - (Antitumour, Golgi Complex Disrupter)

- **(-)-Mycothiazole**
  - (Antitumour)
  - (HIF-1 Inhibitor)

- **(+)-3R-Inthomycin C**
  - (Antitumour)

- **(+)-Prunustatin A**
  - (Antitumour)
  - (GRP78 Inhibitor)

- **(-)-Inthomycin C**
  - (Antitumour)

- **(+)-Kettapeptin**
  - (Antitumour)

- **(+)-Azinothricin**
  - (Antibacterial)

- **(-)-(3R)-Brefeldin A**
  - (Antitumour, Cardiotonic)

- **(+)-Prunustatin A**
  - (Antitumour)

Research Interests of the Hale Group

Total Synthesis of Pharmacologically-Active, Complex, Natural Products of Value to Human Medicine

First Asymmetric Total Synthesis of the Antitumour Natural Product, (+)-Azinothricin


Enantiospecific Total Synthesis of the Antitumour Alkaloid (-)-Agelastatin A

Research Interests of the Hale Group
Chemical Biology and Medicinal Chemistry

ABERRANT UPREGULATED β-CATENIN SIGNALLING IS A MAJOR CONTRIBUTOR
TO THE ONSET AND PROGRESSION OF MANY HUMAN CANCERS


For a detailed account of our chemical biology and med chem efforts on A83586C and its congeners see: Hale, Manaviazar, and George *Chem. Comm.* **2010**, *46*, 4021.

Research Interests of the Hale Group
New Reaction Development

The Tandem Electrophilic Hydrazination-Nucleophilic Cyclisation Method for Piperazic Acid Assembly

How The (+)-A83586C Venture Spurred Development of The Tandem Electrophilic Hydrazination-Nucleophilic Cyclisation Method

How Our Tandem Asymmetric Electrophilic Hydrazination-Nucleophilic Cyclisation Helped Provide New Insights Into Kutzneride Biosynthesis

The above strategy was used to prepare the cis- and trans-5-Cl-Piz reference standards needed to deduce the stereochemistry of KtzP halogenase mediated piperazic acid chlorination during kutzneride biosynthesis.

Following thioesterase release, NMR correlation of the resulting free 5-Cl-Piz with our synthetic 3,5-trans-5-chloropiperazic acid revealed that the kutzneride P halogenase initially installs a cis-3,5-chloropiperazic acid into the kutznerides, and that this then undergoes further enzymatic processing to give the 3,5-trans-5-chloropiperazic acid residues found in the actual natural products themselves.

Research Interests of the Hale Group

New Reaction Development

A New Mild Method For Ketone Enolate C-Acylation

Use of the Ketone Enolate C-Acylation Method with Pentafluorophenyl Carbonates In the Formal Total Synthesis of (-)-Echinosporin

(-)-Echinosporin (Antitumour)


Research Interests of the Hale Group

New Reaction Development

The O-Directed Free Radical Hydrostannation Reaction Of Alkylacetylenes With Ph₃SnH/cat. Et₃B/O₂ in PhMe

Use in a Formal Total Synthesis of (+)-Pumiliotoxin B

Hale's "Trans" N-Trifluoroacetamido-Directed Iodohydroxylation

Use in the Asymmetric Total Synthesis of (-)-(3R)-Inthomycin C

Deployment of a New "Double O-Directed Free Radical Hydrostannation" in a Synthesis of the C(7)-C(22)-Sector of (+)-Acutiphycin
Research Interests of the Hale Group
Physical Organic Chemistry and Elucidating Complex Reaction Mechanisms With Small Molecule Probes

The Definitive Probe Experiment Which Proved That Propargyloxy O-Coordination to the Ph₃SnH Was Responsible for the α-Stannylated Products Preferentially Arising In the O-Directed Free Radical Hydrostannation of Propargically-Oxygenated Alkylacetylenes

At low stannane concentrations uncoordinated Ph₃Sn adds to the acetylene and electronic control is dominant; minimal coordination occurs between the stannane and the propargylic OH.

At high stannane concentrations coordination between the Ph₃SnH the propargylic OH readily occurs and O-coordinative control becomes dominant. It is now the O-coordinated Ph₃Sn⁺ that preferentially adds because it is formed faster and it has greater longevity in solution.

If electronic effects were primarily responsible for determining the regiochemistry of stannyl radical addition, then one would not see the ratio of α : β addition products changing to any significant degree as the concentration of stannane increased. However, it does change very dramatically, as one can see. The only rational explanation of this behaviour is O-coordinative control overriding the inherent electronic preferences of uncoordinated stannyl radical addition.

Research Interests of the Hale Group
Physical Organic Chemistry and Elucidating Complex Reaction Mechanisms With Small Molecule Probes

The Mechanism of the O-Directed Hydrostannation of Alkylacetylenes with Ph₃SnH/cat. Et₃B

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N.B. The allylic O-atom does not coordinate to Sn following α-addition to the acetylene.

X-ray crystallography confirms that no such coordination occurs in the α-(Z)-adducts.

This lack of internal O-coordination in the fully developed α-stannylvinyl radical makes the reverse elimination less favourable, and helps promote formation of the α-(Z)-adduct.

Internal 1,5-H-Atom Abstraction by the Vinyl Radicals Can Also Occur.

Research Interests of the Hale Group
Physical Organic Chemistry and Elucidating Complex Reaction Mechanisms With Small Molecule Probes

The Mechanism of the O-Directed Hydrostannation of Alkylacetylenes with Ph₃SnH/cat. Et₃B

The Disfavoured (E)-α-Vinylstannane Geometric Isomer Isomerises Under the Reaction Conditions

Two Main Mechanisms By Which It Does This:

β-Stanny1 Radical-Addition with C-C Bond Rotation and Elimination

α-(E)-Adduct

α-(E)-Adduct

α-Stanny1 Radical-Addition with C-C Bond Rotation and Elimination

Research Interests of the Hale Group
Physical Organic Chemistry and Elucidating Complex Reaction Mechanisms With Small Molecule Probes

The Mechanism of the O-Directed Hydrostannation of Alkylacetylenes with Ph₃SnH/cat. Et₃B

O-Directed β-C-Addition is Not Favoured Due to Coordinative Elimination of the Intermediary β-Stannyl(alkyl)vinyl Radicals

The O-Directed β-Additive 5-Endo-Dig Cyclization is Less Favoured Stereoelectronically

Strong internal O-coordination in the β-stannylvinyl radical adducts weakens the C-Sn bond and this, along with the geminal repulsion indicated, almost certainly favours the reverse retro-stannation reaction:

i.e. β-elimination of the intermediary vinylstannyl radical to its O-coordinated stannyl radical acetylene precursor.

(+)-Prunustatin A

- Isolated from fermentation broths of *Streptomyces violaceoniger* (4521-SVS3) by Shin-ya et al.\textsuperscript{[1,2]}

- *Streptomyces violaceoniger* (4521-SVS3) is indigenous to soil of the Okinawan island of Kumejima.\textsuperscript{[1,2]}

- (+)-Prunustatin A is a very powerful downregulator of GRP78 expression in stressed (glucose-deprived) HT1080 human fibrosarcoma cells at very low drug concentrations (IC\textsubscript{50} = 11.5 nM) and, at the 100 nM level, it causes full cancer cell apoptosis.\textsuperscript{[1]}

- GRP78 (78 kDa glucose-regulated protein) is an endoplasmic reticulum (ER) protein that is produced in response to cell stress and hostile environments.

- It is massively upregulated in untreatable, drug-resistant, hypoxic solid tumours.

- High GRP78 levels switch on the unfolded protein response within tumours, which renders them recalcitrant to treatment with drugs and radiotherapy.

- As a result of this profile, (+)-prunustatin A might be of potential value for treating currently incurable hypoxic solid human tumours.

- (+)-Prunustatin A has recently been synthesised by the groups of Kawanishi and Usuki.\textsuperscript{[3,4]}

\textsuperscript{[1]} Isolation and Biological Activity: (a) K. Shin-ya et al. *J. Antibiot.* 2005, 58, 206.
(+)-SW-163A was first isolated by Takahashi et al. in 2001 from culture broths of *Streptomyces sp.* SNA15896, a soil microbe from the Yuuki region of Japan.[1,2]

(+)-SW-163A inhibits the immune response of murine splenic lymphocytes with an IC$_{50}$ value of 62 nM.[1]

(+)-SW-163A also inhibits lymphocyte blastogenesis with an IC$_{50}$ value of 48 nM.[1]

Unlike, (-)-FK506 and cyclosporin A, which both suppress the immune response by blocking T-cell function, (+)-SW-163A prevents T-cells and B-cells from simultaneously becoming primed and activated.

As such, (+)-SW-163A is of considerable pharmaceutical interest, since (-)-FK506 and cyclosporin A are both extremely toxic.

The introduction of (+)-SW-163A could potentially herald a new safe era for human transplant surgery and improve many patient outcomes, since (+)-SW-163 is non-cytotoxic towards unstressed eukaryotic cells.

Objectives of the Present Research Programme

![Chemical structures of (+)-Prunustatin A and (+)-SW-163A]

Background

- (+)-Prunustatin A and (+)-SW-163A are both in extremely short supply and presently inaccessible to the wider scientific community and the pharmaceutical industry.

- The producing organisms are also not generally accessible and closely guarded by the owners.

- To enter human clinical development, new, **easily executed**, total syntheses of both agents urgently need to be developed.

- The two recent total syntheses of (+)-prunustatin A by Kawanishi\(^1\) and Usuki\(^2\) are tricky to carry out and, in the case of the 2014 Kawanishi synthesis, no experimental details of the route have ever been reported, making its repetition extremely difficult.

Objectives of Present Research Programme

- To develop a practical, easy to execute, new total synthesis of both compounds to help expedite their future clinical evaluation and allow future med chem refinement.

- To use these new synthetic routes to prepare novel probes and analogues that could allow the biological targets of these agents to be isolated and identified.

Early Defeats! These Outcomes Revealed The Nature of the Problem That We Were Confronting!

2,4,6-Trichlorobenzoyl chloride (10 equiv) Et$_3$N (2 equiv) C$_6$H$_6$
rt, 15 h then dilute with C$_6$H$_6$ and add dropwise to DMAP (7 equiv) in C$_6$H$_6$ at 2-4 °C over 6 h; then stir at rt 7 d; 74%

0.75 g
Single product formed!

0.54 g
Single product formed!

Add hydroxy acid in THF: CH$_2$Cl$_2$ (1:1) dropwise over 9 h

Single product formed!
However, the products from either route were both different!!

(+)-Prunustatin A
Not produced by either route! Rather, diastereoisomers of prunustatin A were synthesized instead!
Our Revised Retrosynthetic Planning for (+)-Prunustatin A

- **Esterify**
- **Macrolactonise**
- **Cleave PMB ether**
- **O-desilylate**

**(+)-Prunustatin A**

- **Selectively O-desilylate and oxidise at C(10)**
- **Esterify**
- **Reduce lactone and selectively O-silylate**
- **Sharpless AD tandem lactonization O-p-methoxybenzylation**
- **Julia-Kocienski (E)-olefination**
Our New Synthetic Route (+)-Prunustatin A


This is best for ADs in sterically-hindered systems such as this alkene here.
Our New Synthetic Route (+)-Prunustatin A

1) t-BuPh₂SiCl (1.1 equiv) Imidazole (3 equiv) DMF, rt, 4 h
2) LiOH·H₂O (2.6 equiv) THF:MeOH (1:1), H₂O rt, 1 h (84%, 2 steps)

2,4,6-Trichlorobenzoyl chloride (1 equiv) Et₃N (1 equiv), THF rt, 2 h
DMAP (2 equiv) C₆H₆, rt, 17 h 93% (2 steps)

NaClO₂ (2.4 equiv) NaH₂PO₄ (3 equiv) t-BuOH, H₂O 2-Methyl-butene rt, 17 h (89%)

TPAP (0.1 equiv) NMO (2 equiv) 4Å MS, CH₂Cl₂ rt, 45 min (98%)

PPTS (0.1 equiv) MeOH, rt, 45 min 98%

(0.62 equiv)
Our New Synthetic Route (+)-Prunustatin A

To H$_2$SO$_4$ (1 M in H$_2$O) and IsoLeu at 0 °C slowly add NaNO$_2$ (1.5 equiv) in H$_2$O over 70 min at 0 °C, then warm to rt over 2 h, stir 68 h (76%)

K$_2$CO$_3$ (1.2 equiv) Bu$_4$NI (1.2 equiv) DMF, rt, 18 h 82%

AllBr (4 equiv) 72%

DDQ (1.5 equiv) CH$_2$Cl$_2$:H$_2$O (18:1) rt, 70 min (90%)

EDCI (1.3 equiv) DMAP (1.6 equiv) CH$_2$Cl$_2$, rt, 18 h 72%

1) TPAP (0.1 equiv) NMO (2.2 equiv) 4Å MS, CH$_2$Cl$_2$ rt, 2.5 h (84%)

(Ph$_3$P)$_4$Pd (0.03 equiv) PhSiH$_3$ (2 equiv) CH$_2$Cl$_2$, rt, 6 h (93%)

DMAP (0.3 equiv) Et$_3$N (1.3 equiv) CH$_2$Cl$_2$, rt, 20.5 h 74%
Completion of Our New Total Synthesis of (+)-Prunustatin A
Soraya Manaviazar
SM-19-87
PROTON
Ref: 0.00 ppm (CDCl3-TMS)

(+)-Prunustatin A
Completion of The First Total Synthesis of (+)-SW-163A

NaBH₄ (3 equiv)
EtOH, rt, 6 h
41%

(+)-Prunustatin A

(+)-SW-163A
Origin of the Stereoselectivity of Reduction
In the Total Synthesis of (+)-SW-163A
For our recent published total synthesis of (+)-prunustatin A and (+)-SW-163A, see:
Retroynthetic Analysis of (+)-Brefeldin A

(+)-Brefeldin A

Antitumour

Regioselective Macrolactonization

Cleavage of the O-p-methoxyphenyl group and ester hydrolysis

Julia-Kocienski (E)-olefination

Underside ketone reduction at C(7)

Double Mitsunobu inversion

O-desilylation alcohol iodination

Vasella reductive ring-cleavage

Base-mediated epimerization at C(9)

Cross-Metathesis

Padwa anionic [3+2]-cycloadditive elimination

Sulfone anion oxidation

Olefin hydrogenation and ketone reduction from less hindered underside

Alcohol iodination

O-desilylation ketone reduction

Antitumour
Our Initial Foray On (+)-Brefeldin A

For our (-)-echnosporin synthesis Padwa [3+2]-cycloadditive elimination, see:

For Padwa’s seminal publications on his allenylsulfone [3+2]-cycloadditive elimination, see:

None of these Padwa publications attempted to apply the [3+2]-cycloadditive elimination on chiral substrates.
Revised Retrosynthetic Analysis of Advanced Iodide

Attempted Implementation and a Quick Evaluation of Double Mitsunobu Inversion Feasibility

Clearly the C(4)-OH in the above di-O-benzoate is far too hindered to undergo Mitsunobu Inversion. C(4)-inversion would thus have to be postponed until after the pyranoside ring had been fragmented.

Revised Retrosynthetic Plan

(+) - Brefeldin A Antitumour

O-Desilylation Selective deesterification at O(4)

Macrolactonization Selective O-Desilylation Mitsunobu Inversion at O(4)

Cleavage of the O-p-methoxyphenyl group and Ester hydrolysis

Vasella reductive Ring-cleavage

Base-mediated Epimerization at C(9)

Cross-Metathesis

Julia-Kocienski (E)-olefination Protecting group Interchange

Revised Retrosynthetic Plan
Attempted Implementation of the C(9)-Aldehyde Epimerisation and Olefin Cross-Metathesis Tactics
Further Revised Retrosynthetic Plan

- **O-Desilylation:** Selective deesterification at O(4)
- **Macrolactonization:** Selective O-Desilylation, Mitsunobu Inversion at O(4)
- **Cleavage of the O-p-methoxyphenyl group and Ester hydrolysis**

**Chemical Structures:**
- (-)-Brefeldin A
- Antitumour
- Reduce and O-Silylate Twice
- Oxidatively Cleave Alkene
- (E)-Selective Wittig Olefination
- Julia-Kocienski (E)-olefination Protecting group Interchange
- Selective O-Desilylation
- Selective deesterification at O(4)
- Ester hydrolysis
- Mitsunobu Inversion at O(4)
- Cleavage of the O-p-methoxyphenyl group
Success in the Stabilised Wittig Strategy for C(1)-C(3) Elaboration

![Chemical structures and reactions](image)
A New Improved Synthesis of the N-Phenyltetrazolylsulfone

prepared in 1 step
R. Fernandes et al
*RSC Adv.* **2015** 5, 42131

- Commercially available

\[
\text{BH}_3\text{Me}_2\text{S} \quad (2 \text{ equiv}) \\
\text{THF, 0 °C, 0.5 h, then rt} \\
2 \text{ h, 35% aq H}_2\text{O}_2, \text{EtOH} \\
\text{THF, 1 M aq NaOH} \\
16 \text{ h (59%)}
\]

\[
\text{MeO-} \quad \text{Me}
\]

\[
\text{BH}_3\text{Me}_2\text{S} \quad (2 \text{ equiv}) \\
\text{THF, 0 °C, 0.5 h, then rt} \\
2 \text{ h, 35% aq H}_2\text{O}_2, \text{EtOH} \\
\text{THF, 1 M aq NaOH} \\
16 \text{ h (59%)}
\]

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\text{MeO-} \quad \text{Me}
\]

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\text{BH}_3\text{Me}_2\text{S} \quad (2 \text{ equiv}) \\
\text{THF, 0 °C, 0.5 h, then rt} \\
2 \text{ h, 35% aq H}_2\text{O}_2, \text{EtOH} \\
\text{THF, 1 M aq NaOH} \\
16 \text{ h (59%)}
\]

\[
\text{MeO-} \quad \text{Me}
\]
Completion of the Total Synthesis of (+)-Brefeldin A

For our recent report on the enantioselective total synthesis of (+)-brefeldin A, see:
$^1$H NMR spectrum of Kim's synthetic (+)-brefeldin A (1) in CD$_3$OD at 500 MHz:

$^1$H NMR spectrum of our synthetic (+)-brefeldin A (1) in CD$_3$OD at 600 MHz: