Stories from Staudinger: Synthesis of Chiral β-Lactams

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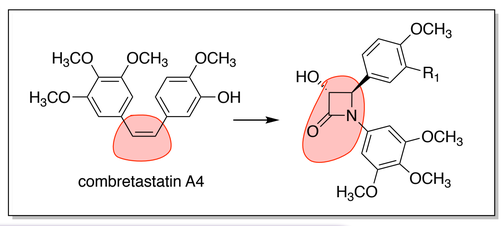
Introduction

Combretastatin A-4 (CA-4) is a potent anticancer drug isolated from the wood of the South African tree *Combretum caffrum* acting by inhibition of tubulin polymerisation. Isomerization of *cis* CA-4 to the *trans* form is observed both during storage and *in vivo* during metabolism. This dramatically reduces antitumour activity. Our group has previously synthesized novel 3-hydroxy-1,4-diaryl-2-azetidinones by Staudinger reaction, inducing *cis*-restriction and overcoming the problem of isomerisation of CA-4. A number of *trans* beta-lactams have shown potent nanomolar antiproliferative activity in MCF-7 breast cancer cells with enhanced activity relative to CA-4.



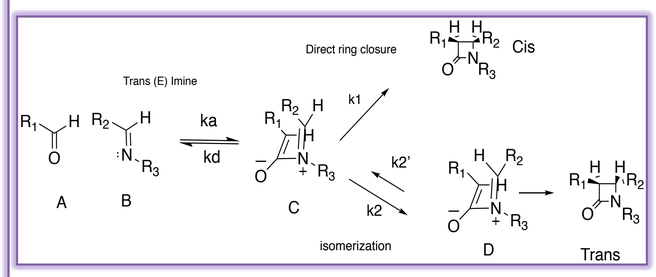
First Aim: *Staudinger Optimisation*

Determine the necessary conditions to **optimise the yield of the *trans* isomer** of 3-hydroxy-1,4-diaryl-2-azetidinones in the Staudinger reaction. *Trans* isomers of 3-substituted-2-azetidinones are up to 50 times more potent than the corresponding *cis* derivatives.

Second Aim: *Enantiomer Resolution*

Isolation of enantiomers of the most potent azetidinones by formation of diastereomers and separation by column chromatography. Levo- and dextro-rotatory enantiomers may differ in biological activity relative to one another.

Pathways for *cis* & *trans* β-lactam isomer product formation during Staudinger syntheses



Relative stereochemistry is **determined** by the **rate of ring closure**; controlled by **two** competing factors:

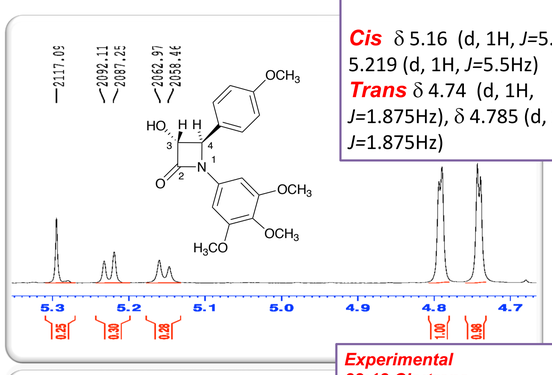
1. Competition for direct ring closure
2. Isomerization of zwitterionic intermediate.
   - Product is *cis* if $k_1 > k_2$.
   - If $k_2 = k_1$ it, product is a mixture of *cis* & *trans*
   - Zwitterionic intermediate must be allowed time to isomerize in situ.
   - An **acid chloride** with an **electron withdrawing group** decreases the nucleophilicity of the enolate anion.
     - In optimised conditions will allow for isomerization (k_2) to allow for trans ring closure.

^{1}H NMR of H_3 & H_4 doublets

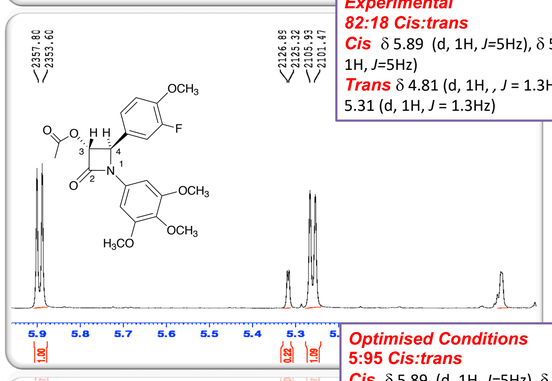
β-Lactam proton integration indicates relative cis:trans ratio; J = 4 Hz for cis; J = 1 Hz for trans

Preliminary
4:6 Cis:trans

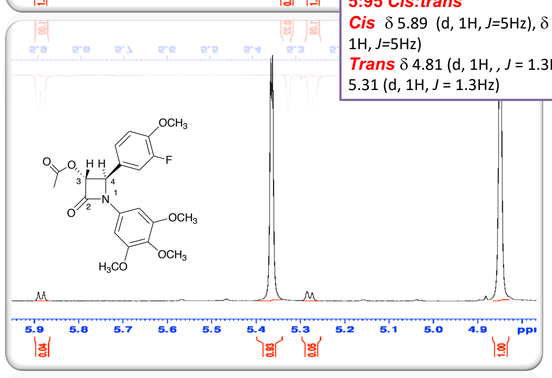
Cis δ 5.16 (d, 1H, *J*=5.5Hz), δ 5.219 (d, 1H, *J*=5.5Hz)
Trans δ 4.74 (d, 1H, *J*=1.875Hz), δ 4.785 (d, 1H, *J*=1.875Hz)

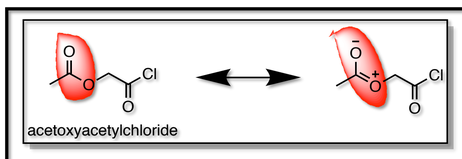


Experimental
82:18 Cis:trans
Cis δ 5.89 (d, 1H, *J*=5Hz), δ 5.25 (d, 1H, *J*=5Hz)
Trans δ 4.81 (d, 1H, , *J* = 1.3Hz), δ 5.31 (d, 1H, *J* = 1.3Hz)

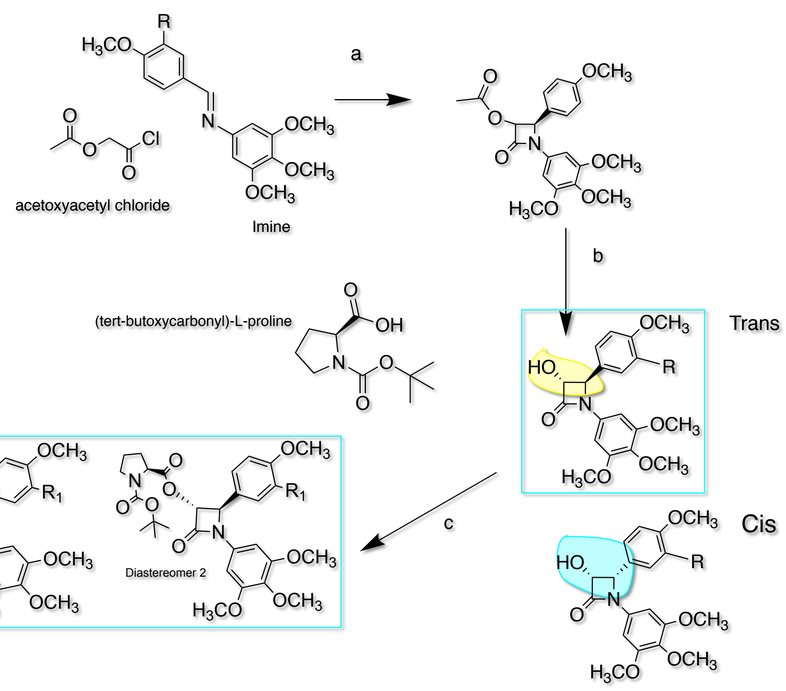


Optimised Conditions
5:95 Cis:trans
Cis δ 5.89 (d, 1H, *J*=5Hz), δ 5.25 (d, 1H, *J*=5Hz)
Trans δ 4.81 (d, 1H, , *J* = 1.3Hz), δ 5.31 (d, 1H, *J* = 1.3Hz)

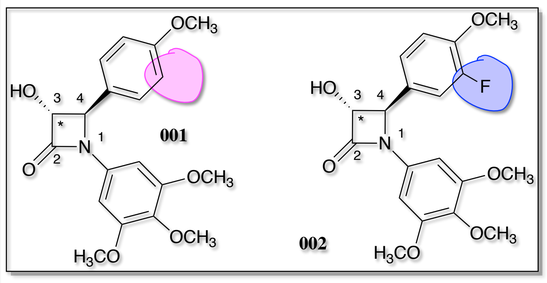




Electron withdrawing acid chlorides favour trans isomers in Staudinger reactions. *Cis* isomers were present in 50-90% yields were evident despite employing an electron withdrawing acid chloride.



Scheme 1: Staudinger Synthesis; General Reaction Scheme. a) Acetoxyacetylchloride (1.4Eq), Triethylamine (1.8Eq), Toluene (30 mL), 100 degrees centrigrade, 5 hours. b) Hydrazine dihydrochoride (2Eq), Triethylamine (2Eq), Methanol, Reflux, 4 hours. c) HBTU (2.2Eq), N-tertbutoxycarbonyl-L-Proline (2Eq), Acetonitrile (30 mL), DIPEA (62Eq), 24 hours



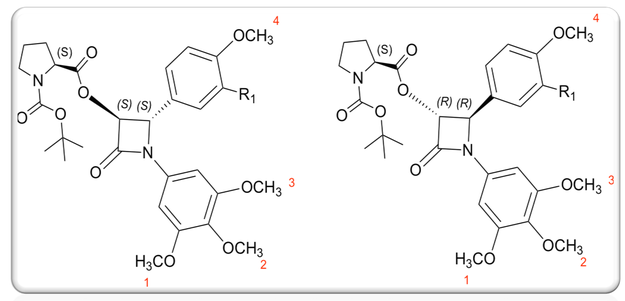
Lead *Trans* 3-Hydroxy-2-Azetidinones with potent MCF-7 antiproliferative activity

Molecular mechanisms for tubulin binding remain obscure due to lack of information regarding absolute configuration at position 3.

Conditions for Staudinger Synthesis	Preliminary (P)	Experimental (E)	Optimized Conditions (OC)
Imine	Added to anhydrous toluene first at zero degrees	Added to anhydrous toluene first (1 Eq) at zero degrees again on ice	Imine added in portions to acetoxyacetylchloride and dry toluene.
Acetoxy acetylchloride	Added to imine in anhydrous toluene at zero degrees	Added in under ice to imine and acetoxyacetylchloride.	Added first to dry toluene without Imine
Duration of imine & acetoxy acetylchloride stirring prior to base addition	0 minutes	0-10 minutes	25 minutes at reflux conditions Colour change from initial deep orange on addition of imine to acetoxyacetylchloride seen → yellow.
Triethylamine	Added immediately after addition of acetoxyacetylchloride at zero degrees.	Added after heating to reflux conditions (100 degrees), dropwise over 10 minutes.	Added in dropwise after 25 minutes of reflux.

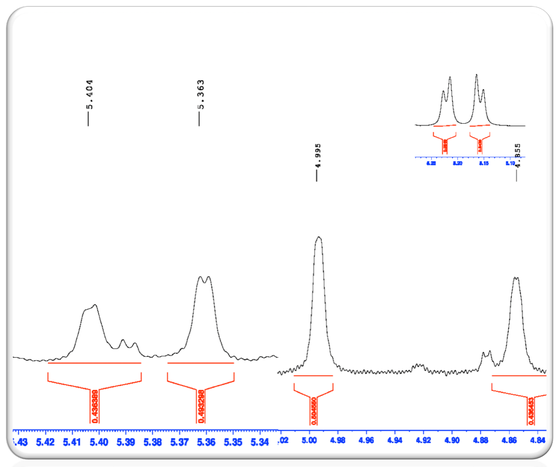
Resolution using N-(*tert*-butoxycarbonyl)-L-Proline

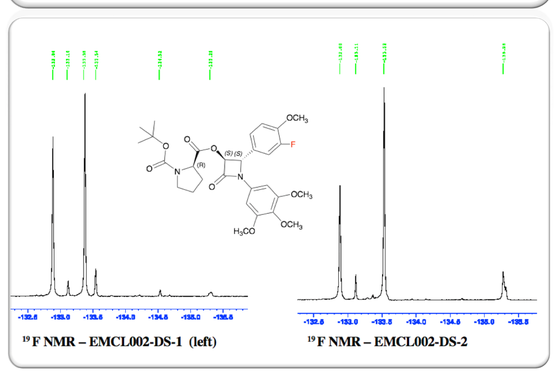
- Diastereomers were separated using flash column chromatography
- Proline was subsequently cleaved using hydrazine dihydrochloride to reveal optically pure enantiomers



^{1}H NMR: H_3 & H_4 splitting pattern indicative of diastereomer rotameric derivatives.

- Two sets of β – Lactam doublets present in pure diastereomer derivative compared to parent compound (top right)
- **^{19}F NMR** illustrated the presence of at least four rotamers with four fluorine signals.

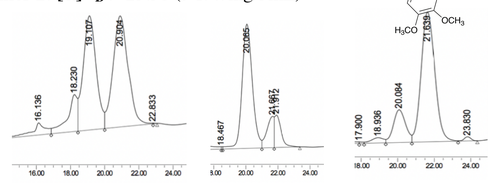




Verification of enantiomeric purity via optical rotation and chiral HPLC

001

Enantiomer 1: $[\alpha]^{20}_D$ **:** +25.14 (*c* 17.9mg/5mL)
Enantiomer 2: $[\alpha]^{20}_D$ **:** -25.38 (*c* 19.7mg/5mL)



001 *trans* racemic mixture **001 *trans* Enantiomer** **001 *trans* Enantiomer 2**

% Peak Area on Chiral HPLC
50:50 **85:15 18:82**

002

Enantiomer 1: $[\alpha]^{20}_D$ **:** +22.15 (*c* 15.8mg/5mL)
Enantiomer 2: $[\alpha]^{20}_D$ **:** -17.6(*c* 15.1mg/5mL) ee (%): 77%

Conclusions & Future Work

1. In order to favour zwitterion isomerization; k2 over direct ring closure; k1, the imine should be left to stir with the acid chloride for a period of time. 25 minutes was chosen as the ideal time.
2. Separation of enantiomers was achieved by synthesis of diastereomers and separation on column chromatography. These will be evaluated for their biochemical activity in future work. Future aims are to clarify the impact of absolute configuration on binding mode with tubulin and therefore bioactivity in MCF-7 cells

Acknowledgements

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