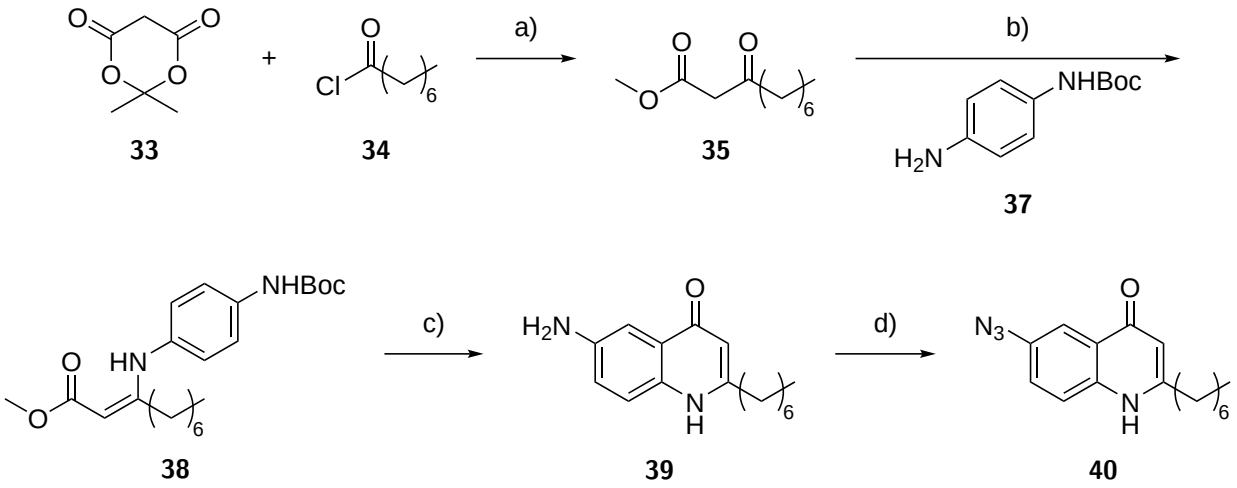
Contents

1 Autoinducer derivatives

1.1 Synthesis of the HHQ derivative

The synthesis of HHQ derivative **40** is shown in Scheme 1 and follows a route devised by Baker.[1] Octanoyl chloride **34** was be converted to β-Ketoester **35** via a Meldrum's acid adduct.[2,3] The β-ketoester **35** was condensed with *N*-Boc-*p*-phenylenediamine **37** to form enamine **38**. The disappointing yield of this step was in part due to the reaction proceeding to an equilibrium state rather than to completion, and hence not all of the starting material being consumed. Starting materials can be recycled to improve the yield. Alternatively, Baker later found a higher-yielding reaction using a $ZrCl_4$ catalyst.

The enamine **38** was cyclised with polyphosphoric acid to form amino-HHQ **39** in good yield. The amine group of amino-HHQ **39** was converted to a diazo group by reaction with $NaNO_2$ and HCl, followed by displacement with NaN_3 to form the final azido-HHQ product **40**.[4]



Scheme 1: The synthesis of **40**. a) i) Pyridine, DCM, 0 °C. ii) MeOH, reflux, 66 % over two steps. b) MeOH, reflux, 19 %. c) Polyphosphoric acid, 120 °C, 72 %. d) i) $NaNO_2$, HCl, H_2O, 0 °C. ii) NaN_3, H_2O, r.t., 46.5 %.

1.2 Synthesis of PQS derivative 51

The synthesis of PQS derivative **51** is shown in Scheme 2, and also follows a route devised by Baker.[1] The Weinreb amide **45**[5] was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide **42** to form 1-chlorononan-2-one **46** following a procedure described by Hodgkinson *et al.*[6]

The synthesis of PQS described by Hodgkinson *et al.*[6] uses a microwave reaction of 1-chlorononan-2-one **46** with anthranilic acid. It was hoped that the azide group could be installed by using 5-nitroanthranilic acid **47** in the place of anthranilic acid in this microwave reaction, so that the nitro group could then be converted to an azide group via an amine. However, the microwave-catalysed reaction fails when 5-nitroanthranilic acid **47** is used.[1] Therefore, a two step process was employed instead.

5-Nitroanthranilic acid **47** was heated with K_2CO_3 to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one **46** to form the ester **48** by S_N2 displacement of the chlorine atom in a procedure adapted from Hlaváč et al.[7] Cyclisation with polyphosphoric acid produced nitro-PQS **49** cleanly.[7,8]

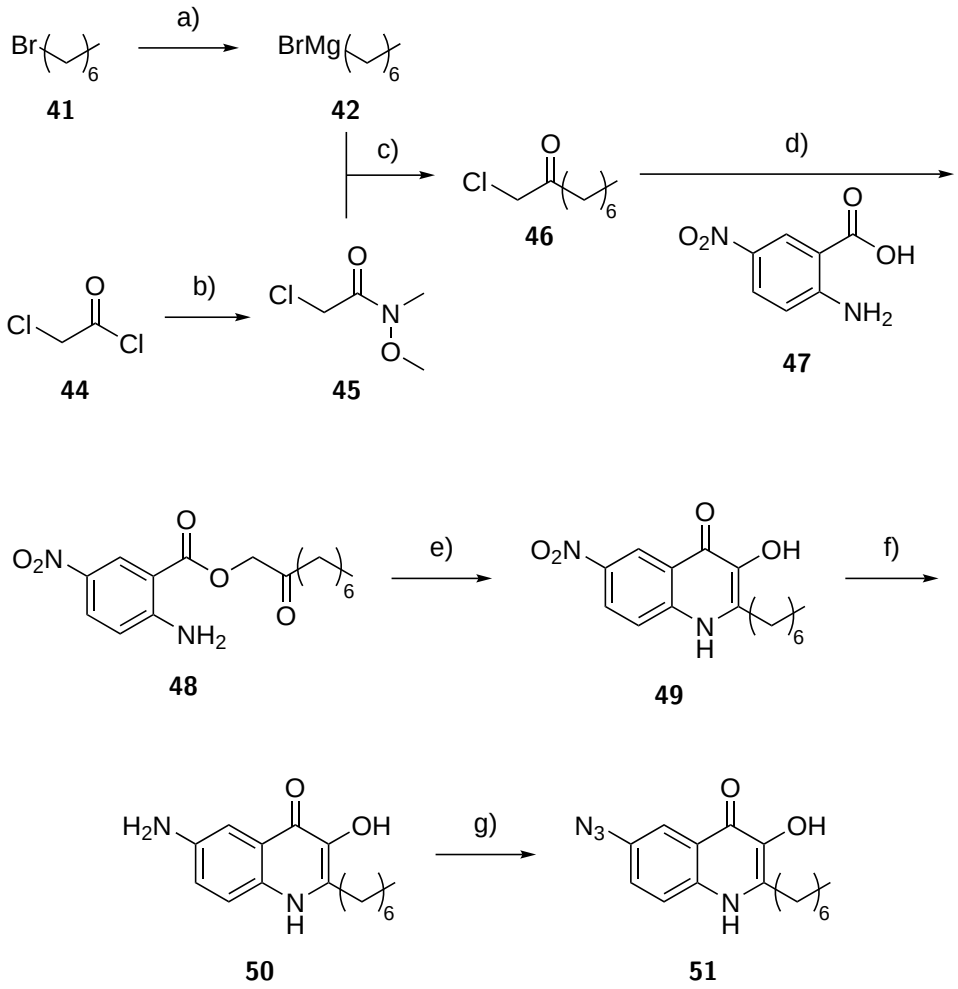
Conditions for the reduction of the nitro group were then compared (see Table 1). Baker initially used Zn and HCl, however this gave a yield over 100 % suggesting coordination of Zn to the amino-PQS **50**[1] (this product was taken through and purified after the next step). She also attempted reduction with Pd/C and H_2 or ammonium formate, but no reaction was observed.

Further conditions were tested in this work in order to obtain a clean sample of amino-PQS **50**. An initial test of reduction with $SnCl_2$ produced no detectable product by LCMS. Catalytic hydrogenation using harsher conditions was then attempted, and it was determined that increasing the pressure to 3 atm using a Paar hydrogenator causes full conversion in 4 h using Pd/C and H_2. Good yields (80 %) were also achieved using PtO_2 as a catalyst, with the advantage that the reaction proceeds more quickly, and at atmospheric pressure and temperature.[9]

Finally, amino-PQS **50** was converted to azido-PQS **51** by reaction with $NaNO_2$ and HCl to form diazo-PQS, followed by displacement of the diazo group using NaN_3 to give the azido-PQS **51**.[4] The yield of this reaction was rather disappointing (28 %), and is probably due to loss of product in the supernatant following precipitation.[1]

Conditions	**Outcome**
H_2, Pd/C, 1 atm, r.t., 18 h	No reaction[1]
NH_4HCO_2, Pd/C, 1 atm, r.t., 18 h	No reaction[1]
Zn, HCl (aq), r.t., 5 min h	Product **50** + Zn, assumed quantitative yield[1]
$SnCl_2.2H_2O$, MeOH, r.t., 18 h	No reaction
H_2, Pd/C, MeOH, 3 atm, r.t., 4 h.	Product **50**, 100 % yield
H_2, PtO_2, MeOH, 1 atm, r.t., 45 min	Product **50**, 80 % yield

Table 1: Conditions attempted for the synthesis of **50**.

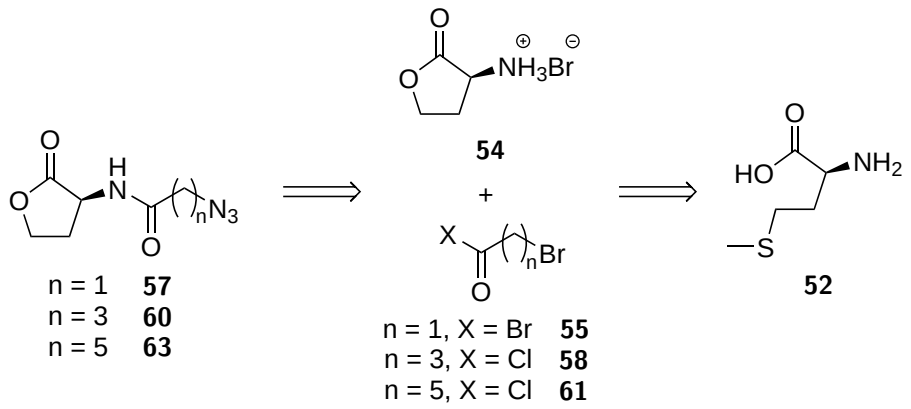


Scheme 2: The synthesis of **51**. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b) *N,O*-dimethylhydroxyl amine hydrochloride, K_2CO_3, toluene, H_2O, - 5 °C to r.t., 30 min, 71 %. c) THF, 0 °C to r.t., 15 h, 96 %. d) **47**, K_2CO_3, DMF, 90 °C, 1 h, then **46**, r.t., 18 h, 100 %. e) Polyphosphoric acid, 90 °C, 5.5 h, 40 %. f) H_2, PtO_2, MeOH, 1 atm, r.t., 45 min, 80 %. g) i) $NaNO_2$, HCl, H_2O, 0 °C, 50 min. ii) NaN_3, H_2O, r.t., 4 h, 28 % over two steps.

1.3 C_4-HSL derivatives

1.3.1 Retrosynthesis of C_4-HSL derivatives 57, 60 and 63

The azido derivative of C_4-HSL with a C_2 chain **57** (see **??**) has previously been prepared by Stacey *et al.*[10] The synthesis uses the cyclisation of L-methionine **52** using bromoacetic acid to form the homoserine lactone HBr salt **54**. This is then converted by a biphasic one-pot process to the azido-C_2 derivative **57** using bromoacetyl bromide **55** and NaN_3. It was hoped that this procedure could also be used to produce the azido-C_4 and C_6 chain derivatives.



Scheme 3: The proposed retrosynthesis of **57**, **60** and **63**.

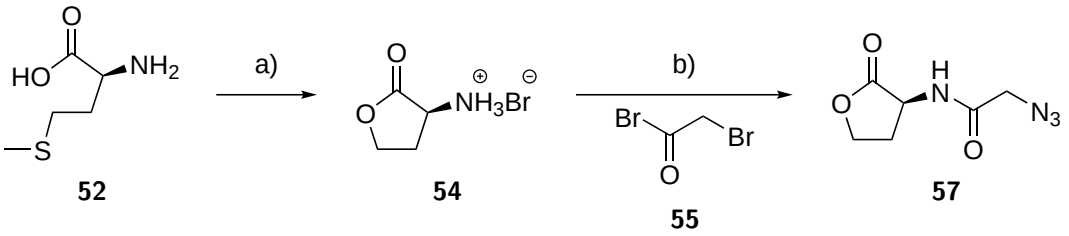
1.3.2 Synthesis of C_4-HSL derivatives 57, 60 and 63

The homoserine lactone HBr salt **54** was synthesised using the procedure developed by Stacey *et al.*,[10] followed by conversion to the azido-C_2 derivative **57** (see Scheme 4). Attempts to convert homoserine lactone **52** to the azido-C_4 derivative using 4-bromobutyryl chloride **58** produced a complex mixture of products. This is likely to be because the S_N2 reaction in which the azide anion displaces bromine is slower for the C_4 derivative as the bromine atom being displaced is no longer adjacent to a carbonyl group. In addition, the longer chain length allows intramolecular cyclisation of the bromide with the secondary amide.

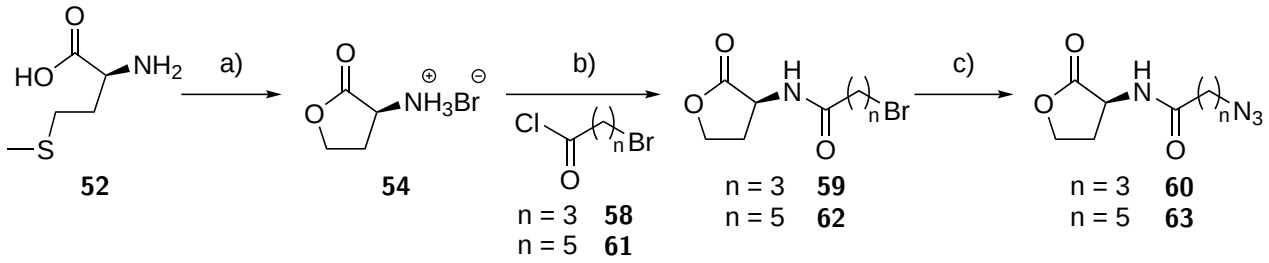
The conversion was therefore carried out as a two-step process, where a bromoacyl chain was initially installed, followed by the S_N2 reaction with NaN_3 (see Scheme 5).

Reaction of the homoserine lactone HBr salt **54** with 4-bromobutyryl chloride **58** or 6-bromohexanoyl chloride **61** produced the bromo-C_4 derivative **59** or bromo-C_6 derivative **62** respectively. Heating with NaN_3 in DMF converted the bromo-C_6 derivative **62** to the azido-C_6 derivative **63**.

Similar conditions were used by Dr. Bin Yu, a visiting PhD student in the Spring group, to convert the bromo-C_4 derivative **59** to the azido-C_4 derivative **60**, and this compound was kindly donated to complete the set.



Scheme 4: The synthesis of **57**. a) Bromoacetic acid, *i*-PrOH:H_2O:AcOH (5:5:2), r.t., 18 h, 41 %. b) NaN_3, $NaHCO_3$, H_2O/CH_2Cl_2, r.t., 18 h, 41 %.



Scheme 5: The synthesis of **60** and **63**. a) Bromoacetic acid, *i*-PrOH:H_2O:AcOH (5:5:2), r.t, 18 h, 41 %. b) $NaHCO_3$, H_2O/CH_2Cl_2, r.t., 18 h, **59** : 80 %, **62** : 66 %. c) NaN_3, DMF, 100 °C, 5 h,**63** : 27 % (donated by Dr. Bin Yu), **63** : 56 %.

1.4 HSL conjugates with cleavable linkers

In addition to the conjugates shown in the previous section, a further collection was synthesised in collaboration with Prof. Eddy Sotelo-Perez, a visiting researcher in the Spring group. Prof. Sotelo-Perez synthesised two alkyne-linked ciprofloxacin derivatives **92** and **93** (see Figure 1), both with cleavable linkers (see **??**).

link this up

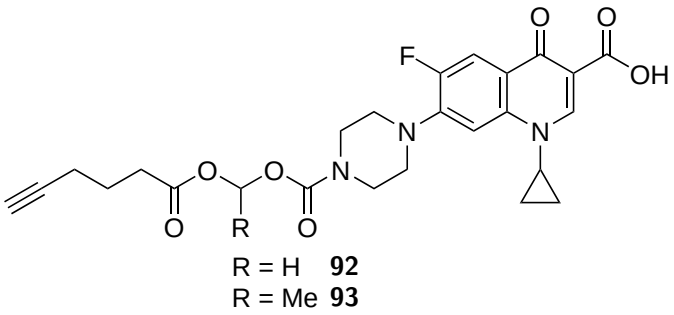
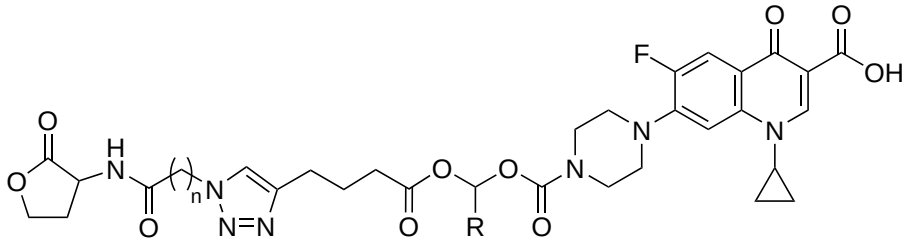


Figure 1: The cleavable alkyne-Cip derivatives synthesised by Prof. Eddy Sotelo-Perez.

Prof. Sotelo-Perez then performed click reactions using the AHL azide derivatives **57**, **60** and **63** shown in 1.3 to form a library of conjugates (see Figure 2). It was hoped that these conjugates would enter the cell and then be cleaved by esterases to release ciprofloxcin (see **??**).

link this up

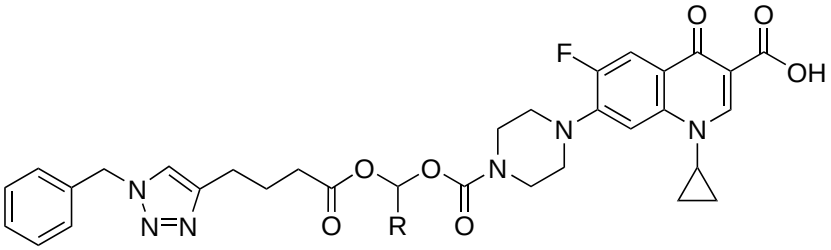
should I show the synthesis?



n = 1, R = H **94**
n = 3, R = H **95**
n = 5, R = H **96**
n = 1, R = Me **97**
n = 3, R = Me **98**
n = 5, R = Me **99**

Figure 2: The cleavable HSL-Cip triazole conjugates synthesised by Prof. Eddy Sotelo-Perez.

In addition, two control compounds **100** and **101** with benzyl head groups were produced by Prof. Sotelo-Perez (see Figure 3). It was hoped that these would show whether the AHL head group is required for activity.



R = H **100**
R = Me **101**

Figure 3: The cleavable Bn-Cip triazole conjugates **100** and **101** synthesised by Prof. Eddy Sotelo-Perez.

2 Autoinducer analogue-ciprofloxacin conjugates

2.1 Inspiration

The formation of biofilms can drastically increase MIC for many antibiotics.[11] For ciprofloxacin in *P. aeruginosa* the MIC increases by 16 fold according to Ceri et al.

Ganguly et al.[12] found the MICs of ciprofloxacin and a BHL analogue-ciprofloxacin **105** (see Figure 4) conjugate under standard planktonic conditions by introducing the compounds to liquid culture. The MICs were found to be ten times lower for ciprofloxacin vs. the conjugate **105** (5 vs 50 um). They then investigated the effect of the compounds on biofilms. The compounds were first cultured at 25um, with PA liquid culture. As expected, the culture failed to grow and form biofilm in the presence of ciprofloxacin, but did grow in the presence of the conjugate **105**. They then cultured biofilm for 24 hours before adding the compounds, and found that, in contrast, the conjugate **105** disrupted the biofilm more effectively than ciprofloxacin. When the biofilm was cultured for 48 or 72 hours the conjugate similarly disruptive effects, whereas ciprofloxacin 'did not show any significant antibacterial activity'.

Ganguly et al. used Bac-Light Live/Dead staining and confocal microscopy to image the biofilms, whereas so far I have used crystal violet staining. Crystal violet does not differentiate between live or dead cells, and so might not pick up on the antibacterial effects of compounds. However, their confocal microscopy results show a quantifiable decrease in biofilm thickness, and it may be possible to detect this using crystal violet.

The conjugate **105** developed by Ganguly et al. contained a thiolactone AHL. The unconjugated thiolactone BHL **158** was shown to have 'either enhanced uptake or functional activity' when compared with BHL **2**. Therefore it seems possible that my compounds may not show enhanced antibiotic activity, where thiolactone analogues might.

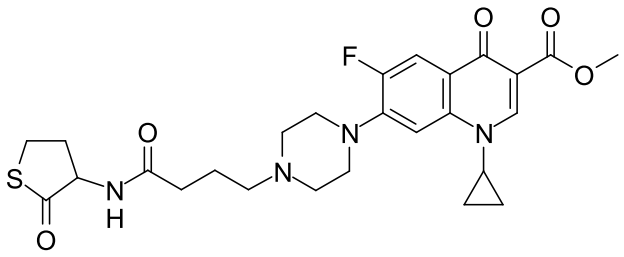
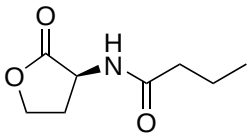


Figure 4

2.2 Library design

Discuss which AHL analogues were picked + why. Might as well make other enan of HOcy5



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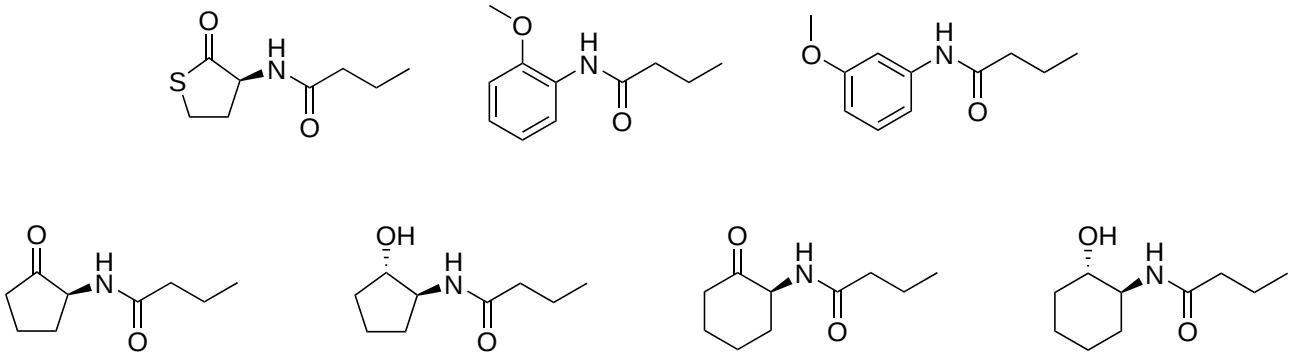
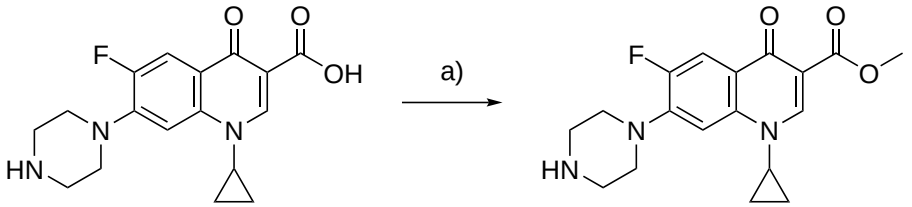


Figure 5

Introduce initial strategy of making bromide then azide, and diverting down the two different paths to make directly linked or triazole linked products.

2.3 Synthesis of the homocysteine thiolactone derivatives

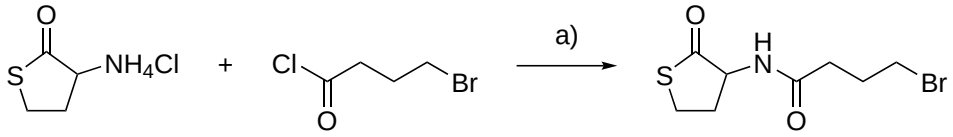
Methyl ciprofloxacin **102** was synthesised from ciprofloxacin **28** and MeOH in very good yield using *para*-toluenesulfonic acid as a catalyst.[13]



28 **102**

Scheme 6: Synthesis of methyl ciprofloxacin **102**. a) *p*-TSA, MeOH, 72 h, reflux, 83.3 %.

Br-C_4-HCTL **104** was synthesised using the Schotten-Baumann conditions employed previously for the Br-C_n-HSL compounds **56**, **59** and **62**. Br-C_4-HCTL **104** was isolated in markedly higher yield than that achieved by Ganguly *et al.*[12] (87.9 % vs. 25.0 %). It is possible that this was due to CH_2Cl_2 being used for the extraction, whereas Ganguly *et al.* used EtOAc.



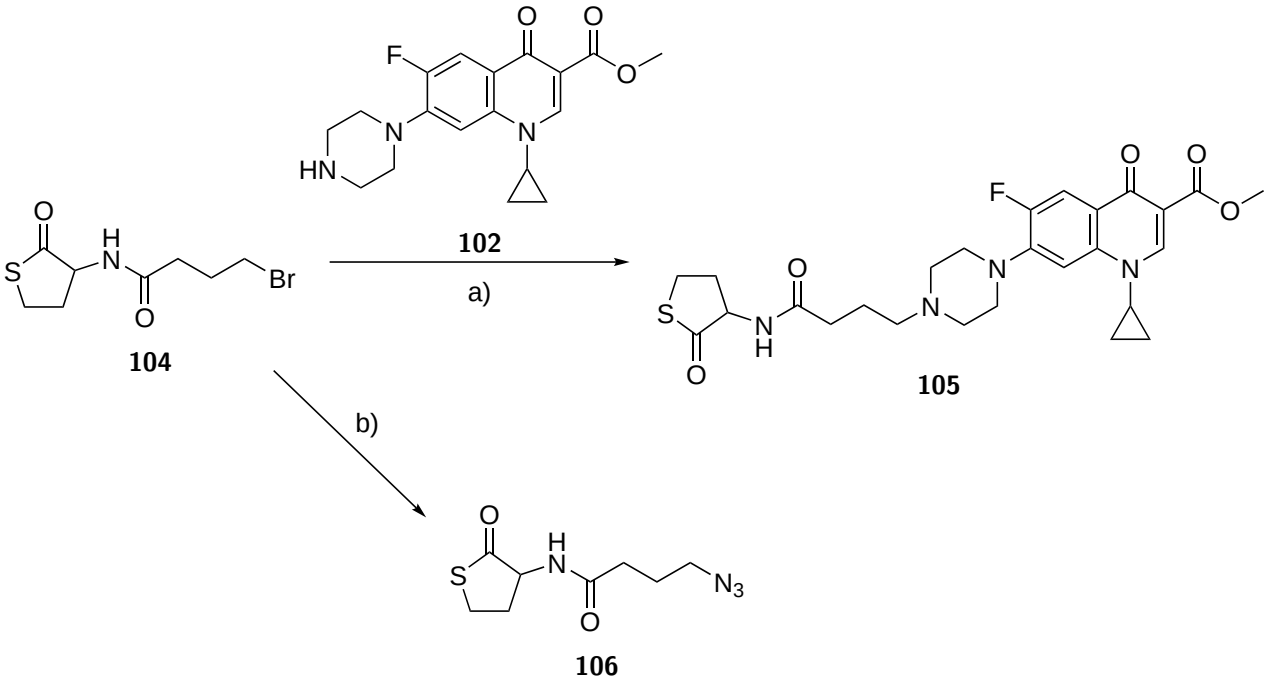
103 **58** **104**

Scheme 7: Synthesis of Br-C_4-HCTL **104**. a) $NaHCO_3$, CH_2Cl_2, H_2O, 0 °C, 1 h, 87.9 %.

The HCTL-CipMe conjugate **105** was synthesised using the procedure outlined by Ganguly *et al.*[12] Monitoring by LCMS showed slow conversion to the product. Br-C_4-HCTL **104** was presumably consumed by side reactions as 4 eq. were required to reach full conversion. Ganguly *et al.* do not quote a yield for comparison[12,14] but it is hoped that the 12.2 % achieved herein could be improved upon. The side reactions led to the production of an unidentified brown, viscous contaminant which made purification by flash column chromatography (as was used by Ganguly *et al.*) challenging. Preparatory HPLC on a partially purified sample gave enough pure HCTL-CipMe conjugate **105** for biological testing.

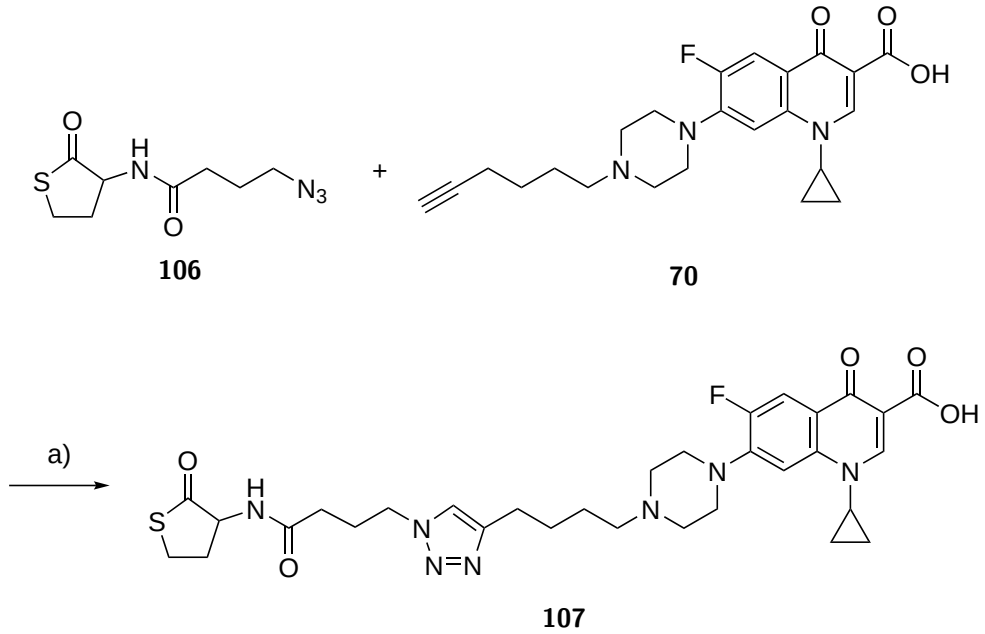
Future optimisation of the synthesis could focus on different routes to the product, e.g. the peptide coupling described in **??**, or different purification methods, e.g. using just preparatory HPLC, or reverse phase flash column chromatography.

Br-C_4-HCTL **104** was also converted into N_3-C_4-HCTL **106**, by an S_N2 reaction with sodium azide which proceeded in excellent yield.



Scheme 8: Synthesis of the HCTL-CipMe conjugate **105**, N_3-C_4-HCTL **106**, and the HCTL-Cip triazole conjugate **107**. a) K_2CO_3, acetonitrile, reflux, 24 h, 12.2 %. b) NaN_3, acetonitrile, reflux, 1.5 h, 89.3 %.

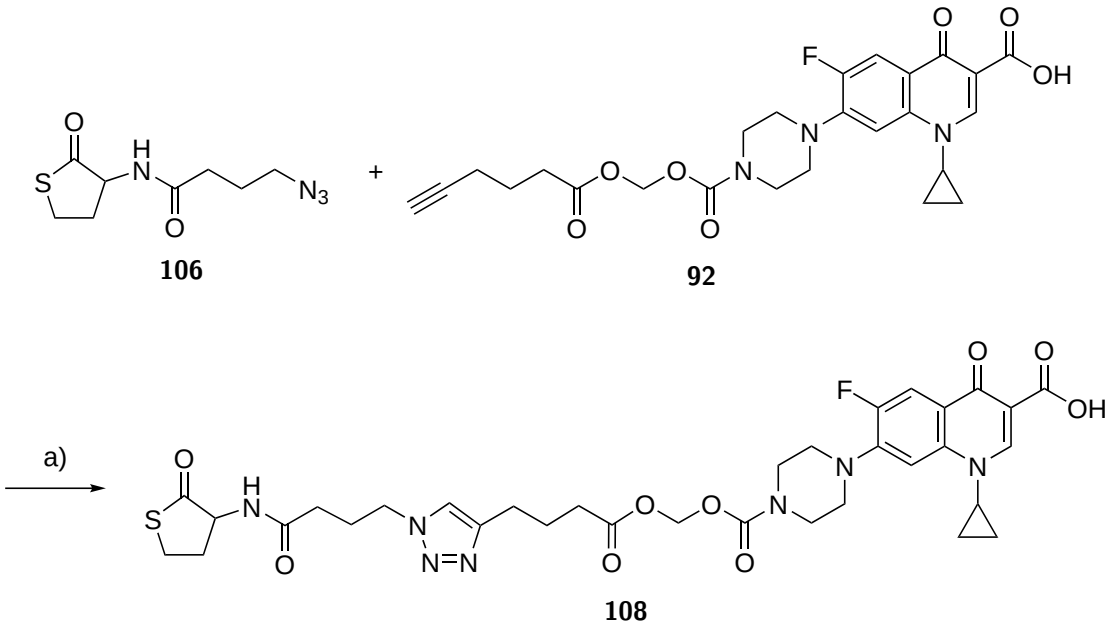
N_3-C_4-HCTL **106** was then subjected to the click reaction conditions optimised previously (see **??**). The reaction proceeded very slowly at first, until it was realised that the azide did not dissolve in the reaction solvent and formed a single solid clump. DMSO was added as a co-solvent, and the reaction began to proceed, albeit still slowly. It is possible that the sulfur atom coordinates to the copper, thus inhibiting its catalytic ability. Nonetheless the HCTL-Cip triazole conjugate **107** was eventually isolated in good yield (see Scheme 9).



Scheme 9: Synthesis of the HCTL-Cip triazole conjugate **107**. a) $CuSO_4$, THPTA, sodium ascorbate, H_2O, *t*-BuOH, DMSO, r.t., 7 d, 70.6 %.

A cleavable conjugate **108** was also synthesised from N_3-C_4-HCTL **106** by reaction with a cleavable alkyne-

Cip derivative **92** synthesised previously by Prof. Eddy Sotelo-Perez (see 1.4). Conditions developed by Prof. Sotelo-Perez were used, but again the reaction proceeded very slowly. The disappointing yield is, however, most likely due to losses during purification.



Scheme 10: Synthesis of the cleavable HCTL-Cip triazole conjugate **108**. a) CuI, DIPEA, CH_2Cl_2, r.t., 3 h, 5.0 %.

3 References

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Todo list