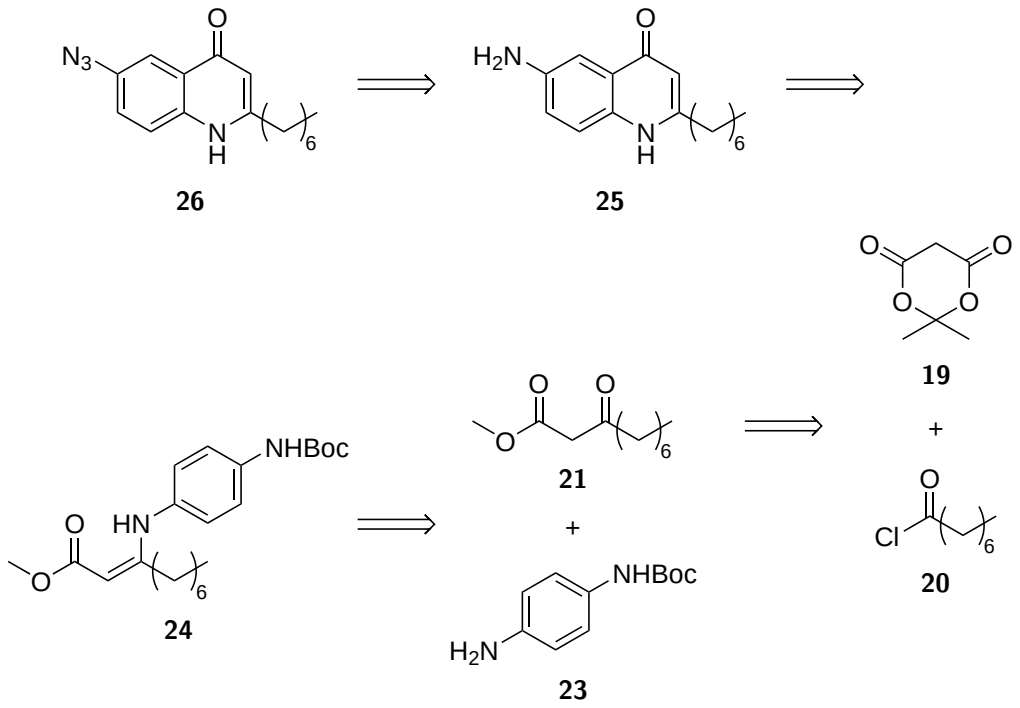
1 Autoinducer analogues

1.1 HHQ derivative

1.1.1 Retrosynthesis of HHQ analogue 26

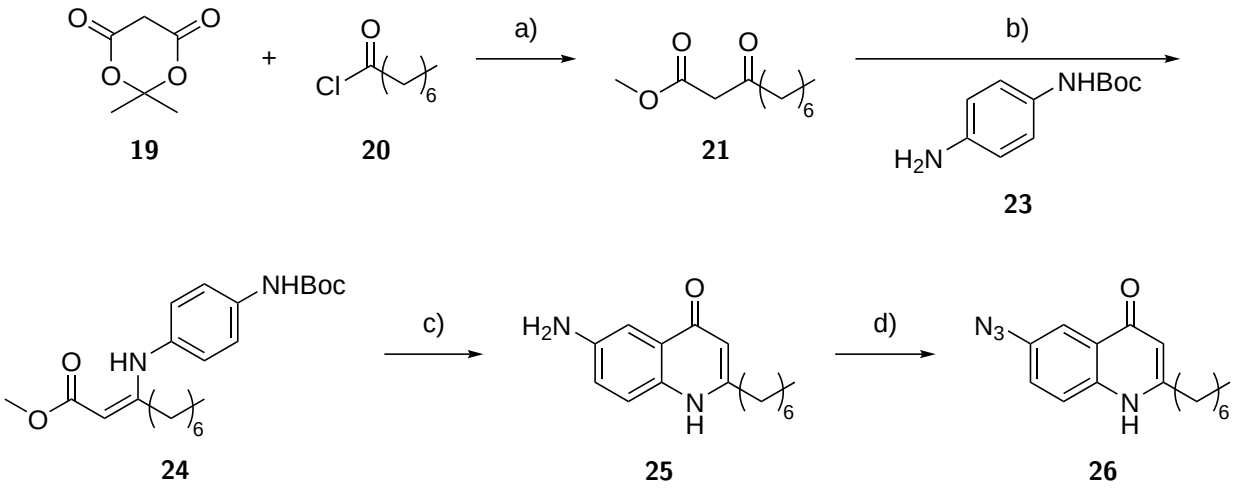
The retrosynthesis of HHQ analogue **26** is shown in Scheme 1 and follows a synthesis devised by Baker.[?] Octonyl chloride **20** can be converted to β-ketoester **21** via a Meldrum's acid adduct. β-ketoester **21** can be condensed with *N*-Boc-*p*-phenylenediamine **23** to form enamine **24**, which can then be cyclised with polyphosphoric acid to form amino-HHQ **25**. The amine group of amino-HHQ **25** could be 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 **26**.[?]



Scheme 1: The retrosynthesis of HHQ analogue **26**.

1.1.2 Synthesis of HHQ analogue 26

Amino-HHQ **25** was synthesised as shown in Scheme 2 and follows the route devised by Baker[?] described above. The final step in the synthesis of **26** will be completed shortly.



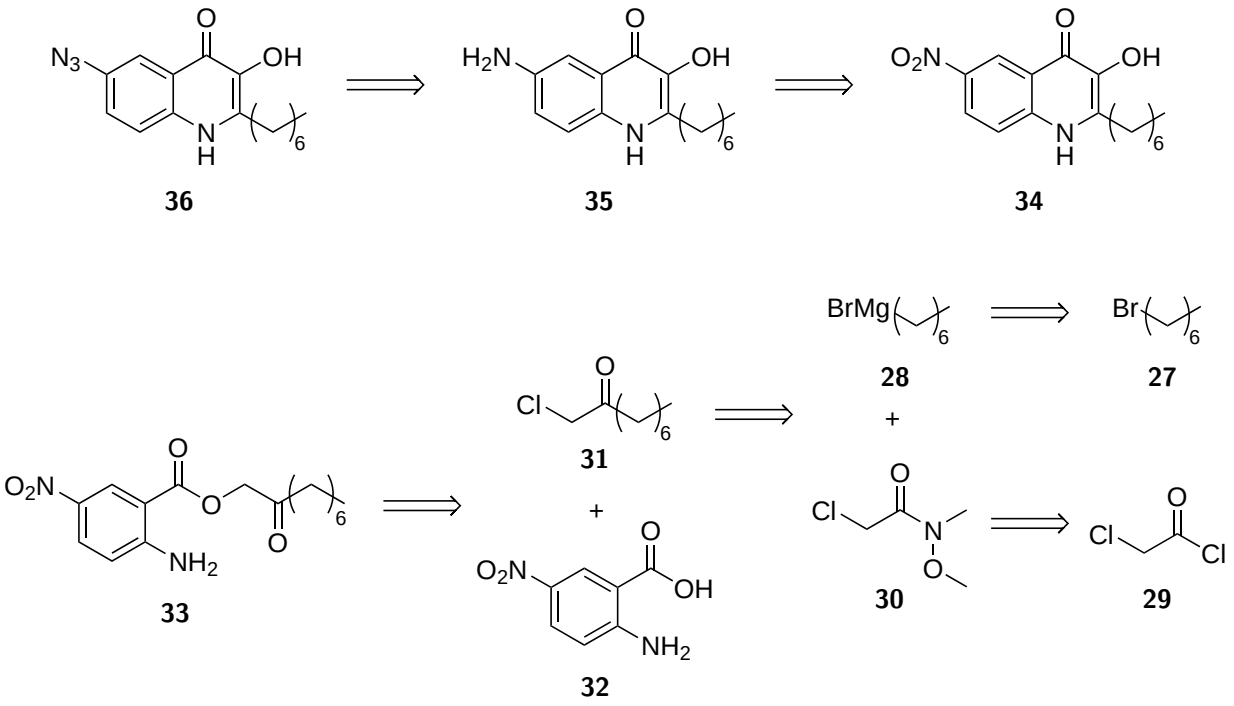
Scheme 2: The synthesis of **26**. a) i) Pyridine, DCM, 0°C. ii) MeOH, reflux, 66 % over two steps. b) MeOH, reflux, 19 %. c) Polyphosphoric acid, 120°C, 72 %. d) i) $NaNO_2$, HCl, H_2O, 0 °C. ii) NaN_3, H_2O, r.t.

azide step

1.2 PQS derivative

1.2.1 Retrosynthesis of PQS derivative 36

The retrosynthesis of PQS analogue **36** is shown in Scheme 3. The synthesis of 1-chlorononan-2-one **31** from heptyl magnesium bromide **28**[?] and the Weinreb amide **30**[?] prepared from chloroacetyl chloride **29** has been previously described by Hodgkinson *et al.*[?] The synthesis of PQS described by Hodgkinson *et al.*[?] uses a microwave reaction of 1-chlorononan-2-one **31** with anthranillic acid. It was hoped that the azide group could be installed by using 5-nitroanthranillic acid **32** in the place of anthranillic 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-nitroanthranillic acid **32** is used.[?] Therefore, a two step process is employed instead. Firstly, ester **33** is formed by S_N2 displacement of the chlorine atom of 1-chlorononan-2-one **31** by the carboxylate group of 5-nitroanthranillic **32**. The ester **33** is then cyclised using a polyphosphoric acid-catalysed reaction developed by Hradil *et al.*[?] to form nitro-PQS **34**. The nitro group can then be hydrogenated to form amino-PQS **35** followed by conversion to azido-PQS **36**.[?]



Scheme 3: The retrosynthesis of PQS analogue **36**.

1.2.2 Synthesis of PQS derivative 36

The Weinreb amide **30** was prepared from chloroacetyl chloride, followed by attack with heptyl magnesium bromide **28** to form 1-chlorononan-2-one **31** (see Scheme 4). 5-Nitroanthranillic acid **32** was heated with K_2CO_3 to deprotonate the carboxylic acid, followed by addition of 1-chlorononan-2-one **31**. Cyclisation to form nitro-PQS **34** was attempted using Eaton's reagent as it has been reported to improve yields when compared with polyphosphoric acid,[?,?] however, this lead to production of a black powder side-product in addition to the desired product (see Table 1). This proved difficult to separate out and thus lowered the yield of the desired quinolone. Cyclisation with polyphosphoric acid produced nitro-PQS **34** cleanly.[?]

Conditions for the reduction of the nitro group were then compared (see Table 2). Baker initially used Zn and HCl, however, this gave a yield over 100 % suggesting coordination of amino-PQS **35** to the Zn.[?] Reduction with $SnCl_2$ was then attempted, however, no product was detected by LCMS. Catalytic hydrogenation was then attempted. We determined that nitro-PQS **34** could not be reduced using H_2 and Pd/C at room temperature and pressure. However, increasing the pressure to 3 atm is sufficient to cause conversion to amino-PQS **35** in 4 h. Achieving 3 atm pressure of H_2 in a lab environment requires the use of a Parr hydrogenator, and it was found to be more convenient to use PtO_2 as a catalyst as this allows the reaction to proceed at room pressure and temperature.[?] Finally, amino-PQS **35** was converted to azido-PQS **36** 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 **36**.[?]

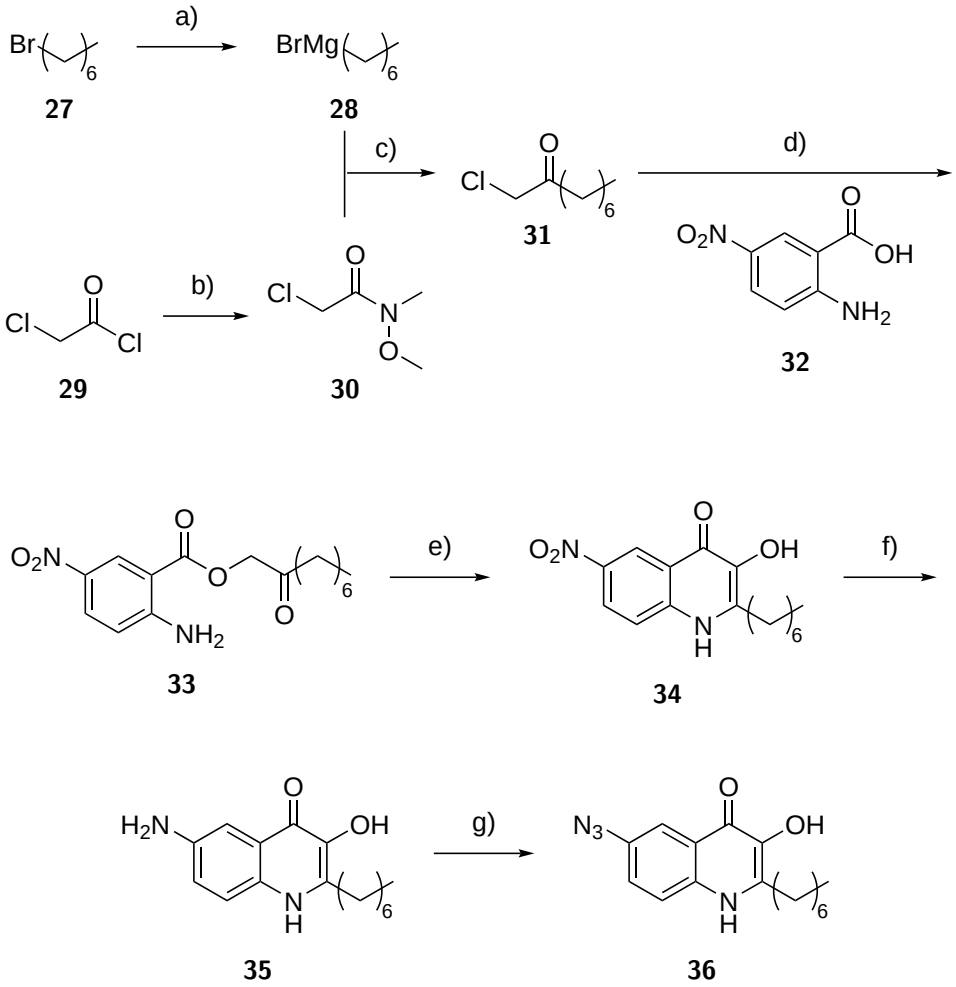
what are we doing with the last 2 compounds??

Conditions	**Outcome**
Eaton's reagent, 70 °C, 10 h.	Product **34** and black powder
Polyphosphoric acid, 90 °C, 5.5 h.	Product **34**

Table 1: Conditions attempted for the synthesis of **34**.

Conditions	Outcome
$SnCl_2.2H_2O$, MeOH, r.t., 18 h	No reaction
H_2, Pd/C, MeOH, 3 atm, r.t., 4 h.	Product **35**, 100 % yield
H_2, PtO_2, MeOH, 1 atm, r.t., 45 min	Product **35**, 80 % yield

Table 2: Conditions attempted for the synthesis of **35**.



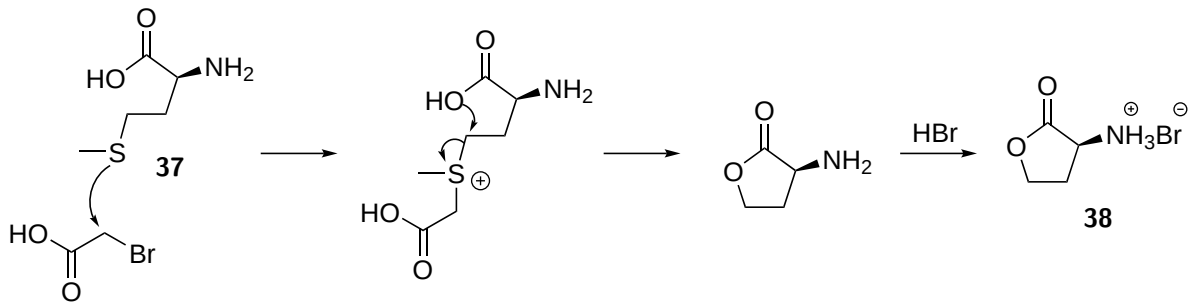
Scheme 4: The synthesis of **36**. a) Mg turnings, THF, r.t., 2 h then reflux, 2 h. b) *N,O*-dimethylhydroxyl amine hydrochloride, K_2CO_3, toluene, H_2O, - 5 °C to r.t., 30 min, 71 %. c) THF, 0 °C to r.t., 15 h, 96 %. d) **32**, K_2CO_3, DMF, 90 °C, 1 h, then **31**, r.t., 18 h, 100 %. e) Polyphosphoric 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

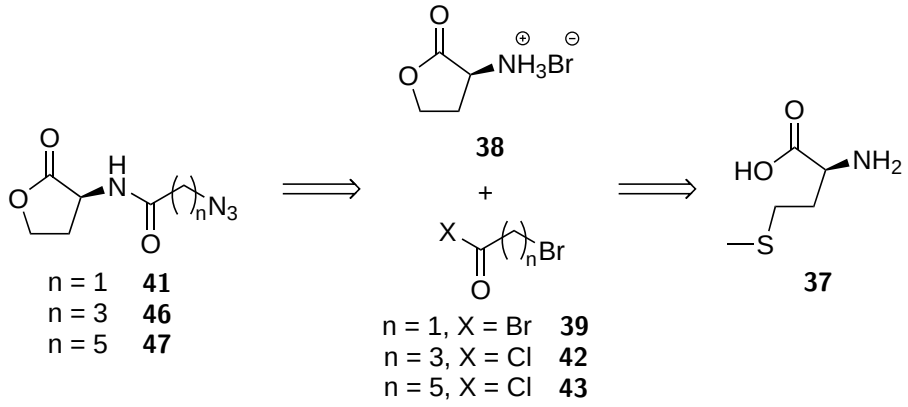
1.3.1 Retrosynthesis of C_4-HSL derivatives 41, 46 and 47

The azido analogue of C_4-HSL with a C_2 chain **41** (see **??**) has previously been prepared by Stacey *et al.*[?] It uses the cyclisation of L-methionine **37** using bromoacetic acid via the mechanism shown in Scheme 5 to form the homoserine lactone HBr salt **38**. This is then converted by a biphasic one-pot process to the azido-C_2 analogue **41** using bromoacetyl bromide **39** and NaN_3. It was hoped that this procedure could also be used to

produce the azido-C_4 and C_6 chain analogues.



Scheme 5: The mechanism of formation of **38**.



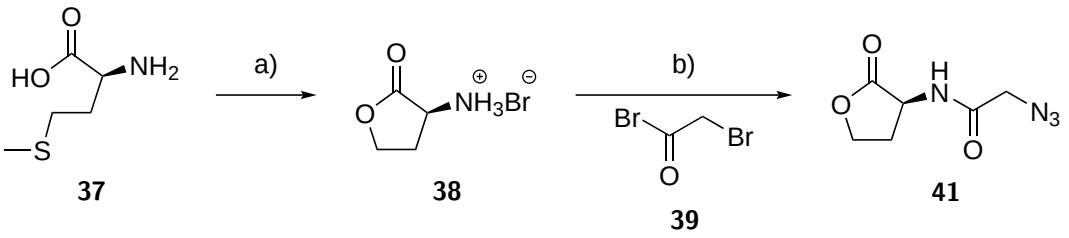
Scheme 6: The retrosynthesis of **41**, **46** and **47**.

1.3.2 Synthesis of C_4-HSL derivatives 41, 46 and 47

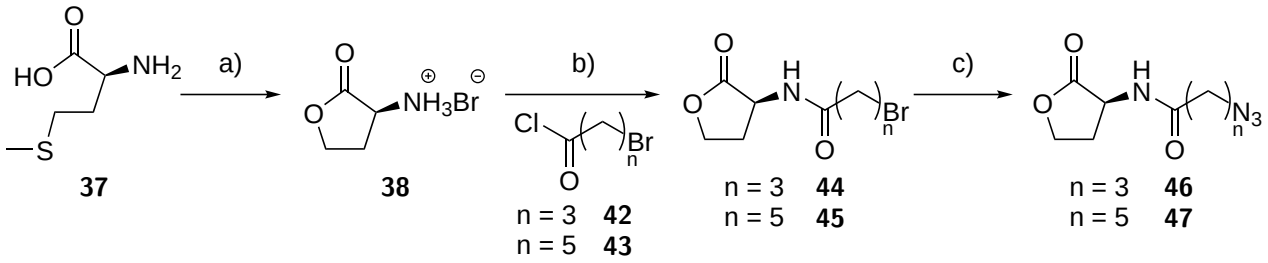
Homoserine lactone HBr salt **38** was synthesised using the procedure developed by Stacey *et al.*,[?] followed by conversion to the azido-C_2 analogue **41** (see Scheme 7). Attempts to convert homoserine lactone **37** to the azido-C_4 analogue using 4-bromobutyryl chloride **42** produced a complex mixture of products. This is likely to be because the S_N2 reaction where the azide anion displaces bromine is slower as the bromine atom being displaced is no longer next to a carbonyl group. Hence, this allows more side reactions to occur instead of the desired reaction. It was therefore decided that the conversion should be carried out as a two-step process, where a bromoacyl chain is first installed, followed by the S_N2 reaction with NaN_3 (see Scheme 8).

Reaction of the homoserine lactone HBr salt **38** with 4-bromobutyryl chloride **42** or 6-bromohexanoyl chloride **43** produced bromo-C_4 analogue **44** or bromo-C_6 analogue **45** respectively. Heating with NaN_3 in DMF converted bromo-C_6 analogue **45** to azido-C_6 analogue **47**.[?] It is hoped that the same conditions can be used to convert bromo-C_4 analogue **44** to azido-C_4 analogue **46** and this will be attempted shortly.

got from Bin Yu



Scheme 7: The synthesis of **41**. 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 8: The synthesis of **46** and **47**. 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, **44** : 80 %, **45** : 66 %. c) NaN_3, DMF, 100 °C, 5 h, **47** : 56 %.

2 Antibiotic analogues

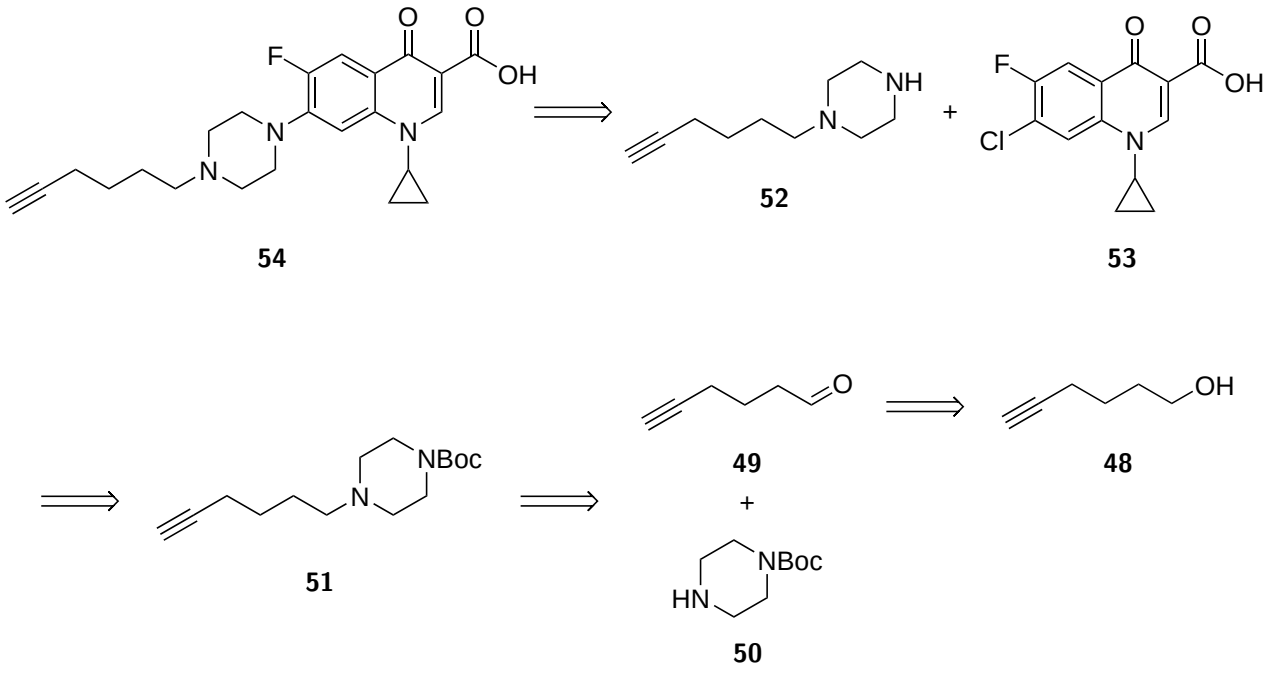
2.1 Ciprofloxacin derivative

Ciprofloxacin **18** (see **??**) is second-generation fluoroquinolone antibiotic used to treat both Gram-positive and Gram-negative bacterial infections.[?] The structure-activity relationships for ciprofloxacin have been investigated[?] and positions 2 and 7 were found not to cause loss of activity. It was therefore decided that alkyne tails would be added at these positions giving two analogues of ciprofloxacin, **54** and **152** (see **??**).

Three derivatives of ciprofloxacin modified at the free piperazine N were synthesised. These contained a six-carbon alkyl chain with a terminal alkyne, a six-carbon acyl chain with a terminal alkyne and a three carbon acyl chain with a terminal alkyne.

2.1.1 Retrosynthesis of ciprofloxacin analogue 54

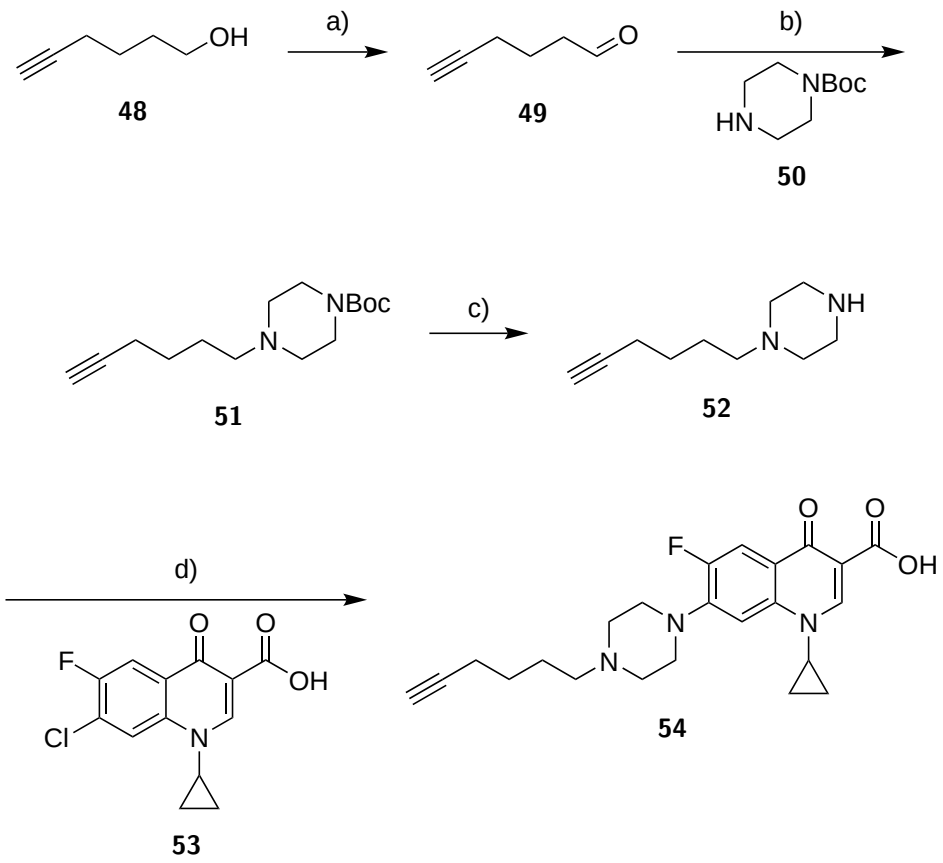
The retrosynthesis of ciprofloxacin analogue **54** is shown in Scheme 9. The analogue has has an alkyne tail attached on the free piperazine N; it is more convenient to attach the alkyne chain to piperazine before coupling of the alkyl piperazine **52** to the ciprofloxacin core **53** as this method is more convergent. This can be achieved by reductive amination of hex-5-ynal **49** with 1-boc-piperazine **50** followed by deprotection to form the alkyl piperazine **52**. This method was found by Renau *et al.* to be "...superior to previous reports which involved alkylation of piperazine with an appropriate alkyl halide.".[?,?] S_NAr coupling of the piperazine derivative with ciprofloxacin precursor **53** leads to the final analogue **54**.



Scheme 9: The retrosynthesis of **54**.

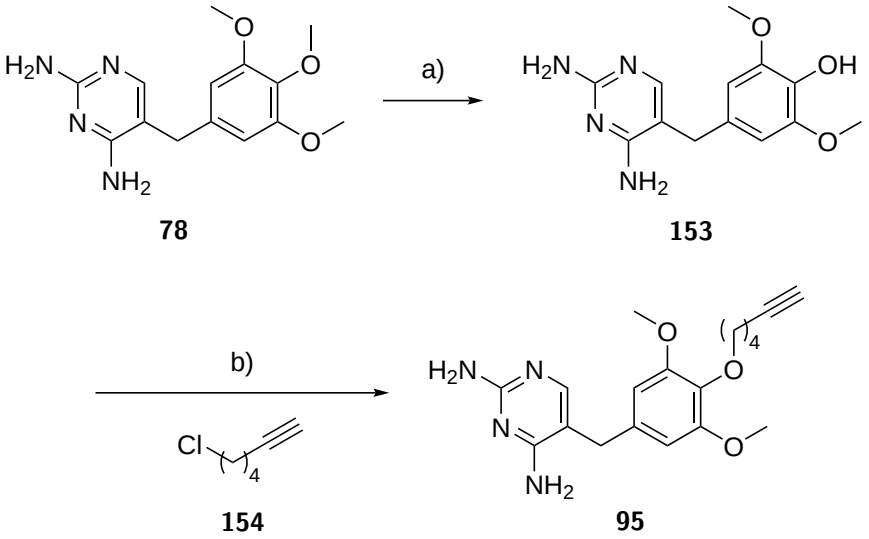
2.1.2 Synthesis of ciprofloxacin analogue 54

The synthesis of **54** follows the strategy followed by Renau *et al.*[?] Unlike the aldehydes and ketones used by Renau *et al.*,[?] hex-5-ynal **49** is not commercially available and so was successfully prepared by PCC oxidation of hex-5-ynol **48** according to the procedure described by Kocsis *et al.*[?] Renau *et al.*[?] used sodium cyanoborohydride to facilitate the reductive amination of hex-5-ynal **49** and 1-Boc-piperazine **50**. However, it was decided to attempt this transformation using the less toxic sodium triacetoxyborohydride following a procedure reported by Abdel-Magid *et al.*[?] This reaction yielded compound **51**, which was deprotected using TFA using the procedure described by Renau *et al.*[?] to give compound **52**. This was refluxed in MeCN with the commercially available ciprofloxacin precursor **53** according to the procedure described by Renau *et al.*,[?] however the reaction did not proceed. Addition of NEt_3 did not lead to reaction, however it was found that refluxing in neat NEt_3 lead to conversion to the final ciprofloxacin analogue **54**.



Scheme 10: The synthesis of **54**. a) Pyridinium chlorochromate, CH_2Cl_2, r.t., 5 h, 72 %. b) $NaBH(AcO)_3$, 1,2-dichloroethane, r.t., 10.5 h, 99 %. c) TFA, r.t., 1 h, 100 %. d) NEt_3, reflux, 15 h, 21 %.

2.2 Trimethoprim derivative



Scheme 11: The synthesis of **95**.

discuss this

3 *P. aeruginosa* autoinducer-antibiotic conjugates

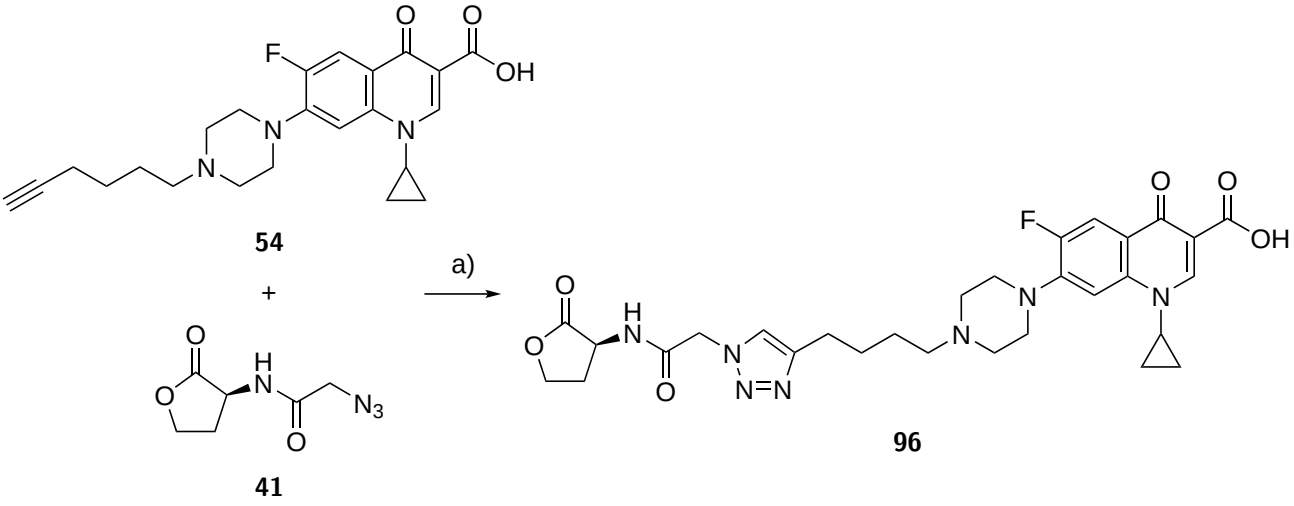
3.1 Synthesis of autoinducer-antibiotic conjugate 96

Test reactions using C_4-HSL analogue **41** and ciprofloxacin analogue **54** were performed to find conditions for the click reactions between the azido autoinducers and the alkynyl antibiotics (see Scheme 12 and Table 3). Stirring at r.t. had no effect even with an extended reaction time. Heating to 50 °C did lead to slow formation of the product, but a mixture of the 1,4 **96** and 1,5 **153** isomers was observed . Use of the ligand tris[(1-benzyl-*1H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) **154** lead to some conversion at room temperature, however the reaction stopped before completion, probably due to oxidation of the Cu(I) catalytic species. When degassed solvent and an argon atmosphere were used the reaction proceeded to completion at room temperature in around 3 h.

check and include ratio from NMR? diagram, isomers diagram

Conditions	**Outcome**
$CuSO_4 \cdot H_2O$, sodium ascorbate, H_2O, *t*-BuOH, air, r.t., 7 d.	No reaction
$CuSO_4 \cdot H_2O$, sodium ascorbate, H_2O, *t*-BuOH, air, 50 °C, 5 d.	1,3-Triazole product **96** and 1,5 triazole impurity **155**
$CuSO_4 \cdot H_2O$, sodium ascorbate, TBTA, H_2O, *t*-BuOH, air, r.t., 3 h.	1,3-Triazole product **96** and starting materials **41** and **54**
$CuSO_4 \cdot H_2O$, sodium ascorbate, TBTA, H_2O, *t*-BuOH, Ar, r.t., 3 h.	1,3-Triazole product **96**

Table 3: Conditions attempted for the synthesis of **96** (see Scheme 12).



Scheme 12: Synthesis of **96**. a) see Table 3.

3.2 Synthesis of the initial triazole-linked library

Once conditions had been found for the click reaction, the synthesis of other conjugates was attempted. Synthesis of some conjugates proved more difficult than expected; AHLs hydrolysed upon HPLC purification, the 3-oxo-C_{12}-HSL conjugate degraded when subjected to column chromatography, and quinolones coordinated copper,

thus inhibiting the click reactions. Nonetheless, several conjugates were produced for testing. The results of the reactions are shown in Table 4, Table 5, Table 6 and Table 7.

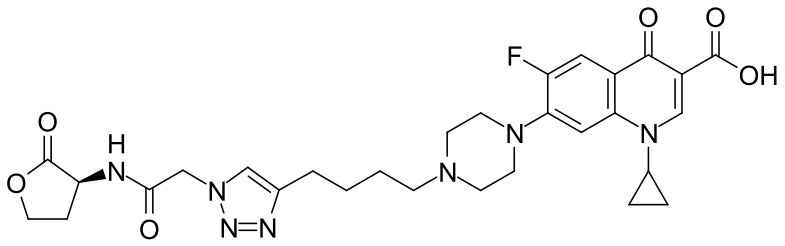
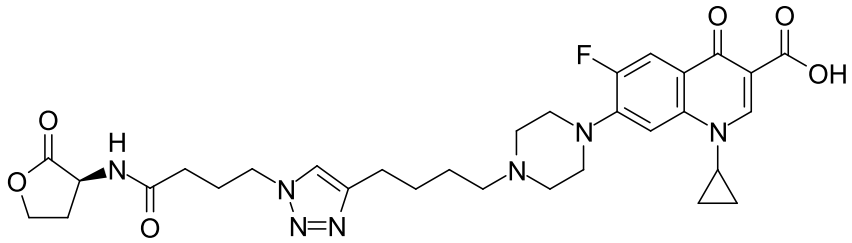
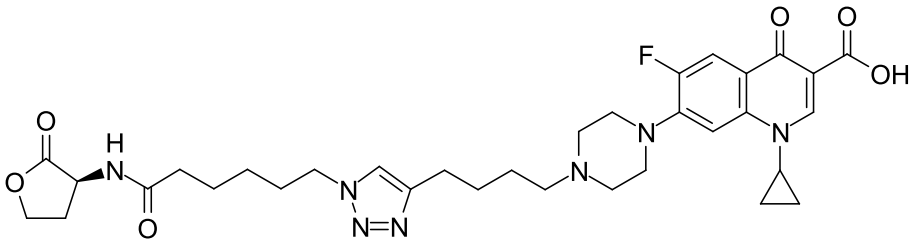
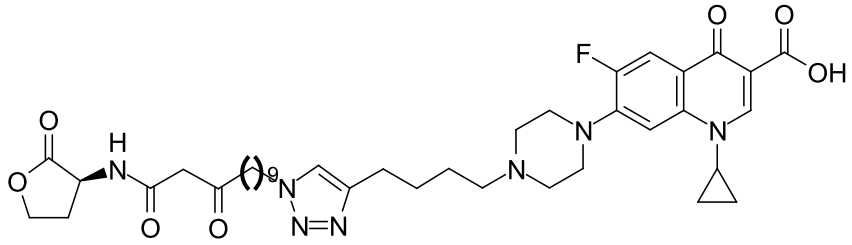
Product	**Outcome**
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2, 0 - 20 % $MeOH/CH_2Cl_2$).
	✗ Reaction complete by LCMS in 3.5 h, but product degraded when subjected to column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).

Table 4: Click reactions attempted.

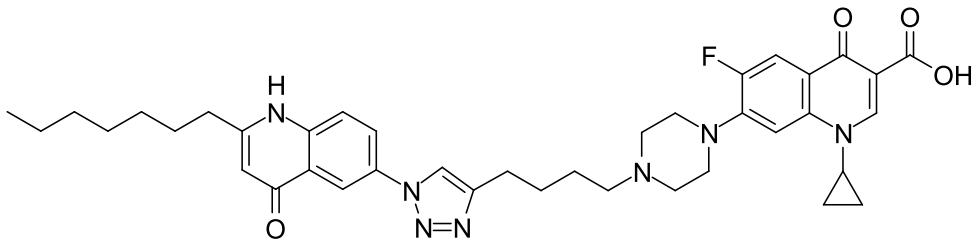
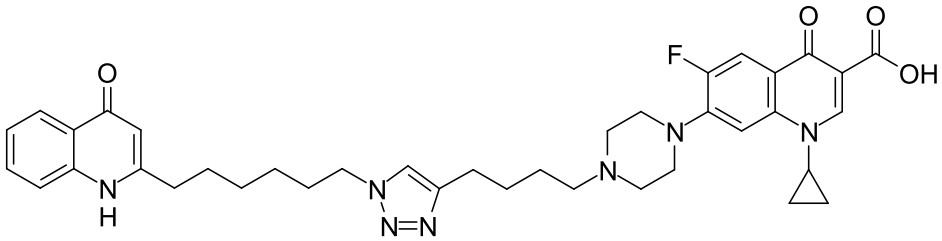
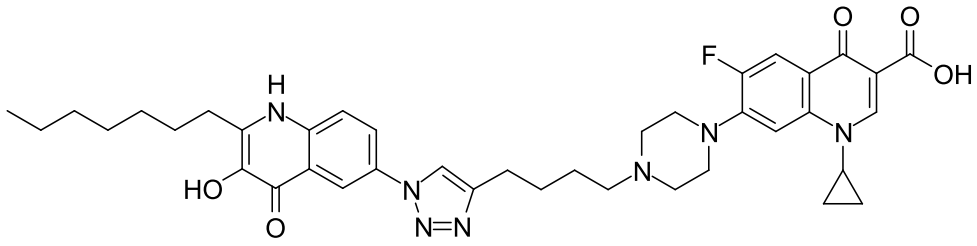
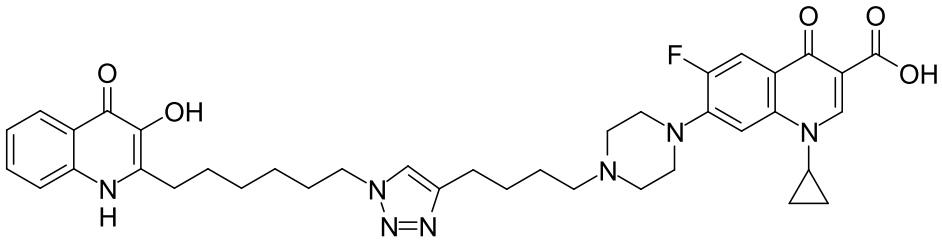
Product	Outcome
	✓ Reaction complete by LCMS. Purified by prep. HPLC.
	✗ Reaction not attempted due to lack of starting material.
	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.
	✗ No reaction.

Table 5: Click reactions attempted.

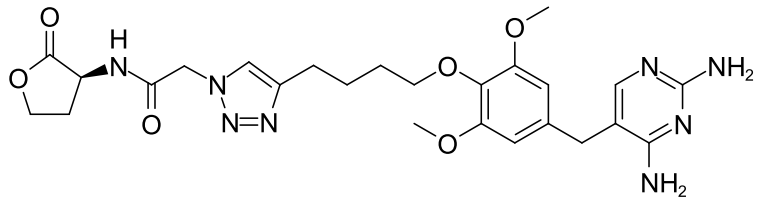
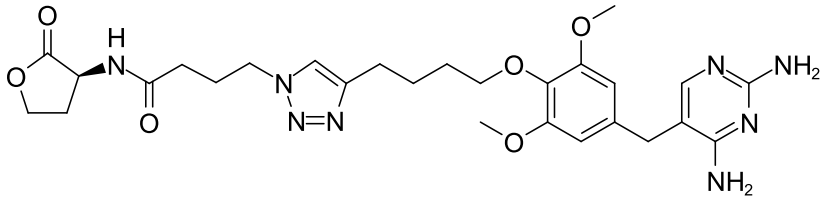
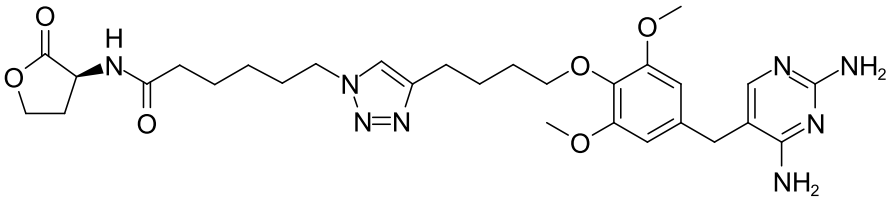
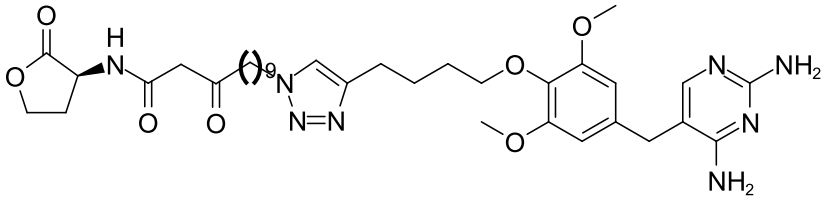
Product	Outcome
	✗ Reaction complete by LCMS in 2 h, but lactone hydrolysed on HPLC column in acidic conditions.
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✓ Reaction complete by LCMS. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$).
	✗ Degraded.

Table 6: Click reactions attempted.

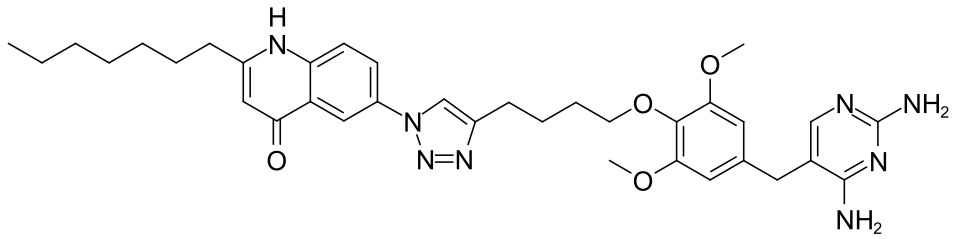
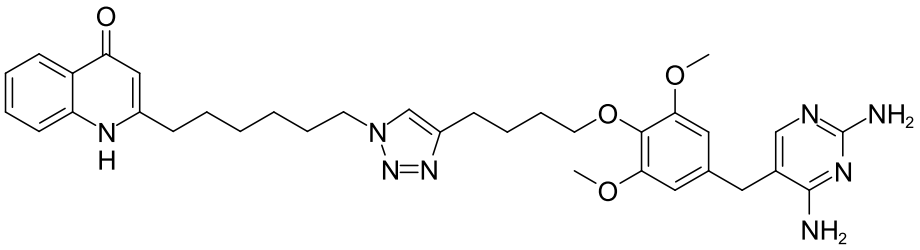
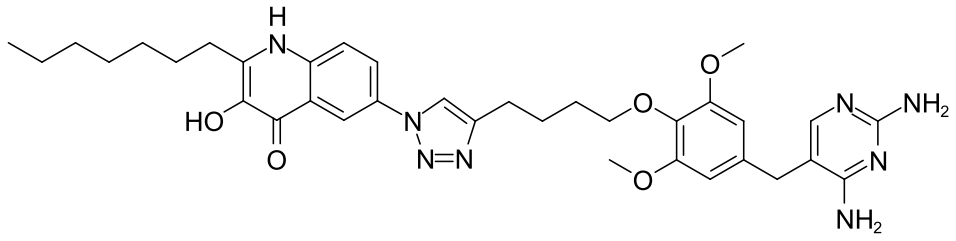
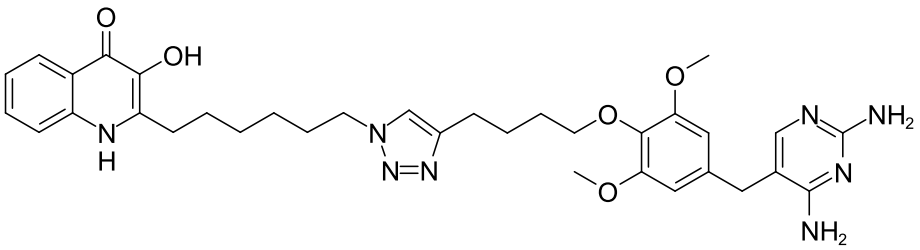
Product	Outcome
	✓ Reaction complete by LCMS in 1.5 h. Purified by prep. HPLC.
	✗ Reaction not attempted due to lack of starting material.
	✗ Reaction did not go to completion by LCMS. Attempted purification by prep. HPLC but unsuccessful.
	✓ Reaction complete by LCMS in 3 h. Purified by column chromatography (SiO_2, 20 % $MeOH/CH_2Cl_2$)

Table 7: Click reactions attempted.

Todo list

diagrams, discussion, saying what I got from Yssy, why didn't I do them all