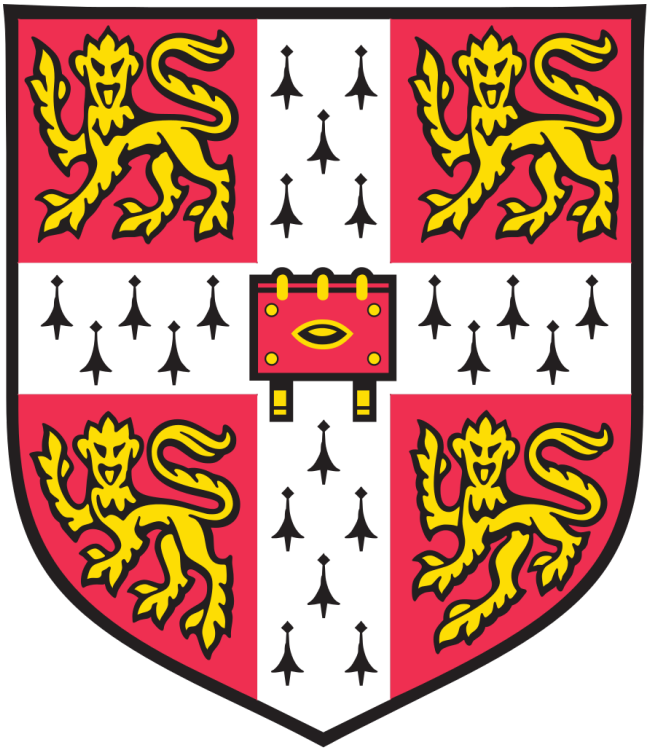
The synthesis and biological evaluation of a library of autoinducer-antibiotic conjugates

Lois Overvoorde



Sidney Sussex College

University of Cambridge

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Supervised by Professor David Spring

This dissertation is submitted for the degree of Doctor of Philosophy

Contents

1 Declaration

This dissertation describes work carried out in the Department of Chemistry, University of Cambridge under the supervision of Professor David Spring, and in the Department of Biochemistry, University of Cambridge under the supervision of Dr Martin Welch. This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except as specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution, except those parts which were included in my CPGS dissertation. The dissertation does not exceed the word limit specified by the Physics and Chemistry Degree Committee.

Lois Overvoorde
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2 Abstract

Bacterial resistance to antibiotics is becoming a serious global health threat, and the discovery of new, safe and effective antibiotics is required urgently.[1–3] A new class of antibiotic, namely sideophore-antibiotic conjugates, has shown promise in initial studies.[4,5] Siderophores are used by bacteria for iron uptake, and so attaching antibiotics to them allows the antibiotic to be carried across cell membranes. This study investigates conjugates designed using a similar approach, but using bacterial autoinducers[6] instead of siderophores. Autoinducers are required for coordination of bacterial behaviours and are involved in the control of swarming, virulence factor production and biofilm formation.[7]

The library was synthesised in two halves which were then coupled together using a copper(I)-catalysed azide-alkyne cycloaddition.[8,9] The autoinducers were functionalised with azide groups and the antibiotics were functionalised with alkynes. The quorum sensing molecules produced by *Pseudomonas aeruginosa* were investigated as it is a significant human pathogen[10] which displays high resistance to many antibiotics[11] and uses quorum sensing to coordinate its group behaviours.[12] Azido analogues of these autoinducers were coupled with alkyne analogues of ciprofloxacin, which was chosen as it is commonly used against *P. aeruginosa*[13] but resistance to it is developing,[14] and trimethoprim. It was hoped that the autoinducers would aid retention of the antibiotic in the cell, thus potentially increasing its potency or even restoring its efficacy against resistant strains.

analogues

3 Conclusions

Clicks

clicks were all crap because dilute Hong2009

SHL

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.

HOcy5

Direct comparisons of routes are not possible without repeating syntheses using this new method, but if it is assumed that peptide coupling of homocysteine thiolactone hydrochloride **101** to carboxylic acid **148** would have a similar yield to the coupling with (1*R*,2*R*)-2-aminocyclopentan-1-ol **122**, approximate comparisons can be made. The synthesis described in **??** has an overall yield of 11%, whereas the route shown in **??** for **127** has an overall yield of 26%. Moreover, if the yield starting from the head group (which may be expensive, difficult to synthesise and/or unstable) is considered, the yield is 55% vs. 11%. Therefore, this route is recommended for further investigation if the library is to be expanded.

A downside to this route is that it cannot branch towards the triazole-coupled library in the same way that the route in **??**. A carboxylic acid intermediate with a triazole in the chain could presumably be synthesised, but this would be rather pointless given that the triazole library was initially proposed so that the two sides could be joined by the click reaction.

No, I didn't try the one-pot synthesis without TBS. No worries, I wonder if it would have worked. Could be one for the conclusions?

Not C4 chain - massive pain due to internal ring formation.

4 Future work

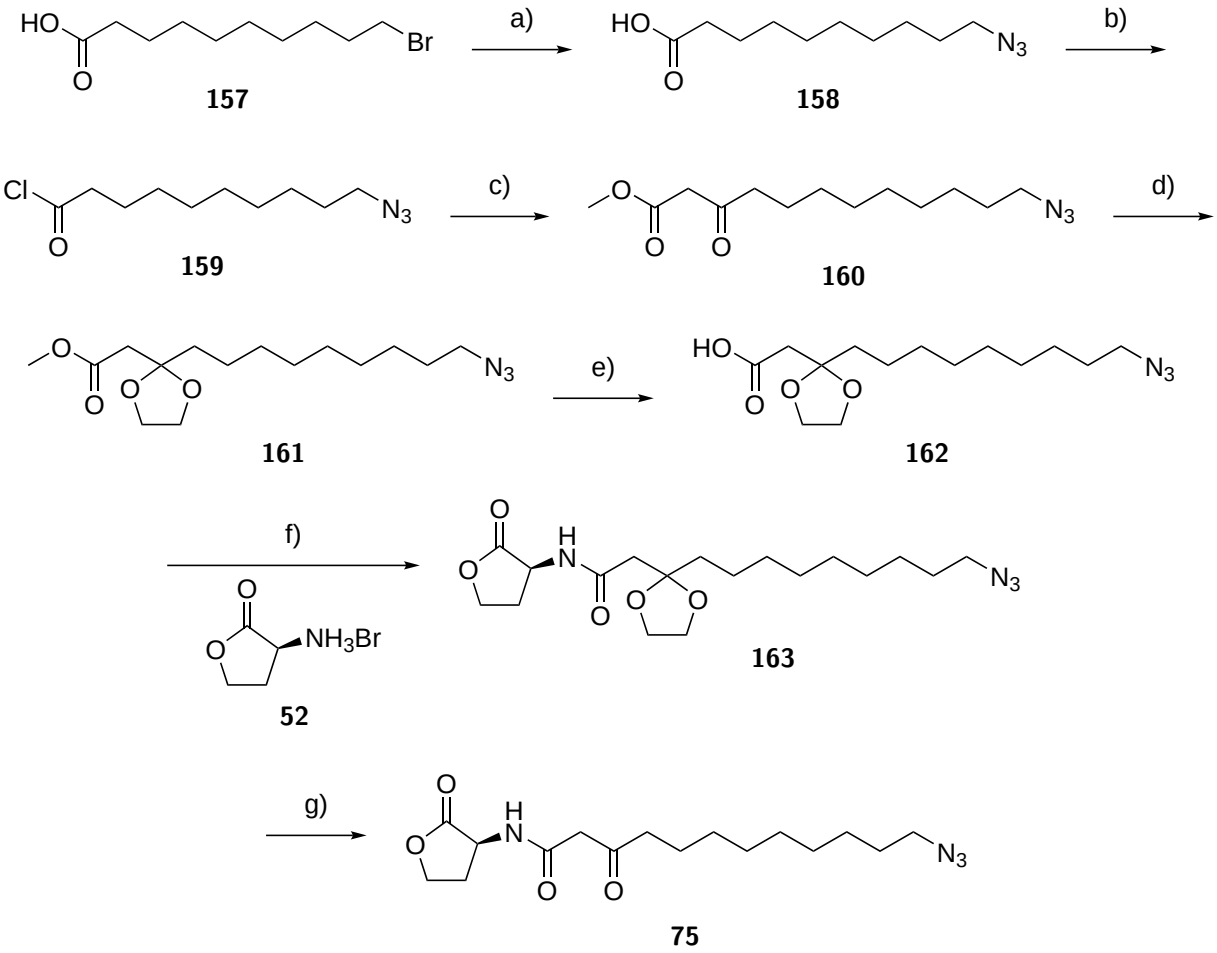
This section begins with discussion of further azido autoinducers and alkynyl antibiotics which could be used in future conjugates, some have which have already been partially or fully synthesised by myself or other members of the Spring group. Further HSL analogues which could be used in conjugates are then discussed. Finally plans for further biological testing of the conjugates synthesised in this study are presented.

4.1 Autoinducer derivatives

4.1.1 3-oxo-C_{12}-HSL derivative 75

N_3-3-oxo-C_{12}-HSL **75** (see Scheme 1) was synthesised by Ryan Howard, a master's student under my supervision. The synthesis was based on a synthesis of 3-oxo-C_{12}-HSL **20** reported by Hodgkinson *et al.*[15] Conjugates of this compound was not included in the library as it degraded during the click reaction. However, reaction conditions could be further optimised, or the acetal protected azide **163** could be used in the click reaction, followed by deprotection.

This compound would be a useful addition to the library as it would demonstrate whether the 3-oxo group and/or longer alkyl chain are required for activity. As the head group is added fairly late in the synthesis it would also be easy to swap it for the other head groups described in **??**, thus expanding the library further.



Scheme 1: The synthesis of N_3-3-oxo-C_{12}-HSL **75** carried out by Ryan Howard. a) NaN_3, DMF, 60 °C, 6 h, 93%. b) Oxalyl chloride, DMF, CH_2Cl_2, 3 h, r.t.. c) MeOAc, *N*-methyl imidazole, $TiCl_4$, DIPEA, toluene, r.t., 2 h, 43% over two steps. d) $HO(CH_2)_2OH$, TsOH, $CH(OMe)_3$, r.t., 5 h, 78%. e) NaOH, water, r.t., 6 h, 85%. f) EDC, DMAP, CH_2Cl_2, r.t., 16 h. g) TFA, r.t., 5 h, 29% over two steps.

4.1.2 AI-2 derivatives

AI-2 **23** is perhaps a more attractive choice of autoinducer for inclusion in conjugates than the others used in this study as it is actively transported into cells[16] and used by a wide range of bacterial species.[17] The synthesis of conjugates of AI-2 **23** with ciprofloxacin **24** and trimethoprim **25** has been attempted in the Spring group by Dr Jamie Stokes. However, the protected azido AI-2 derivative **164** synthesised was found to be unstable, and the click reactions attempted were unsuccessful.[18] AI-2 **23** is known to interconvert between multiple forms (including forming a furanosyl borate diester)[19] so it is to be expected that syntheses involving it might be challenging. If a more stable azido AI-2**23** derivative cannot be developed, another approach would be to use an azido AI-2 **23** analogue which is capable of being taken up by the same active transport mechanism.

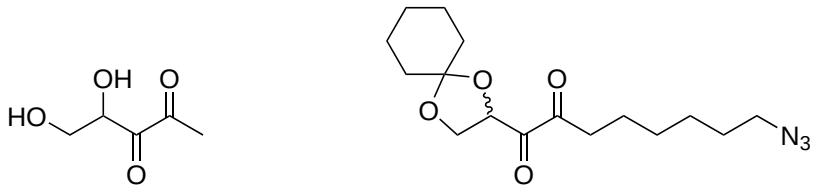


Figure 1: AI-2 **23** in its DPD form and the protected azido AI-2 derivative **164** synthesised by Dr Jamie Stokes.

Two types of AI-2 **23** receptors have been identified: LuxP, present in *Vibrio sp.*,[20] and LsrB, first discovered in *Salmonella enterica* serovar Typhimurium.[21] LuxP is a periplasmic binding protein that relays the signal, but not the actual AI-2 **23** molecule into the cell, and hence is not a useful target.[22] LsrB is the ligand binding protein of a system that transports AI-2 **23** into the cell,[16] and hence can be targeted. LsrB orthologs are found in a wide range of bacterial families including *Enterobacteriaceae*, *Rhizobiaceae*, and *Bacillaceae*.[23] In addition, several bacterial species, including *P. aeruginosa*, are known to respond to AI-2 **23** but do not have either of these two known types of receptors, and thus the discovery of new receptor types is expected.[23] Any postulated receptor would need to internalise the AI-2 analogue in order for conjugates to be effective against the bacterium.

One example of an AI-2 analogue which could be derivatised is a geminal dibromo compound **165** synthesised by Guo *et al.*[19] (see Figure 2). It is as potent as AI-2 at dissociating the LsrR repressor from the promotor region in a reporter strain, and may be more stable. It is also esterified, making it less volatile and thus easily purified using column chromatography. The esters are presumably cleaved by cellular esterases as the compounds can me used in QS assays without deprotection.[24]

A possible azido derivative **166** of this analogue is shown in Figure 2. If a route to it could be found, it appears to be a promising partner in future conjugates given the known properties of AI-2 **23**.

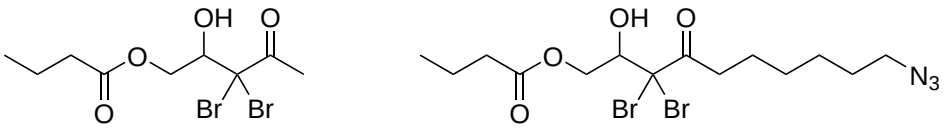


Figure 2: An AI-2 analogue **165** synthesised by Guo *et al.* and the proposed azido AI-2 analogue derivative **166**.

4.2 Antibiotic derivatives

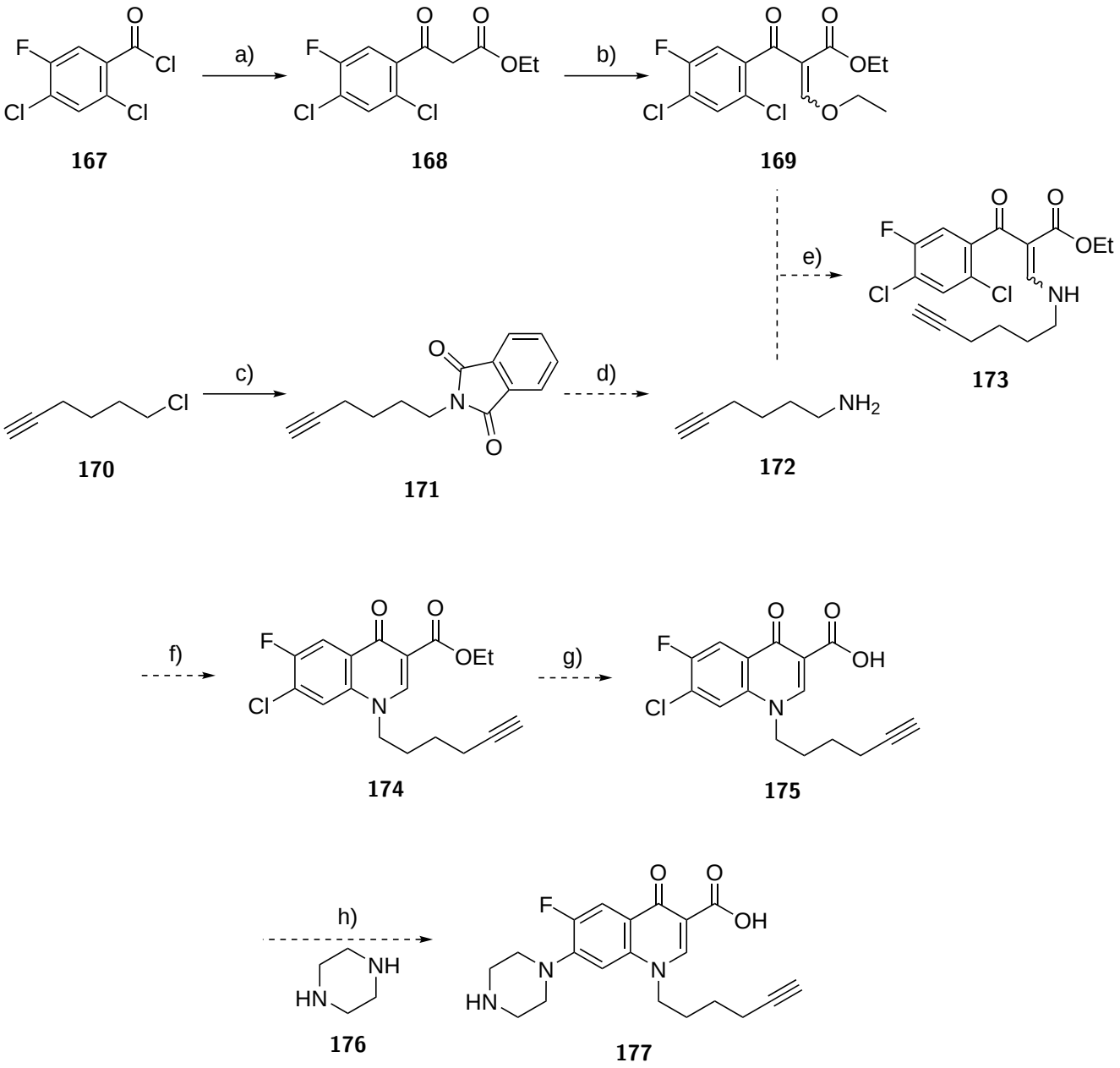
4.2.1 Ciprofloxacin derivative 177

A second alkynyl ciprofloxacin derivative **177** was planned and partially synthesised during this project, and finishing this synthesis would provide a useful intermediate for future conjugates.

The derivative **177** has an alkyne tail attached in place of the cyclopropane ring as it has been shown that bulkier groups in this position can be tolerated.[25,26] This synthesis follows a conventional route to ciprofloxacin similar to that reported by Mitscher *et al.*[25] but using hex-5-yn-1-amine **172** instead of cyclopropylamine.

The $TiCl_4$-catalysed crossed Claisen condensation of the acid chloride **167** and ethyl acetate described by Hashimoto *et al.*[27] was used to produce the β-ketoester. The ethoxymethylene group in **169** was installed by the

reaction of β-ketoester **168** and triethyl orthoformate to give a mixture of the E and Z isomers.[25,28] Hex-5-yn-1-amine **172** was prepared using a Gabriel synthesis[29] described by Rożkiewicz *et al.*[30] Unfortunately the amine was surprisingly volatile and was lost on evaporation of the reaction solvent. If a better purification method could be found the rest of the synthesis could be performed and the resulting alkynyl ciprofloxacin derivative **177** could be used to form more triazole-linked conjugates.

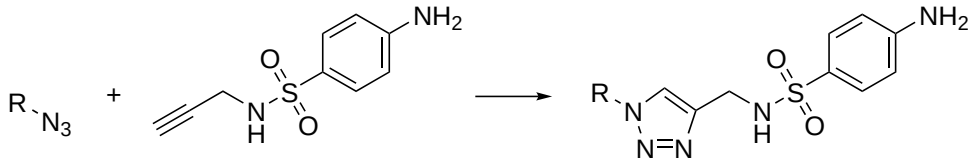


Scheme 2: The synthesis of **177**. a) EtOAc, $TiCl_4$, DIPEA, N-methyl imidazole, toluene, r.t., 30 min. b) Triethyl orthoformate, Ac_2O, reflux, 2 h. c) Potassium phthalimide, potassium iodide, DMF, 80 °C, 18 h. d) $N_2H_2 \cdot H_2O$, EtOH, reflux, 18 h. e) EtOH. f) NaH, dioxane. g) KOH, THF. h) DMSO.

4.2.2 Sulfanilamide derivatives

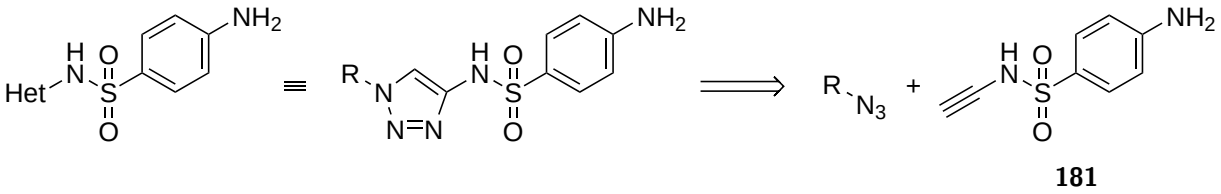
Sulfanilamide antibiotics were the first class of antibiotics to be widely used.[31,32] They are all derivatives of 4-aminobenzenesulfonamide, very commonly with the sulfonamide nitrogen linking to a heterocycle. Sulfanilamide antibiotics function by inhibiting bacterial synthesis of folic acid.

Derivatives of 4-aminobenzenesulfonamide **179** have previously been synthesised using copper(I)-catalyzed alkyne-azide cycloaddition reactions to append various groups[33] (see Scheme 3). However, if one considers sulfonamide antibiotics already in use, nearly all have a heterocycle linked directly to the sulfur atom, rather than with a methylene group in between.



Scheme 3: The sulfanilamide derivatives synthesised using click chemistry by Wang et al.[33]

Therefore, it was postulated that a 1,2,3-triazole could be introduced in the position occupied by a heterocycle in other known sulfonamide antibiotics by attachment of an alkyne directly to the sulfonamide nitrogen to form an alkynyl sulfanilamide derivative **181** or a protected version of it (see Scheme 4).



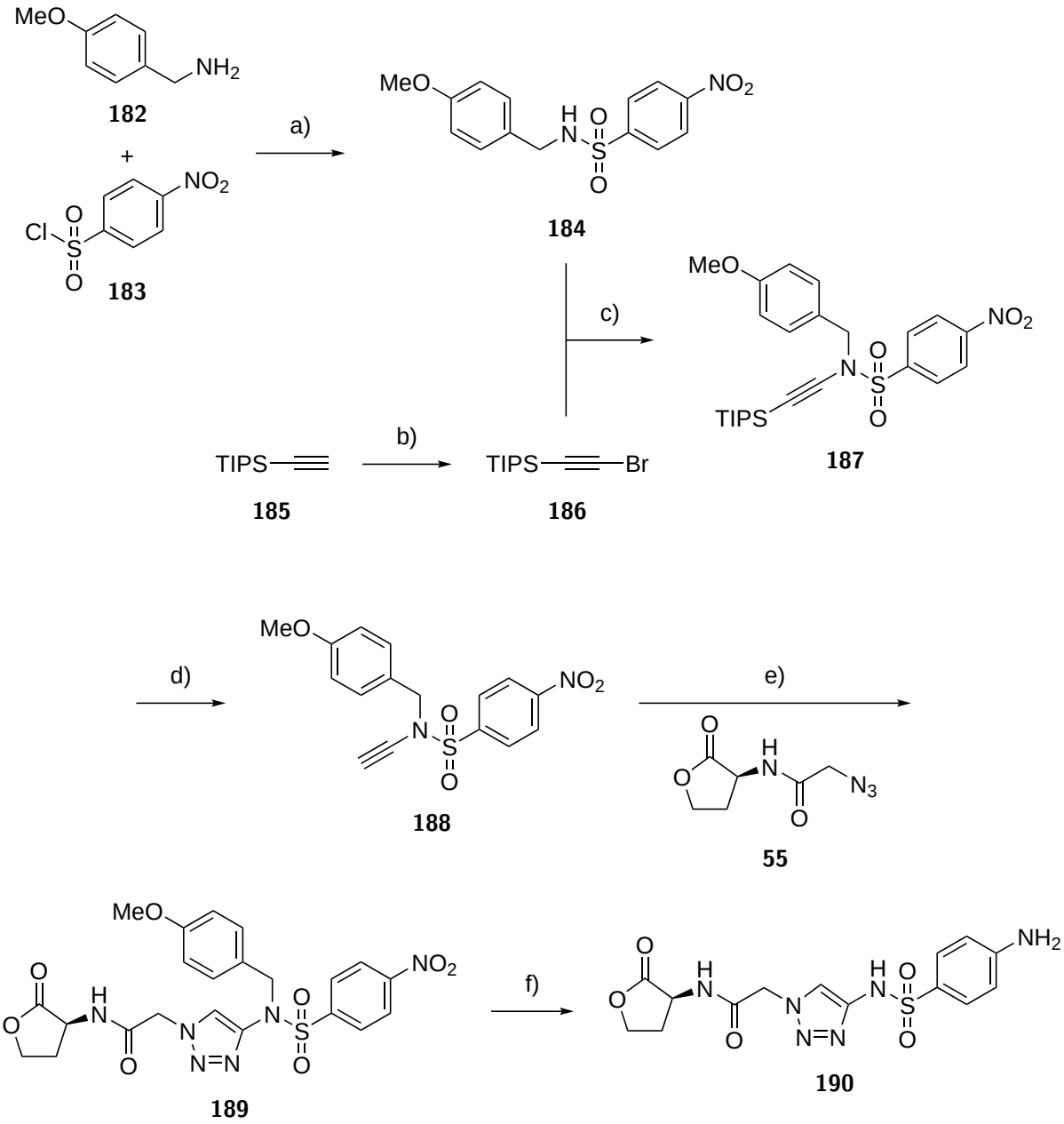
181

Scheme 4: Retrosynthesis of a 1,2,3-triazole-containing autoinducer-sulfonamide conjugate. R = autoinducer.

It was hoped that sulfanilamide derivative **181** could be synthesised and reacted with the azido autoinducer derivatives directly. However, it appears that no secondary ynamides have been synthesised to date. Conversely, the synthesis of tertiary ynamines has been studied more widely.[34] In particular, tertiary ynamides have been shown to be relatively stable and easy to work with in a variety of reactions including copper(I)-catalyzed alkyne-azide cycloadditions.[35,36]

The study of copper(I)-catalyzed alkyne-azide cycloadditions of ynamides by IJsselstijn et al.[35] includes terminal ynamides protected using a benzyl and a tosyl group. Although their click reactions proceed with high yield, they fail to present the deprotection of their final compounds. However, these reactions provided a promising suggestion that click reactions between a protected alkynyl sulfanilamide derivative and the azido autoinducer derivatives were feasible. The tosyl group used by IJsselstijn et al.[35] to protect their ynamide is very similar to the *p*-aminobenzenesulfonyl group needed in the alkynyl-sulfanilamide derivative. However, because installation of the alkyne could be problematic in the presence of a second amine, the NH_2 group was installed as a NO_2 group and reduced after the click reaction.

The synthesis proceeded as shown in **??**.[35,37,38] It was hoped that the methoxybenzyl group could be removed and the nitro group converted to an amine simultaneously by reduction in the last step, but unfortunately the methoxybenzyl group proved difficult to remove. On reflection, methoxybenzene was a poor choice of protecting group, and a more reduction-labile group such as benzyl or diphenylmethyl should have been chosen.[39] This reaction could be repeated with a different choice of protecting group to provide another set of autoinducer-antibiotic conjugates.



Scheme 5: Synthesis of a 1,2,3-triazole-containing sulfonamide antibiotic-autoinducer hybrid. a) CH_2Cl_2, r.t., 24 h. b) $AgNO_3$, acetone, r.t., 3 h. c) $CuSO_4 \cdot 5\,H_2O$, 1,10-phenanthroline, K_2CO_3, toluene, 80 °C, 48 h. d) TBAF, THF, −78 °C, 3 h. e) $Cu(OAc)_2$, sodium ascorbate, CH_2Cl_2, *t*-BuOH, water, r.t., 16 h. f) H_2, PtO_2, MeOH, 1 atm, r.t., 3 h.

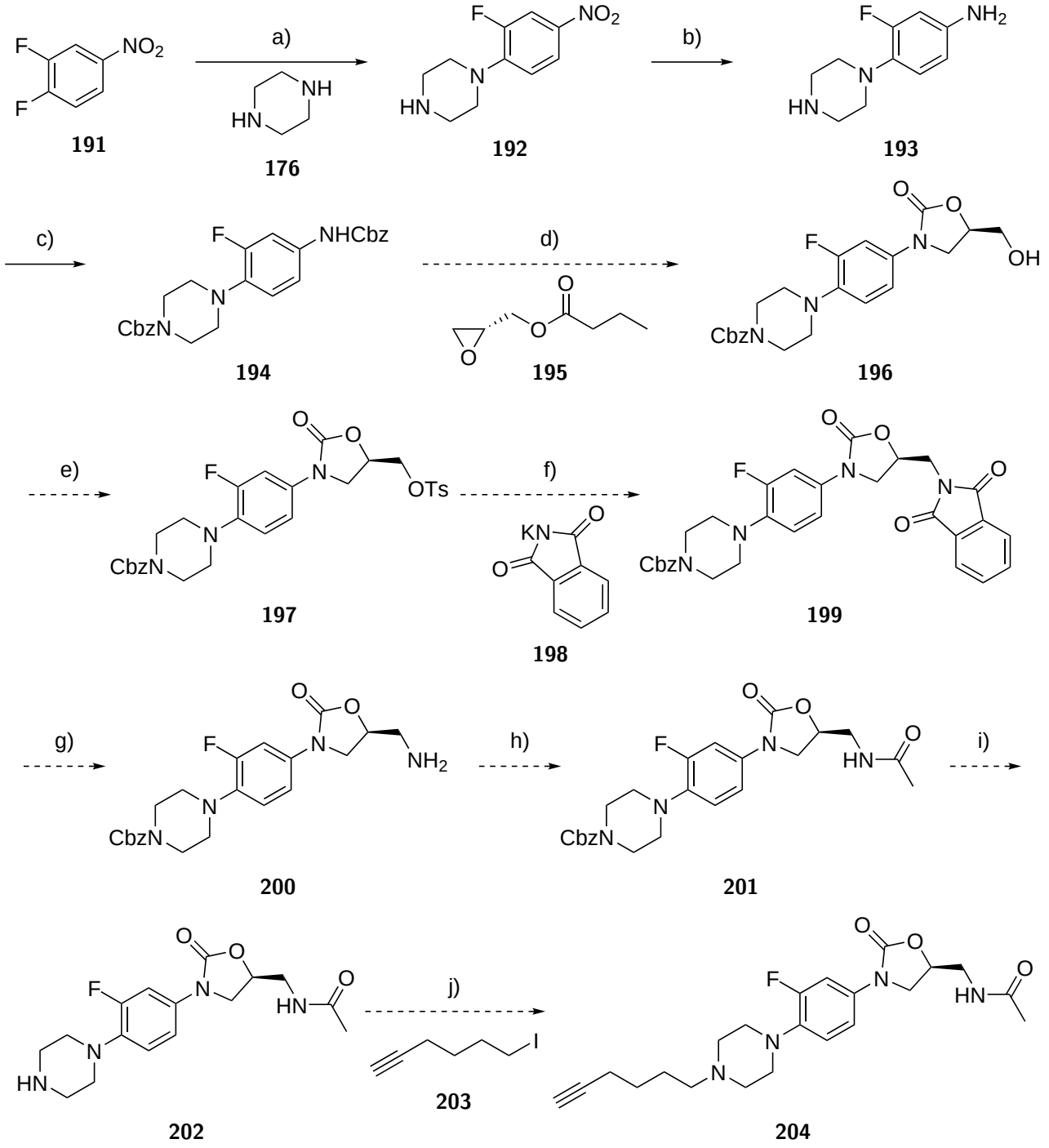
4.2.3 Linezolid derivative 204

Linezolid is a monoamine oxidase inhibitor used for the treatment of infections caused by Gram-positive bacteria. Gram-negative bacteria, including *P. aeruginosa* are resistant to linezolid due to the activity of efflux pumps, and hence it might be possible to increase its activity in such organisms by increasing its uptake and/or retention by conjugation to an autoinducer.

An alkynyl linezolid derivative **211** was partially synthesised by Ryan Howard (see Scheme 6). The route follows a literature procedure described by Phetsang *et al*[40] where the morpholine ring of linezolid is replaced by piperazine, allowing an alkynyl tail to be attached to the molecule.

The first three steps were carried out on a large scale, producing 55.7 g of **194**. As all steps except the final

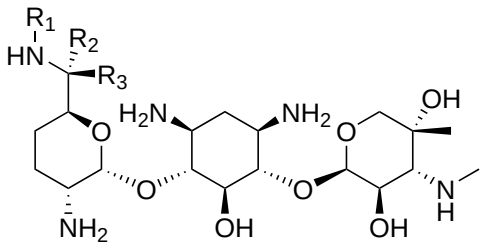
one are reported in the literature[40,41] it is hoped that the alkynyl linezolid derivative **212** could be synthesised fairly straightforwardly.



Scheme 6: Proposed and partially completed synthesis of linezolid derivative **212**.[40] a) MeCN, reflux, 3 h, 91%. b) H_2, 10% Pd/C, THF, 40 psi, <50 °C, 1.5 h, 95%. c) CbzCl, Na_2CO_3, acetone, water, 5 °C, 1 h then r.t., 16 h, 56%. d) *n*-BuLi, THF, -78 °C, 1 h then add epoxide then -78 °C to r.t., 5 h. e) TsCl, TEA, CH_2Cl_2, 0 °C to r.t. 4.5 h. f) Acetonitrile, water, reflux, 48 h. g) $MeNH_2$, EtOH, water, reflux, 5.5 h. h) Ac_2O, pyridine, 0 °C to r.t., 16 h. i) H_2, 10% Pd/C, MeOH/CH_2Cl_2, 1 atm, r.t., 16 h. j) NEt_3, EtOH, reflux.

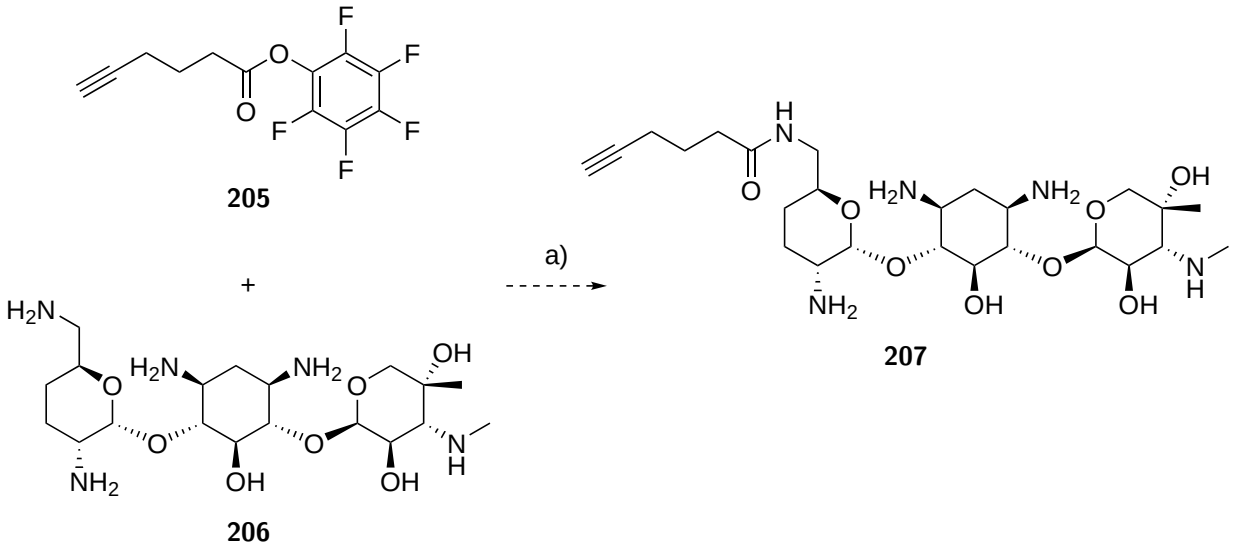
4.2.4 Gentamicin derivative 207

Gentamicin is an aminoglycoside antibiotic used to treat many bacterial infections, particularly those caused by Gram-negative organisms, by binding to the bacterial ribosome. Gentamicin is actually a mixture of components (see Figure 3) synthesised by *Micromonospora sp.*, a genus of Gram-positive bacteria. Separation of the gentamicin components has been achieved by Grote *et al.*[42] by reaction with benzyl chloroformate followed by HPLC and hydrogenolysis of the protecting groups. Gentamicin C1a **206** was isolated pure, and is particularly useful because it the only component which contains a CH_2NH_2 group. This group is less hindered than all other amine groups in gentamicin C1a **206** and hence it is possible to selectively derivatise the molecule at this position. Grote *et al.* attached a tag needed for an immunoassay using a pentafluorophenyl ester.[43] Hence, it may be possible to achieve selective reaction of this site with the pentafluorophenyl ester of 5-hexynoic acid **205** (see Scheme 7). It may even be possible to react the original gentamicin mixture with the pentafluorophenyl ester **205** and then separate out the desired component.



Gentamicin	R_1	R_2	R_3
C1	Me	Me	H
C1a	H	H	H
C2	H	Me	H
C2a	H	H	Me
C2b	Me	H	H

Figure 3: Gentamicin components.

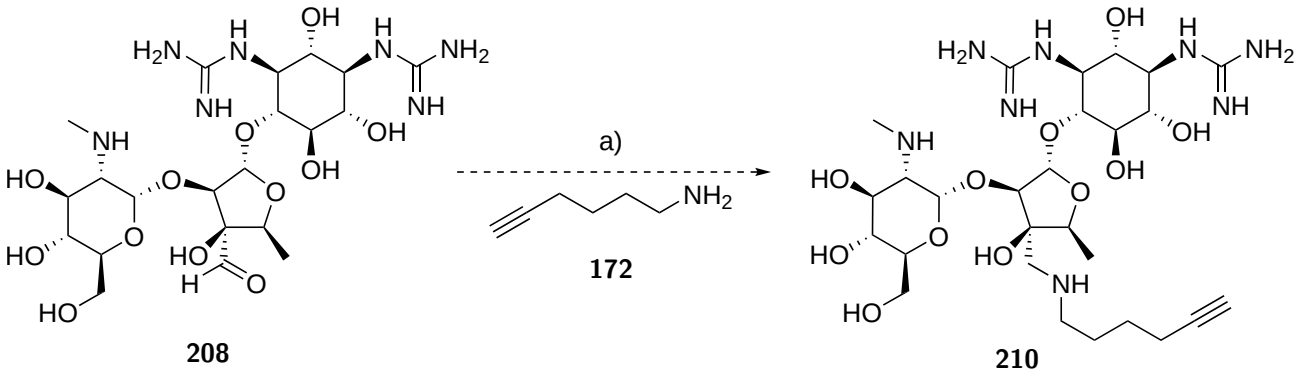


Scheme 7: Proposed synthesis of gentamicin C1a derivative **207**. a) DIPEA, DMF, - 55 °C.

4.2.5 Streptomycin derivative 210

Streptomycin **208** is an aminoglycoside antibiotic used to treat *Mycobacterium tuberculosis* and *S. aureus* which works by binding to the bacterial ribosome. There is limited SAR data on streptomycin but it is known that conversion of the aldehyde to a carboxylic acid destroys activity, whereas conversion an alcohol retains it.[44]

Reductive amination can be used to install an alkyne group by reaction of the aldehyde with an amine such as hex-5-yn-1-amine **172** (see Scheme 8). This approach has been used by Zhang *et al.*[45] to form a conjugate of streptomycin **208** and chitosan which was active against biofilms. Reductive amination replaces the aldehyde O with NH; it is known that an OH is tolerated at this position so it makes sense that NH is as well.



Scheme 8: Proposed synthesis of streptomycin derivative **210**. a) $NaBH_3CN$, water, r.t..

4.3 Autoinducer analogue derivatives

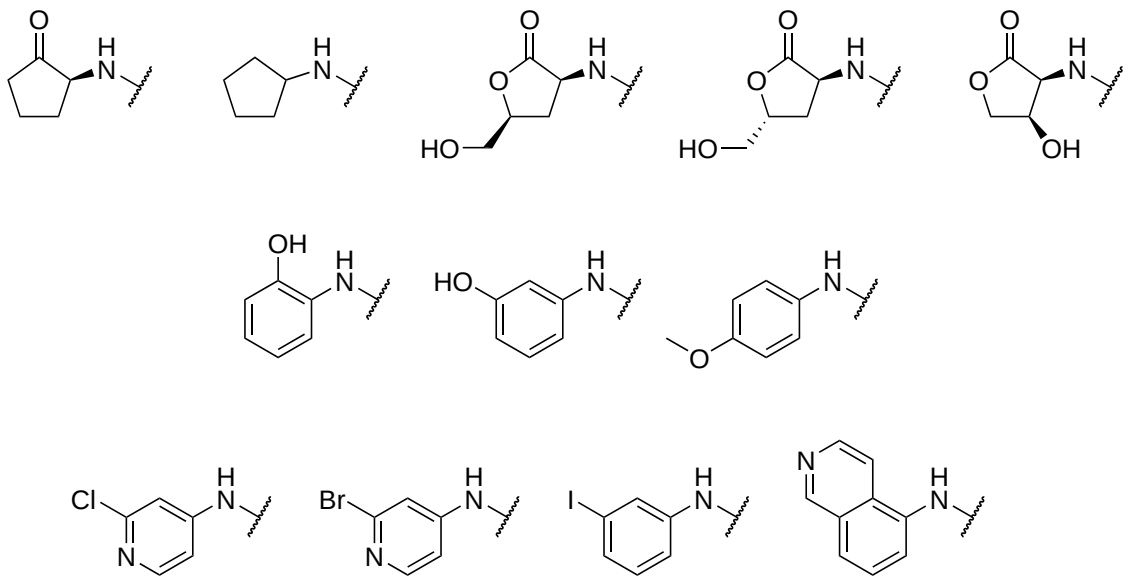


Figure 4: HSL analogue head groups for use in future conjugates.

4.4 Biology

5 References

[1] U.S. Centers for Disease Control and Prevention, *Antibiotic Resistance Threats in the United States*. 2013.

[2] S. C. Davies. *The Drugs Don't Work: A Global Threat*. Penguin Books Limited, 2013.

[3] K. M. G. O'Connell, J. T. Hodgkinson, H. F. Sore, M. Welch, P. George, C. Salmond, D. R. Spring and G. P. C. Salmond. Combating multidrug-resistant bacteria: current strategies for the discovery of novel antibacterials. *Angewandte Chemie International Edition*, 52(41):10706–10733, 2013.

[4] M. G. P. Page. Siderophore conjugates. *Annals of the New York Academy of Sciences*, 1277:115–126, 2013.

[5] I. J. Schalk and G. L. A. Mislin. Bacterial iron uptake pathways: gates for the import of bactericide compounds. *Journal of Medicinal Chemistry*, 60(11):4573–4576, 2017.

[6] C. M. Waters and B. L. Bassler. Quorum sensing: cell-to-cell communication in bacteria. *Annual Review of Cell and Developmental Biology*, 21:319–346, 2005.

[7] M. B. Miller and B. L. Bassler. Quorum sensing in bacteria. *Annual Review of Microbiology*, 55:165–199, 2001.

[8] C. W. Tornøe, C. Christensen and M. Meldal. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *The Journal of Organic Chemistry*, 67(9):3057–3064, 2002.

[9] V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angewandte Chemie International Edition*, 41(14):2596–2599, 2002.

[10] G. P. Bodey, R. Bolivar, V. Fainstein and L. Jadeja. Infections caused by *Pseudomonas aeruginosa*. *Reviews of Infectious Diseases*, 5(2):279–313, 1983.

[11] K. Poole. Efflux-mediated multiresistance in Gram-negative bacteria. *Clinical Microbiology and Infection*, 10(1):12–26, 2004.

[12] J.-F. Dubern and S. P. Diggle. Quorum sensing by 2-alkyl-4-quinolones in *Pseudomonas aeruginosa* and other bacterial species. *Molecular BioSystems*, 4(9):882–888, 2008.

[13] A. P. Macgowan, M. Wootton and H. A. Holt. The antibacterial efficacy of levofloxacin and ciprofloxacin against *Pseudomonas aeruginosa* assessed by combining antibiotic exposure and bacterial susceptibility. *Journal of Antimicrobial Chemotherapy*, 43:345–349, 1999.

[14] H.-C. Su, K. Ramkissoon, J. Doolittle, M. Clark, J. Khatun, A. Secrest, M. C. Wolfgang and M. C. Giddings. The development of ciprofloxacin resistance in *Pseudomonas aeruginosa* involves multiple response stages and multiple proteins. *Antimicrobial Agents and Chemotherapy*, 54(11):4626–4635, 2010.

[15] J. T. Hodgkinson. The synthesis of *Pseudomonas* quinolone signal analogues and their effects on quinolone signalling in *Pseudomonas aeruginosa*. PhD thesis, University of Cambridge, 2011.

[16] M. E. Taga, S. T. Miller and B. L. Bassler. Lsr-mediated transport and processing of AI-2 in *Salmonella typhimurium*. *Molecular Microbiology*, 50(4):1411–1427, 2003.

[17] C. S. Pereira, J. A. Thompson and K. B. Xavier. AI-2-mediated signalling in bacteria. *FEMS Microbiology Reviews*, 37(2):156–181, 2013.

[18] J. Stokes. Synthesis of antibiotic-AI-2 conjugates. Unpublished report. 2017.

[19] M. Guo, Y. Zheng, J. L. Terell, M. Ad, C. Opoku-Temeng, W. E. Bentley and H. O. Sintim. Geminal dihalogen isosteric replacement in hydrated AI-2 affords potent quorum sensing modulators. *Chemical Communications*, 51(13):2617–2620, 2015.

[20] X. Chen, S. Schauder, N. Potier, A. V. Dorsselaer, Â. Pelczer, B. L. Bassler and F. M. Hughson. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature*, 415:545–549, 2002.

[21] S. T. Miller, K. B. Xavier, S. R. Campagna, M. E. Taga, M. F. Semmelhack, B. L. Bassler and F. M. Hughson. *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Molecular Cell*, 15:677–687, 2004.

[22] M. B. Neiditch, M. J. Federle, S. T. Miller, B. L. Bassler and F. M. Hughson. Regulation of LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. *Molecular Cell*, 18(5):507–518, 2005.

[23] C. S. Pereira, A. K. D. Regt, P. H. Brito, S. T. Miller and K. B. Xavier. Identification of functional LsrB-like autoinducer-2 receptors. *Journal of Bacteriology*, 191(22):6975–6987, 2009.

[24] M. Guo, S. Gamby, S. Nakayama, J. Smith and H. O. Sintim. A pro-drug approach for selective modulation of AI-2-mediated bacterial cell-to-cell communication. *Sensors*, 12:3762–3772, 2012.

[25] L. A. Mitscher, P. N. Sharma, D. T. W. Chu, L. L. Shen and A. G. Pernett. Chiral DNA gyrase inhibitors. 1. Synthesis and antimicrobial activity of the enantiomers of 6-fluoro-7-(1-piperazinyl)-1-(2'-*trans*-phenyl-1'-cyclopropyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid. *Journal of Medicinal Chemistry*, 29:2044–2047, 1986.

[26] D. T. W. Chu, P. B. Fernandes, A. K. Claiborne, E. Pihuleac, C. W. Nordeen, R. E. Maleczka and A. G. Pernet. Synthesis and structure-activity relationships of novel arylfluoroquinolone antibacterial agents. *Journal of Medicinal Chemistry*, 28(11):1558–1564, 1985.

[27] N. Hashimoto, T. Funatomi, T. Misaki and Y. Tanabe. Practical method for the synthesis of (*R*)-homopipecolinic acid and (*R*)-homoproline esters from ω-chloroalkanoic acids and available chiral amines. *Tetrahedron*, 62(10):2214–2223, 2006.

[28] P. Senthilkumar, M. Dinakaran, P. Yogeeswari, D. Sriram, A. China and V. Nagaraja. Synthesis and antimycobacterial activities of novel 6-nitroquinolone-3-carboxylic acids. *European Journal of Medicinal Chemistry*, 44(1):345–358, 2009.

[29] S. Gabriel. Ueber eine Darstellungsweise primarer Amine aus den entsprechenden Halogenverbindungen. *Berichte der Deutschen Chemischen Gesellschaft*, 20(2):2224–2236, 1887.

[30] D. I. Rożkiewicz, D. Jańczewski, W. Verboom, B. J. Ravoo and D. N. Reinhoudt. "Click" chemistry by microcontact printing. *Angewandte Chemie*, 45(32):5292–5296, 2006.

[31] H. Otten. Domagk and the development of the sulphonamides. *Journal of Antimicrobial Chemotherapy*, 17:689–696, 1986.

[32] M. Wainwright and J. E. Kristiansen. On the 75th anniversary of Prontosil. *Dyes and Pigments*, 88(3):231–234, 2011.

[33] X.-L. Wang, K. Wan and C.-H. Zhou. Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities. *European Journal of Medicinal Chemistry*, 45(10):4631–9, 2010.

[34] J. Ficini. Ynamine: a versatile tool in organic synthesis. *Tetrahedron*, 32:1449–1486, 1976.

[35] M. IJsselstijn and J.-C. Cintrat. Click chemistry with ynamides. *Tetrahedron*, 62(16):3837–3842, 2006.

[36] G. Evano, A. Coste and K. Jouvin. Ynamides: versatile tools in organic synthesis. *Angewandte Chemie (International Edition in English)*, 49(16):2840–59, 2010.

[37] M. Bendikov, H. M. Duong, F. Wudl and E. Bolanos. An unexpected two-group migration involving a sulfonynamide to nitrile rearrangement. Mechanistic studies of a thermal N → C tosyl rearrangement. *Organic Letters*, 7(5):783–786, 2005.

[38] L. V. Graux, H. Clavier and G. Buono. Palladium-catalyzed addition of 1,3-diones to ynamides: an entry to alkoxy-substituted enamides. *ChemCatChem*, 6:2544–2548, 2014.

[39] P. G. M. Wuts and T. W. Greene. *Greene's Protective Groups in Organic Synthesis*. John Wiley & Sons, Inc., 4th edition, 2007.

[40] W. Phetsang, M. A. T. Blaskovich, M. S. Butler, J. X. Huang, J. Zuegg, S. K. Mamidyala, S. Ramu, A. M. Kavanagh and M. A. Cooper. An azido-oxazolidinone antibiotic for live bacterial cell imaging and generation of antibiotic variants. *Bioorganic & Medicinal Chemistry*, 22(16):4490–4498, 2014.

[41] A. Khalaj, M. Nakhjiri, A. S. Negahbani, M. Samadizadeh, L. Firoozpour, S. Rajabalian, N. Samadi, M. A. Faramarzi, N. Adibpour, A. Shafiee and A. Foroumadi. Discovery of a novel nitroimidazolyl-oxazolidinone hybrid with potent anti Gram-positive activity: synthesis and antibacterial evaluation. *European Journal of Medicinal Chemistry*, 46(1):65–70, 2011.

[42] J. Grote, R. Himmelsbach and D. Johnson. Methodology for the rapid separation of gentamicin components and regiospecific synthesis of gentamicin conjugates. *Tetrahedron Letters*, 53(50):6751–6754, 2012.

[43] P. Cheshev, L. Morelli, M. Marchesi, Č. Podlipnik, M. Bergström and A. Bernardi. Synthesis and affinity evaluation of a small library of bidentate cholera toxin ligands: towards nonhydrolyzable ganglioside mimics. *Chemistry*, 16(6):1951–67, 2010.

[44] T. L. Lemke and D. A. Williams. *Foye's Principles of Medicinal Chemistry*. Wolters Kluwer Health, 2012.

[45] A. Zhang, H. Mu, W. Zhang, G. Cui, J. Zhu and J. Duan. Chitosan coupling makes microbial biofilms susceptible to antibiotics. *Scientific Reports*, 3:1–7, 2013.

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