Journal Name

ARTICLE

Binaphthyl-1,2,3-Triazole Peptidomimetics with Activity Against *Clostridium difficile* and Other Pathogenic Bacteria

Steven M. Wales,[a] Katherine A. Hammer,[b] Amy M. King,[c] Andrew J. Tague,[a] Dena Lyras,[c] Thomas V. Riley,[b] Paul A. Keller*[a] and Stephen G. Pyne*[a]

Clostridium difficile (C. difficile) is a problematic Gram positive bacterial pathogen causing moderate to severe gastrointestinal infections. Based on a lead binaphthyl-tripeptide dicationic antimicrobial, novel mono-, di-and tri-peptidomimetic analogues targeting *C. difficile* were designed and synthesized incorporating one, two or three D-configured cationic amino acid residues, with a common 1,2,3-triazole ester isostere at the C-terminus. Copper- and ruthenium-click chemistry facilitated the generation of a 46 compound library for *in vitro* bioactivity assays, with structure-activity trends over the largest compound subset revealing a clear advantage to triazole-substitution with a linear or branched hydrophobic group. The most active compounds were dicationic-dipeptides where the triazole was substituted with a 4- or 5-cyclohexylmethyl or 4,5-diphenyl moiety, providing MICs of 4 µg mL^{-1} against three human isolates of *C. difficile*. Further biological screening revealed significant antimicrobial activity for several compounds against other common bacterial pathogens, both Gram positive and negative, including *S. aureus* (MICs ≥2 µg mL^{-1}), *S. pneumoniae* (MICs ≥1 µg mL^{-1}), *E. coli* (MICs ≥4 µg mL^{-1}), *A. baumannii* (MICs ≥4 µg mL^{-1}) and vancomycin-resistant *E. faecalis* (MICs ≥4 µg mL^{-1}).

Introduction

Unique antibacterial molecules with new modes of action are currently in high demand for the treatment of antibiotic-resistant bacterial infections.[1] In particular, the Gram positive, gastric anaerobe *Clostridium difficile* has been recently listed as the number one bacterial threat in the USA.[2] *C. difficile* produces potent toxins which cause symptoms such as diarrhea, abdominal pain and pseudomembranous colitis (PMC) upon gastrointestinal infection.[3–5] *C. difficile* infection (CDI) occurs in up to 20% of patients given conventional oral antibiotic therapy due to elimination of the commensal, gastrointestinal microflora.[3,4] High morbidity and up to an 8% mortality rate highlights the seriousness of this disease, with both the severity and incidence increasing due to epidemics of hypervirulent strains.[3–5] Further, the nosocomial nature of the infection and the persistence of *C. difficile* spores results in a major health problem and a serious financial burden to the healthcare sector (>$1 billion/year in the USA alone).[5]

Traditional medications for the treatment of CDI (vancomycin and metronidazole) are inadequate as reinfection occurs in 15–35% of patients.[3,4] Fidaxomicin was approved by

the Food and Drug Administration in 2011 for the treatment of CDI, exhibiting a decrease in recurrence relative to that of vancomycin and metronidazole.[6,7] Potential CDI chemotherapeutics are currently under various stages of development, these include REP3123,[8] nitazoxanide,[9] fusidic acid,[5] teicoplanin,[5,10] LFF571,[11] CB-183 315[12] and cationic nylon-3 polymers.[13] Furthermore, monoclonal antitoxin antibodies are under investigation as potential treatments for CDI recurrence.[7] This intense interest in novel CDI therapeutics is a direct result of the inadequacy of current medications, the continuing cost to the healthcare sector and the emergence of hypervirulent strains.

Previous research in our laboratory has led to the development of a promising new class of antimicrobials based upon a binaphthyl-tripeptide structure (e.g., **1**, Fig. 1).[14–16] These derivatives exhibit selective inhibition of Gram negative bacteria, as well as broad-spectrum activity against Gram positive bacteria including vancomycin-, linezolid- and methicillin-resistant strains.[14–16] Efficacy has been translated *in vivo*, both topically and systemically, for infections of the latter.[14] A rudimentary pharmacophore has emerged comprising an anchoring (semi)rigid aromatic core (binaphthyl/biphenyl), two cationic residues and a hydrophobic moiety at the peptide C-terminus.[14–16]

With a continuing interest in expanding the applications of these binaphthyl-based antimicrobials, we were inspired to investigate their potential as chemotherapeutics for CDI. We envisioned that the non-drug-like features and high molecular weight of these molecules would ensure limited oral

a. *School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia. E-mail: keller@uow.edu.au; spyne@uow.edu.au*
b. *School of Pathology and Laboratory Medicine, The University of Western Australia, Crawley, WA 6009, Australia.*
c. *Department of Microbiology, Monash University, Clayton, VIC 3800, Australia.*

COMMUNICATION

bioavailability, making them ideal candidates for the treatment of gastrointestinal infections. Initial inspiration came from preliminary *in vitro* testing of **1** against *C. difficile*, which exhibited a promising minimum inhibitory concentration (MIC) of 8 μg mL^{-1} against two problematic ribotype 027 strains. This is the first example of this class of compounds inhibiting an anaerobic bacterial strain. Therefore, this lead compound formed the basis of this current study, which aimed to replace the terminal ester with a peptidomimetic 1,2,3-triazole moiety[17] for increased metabolic stability in the gut.[18] We report here the synthesis and antimicrobial activities of forty six novel binaphthyl-1,2,3-triazole peptide derivatives, resulting in the identification of several promising compounds with improved activity against *C. difficile* and various other bacterial pathogens relative to compound **1**.
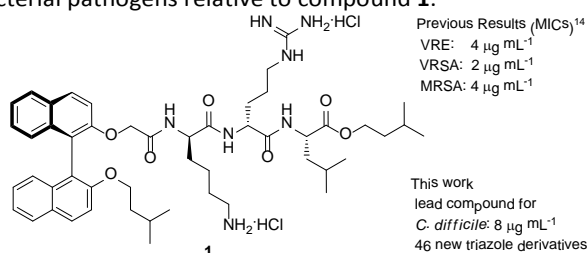

Fig. 1 Antibacterial compound **1** developed previously in our laboratory.[14]

Results and Discussion

The synthetic binaphthyl-1,2,3-triazole peptides for this investigation were divided into three target classes (A–C) based on the number of embedded amino acid residues (Fig. 2). Class A was the starting point for our molecular design, given that is an isostere analogue of our existing broad-spectrum antibacterials which incorporate three amino acid residues (e.g., ester **1**). Smaller peptide classes B and C, bearing two and one amino acid residues respectively, were chosen as additional targets to establish the necessary components for activity against *C. difficile* and other bacterial pathogens. Within classes A and C, we also planned to perform minor variations in the amino acid identity at AA^1 and AA^3. A cationic arginine was to be maintained as the second residue (AA^2) throughout.[14–16] Our previous studies have shown that all stereoisomers of **1** have similar activity,[14] thus we aimed to incorporate the unnatural D-configured cationic residues due to an expected increase in enzymatic stability.[19]
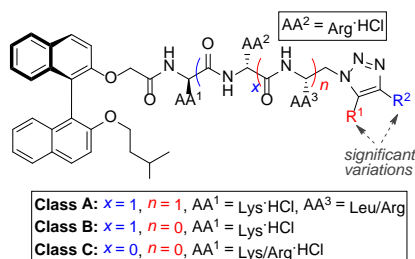

Fig. 2 Binaphthyl-triazole peptide classes (A–C) synthesized in this study.

An overview of our proposed synthetic strategy is shown in Scheme 1, exemplified with a Class B scaffold. The peptide backbone would be assembled from the N-terminus by the iterative coupling of protected amino acid and amino azide components, resulting in a net-replacement of the C-terminus with an azide moiety. This modular approach would allow variation of the number and type of amino acid residues incorporated, enabling straightforward access to all three target classes. The terminal triazole ester isostere, common to all target peptides, could then be assembled by cycloadditions with alkynes and arynes. Finally, basic amino functionalities would be revealed as their hydrochloride salts by acidolysis of the Boc and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) protecting groups.
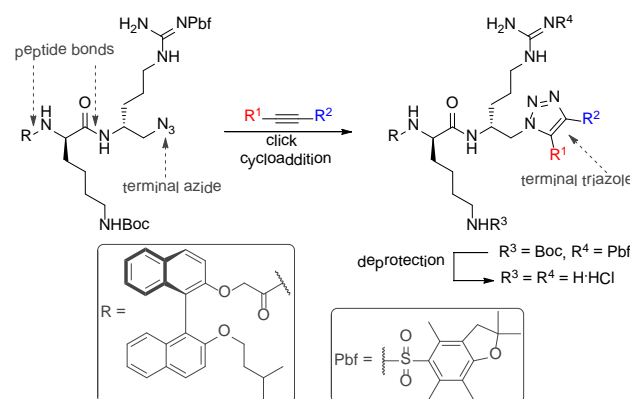
Scheme 1 Brief overview of the synthesis of cationic triazole peptides, exemplified with a Class B scaffold.

In addition to the prevalence of 1,2,3-triazole-containing antibacterials,[20] this heterocyclic motif offers clear advantages in terms of synthetic possibilities, given its reliable access via metal-catalyzed azide-alkyne click chemistry.[21] This atom economical cycloaddition is arguably the most efficient of all click processes, offering a high yielding, regioselective and functionally tolerant entry to 1,2,3-triazoles. Thus, strategic incorporation of click chemistry at the penultimate stages of our syntheses should permit rapid, divergent access to new heterocyclic derivatives based on readily available alkynes, enabling a thorough structure-activity exploration at the peptide termini (R^1 and R^2). A variety of conditions (A–E, Fig. 3) were adapted from the literature for the cycloadditions conducted throughout this study, including ruthenium-catalyzed variants to reverse the regiochemistry obtained by conventional copper catalysis.[22–26]

A[22]: alkyne (3 equiv), Cu(OAc)$_2$ (20 mol %), Na·ascorbate (40 mol %), *t*-BuOH:H$_2$O (4:1), rt
B[23]: alkyne (2 equiv), Cp*RuCl(PPh$_3$)$_2$ (5 mol %), dioxane, 60 °C
C[24]: alkyne (5–10 equiv), toluene, 100 °C
D[25]: alkyne (1.8 equiv), CuI (10 mol %), NEt$_3$, THF, rt
E[26]: aryne precursor (2 equiv), CsF (4 equiv), MeCN, rt

Fig. 3 Conditions for azide−alkyne cycloadditions used in this study.[22–26]

The synthesis of Class A compounds began with the coupling of lysine-based acid **2**[14] and protected arginine ester **3**,[27] giving dipeptide **4** in 92% yield after saponification (Scheme 2a). This intermediate was coupled with leucine-derived amino azide **5**[28] and separately, the novel arginine-

derived amino azide **14** (Scheme 2b) to give terminal azides **6** and **15**, respectively, in near quantitative yields. Excess amine (1.5–2.0 equiv) was necessary during these reactions to avoid competitive consumption of **4** via intramolecular attack of its protected guanidine on the activated acid, which occurred as a side reaction under stoichiometric conditions.

With azide **6** in hand, copper-catalyzed click reactions (conditions A, Fig. 3) with selected terminal alkynes proceeded smoothly to produce triazoles **7**–**9** in 63–93% yields (Scheme 2a). The hydrophobic nature of the binaphthyl-azide constituted the need for a 4:1 t-BuOH:H$_2$O binary solvent, as

opposed to the standard 1:2 t-BuOH:H$_2$O more polar medium.[22] The quantities of inexpensive reagents: alkyne, Cu(OAc)$_2$ and Na·ascorbate were not optimized due to the small scale of the reactions (<0.1 mmol of azide). Prior to deprotection of the leucine-containing Class A compounds, alcohol **7** was converted to its methyl ether derivative **10**. Subsequent treatment of **8**–**10** with TFA, followed by anion exchange with ethereal HCl, afforded dihydrochloride salts **11**–**13** (Scheme 2a; insert). Similarly, a tricationic Class A derivative **17** was prepared in good overall yield via cycloaddition of **15** with 5-methyl-1-hexyne (Scheme 2b).

a)

b)
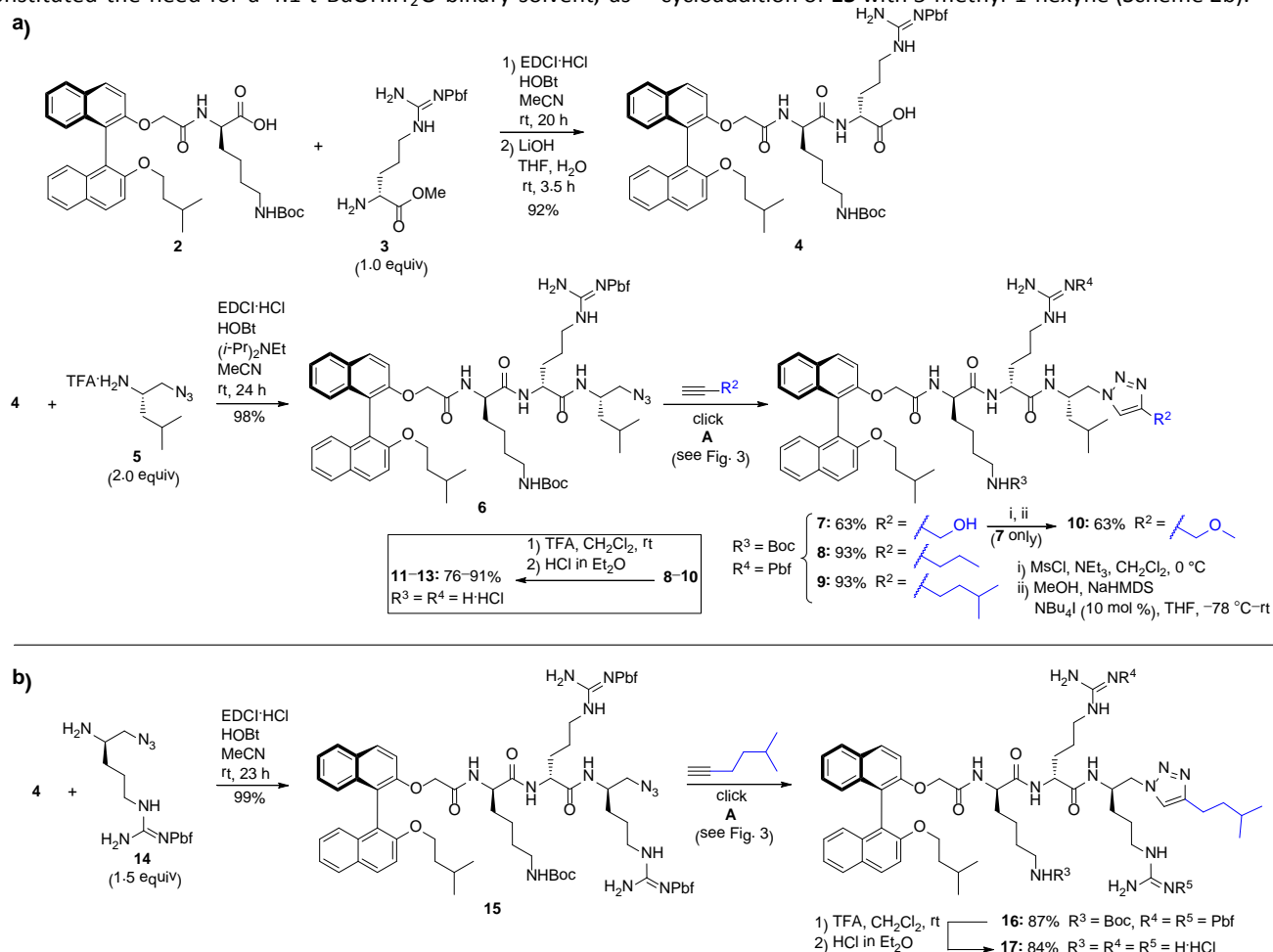
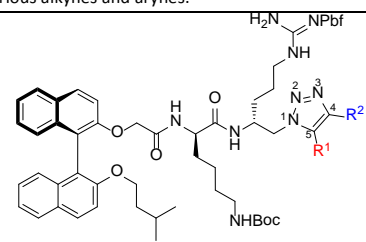**Scheme 2** Synthesis of Class A triazoles bearing three amino acid residues.
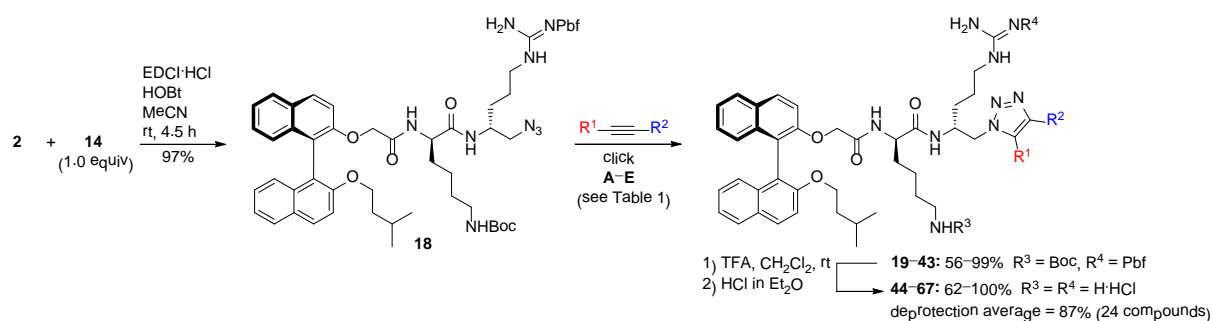
Scheme 3 Synthesis of Class B triazoles bearing two amino acid residues. See Table 1 for specific click conditions and yields.

COMMUNICATION
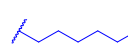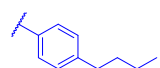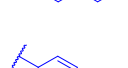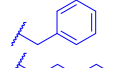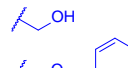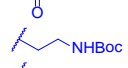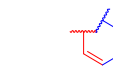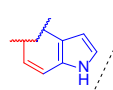
Class B triazoles precursor **18** was obtained in multigram quantities by the coupling of acid **2**[14] with **14** (Scheme 3). Cycloaddition reactions were then carried out under a range of conditions as summarized in Table 1. All unsymmetrically substituted non-fused triazoles (R[1] ≠ R[2]) were formed with >95% regiochemical purity as determined by NMR analysis. A diverse set of 1,4-disubstituted triazoles (R[1] = H, R[2] ≠ H) were obtained by copper-catalyzed conditions A,[22] which accommodated a number of terminal alkynes including functionalized versions and those substituted with primary alkyl, tertiary alkyl, cyclic and aryl hydrocarbons. Under conditions A, good to excellent yields were obtained throughout, with the exception of the electron-poor methyl propiolate, which afforded triazole ester **38** in a moderate 56% yield (Table 1, entry 20). Silylacetylenes reacted slowly under the copper catalyzed conditions and afforded mixtures containing de-silylated products. Therefore, thermal Huisgen cycloaddition was used for these substrates, allowing the sterically controlled installation of trimethylsilyl (TMS) and dimethylphenylsilyl groups at R[2] in high yields (conditions C, entries 9 and 10).[24]

The 1,5-regioisomers (R[1] ≠ H, R[2] = H) of selected Class B triazoles were prepared by Ru(II) catalysis in 70–82% yield (conditions B, Table 1, entries 3, 7 and 12).[23] Notable differences in the NMR spectra were observed in comparison to their previously prepared regioisomers. For example, the lone triazole proton (R[2] = H) of compound **30** (entry 12) appeared as a singlet at 7.69 ppm in the ^{1}H NMR spectrum, in contrast to its 1,4-regioisomer **29** (entry 11) in which this proton (R[1] = H) resonated at 8.06 ppm. Ru(II) activation was also suitable for internal alkyne diphenylacetylene,[29] providing a fully substituted triazole **40** in excellent yield (entry 22). Additionally, a halogen was introduced at C-5 (R[1]) of the triazole ring through CuI mediated cycloaddition of 1-iodo-phenylacetylene and **18** (conditions D, entry 23).[25]

Fused benzotriazole **42** was synthesized via the cycloaddition of azide **18** with in situ generated benzyne (conditions E, Table 1, entry 24).[26] This protocol was also extended to a heteroaryne, producing the indole-fused triazole **43** in a reasonable 58% yield, albeit as an approximate 2:1 mixture of regioisomers (entry 25). The structure of the major isomer could not be determined due to poor resolution of regioisomeric peaks in the ^{1}H NMR spectrum, however it is noteworthy that a previous indolyltriazole formed in this manner showed moderate preference for azide attack (triazole N-1) at the indole 5-position (2.4:1 isomeric ratio).[30] Commercially available pyridyne precursors were also screened in this (hetero)aryne cycloaddition, however, with the need to utilize azide **18** as the limiting reagent, pyridine polymerization[31] occurred almost exclusively over the desired cycloaddition.

Table 1 Conditions and yields for the preparation of Class B triazoles via cycloaddition of azide **18** with various alkynes and arynes.

Entry	R[1]	R[2]	Cond[a]	Prod	Yield (%)
1	H	(alkyl)	A	**19**	90
2	H	(alkyl)	A	**20**	67
3	(alkyl)	H	B	**21**	74
4	H	(alkyl)	A	**22**	94
5	H	(cyclohexyl)	A	**23**	82
6	H	(cyclohexyl)	A	**24**	93
7	(cyclohexyl)	H	B	**25**	70
8	H	(t-Bu)	A	**26**	99
9	H	(Si)	C	**27**	93
10	H	(SiPh)	C	**28**	96
11	H	(Ph)	A	**29**	90
12	(Ph)	H	B	**30**	82
13	H	(aryl)	A	**31**	87
14	H	(aryl-OMe)	A	**32**	79
15	H	(benzyl)	A	**33**	85
16	H	(benzyl)	A	**34**	81
17	H	(OH)	A	**35**	92
18	H	(OBn)	A	**36**	75
19	H	(OEt)	A	**37**	87
20	H	(CO$_2$Me)	A	**38**	56
21	H	(NHBoc)	A	**39**	78
22	(Ph)	(Ph)	B	**40**	94
23	I	(Ph)	D	**41**	85
24[b]		(benzyne)	E	**42**	81
25[b]		(indole) 2:1 regioisomers	E	**43**	58

[a] See Fig. 3 for reaction conditions. [b] Prepared by reaction with the appropriate (hetero)aryne.

Journal Name

COMMUNICATION

Although the preferred regiochemical outcome of conditions A–D could be confidently inferred from literature precedent,[22–25] we were able to confirm the expected orientations of representative triazoles **28**, **30**, **31** and **41** by two-dimensional HSQC and HMBC NMR experiments (Fig. 4). In each case, a through-bond correlation between the methylene hydrogens adjacent to the triazole and C-5 of the ring provided unequivocal evidence of the assigned structure.
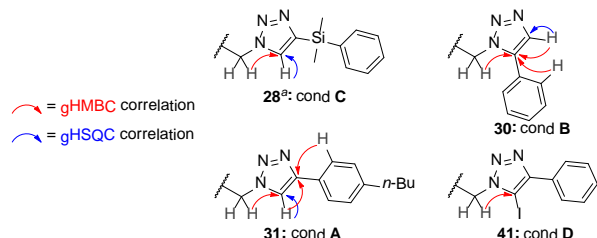
Fig. 4 Key NMR through-bond H–C correlations used to confirm the regiochemistry of representative Class B triazoles. [a] Spectra acquired for deprotected derivative.

With the exception of triazole ester **38**, all Class B triazoles documented in Table 1 were converted to their corresponding hydrochloride salts **44**−**67** in an average yield of 87% (Scheme 3). Selected click products from this Class B set (in protected form) were also used to access further derivatives as shown in Schemes 4−6. Unsubstituted triazole **69** was obtained through the desilylation of compound **27** with TBAF (Scheme 4). Removal of the TMS group was extremely slow, requiring 7 d at rt to give intermediate **68** in 91% yield.
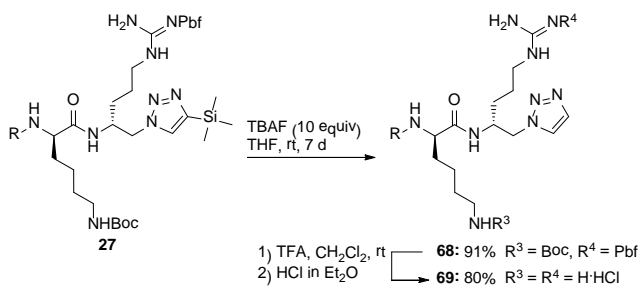
Scheme 4 Synthesis of unsubstituted Class B triazole **69**.

Further Class B ether derivatives **77**−**83**, unattainable from commercially available alkynes via click chemistry, were synthesized in a divergent fashion from alcohol **35** (Scheme 5). The mesylate ester derivative was treated with preformed alkoxides or phenoxide using NBu_4I as an additive, giving penultimate protected ethers **70**−**76** in 41−85% yield. The efficiency of this etherification generally decreased with increasing length of the alkyl chain. It should be noted that compound **75** was also prepared in 79% yield by the copper catalyzed click reaction of azide **18** with pre-synthesized *i*-heptyl propargyl ether (reaction not shown). This more convergent approach could prove superior for future scale-up of a selected compound, but would have proven synthetically more cumbersome if used to access all desired ether derivatives.

Finally, Class B ester **38** was used for the synthesis of amide derivatives after saponification to carboxylic acid **84** (Scheme 6). Initially, we were surprised to find that neither our standard EDCI·HCl/HOBt coupling conditions nor a HATU/(*i*-

Pr)$_2$NEt system were successful for amide bond formation, resulting in recovery of unreacted starting materials. Eventually, we found that acid **84** could be activated as a mixed anhydride,[32] although excess reagents were necessary to obtain synthetically useful yields of the desired amides.
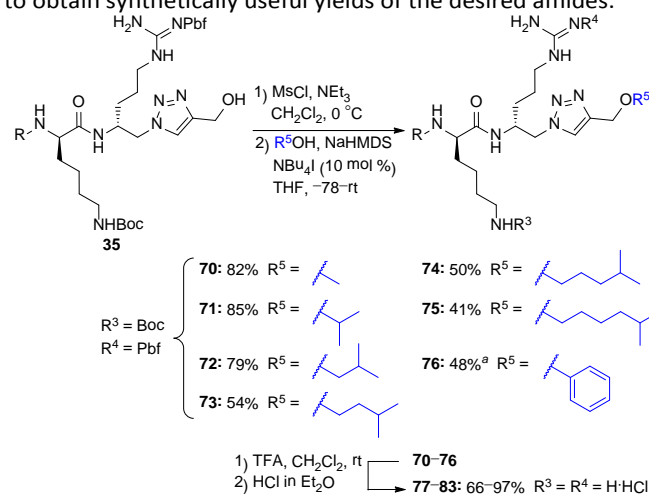
Scheme 5 Synthesis of additional Class B triazole ethers. [a] Etherification conditions: PhOH, Cs_2CO_3, MeCN, rt.
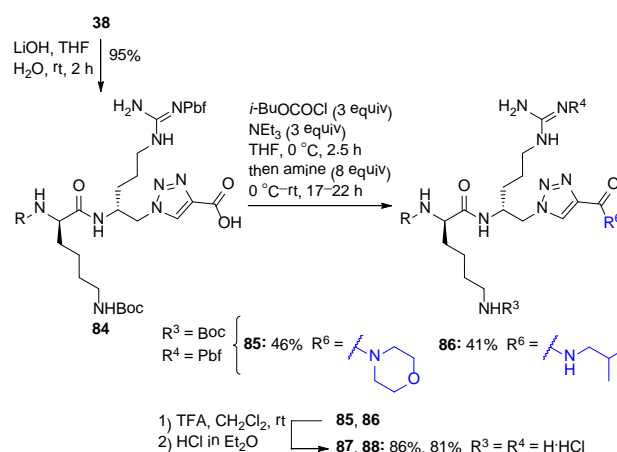
Scheme 6 Synthesis of Class B triazole amides.

After establishing an extensive set of Class B triazoles, we turned our attention to the synthesis of monocationic Class C compounds; details are given in Scheme 7. The required lysine and arginine-based azides **91** and **98** were readily obtained by the coupling of acid **89**[14] with amino azides **90**[33] and **14**, respectively. Each azide was then subjected to copper catalyzed cycloadditions with a small set of previously employed hydrophobic alkynes (Table 2). Interestingly, cycloadditions of lysine-derived azide **91** with benzyl and isopentyl-substituted alkynes were rather slow; heating to 35 °C for 46−48 h was required for complete azide consumption (Table 2, entries 2 and 3), in contrast to the arginine analogue **98**, which reacted completely at rt within 24 h. This reactivity difference revealed a theretofore hidden accelerating effect of the remote guanidine moiety, presumably by coordination to the copper catalyst.

Journal Name

ARTICLE
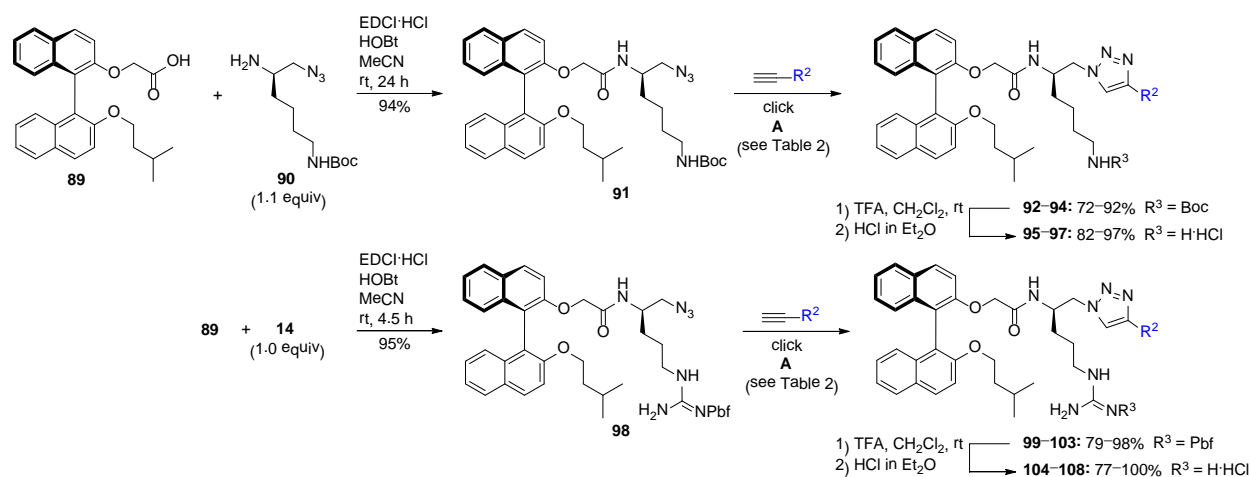
Scheme 7 Synthesis of Class C triazoles bearing one amino acid residue. See Table 2 for specific click conditions and yields.

Table 2 Conditions and yields for the preparation of Class C triazoles via cycloaddition of azides **91** and **98** with various alkynes.

Entry	AA^1	R^2	Cond^a	Prod	Yield (%)
1	Lys(Boc)		A	**92**	92
2	Lys(Boc)		A^b	**93**	79
3	Lys(Boc)		A^b	**94**	72
4	Arg(Pbf)		A	**99**	98
5	Arg(Pbf)		A	**100**	85
6	Arg(Pbf)		A	**101**	98
7	Arg(Pbf)		A	**102**	79
8	Arg(Pbf)		A	**103**	89

^{a} See Fig. 3 for reaction conditions. ^{b} Reaction temp = 35 °C.

In vitro bacterial testing results for the three triazole classes A–C are presented in Tables 3–5, respectively. Lead compound **1** was included as a positive control throughout, in addition to vancomycin and chloramphenicol for Gram positive and Gram negative strains, respectively. Three *C. difficile* human isolates were evaluated including 027 ribotypes from Canada (M7404) and the UK (R20291).[34]

Our initial results with Class A tripeptides **11**–**13** were encouraging (Table 3, entries 2–4) and established that antibacterial activity across the eight strains tested was not adversely affected by incorporation of the triazole ester isostere. A direct comparison between leucine-containing triazole **13** and ester **1**, which both contain a terminal isopentyl group, revealed similar or identical potency against *C. difficile* and the majority of other strains tested (entry 4 versus entry 1). Our laboratory had yet to examine the effect of a third cationic residue incorporated into the peptide backbone, however this modification ultimately resulted in a slight decrease in *C. difficile* activity (entry 5).
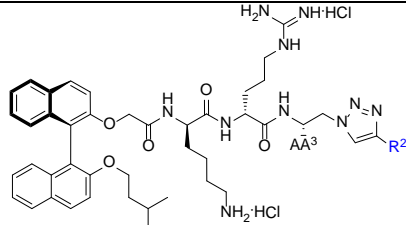
Improved antimicrobial activity was observed for several Class B dipeptides in comparison to their Class A counterparts. Good to excellent activity was observed across Gram positive *S. aureus*, *E. faecalis* and *S. pneumoniae* strains (Table 4, entries 2–35), with equivalent potency to previously optimized lead compound **1** in many cases. 1,4-Disubstituted triazoles and their 1,5-regioisomers generally exhibited comparable activity; for example, cyclohexylmethyl regioisomers **49** and **50** returned identical MICs across seven of the eight strains tested (Table 4, entries 8 and 9). A notable structure-activity trend was observed for dialkyl ether derivatives **77**–**82** (entries 20–25). Namely, when the length of the terminal alkyl chain was increased beginning with a methyl ether (entry 20), a corresponding increase in activity was observed across all strains until reaching optimal potency with an isopentyl or isohexyl terminus (entries 23 and 24). No further benefit was accrued from further elongation of the alkyl chain (entry 25).

Analysis of *C. difficile* testing results from Class B derivatives revealed a clear activity dependency upon triazole substitution with a hydrophobic moiety (Table 4). Aryl, alkyl and silyl-based groups were all conducive to good activity, while the

Organic & Biomolecular Chemistry Accepted Manuscript

incorporation of polar functional groups such as an alcohol (Table 4, entry 19), amides (entries 29–30) and an ammonium cation (entry 31) led to decreased *C. difficile* inhibition. A notable exception to this trend was a 4-butylphenyl substituent (entry 15), which showed relatively weak activity overall. Reduced *C. difficile* activity was also observed with unsubstituted triazole **69** (entry 2) and the propyl derivative **44** (entry 3), highlighting the general requirement of a larger and/or branched hydrophobic group. The most potent Class B

triazoles were **49**, **50** and **64**, bearing 4-cyclohexylmethyl, 5-cyclohexylmethyl and 4,5-diphenyl substituents, respectively (Table 4, entries 8, 9 and 32). These compounds inhibited *C. difficile* growth at a concentration of only 4 μg mL^{-1} across all three strains examined, thus approaching the efficiency observed for vancomycin (MIC = 2 μg mL^{-1}, Table 4, entry 36). None of the ether derivatives tested had MICs lower than 8 μg mL^{-1} against *C. difficile* (entries 20–27).

Table 3 Antibacterial activity of Class A triazoles as minimum inhibitory concentrations (MICs) in μg mL^{-1}.

				C. difficile			*E. coli*	*S. aureus*		*E. faecalis*	*S. pneumoniae*
Entry	Compound	AA^3	R^2	M7404 (RT027)	R20291 (RT027)	1470 (RT017)	ATCC 25922	ATCC 29213	NCTC 10442	ATCC 29212	ATCC 49619
1	**1**	-	-	8	8	8	16	2	2	2	2
2	**11**	Leu	CH$_2$OMe	16	16	16	16	4	4	4	8
3	**12**	Leu	Propyl	8	8	8	8	2	4	4	8
4	**13**	Leu	*i*-Pentyl	8	8	8	16	4	4	4	8
5	**17**	D-Arg·HCl	*i*-Pentyl	16	8	16	16	2	4	4	8
6	vancomycin	-	-	2	2	2	-	1	1	4	0.5
7	chloramphenicol	-	-	-	-	-	4	-	-	-	-

A related dependence on hydrophobic termini also emerged from the testing of Class B compounds against Gram negative *E. coli* (Table 4). Triazole substituents such as isopentyl, cyclohexyl, TMS and benzyl provided optimal MICs of 4 μg mL^{-1} (entries 4, 5, 7, 11 and 17), representing a four-fold increase in potency relative to that of compound **1**.

Class C compounds with a single amino acid residue were also active against *C. difficile*, with MICs ranging from 4–16 μg mL^{-1} (Table 5, entries 2–9). Strong bacterial inhibition was also maintained for Gram positive *S. aureus* and *E. faecalis* strains. Class C triazoles derived from arginine were generally more active than their lysine-based analogues; for instance, comparison of phenyl-substituted triazoles **95** and **104** derived from lysine and arginine, respectively, showed at least a two-fold increase in potency for the latter for six out of the eight strains tested (Table 5, entry 2 versus entry 5). Notably, Class C triazoles were completely inactive against *E. coli*, establishing the need for two basic side chains for Gram negative activity.

Selected Class B compounds bearing hydrophobic termini were also tested against additional bacterial isolates (Table 6). Similar to compound **1**, good to excellent activity was observed across all strains including Gram negative *A. baumannii* and Gram positive vancomycin-resistant *E. faecalis* (VRE) strains. Comparison of Class B data for VRE (Table 6) to that obtained for vancomycin-susceptible *E. faecalis* (Table 4), shows that MICs are largely the same or one dilution different,

suggesting that vancomycin resistance does not have a significant impact on susceptibility to the triazole compounds.

The ease of synthetic access to this class of binaphthyl-triazole peptides has allowed ready scale-up for impending *in vivo* studies on CDI. For instance, 0.56 g of Class B triazole **49** and 0.67 g of Class C triazole **104** have been prepared. Preliminary toxicity screening has also provided promising results. Our most active compounds (i.e., **49**, **50** and **64**, Table 4) exhibited <3% hemolysis of sheep erythrocytes at concentrations above their *C. difficile* MICs (5 μg mL^{-1}, Tables S3–S5 in ESI†).

Although the precise antimicrobial mechanism of our binaphthyl-peptides has not yet been fully elucidated, the observation that all three compound classes A–C were active suggests a general mode of action. Related cationic peptides have been shown to operate via a membrane depolarization mechanism.[35] Resistance studies with *C. difficile* are in progress within our laboratories to identify a potential binding target for our newly developed molecules.

Journal Name

ARTICLE

Table 4 Antibacterial activity of Class B triazoles as minimum inhibitory concentrations (MICs) in µg mL^{-1}.

Entry	Compound	R^1	R^2	C. difficile M7404 (RT027)	C. difficile R20291 (RT027)	C. difficile 1470 (RT017)	E. coli ATCC 25922	S. aureus ATCC 29213	S. aureus NCTC 10442	E. faecalis ATCC 29212	S. pneumoniae ATCC 49619
1	**1**	-	-	8	8	8	16	2	2	2	2
2	**69**	H	H	32	32	32	64	4	4	8	2
3	**44**	H	Propyl	16	16	16	16	2	2	4	8
4	**45**	H	i-Pentyl	16	16	8	4	2	2	2	1
5	**46**	i-Pentyl	H	8	8	8	4	2	2	2	4
6	**47**	H	n-Hexyl	8	4	8	8	2	2	2	4
7	**48**	H	Cy	8	8	8	4	2	2	2	2
8	**49**	H	CH$_2$Cy	4	4	4	8	2	2	2	4
9	**50**	CH$_2$Cy	H	4	4	4	8	2	4	2	4
10	**51**	H	t-Bu	8	8	8	8	2	2	4	4
11	**52**	H	SiMe$_3$	8	8	8	4	2	2	2	1
12	**53**	H	SiMe$_2$Ph	8	8	8	8	2	4	2	4
13	**54**	H	Ph	16	16	16	8	2	2	2	2
14	**55**	Ph	H	8	8	8	4	2	2	2	4
15	**56**	H	4-(n-Bu)-Ph	32	32	16	64	8	4	4	16
16	**57**	H	4-OMe-Ph	8	16	8	16	2	2	4	4
17	**58**	H	Bn	8	8	8	4	2	2	2	4
18	**59**	H	CH$_2$CH$_2$Ph	8	8	8	8	2	4	2	4
19	**60**	H	CH$_2$OH	32	32	32	64	8	8	8	8
20	**77**	H	CH$_2$OMe	32	32	32	32	8	8	8	8
21	**78**	H	CH$_2$O(i-Pr)	32	32	32	32	4	4	8	4
22	**79**	H	CH$_2$O(i-Bu)	16	16	16	8	2	2	4	4
23	**80**	H	CH$_2$O(i-Pent)	8	8	8	4	2	2	2	4
24	**81**	H	CH$_2$O(i-Hex)	8	8	8	4	2	2	2	8
25	**82**	H	CH$_2$O(i-Hept)	8	8	8	16	4	4	4	8
26	**83**	H	CH$_2$OPh	8	8	8	8	4	2	4	4
27	**61**	H	CH$_2$OBn	16	16	16	4	2	2	2	2
28	**62**	H	OEt	16	16	16	16	4	4	4	4
29	**87**	H	CO(Morph)	16	16	16	16	4	4	8	8
30	**88**	H	CONH(i-Bu)	16	16	16	8	2	4	4	4
31	**63**	H	(CH$_2$)$_2$NH$_2$·HCl	32	32	16	64	4	4	8	4
32	**64**	Ph	Ph	4	4	4	16	4	4	4	4
33	**65**	I	Ph	8	8	8	16	4	4	4	4
34	**66**		Benzo(triazole)^a	16	16	16	8	2	2	2	4
35	**67**		Indolyl(triazole)^b	16	8	8	16	2	4	4	8
36	vancomycin	-	-	2	2	2	-	1	1	4	0.5
37	chloramphenicol	-	-	-	-	-	4	-	-	-	-

^{a} See Table 1, entry 24 for compound structure. ^{b} See Table 1, entry 25 for compound structure.

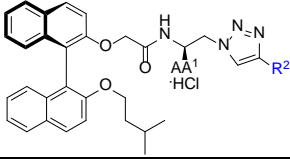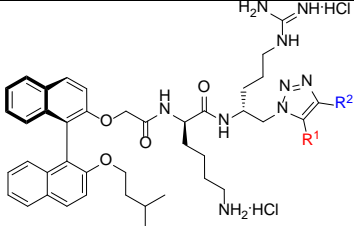**Table 5** Antibacterial activity of Class C triazoles as minimum inhibitory concentrations (MICs) in µg mL^{-1}.

Entry	Compound	AA^1	R^2	C. difficile M7404 (RT027)	C. difficile R20291 (RT027)	C. difficile 1470 (RT017)	E. coli ATCC 25922	S. aureus ATCC 29213	S. aureus NCTC 10442	E. faecalis ATCC 29212	S. pneumoniae ATCC 49619
1	**1**	-	-	8	8	8	16	2	2	2	2
2	**95**	Lys	Ph	16	16	16	>128	4	8	4	32
3	**96**	Lys	Bn	8	8	8	>128	4	4	4	32
4	**97**	Lys	i-Pentyl	16	16	16	128	8	8	4	32
5	**104**	Arg	Ph	8	8	4	>128	2	2	4	8
6	**105**	Arg	Bn	8	8	8	128	2	2	4	16
7	**106**	Arg	i-Pentyl	8	8	8	128	2	2	4	16
8	**107**	Arg	Cy	8	8	8	>128	2	2	4	8
9	**108**	Arg	CH$_2$Cy	16	16	16	>128	4	4	4	8
10	vancomycin	-	-	2	2	2	-	1	1	4	0.5
11	chloramphenicol	-	-	-	-	-	4	-	-	-	-

Table 6 Further biological screening of selected Class B compounds. Results given as minimum inhibitory concentrations (MICs) in µg mL^{-1}.

Entry	Compound	R^1	R^2	A. baumannii ATCC 19606	A. baumannii ATCC 15308	S. aureus Mu50 (VISA)	S. epidermidis ATCC 11047	E. faecalis ATCC 51299 (VRE)	E. faecalis clinical (VRE)
1	**1**	-	-	4	8	2	2	4	4
2	**45**	H	i-Pentyl	4	4	2	2	4	4
3	**46**	i-Pentyl	H	4	4	2	2	4	4
4	**47**	H	n-Hexyl	4	4	2	2	4	4
5	**49**	H	CH$_2$Cy	8	4	2	2	4	4
6	**52**	H	SiMe$_3$	8	8	4	1	8	4
7	**54**	H	Ph	8	8	2	2	4	4
8	**55**	Ph	H	4	4	4	2	8	8
9	**59**	H	CH$_2$CH$_2$Ph	8	8	2	2	8	4
10	**81**	H	CH$_2$O(i-Hex)	4	4	4	2	4	4
11	**61**	H	CH$_2$OBn	8	4	4	2	8	8
12	**64**	Ph	Ph	4	4	2	4	8	4
13	**65**	I	Ph	8	8	2	4	4	4
14	vancomycin	-	-	-	-	8	2	128	64
15	ciprofloxacin	-	-	1	0.25	-	-	-	-

Conclusions

In conclusion, we have prepared forty six triazole ester isostere analogues of the antibacterial dicationic tripetide **1**, with the aim of developing metabolically more stable molecules suitable for targeting *C. difficile* infections in the gut. These analogues were synthesized employing Cu- or Ru-catalyzed click chemistry which allowed for regioselective variations of substituents at C-4 and C-5 (R^1 or R^2) of the triazole moiety. Dicationic-tripeptide and dipeptide mimetics were prepared

along with a series of monocationic-monopeptide analogues, incorporating unnatural D-amino acids in most cases.

Significantly, the dicationic-tripeptide analogues (Class A compounds) had similar antibacterial activity as the lead compound **1** against a select panel of Gram positive and Gram negative bacteria and were effective (MICs of 8 µg mL^{-1}) against three strains of *C. difficile*, including two problematic ribotype 027 strains. The larger set of dicationic-dipeptide analogues (Class B compounds) provided useful SAR data and identified three significant compounds (**49** (R^2 = CH$_2$Cy), **50** (R^1 = CH$_2$Cy) and **64** (R^1 = R^2 = Ph)) with MICs of 4 µg mL^{-1} against

the three *C. difficile* strains, only one broth dilution different from the positive control vancomycin (MIC of 2 μg mL^{-1}). SAR studies indicated that linear or branched hydrocarbon chain substituents (>4 carbons) at C-4 and/or C-5 (R^1 or R^2) of the triazole provided compounds with the highest activities against the eight bacterial strains, while compounds with more polar substituents (short chain ethers, alcohol, amide or ammonium ion) were less effective. Interestingly, some of the structurally much simpler monocationic-monopeptides (Class C compounds) were just as active as compound **1** against most Gram positive strains, while a complete loss of Gram negative *E. coli* activity was observed for all monocationic compounds.

In addition to the promising antimicrobial activity of these peptidomimetics, their straightforward synthetic access via amide coupling and click chemistry, based on an inexpensive binaphthyl scaffold, makes them attractive candidates for the treatment of CDI. These encouraging results have prompted *in vivo* studies of these compounds in mouse models which are underway. Additionally, the significant *C. difficile* inhibition observed for diphenyl triazole **64**, in particular, presents further synthetic opportunities to be explored including click chemistry with other internal alkynes, both symmetrical and unsymmetrical,[23] to produce a wider range of fully substituted triazoles. Transition metal-catalyzed cross-coupling[36] using halogenated derivatives such as **41** should also provide modular access to numerous second-generation drug candidates for screening and optimization purposes. Further developments in these areas will be reported in due course.

Experimental section

Synthesis and characterization methods. All reactions were carried out in standard laboratory glassware with magnetic stirring. Thin layer chromatography (TLC) was performed on aluminum-backed 0.20 mm silica gel plates. Visualization was accomplished with UV light, a ninhydrin staining solution in *n*-butanol and/or an aqueous ceric ammonium molybdate solution. Flash chromatography and silica pipette plugs were performed under positive air pressure using Silica Gel 60 of 230–400 mesh (40–63 μm). Optical Rotations were measured at 25 °C in the specified solvent with a path length of 1.0 dm on a Jasco P-2000 Digital Polarimeter (λ = 589 nm). Concentrations (*c*) are given in g/100 mL. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian Mercury 300 MHz spectrometer, a Varian Inova 500 MHz spectrometer or a Varian VNMRS PS54 500 MHz spectrometer. Spectra aquired in CDCl$_3$ are reported relative to tetramethylsilane (^{1}H: δ = 0.00 ppm) and solvent resonance (^{13}C: δ = 77.0 ppm). Spectra acquired in CD$_3$OD are reported relative to solvent resonance (^{1}H: δ = 3.31 ppm; ^{13}C: δ = 49.0 ppm). ^{1}H NMR data are reported as follows: chemical shift, multiplicity (abbreviations: s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, app. d = apparent doublet, dd = doublet of doublets, t = triplet, app. t = apparent triplet, q = quartet, ABq = AB quartet, quin = quintet, sex = sextet, sep = septet, m = multiplet and bm = broad multiplet), coupling constant (Hz) and integration. Infrared (IR) spectra were

obtained on a Shimadzu IRAffinity-1 FTIR Spectrometer. IR samples were analyzed as neat solids or oils. Low resolution mass spectrometry (MS) was performed on a Shimadzu LC-2010 Electrospray Ionization (ESI) Mass Spectrometer. All samples were prepared in HPLC grade methanol with a trace of formic acid. High resolution mass spectrometry (HRMS) was performed on a Waters Quadrupole-Time of Flight (QTOF) Xevo Spectrometer via ESI with Leucine-Enkephalin as an internal standard. For isolated hydrochloride salts of basic amino compounds, "M" refers to the mass of the corresponding *neutral* molecule. High performance liquid chromatography (HPLC) was performed on a reverse-phase phenomenex Synergi 4u Fusion-RP 80Å column (φ = 4.6 × 150 mm) at a wavelength (λ) of 280 nm using water/acetonitrile (both containing 0.1% TFA) as the mobile phase. All samples were injected at a concentration of ~1 mg mL^{-1} in HPLC grade MeOH (injection volume = 20 μL).

Synthesis materials. Nitrogen (N$_2$) was dried by passage through self-indicating silica gel (2–4 mm bead size). Unless otherwise noted, anhydrous solvents (obtained from commercial sources) were utilized. Known reagents and alkynes that were not commercially available were prepared according to literature procedures cited within the ESI†. All other reagents were purchased reagent grade and used as received.

General synthetic procedures

General procedure 1 for peptide coupling. A reaction vessel was charged in air with the carboxylic acid (1.0 equiv), EDCI·HCl (1.2 equiv), HOBt (1.2 equiv) and the specified equivalents of the amine. If the latter was an ammonium salt, a slight excess of (*i*-Pr)$_2$NEt was also added as noted. To this was added the specified volume of HPLC grade MeCN (not pre-dried) and the resulting mixture was stirred at rt in an air atmosphere for the time specified. After removal of the solvent under reduced pressure (for reactions with less than 5 mL of MeCN this is not necessary), the residue was dissolved in EtOAc (20 mL for reactions with ≤1 mmol of acid; or 20 mL/mmol of acid for larger scale) and washed sequentially with 1 M HCl (2×20 mL; to remove any excess amine, EDCI and the urea by-product), saturated NaHCO$_3$ (2×20 mL; to remove HOBt) and brine (20 mL). The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure, yielding in most cases the analytically pure peptide. Purification was carried out by flash chromatography with the indicated eluent if required.

General procedure 2 for copper-catalyzed azide–alkyne cycloaddition. A reaction vessel was charged in air with the azide (1.0 equiv), Cu(OAc)$_2$·H$_2$O (0.2 equiv), Na·ascorbate (0.4 equiv), and the neat alkyne (3.0 equiv). To this was added *t*-BuOH (20 mL/mmol of azide) and H$_2$O (5 mL/mmol of azide). The mixture was sonicated briefly (<1 min) and the resulting suspension stirred at rt in an air atmosphere for the time specified. The mixture was diluted with EtOAc (20 mL for reactions with ≤1 mmol of azide; or 20 mL/mmol of azide for larger scale) and shaken with a 1:1 mixture of 32% aqueous

NH$_3$:brine (20 mL). The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure. If required, the residue was purified using either a small silica pipette plug or conventional flash chromatography as specified, to give the desired 1,4-substituted triazole.

General procedure 3 for ruthenium-catalyzed azide–alkyne cycloaddition. An oven-dried vial was charged in air with the azide (1.0 equiv), Cp*RuCl(PPh$_3$)$_2$ (5 mol %) and the neat alkyne (2.0 equiv). The vial was fitted with a rubber septum, evacuated and refilled with N$_2$ (single cycle), then anhydrous 1,4-dioxane (10 mL/mmol of azide) was added. The sealed vessel was heated at 60 °C under N$_2$ for the specified time. After cooling to rt, the reaction mixture (with solvent) was directly subjected to flash chromatography with the specified eluent to provide the desired 1,5-disubstituted- or 1,4,5-trisubstituted triazole.

General procedure 4 for the preparation of alkyl ethers. To the neat alkyl alcohol (R'OH, 10 equiv) under N$_2$ at −78 °C was added a solution of NaHMDS (1 M in THF, 5 equiv) and the solution was stirred at −78 °C for 15 min to generate the sodium alkoxide. To this was added a solution of the mesylate (1 equiv) and NBu$_4$I (0.1 equiv) in THF and the external cooling bath was allowed to warm to rt with stirring for the indicated time. The mixture was diluted with EtOAc (20 mL) and washed with 1 M HCl (20 mL) and saturated NaHCO$_3$ (20 mL), then dried (MgSO$_4$) and concentrated under reduced pressure. If required, purification was carried out by flash chromatography to provide the desired alkyl ether.

General procedure 5 for TFA mediated deprotection. To a solution of the *N*-protected peptide in reagent grade CH$_2$Cl$_2$ (3.3 mL/0.1 mmol of substrate) was added TFA (3.3 mL/0.1 mmol of substrate) and the solution was stirred at rt in an air atmosphere for the time specified. The solvents were removed under reduced pressure and the residue dried under high vacuum. This was taken up in CH$_2$Cl$_2$ (~0.5 mL) and an aliquot of excess ethereal HCl (2 M in Et$_2$O, 1.6 mL/0.1 mmol of substrate) was added to exchange the TFA anion with chloride. The mixture was again concentrated and dried under reduced pressure. The remaining sticky solid was dissolved in minimal MeOH (≤10 drops from a Pasteur pipette for ≤0.05 mmol of product) and reagent grade Et$_2$O (5 mL) was added, resulting in instantaneous precipitation of the product. The precipitate was collected via vacuum filtration and the original vessel (containing significant product deposited on the glass) and filter cake were washed with Et$_2$O (3×10 mL). The filter cake was transferred back into the original vessel (containing the remainder of the product) via dissolution with MeOH (~10 mL). Concentration and drying under reduced pressure provided the desired hydrochloride salts as thin films which routinely gave easily-handled powders upon scratching with a spatula.

Representative synthesis of compound 47 from acid 2

***tert*-Butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-(((*S*)-2'-(isopentyloxy)-[1,1'-binaphthalen]-2-yl)oxy)acetamido)-6-oxohexyl)carbamate (18).** This compound was prepared according to *general procedure 1*

using the known acid **2**[14] (1.806 g, 2.81 mmol), EDCI·HCl (646 mg, 3.37 mmol), HOBt (455 mg, 3.37 mmol), amine **14** (1.266 g, 2.89 mmol) and MeCN (28 mL) with a 4.5 h reaction time. Work-up as described gave **18** (2.899 g, 97%) as an off-white solid. TLC (10% MeOH/CH$_2$Cl$_2$) R_F = 0.60, (75% EtOAc/pet. ether) R_F = 0.68; $[\alpha]_D^{25}$ −24.0 (*c* 5.31, CH$_2$Cl$_2$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.97 (app. t, *J* = 9.4 Hz, 2H), 7.92–7.83 (m, 2H), 7.47 (d, *J* = 9.0 Hz, 1H), 7.41–7.20 (m, 5H), 7.18–7.09 (m, 2H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.17 (d, *J* = 7.6 Hz, 1H), 6.07 (bs, 2H), 4.59 (t, *J* = 5.7 Hz, 1H), 4.45 (ABq, Δδ$_{AB}$ = 0.06, *J* = 14.5 Hz, 2H), 4.09–3.96 (m, 2H), 3.96–3.85 (m, 2H), 3.30 (dd, *J* = 12.4, 4.5 Hz, 1H), 3.23 (dd, *J* = 12.4, 5.8 Hz, 1H), 3.17–3.02 (m, 2H), 3.03–2.84 (m, 4H), 2.56 (s, 3H), 2.50 (s, 3H), 2.08 (s, 3H), 1.51–1.31 (m, 20H), 1.31–1.06 (m, 5H), 0.97–0.75 (m, 3H), 0.55 (d, *J* = 6.5 Hz, 3H), 0.50 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 171.4, 168.8, 158.6, 156.1, 155.9, 154.4, 152.1, 138.2, 133.8, 133.6, 133.2, 132.1, 129.8, 129.75, 129.68, 129.2, 128.0, 127.9, 126.6, 126.5, 125.4, 124.9, 124.4, 124.1, 123.7, 120.4, 119.3, 117.3, 115.9, 114.2, 86.2, 78.9, 68.3, 67.9, 54.6, 52.6, 48.8, 43.2, 40.5, 40.0, 37.9, 31.1, 29.1, 28.5, 28.4, 25.6, 24.5, 22.4, 22.20, 22.16, 22.0, 19.2, 17.9, 12.4; IR (cm^{-1}) υ 3330, 2929, 2097, 1653, 1507, 1244, 1087, 808; MS (ES$^+$) *m/z* 1085 (24%, M+Na), 1063 (100%, M+H); HRMS (ES$^+$) Calcd. for C$_{57}$H$_{75}$N$_9$NaO$_9$S: 1084.5306 (M+Na), Found: 1084.5342.

***tert*-Butyl ((*R*)-6-(((*R*)-1-(4-hexyl-1*H*-1,2,3-triazol-1-yl)-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-(((*S*)-2'-(isopentyloxy)-[1,1'-binaphthalen]-2-yl)oxy)acetamido)-6-oxohexyl)carbamate (22).** This compound was prepared according to *general procedure 2* using azide **18** (50.0 mg, 0.047 mmol), Cu(OAc)$_2$·H$_2$O (1.9 mg, 0.0094 mmol), Na·ascorbate (3.7 mg, 0.019 mmol), 1-octyne (15.6 mg, 0.14 mmol), *t*-BuOH (1.0 mL) and H$_2$O (0.25 mL) with a 24 h reaction time. Work-up as described gave **22** (52.0 mg, 94%) as an off-white solid. TLC (100% EtOAc) R_F = 0.10; $[\alpha]_D^{25}$ −21.9 (*c* 2.45, CH$_2$Cl$_2$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.98–7.90 (m, 2H), 7.85 (app. t, *J* = 7.6 Hz, 2H), 7.44 (d, *J* = 8.9 Hz, 1H), 7.38 (s, 1H), 7.37–7.26 (m, 3H), 7.26–7.17 (m, 3H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.10 (d, *J* = 8.5 Hz, 1H), 6.27 (bs, 2H), 6.18 (bd, *J* = 5.7 Hz, 1H), 4.90 (bs, 1H), 4.48 (d, *J* = 14.5 Hz, 1H), 4.41–4.27 (m, 3H), 4.25–4.16 (m, 1H), 4.07–3.95 (m, 2H), 3.93–3.85 (m, 1H), 3.15–3.04 (m, 2H), 2.97–2.84 (m, 4H), 2.67 (t, *J* = 7.5 Hz, 2H), 2.55 (s, 3H), 2.48 (s, 3H), 2.07 (s, 3H), 1.69–1.59 (m, 2H), 1.52–1.08 (m, 29H), 0.94–0.76 (m, 4H), 0.75–0.63 (m, 2H), 0.53 (d, *J* = 6.3 Hz, 3H), 0.49 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 171.5, 168.8, 158.6, 156.2, 156.0, 154.3, 152.1, 148.1, 138.1, 133.8, 133.6, 133.0, 132.1, 129.7, 129.6, 129.1, 128.0, 127.9, 126.6, 126.5, 125.4, 124.9, 124.5, 124.1, 123.7, 121.9, 120.3, 119.3, 117.4, 115.9, 114.2, 86.3, 78.8, 68.2, 67.9, 52.8, 52.6, 49.2, 43.2, 40.5, 39.9, 37.9, 31.5, 31.0, 29.4, 29.02, 28.95, 28.5, 28.4, 25.6, 25.3, 24.5, 22.5, 22.4, 22.2, 22.0, 19.2, 17.9, 14.0, 12.4; MS (ES$^+$) *m/z* 1195 (100%, M+Na), 1173 (92%, M+H); HRMS (ES$^+$) Calcd. for C$_{65}$H$_{89}$N$_9$NaO$_9$S: 1194.6402 (M+Na), Found: 1194.6436.

(*R*)-6-Amino-*N*-((*R*)-5-guanidino-1-(4-hexyl-1*H*-1,2,3-triazol-1-yl)pentan-2-yl)-2-(2-(((*S*)-2'-(isopentyloxy)-[1,1'-binaphthalen]-2-

yl)oxy)acetamido)hexanamide·dihydrochloride (47). This compound was prepared according to *general procedure 5* using **22** (47.8 mg, 0.041 mmol), CH_2Cl_2 (1.34 mL) and TFA (1.34 mL) with a 24 h reaction time. Work-up and treatment with HCl (2 M in Et_2O, 0.67 mL) as described gave **47** (34.2 mg, 94%) as a tan solid. $[\alpha]_D^{25}$ –15.1 (*c* 0.95, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (s, 1H), 8.03 (app. d, *J* = 9.0 Hz, 2H), 7.96–7.89 (m, 2H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.49 (d, *J* = 9.0 Hz, 1H), 7.39–7.32 (m, 2H), 7.23 (app. t, *J* = 7.5 Hz, 2H), 7.12–7.02 (m, 2H), 4.70–4.41 (m, 4H), 4.34–4.25 (m, 1H), 4.18–4.08 (m, 1H), 4.02–3.91 (m, 2H), 3.23–3.11 (m, 2H), 2.86–2.70 (m, 4H), 1.80–1.47 (m, 8H), 1.46–1.10 (m, 10H), 1.07–0.97 (m, 1H), 0.96–0.79 (m, 5H), 0.58 (d, *J* = 6.5 Hz, 3H), 0.52 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.7, 170.9, 158.5, 155.9, 154.1, 146.5, 135.2, 135.1, 131.4, 130.8, 130.7, 129.3, 129.1, 127.6, 127.5, 126.3, 126.0, 125.2, 124.8, 121.7, 120.6, 116.8, 116.0, 69.3, 69.0, 56.8, 53.9, 50.5, 41.8, 40.4, 39.3, 32.5, 32.2, 29.8, 29.6, 29.5, 27.7, 26.1, 25.6, 24.7, 23.6, 23.5, 22.8, 22.5, 14.4; IR (cm^{-1}) υ 2923, 1653, 1506, 1214, 1149, 1048, 811, 747; MS (ES$^+$) *m/z* 821 (<5%, M+H), 411 (100%, M+2H); HRMS (ES$^+$) Calcd for $C_{47}H_{66}N_9O_4$: 820.5238 (M+H), Found: 820.5256.

Acknowledgements

References

1   (*a*) L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen, K. Lewis, *Nature*, 2015, 517, 455; (*b*) P. M. Wright, I. B. Seiple, A. G. Myers, *Angew. Chem. Int. Ed.*, 2014, 53, 8840; (*c*) J. Ziemska, A. Rajnisz, J. Solecka, *Cent. Eur. J. Biol.*, 2013, 8, 943.

2   Office of the Associate Director for Communication, Digital Media Branch, Division of Public Affairs. *Antibiotic Resistance Threats in the United States, 2013;* Centers for Disease Control and Prevention: Atlanta, GA, Sep 16, 2013; http://www.cdc.gov/features/AntibioticResistanceThreats/.

3   (*a*) D. Lyras, J. R. O'Connor, P. M. Howarth, S. P. Sambol, G. P. Carter, T. Phumoonna, R. Poon, V. Adams, G. Vedantam, S. Johnson, D. N. Gerding, J. I. Rood, *Nature*, 2009, 458, 1176; (*b*) D. A. Leffler, J. T. Lamont, *Gastroenterology*, 2009, 136, 1899.

4   (*a*) C. Slimings, T. V. Riley, *J. Antimicrob. Chemother.*, 2014, 69, 881; (*b*) S. R. Eaton, J. E. Mazuski, *Crit. Care Clin.*, 2013, 29, 827.

5   J. Joseph, S. Singhal, G. M. Patel, S. Anand, *Am. J. Ther.*, 2014, 21, 385.

6   C. J. Hostler, L. F. Chen, *Exp. Opin. Pharmacother.*, 2013, 14, 1529.

7   A. S. Ritter, W. A. Petri, *Curr. Opin. Infect. Dis.*, 2013, 26, 461.

8   I. A. Critchley, L. S. Green, C. L. Young, J. M. Bullard, R. J. Evans, M. Price, T. C. Jarvis, J. W. Guiles, N. Janjic, U. A. Ochsner, *J. Antimicrob. Chemother.*, 2009, 63, 954.

9   D. M. Musher, N. Logan, R. J. Hamill, H. L. DuPont, A. Lentnek, A. Gupta, J. F. Rossignol, *Clin. Infect. Dis.*, 2006, 43, 421.

10 J. Wistrom, R. Lundholm, M. Prag, E. Tornkvist, R. Maller, S. Ansehn, P. E. Abom, B. Hoorn, G. Nystrom, L. O. Mentzing, E. Myhre, C. Schalen, S. R. Norrby, C. Holmen, J. H. Larsen, S. A. Hedstrom, S. Eriksson, A. Stromberg, B. Wretlind, G. Bondesson, I. Harding, C. E. Nord, *Scand. J. Infect. Dis.*, 1994, 26, 309.

11 M. J. LaMarche, J. A. Leeds, A. Amaral, J. T. Brewer, S. M. Bushell, G. J. Deng, J. M. Dewhurst, J. Ding, J. Dzink-Fox, G. Gamber, A. Jain, K. Lee, L. Lee, T. Lister, D. McKenney, S. Mullin, C. Osborne, D. Palestrant, M. A. Patane, E. M. Rann, M. Sachdeva, J. Shao, S. Tiamfook, A. Trzasko, L. Whitehead, A. Yifru, D. H. Yu, W. L. Yan, Q. M. Zhu, *J. Med. Chem.*, 2012, 55, 2376.

12 C. T. M. Mascio, L. I. Mortin, K. T. Howland, A. D. G. Van Praagh, S. X. Zhang, A. Arya, C. L. Chuong, C. F. Kang, T. C. Li, J. A. Silverman, *Antimicrob. Agents Chemother.*, 2012, 56, 5023.

13 R. Liu, J. M. Suárez, B. Weisblum, S. H. Gellman, S. M. McBride, *J. Am. Chem. Soc.*, 2014, 136, 14498.

14 J. B. Bremner, P. A. Keller, S. G. Pyne, T. P. Boyle, Z. Brkic, D. M. David, A. Garas, J. Morgan, M. Robertson, K. Somphol, M. H. Miller, A. S. Howe, P. Ambrose, S. Bhavnani, T. R. Fritsche, D. J. Biedenbach, R. N. Jones, R. W. Buckheit Jr., K. M. Watson, D. Baylis, J. A. Coates, J. Deadman, D. Jeevarajah, A. McCracken, D. I. Rhodes, *Angew. Chem. Int. Ed.*, 2010, 49, 537.

15 J. B. Bremner, P. A. Keller, S. G. Pyne, T. P. Boyle, Z. Brkic, D. M. David, M. Robertson, K. Somphol, D. Baylis, J. A. Coates, J. Deadman, D. Jeevarajah, D. I. Rhodes, *Bioorg. Med. Chem.*, 2010, 18, 2611.

16 J. B. Bremner, P. A. Keller, S. G. Pyne, T. P. Boyle, Z. Brkic, J. Morgan, K. Somphol, J. A. Coates, J. Deadman, D. I. Rhodes, *Bioorg. Med. Chem.*, 2010, 18, 4793.

17 I. E. Valverde, T. L. Mindt, *Chimia*, 2013, 67, 262.

18 A. Massarotti, S. Aprile, V. Mercalli, E. Del Grosso, G. Grosa, G. Sorba, G. C. Tron, *ChemMedChem*, 2014, 9, 2497.

19 L. Gentilucci, R. De Marco, L. Cerisoli, *Curr. Pharm. Des.*, 2010, 16, 3185.

20 (*a*) B. Garudachari, A. M. Isloor, M. N. Satyanarayana, H.-K. Fun, G. Hegde, *Eur. J. Med. Chem.*, 2014, 74, 324; (*b*) A. Thirunarayanan, S. Raja, G. Mohanraj, P. Rajakumar, *RSC Adv.*, 2014, 4, 41778; (*c*) D. T. G. Gonzaga, D. R. da Rocha, F. d. C. da Silva, V. F. Ferreira, *Curr. Top. Med. Chem.*, 2013, 13, 2850; (*d*) B. F. Abdel-Wahab, E. Abdel-Latif, H. A. Mohamed, G. E. A. Awad, *Eur. J. Med. Chem.*, 2012, 52, 263; (*e*) H. Behbehani, H. M. Ibrahim, S. Makhseed, H. Mahmoud, *Eur. J. Med. Chem.*, 2011, 46, 1813; (*f*) S. G. Agalave, S. R. Maujan, V. S. Pore, *Chem. Asian J.*, 2011, 6, 2696; (*g*) A. Gupte, H. I. Boshoff, D. J. Wilson, J. Neres, N. P. Labello, R. V. Somu, C. Xing, C. E. Barry, C. C. Aldrich, *J. Med. Chem.*, 2008, 51, 7495; (*h*) F. Reck, F. Zhou, M. Girardot, G. Kern, C. J. Eyermann, N. J. Hales, R. R. Ramsay, M. B. Gravestock, *J. Med. Chem.*, 2005, 48, 499.

21 (*a*) A. Lauria, R. Delisi, F. Mingoia, A. Terenzi, A. Martorana, G. Barone, A. M. Almerico, *Eur. J. Org. Chem.*, 2014, 3289; (*b*) H. Li, R. Aneja, I. Chaiken, *Molecules*, 2013, 18, 9797; (*c*) H. C. Kolb, K. B. Sharpless, *Drug Discovery Today*, 2003, 8, 1128.

22 V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, 41, 2596.

23 B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia, V. V. Fokin, *J. Am. Chem. Soc.*, 2008, 130, 8923.

24 J. Huang, S. J. F. Macdonald, J. P. A. Harrity, *Chem. Commun.*, 2009, 436.

25 J. E. Hein, J. C. Tripp, L. B. Krasnova, K. B. Sharpless, V. V. Fokin, *Angew. Chem. Int. Ed.*, 2009, 48, 8018.

26 F. Shi, J. P. Waldo, Y. Chen, R. C. Larock, *Org. Lett.*, 2008, 10, 2409.

Organic & Biomolecular Chemistry Accepted Manuscript

27 B. Kolesinska, S. Grabowski, W. Kaca, Z. J. Kaminski, *Acta Pol. Pharm.,* 2006, 63, 265.

28 S. Mosca, J. Keller, N. Azzouz, S. Wagner, A. Titz, P. H. Seeberger, G. Brezesinski, L. Hartmann, *Biomacromolecules*, 2014, 15, 1687.

29 L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin, G. Jia, *J. Am. Chem. Soc.*, 2005, 127, 15998.

30 S. M. Bronner, K. B. Bahnck, N. K. Garg, *Org. Lett.*, 2009, 11, 1007.

31 N. Saito, K.-I. Nakamura, Y. Sato, *Heterocycles*, 2014, 88, 929.

32 N. S. Chakor, L. Musso, S. Dallavalle, *J. Org. Chem.*, 2009, 74, 844.

33 P.-Y. Yang, H. Wu, M. Y. Lee, A. Xu, R. Srinivasan, S. Q. Yao, *Org. Lett.*, 2008, 10, 1881.

34 G. P. Carter, G. R. Douce, R. Govind, P. M. Howarth, K. E. Mackin, J. Spencer, A. M. Buckley, A. Antunes, D. Kotsanas, G. A. Jenkin, B. Dupuy, J. I. Rood, D. Lyras, *PLoS Pathog.*, 2011, 7, e1002317.

35 (*a*) J.-J. Koh, S. Lin, T. T. Aung, F. Lim, H. Zhou, Y, Bai, J. Li, H. Lin, L. M. Pang, W. L. Koh, S. M. Salleh, R. Lakshminarayanan, L. Zhou, S. Qiu, K. Pervushin, C. Verma, D. T. H. Tan, D. Cao, S. Liu, R. W. Beuerman, *J. Med. Chem.*, 2015, 58, 739; (*b*) C. Ghosh, G. B. Manjunath, P. Akkapeddi, V. Yarlagadda, J. Hoque, D. S. S. M. Uppu, M. M. Konai, J. Haldar, *J. Med. Chem.*, 2014, 57, 1428; (*c*) V. Yarlagadda, P. Akkapeddi, G. B. Manjunath, J. Haldar, *J. Med. Chem.*, 2014, 57, 4558; (*d*) K. A. Brogden, *Nat. Rev. Microbiol.*, 2005, 3, 238; (*e*) K. V. R. Reddy, R. D. Yedery, C. Aranha, *Int. J. Antimicrob. Agents*, 2004, 24, 536.

36 J. Deng, Y.-M. Wu, Q.-Y. Chen, *Synthesis*, 2005, 2730.