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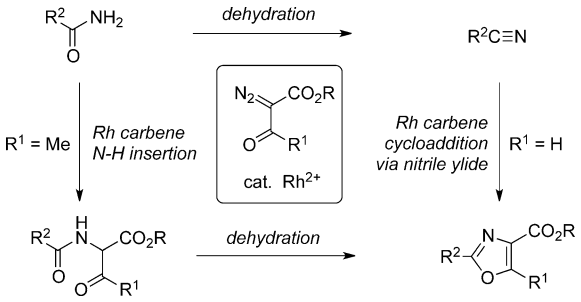
Total Synthesis of the Posttranslationally Modified Polyazole Peptide Antibiotic Plantazolicin A

*Hiroki Wada, Huw E. L. Williams, and Christopher J. Moody**

Abstract: *The power of rhodium–carbene methodology in chemistry is demonstrated by the synthesis of a structurally complex polyazole antibiotic. Plantazolicin A, a novel soil-bacterium metabolite, comprises a linear array of 10 five-membered rings in two pentacyclic regions that derive from ribosomal peptide synthesis followed by extensive posttranslational modification. The compound possesses potent antimicrobial activity, and is selectively active against the anthrax-causing organism. A conceptually different synthesis of plantazolicin A is reported in which the key steps are the use of rhodium(II)-catalyzed reactions of diazocarbonyl compounds to generate up to six of the seven oxazole rings of the antibiotic. NMR spectroscopic studies and molecular modeling reveal a likely dynamic hairpin conformation with a hinge region around the two isoleucine residues. The compound has modest activity against methicillin-resistant Staphylococcus aureus (MRSA).*

In the one and a half centuries since August Kekulé and Archibald Scott Couper independently proposed that a carbon atom could form four bonds to other atoms (including other carbon atoms),[1,2] the existence of divalent carbon species with a six-electron valence shell has intrigued chemists. Once regarded as mechanistic curiosities or fleeting intermediates, such divalent species, now known as carbenes, have moved center stage as a result of the isolation of the first stable carbene in the late 1980s,[3] to be followed by the now familiar stable N-heterocyclic carbenes,[4] which as ligands have revolutionized transition-metal catalysis.[5] Also metallocarbene intermediates, derived from diazo compounds, participate in a plethora of reactions that are useful in chemical synthesis.[6] We now demonstrate the power of carbene chemistry in the synthesis of the structurally unique, complex polyazole antibiotic plantazolicin A, in which up to six of the seven five-membered oxazole rings of the natural product are formed from simple precursors, such as carboxamides or nitriles, as facilitated by carbene methodology (Scheme 1).[7–10]

Plantazolicin A (**1**) and plantazolicin B (**2**) are novel metabolites isolated from the soil bacterium *Bacillus amyloliquefaciens* FZB42.[11,12] The structures consist of a linear array of five-membered rings (azoles) that derive biosynthetically from amino acids by ribosomal peptide synthesis followed by wide-ranging posttranslational modification.[13–15] However, it is the potent antimicrobial activity of plantazolicin A against Gram-positive organisms that has attracted much attention. In particular, the compound is selectively active against the anthrax-causing organism *Bacillus anthracis* (Sterne).[11,13] Although the polyazole nature of plantazolicin A is highly reminiscent of the thiopeptide antibiotics,[16–18] a synthetic derivative of which has entered the clinic against *Clostridium difficile* infections,[19,20] there are key differences. Specifically, the linear nature of the antibiotic with its two pentacyclic regions represents a challenge for chemical synthesis that, in combination with the antimicrobial activity, makes plantazolicin A highly attractive for further study. The first total synthesis of plantazolicin A was reported by Süssmuth and co-workers in 2013,[21] and very recently, a second total synthesis was reported by Ley and co-workers.[22] Both syntheses relied on classical peptide coupling. We now report a conceptually different synthesis of plantazolicin A on the basis of carbene chemistry.

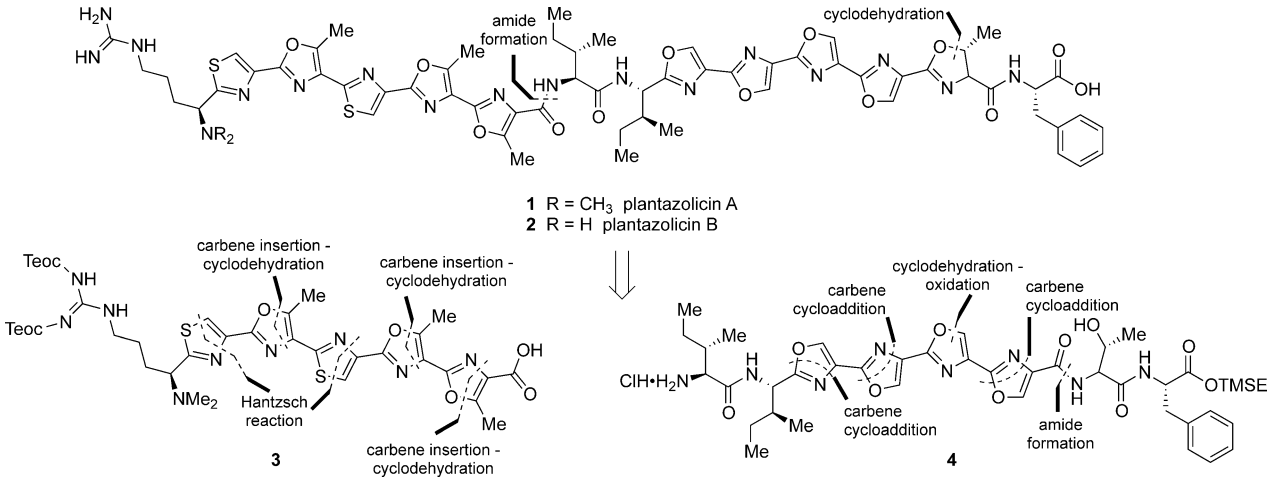


Scheme 1. Synthesis of oxazoles from carboxamides and nitriles via rhodium carbenes.

Our strategy was to construct the azole rings by using carbene intermediates, and hence our retrosynthetic analysis divided the molecule into two fragments, **3** and **4**, each adorned with appropriate protecting groups, with the sensitive oxazoline ring in the C-terminus right-hand fragment **4** to be formed by a late-stage cyclodehydration reaction (Scheme 2). In contrast with other approaches, we elected to introduce the guanidine moiety later in the synthesis. Thus, the starting point for our synthesis was the known ornithine-derived thiazole-4-ester **5**,[23] which was readily converted into the corresponding carboxamide **6** to set the scene for our first step involving a carbene (Scheme 3). The key metallocarbene N–H insertion was carried out by heating a mixture of methyl 2-diazo-3-oxobutanoate and amide **6** in the presence of rhodium(II) acetate dimer (2.5 mol%) in dichloromethane in a microwave reactor (200 W, 80 °C), to give the ketoamide

[*] H. Wada, Dr. H. E. L. Williams, Prof. C. J. Moody
School of Chemistry, University of Nottingham
Nottingham NG7 2RD (UK)
E-mail: c.j.moody@nottingham.ac.uk

Supporting information for this article, including experimental procedures, characterization data for all compounds, and copies of the ^{1}H and ^{13}C NMR spectra, is available on the WWW under http://dx.doi.org/10.1002/anie.201507062.



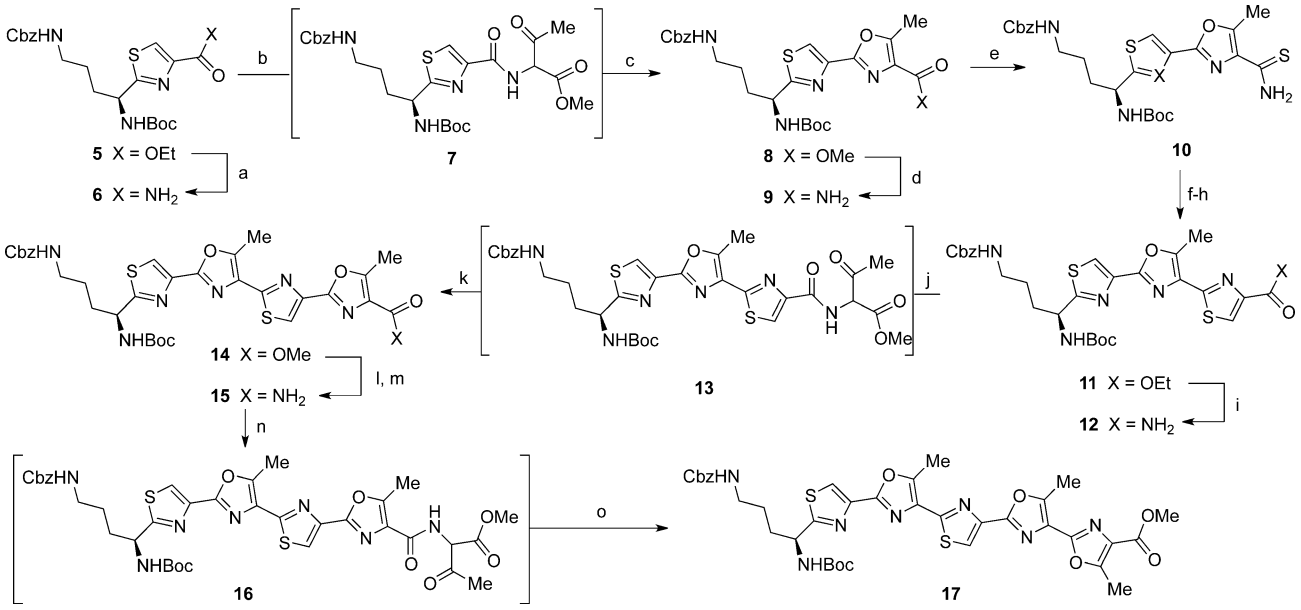
Scheme 2. Retrosynthetic analysis of plantazolicin A.

7, which was immediately cyclized to oxazole **8** by cyclodehydration with triphenylphosphine and iodine in the presence of a base.[24] The difficulties associated with the isolation of the product from triphenylphosphine oxide were largely overcome by the use of polymer-supported triphenylphosphine. The ester **8** was converted into the corresponding carboxamide **9** and then into the thiocarboxamide **10** by treatment with the Lawesson reagent. A Hantzsch reaction formed the next ring and delivered the three-azole array **11** (Scheme 3). With two further oxazole rings to install, we instigated an iterative carbene carboxamide N–H insertion–cyclodehydration sequence. Thus, ester **11** was converted into carboxamide **12**, which underwent the desired carbene insertion to give ketoamide **13**, the cyclodehydration of which gave tetra-azole **14** in 52% yield over two steps. A second iteration via carboxamide **15** and ketoamide **16** produced the desired penta-azole **17**, although the yield of this last ring-forming step was poor.

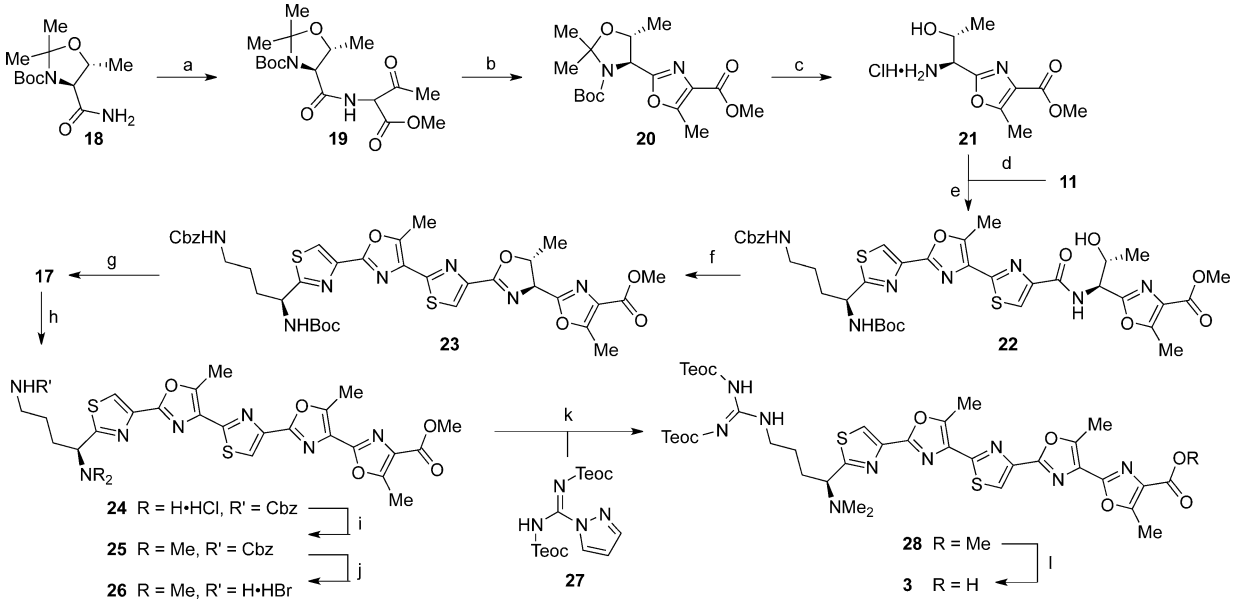
In view of the unsatisfactory formation of the fifth and final ring in the penta-azole **17**, we sought an alternative, while still satisfying our desire to use carbene methodology to construct the azole rings. Thus, the protected threoninamide **18** underwent rhodium(II)-catalyzed N–H insertion followed by cyclodehydration to give oxazole ester **20** in good yield, the deprotection of which resulted in oxazole **21** with a threonine-derived free amine (Scheme 4). This amine was then coupled with the thiazolecarboxylate formed by simple hydrolysis of the bis(thiazolyl)oxazole **11**, thus resulting in the formation of the linear tetra-azole **22**. Subsequent ring closure of the threonine fragment by the use of DAST methodology gave oxazoline **23**,[25] the dehydrogenative aromatization of which with bromotrichloromethane in the presence of a base[26] yielded the previously prepared penta-azole **17**.

With an improved route available to the key penta-azole, the synthesis rapidly progressed towards the complete left-hand fragment **3** of the antibiotic. Acid-mediated removal of the Boc group allowed for installation of the dimethylamino group by a reductive amination reaction with formaldehyde to give **25**, which underwent cleavage of the remaining N-protecting group in preparation for the introduction of the 2-trimethylsilylethoxycarbonyl (Teoc)-protected guanidine moiety onto amine **26**. To this end, we developed a new reagent, the pyrazole carboxamidine **27**, which successfully incorporated the protected guanidine to give penta-azole **28**. Hydrolysis of the ester finally gave the desired fragment **3** (Scheme 4) in 1.2% overall yield over 17 steps.

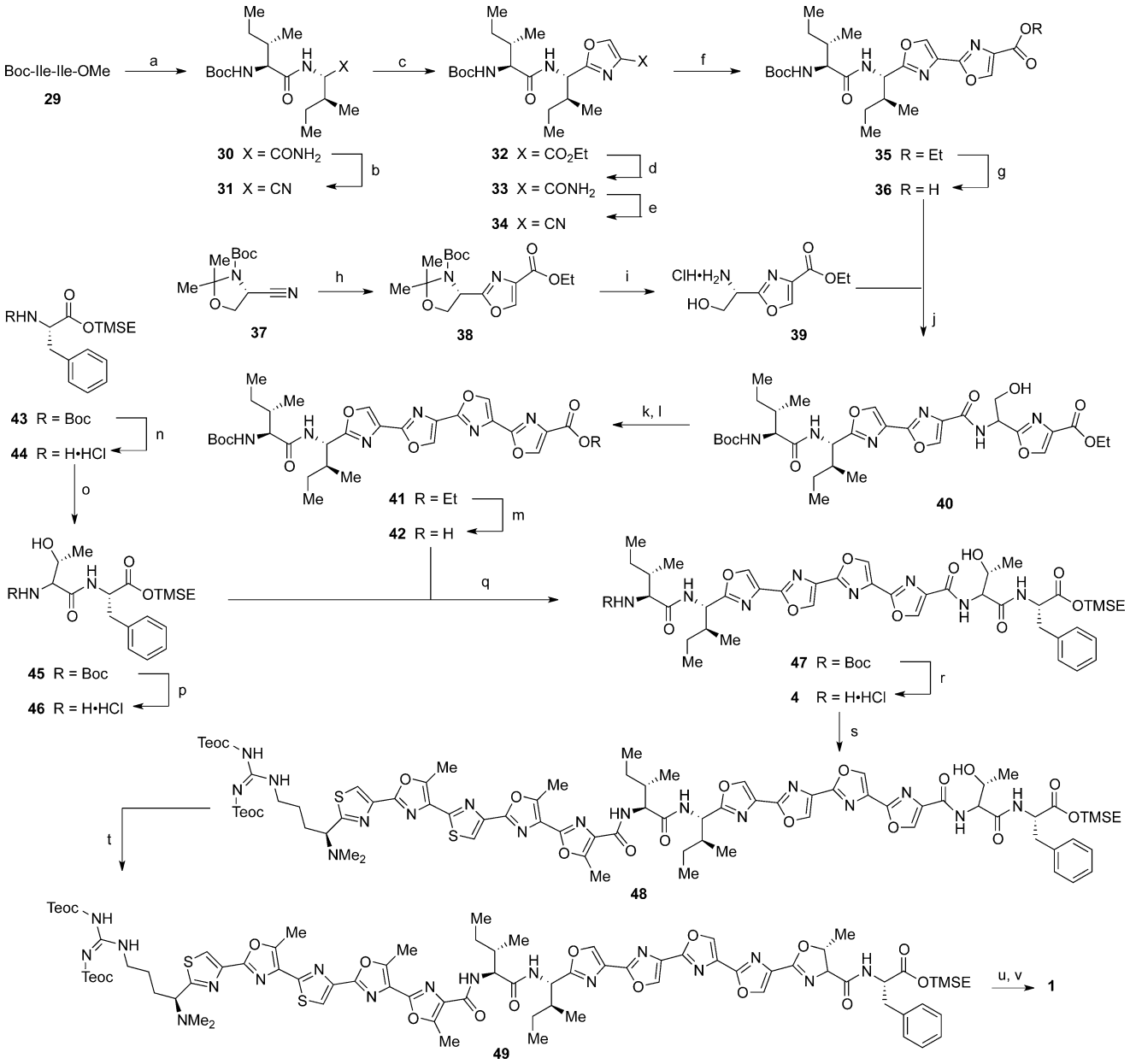
The presence of four C5-unsubstituted oxazoles in the C-terminal fragment of the antibiotic, in contrast to the three 5-methyloxazoles present in the N-terminal fragment **3**, necessitated a slight change in methodology. Thus, the order of steps was reversed, and the carboxamide was initially dehydrated to the nitrile, with subsequent direct conversion into the oxazole in the rhodium-catalyzed carbene cycloaddition step (Scheme 1).[10,27] Hence, the synthesis of fragment **4** started with the known isoleucine–isoleucine dipeptide **29**,[28] which was converted into the carboxamide **30** and then the required nitrile **31** (Scheme 5). These transformations set the stage for the direct installation of the first oxazole **32** by a dirhodium(II)-catalyzed reaction with the formyl diazo compound ethyl 2-diazo-3-oxopropanoate, although a change in catalyst to dirhodium tetrakis(heptafluorobutyramide) was beneficial.[27] The sequence was iterated to deliver the bisoxazole ester **35** by the conversion of oxazole ester **32** into nitrile **34** and thereafter a second rhodium–carbene step. Meanwhile, nitrile **37** also underwent rhodium-catalyzed carbene cycloaddition to give oxazole **38**,[10] which was deprotected to give amine **39** in preparation for union with the bisoxazole fragment. Bisoxazole ester **35** was hydrolyzed to acid **36**, and subsequently coupled to amine **39** by use of the HBTU protocol to give adduct **40**. A DAST-mediated cyclodehydration and aromatization completed the linear array **41** of four oxazole rings, and this ester was subsequently hydrolyzed to the corresponding carboxylic acid **42** for further elaboration (Scheme 5). Separately, Boc-Phe-OH was protected as its 2-trimethylsilylethyl (TMSE) ester **43**, the Boc group was cleaved, and the ensuing amine **44** was united with *N*-Boc-protected allothreonine to give dipeptide **45**. The dipeptide was deprotected in acid, and the resulting amine **46** underwent coupling with the tetra-oxazole acid **42** to give, after removal of the Boc group, the complete C-terminal fragment **4** in 1.7% overall yield over 13 steps from the isoleucine dipeptide **29**.



Scheme 3. Synthesis of intermediate penta-azole **17**: a) 35% aqueous NH_3, EtOH, room temperature, 93%; b) methyl 2-diazo-3-oxobutanoate (1.5 equiv), rhodium(II) acetate dimer (2.5 mol%), CH_2Cl_2, 80°C, microwave (200 W); c) polymer–Ph_3P (1.6 equiv), I_2 (1.6 equiv), Et_3N (3.2 equiv), CH_2Cl_2, room temperature, 72% (2 steps); d) 35% aqueous ammonia, MeOH, THF, room temperature, 80%; e) Lawesson reagent (0.7 equiv), $CHCl_3$, room temperature, 55%; f) ethyl bromopyruvate (5.0 equiv), $KHCO_3$ (10 equiv), DME, −10°C; g) trifluoroacetic anhydride (5 equiv), 2,6-lutidine (10 equiv), DME, −10°C; h) K_2CO_3 (5.0 equiv), EtOH, H_2O, room temperature, 71% (3 steps); i) 35% aqueous NH_3, EtOH, room temperature, 84%; j) methyl 2-diazo-3-oxobutanoate (1.4 equiv), rhodium(II) acetate dimer (2.5 mol%), $CHCl_3$, 60°C; k) polymer–Ph_3P (3.5 equiv), I_2 (3.5 equiv), Et_3N (7.0 equiv), CH_2Cl_2, room temperature, 52% (2 steps); l) LiOH, MeOH/THF/H_2O (1:5:5), room temperature, 93%; m) EtO_2CCl, Et_3N, THF, 35% aqueous NH_3, room temperature, 57%; n) methyl 2-diazo-3-oxobutanoate (1.5 equiv), rhodium(II) acetate dimer (2.5 mol%), $CHCl_3$, 60°C; o) polymer–Ph_3P (4.0 equiv), I_2 (4.0 equiv), Et_3N (8.0 equiv), CH_2Cl_2, room temperature, 11% (2 steps). Boc = *tert*-butoxycarbonyl, Cbz = benzyloxycarbonyl, DME = 1,2-dimethoxyethane.



Scheme 4. Synthesis of left-hand penta-azole fragment **3**: a) methyl 2-diazo-3-oxobutanoate (1.4 equiv), rhodium(II) acetate dimer (2.5 mol%), $CHCl_3$, 70°C, 16 h; b) Ph_3P (2.0 equiv), I_2 (2.0 equiv), Et_3N (4.0 equiv), CH_2Cl_2, room temperature, 55% (2 steps); c) 4 M HCl in 1,4-dioxane, room temperature, 6 h, quantitative; d) LiOH, MeOH/THF/H_2O (1:5:5), room temperature, 15 h, 82%; e) HBTU, Et_3N, CH_2Cl_2, room temperature, 92%; f) DAST, K_2CO_3, CH_2Cl_2, −78°C; g) $BrCCl_3$, DBU, CH_2Cl_2, 0°C→RT, 32%; h) 4 M HCl in dioxane, room temperature, quantitative; i) NaOAc·$3H_2O$ (56 equiv), formaldehyde (37% solution in H_2O, 30 equiv), $NaBH_3CN$ (23 equiv), THF, room temperature, 63%; j) HBr (33% solution in AcOH; 100 equiv), room temperature, quantitative; k) **27** (1.4 equiv), Et_3N (2.8 equiv), $CHCl_3$, 40%; l) Me_3SnOH (10 equiv), DCE, 80°C, quantitative. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DCE = 1,2-dichloroethane, DAST = dimethylamino sulfur trifluoride, HBTU = (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.



Scheme 5. Synthesis of right-hand tetra-azole fragment **4**: a) 35% aqueous ammonia, MeOH, THF, room temperature, 76%; b) DBU (5.0 equiv), ethyl dichlorophosphate (3.0 equiv), CH_2Cl_2, 0°C, 80%; c) ethyl 2-diazo-3-oxopropanoate (3.0 equiv), rhodium(II) perfluorobutyramide dimer (2.5 mol%), $CHCl_3$, 60°C, 53%; d) 35% aqueous ammonia, EtOH, THF, room temperature, 89%; e) DBU (5.0 equiv), ethyl dichlorophosphate (3.0 equiv), CH_2Cl_2, 0°C, 79%; f) ethyl 2-diazo-3-oxopropanoate (3.0 equiv), rhodium(II) perfluorobutyramide dimer (2.5 mol%), $CHCl_3$, 60°C, 59%; g) LiOH (58 equiv), EtOH/THF/H_2O (1:5:5), room temperature, 65%; h) ethyl 2-diazo-3-oxopropanoate (3.0 equiv), rhodium(II) perfluorobutyramide dimer (2.5 mol%), $CHCl_3$, 60°C, 51%; i) 2 M HCl in ether, room temperature, quantitative; j) HBTU (1.5 equiv), **39** (1.5 equiv), Et_3N (2.0 equiv), CH_2Cl_2/DMF (1:1), room temperature, 76%; k) DAST (1.7 equiv), K_2CO_3 (5.0 equiv), CH_2Cl_2, −78°C, quantitative; l) $BrCCl_3$ (4.0 equiv), DBU (4.0 equiv), CH_2Cl_2, 0°C, 59% (2 steps); m) LiOH (29 equiv), EtOH/THF/H_2O (1:5:5), room temperature, 76%; n) 4 M HCl in dioxane, room temperature, 56%; o) Boc-protected allothreonine (1.0 equiv), HBTU (2.0 equiv), **44** (2.0 equiv), Et_3N (4.0 equiv), CH_2Cl_2/DMF (1:1), room temperature, 65%; p) 4 M HCl in 1,4-dioxane, room temperature, 79%; q) HBTU (1.5 equiv), **46** (1.5 equiv), Et_3N (3.0 equiv), CH_2Cl_2/DMF (1:1), room temperature, 58%; r) 4 M HCl in 1,4-dioxane, room temperature, quantitative; s) HBTU (1.8 equiv), **3**, Et_3N (3.7 equiv), CH_2Cl_2, room temperature, 50%; t) DAST (30.0 equiv), CH_2Cl_2, −78°C, 52%; u) TASF (30.0 equiv), DMSO, room temperature; v) HFIP, room temperature, 31%. DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulfoxide, TMSE = 2-trimethylsilylethyl, TASF = tris(dimethylamino)sulfonium difluorotrimethylsilicate, HFIP = hexafluoroisopropanol.

Having successfully obtained both the N- and C-terminal halves of the antibiotic, we coupled polyazoles **3** and **4** in the presence of HBTU and Et_3N to give **48**. The threonine moiety of the intermediate **48** was cyclized in the presence of DAST to afford the oxazoline **49**, the ^1H NMR and ^{13}C NMR spectroscopic data of which matched those of an intermediate prepared in an earlier synthesis.[21] All that remained was to remove the three silicon-based protecting groups, and in common with previous workers, we found that the removal of these groups was not completely straightforward, and

required a two-stage strategy involving the sequential use of two fluoride-based reagents (Scheme 5). Following purification by HPLC, the NMR spectroscopic data of our synthetic material matched those reported for the natural antibiotic (see Tables S1 and S2 in the Supporting Information); the compound also co-eluted on HPLC with authentic material (see Figures S1–S3). Our synthetic material also exhibited modest potency against methicillin-resistant *Staphylococcus aureus* (MRSA) with a minimum inhibitory concentration (MIC) of $>33\,\mu g\,mL^{-1}$ (compare with the value of $>128\,\mu g\,mL^{-1}$ found for the natural material[13]).

Finally, we were interested in the conformation of plantazolicin A. NMR spectroscopy NOESY and TOCSY experiments were carried out (see the Supporting Information) along with molecular modeling. The lack of long-range NOEs suggests a moderately dynamic molecule with rigid oxazole/thiazole arms that are not in close contact for any appreciable time. However, the strong NOEs around the central two isoleucine residues suggest that this portion of the molecule could act as a hinge region leading to a dynamic hairpin conformation. A structure consistent with these data is shown in Figure 1.

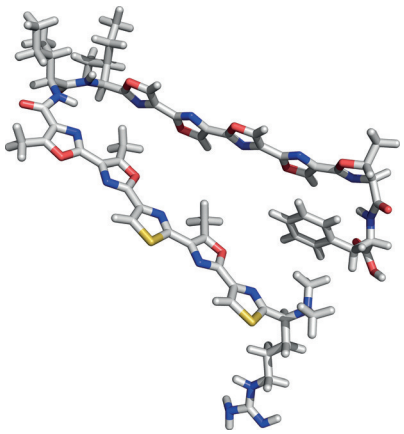


Figure 1. Most probable conformation of plantazolicin A on the basis of NMR spectroscopy and molecular modeling.

The carbene-based synthesis described above is not only a practical route that could be used to provide further quantities of the fascinating antimicrobial agent plantazolicin A, but it also makes available a wide range of fragment structures and analogues that are not made available by Nature's biosynthetic machinery. The full biological evaluation of these novel structures is under way.

Acknowledgements

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