Mutasynthesis approaches to the preparation of streptorubin B analogues

Stuart W. Haynes

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

University of Warwick
Department of Chemistry

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Stereochemical elucidation of streptorubin B by mutasynthesis with stereospecifically deuterium labelled 2-undecylpyrrole

Synthesis of (R)- and (S)-[C-4′-D]2-undecylpyrrole

Feeding of stereoselectively labelled 2-undecylpyrroles to S. coelicolor W116

Investigation of why streptorubin B diastereoisomers are produced and absolute stereochemical assignment of streptorubin B

Conclusions and perspectives

Mutasynthesis of streptorubin B analogues – probing substrate tolerances of RedH and RedG

Mutasynthesis of streptorubin B analogues – access to new compounds versus total synthesis

In vitro application of RedH and RedG

Probing the mechanism of carbocyclisation by RedG

Further experiments to confirm the absolute stereochemistry of streptorubin B

Experimental Section

General Experimental

(5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine

4-Methoxy-1H,1′H-[2,2′]bipyrrrolyl-5-carbaldehyde

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Declaration

The experimental work reported in this thesis is original research carried out by the author, unless otherwise stated, in the Department of Chemistry, University of Warwick, between September 2006 and December 2009. No material contained herein has been submitted for any other degree, or at any other institution.

Results from other authors are referenced in the usual manner throughout the text.

_________________________________________ Date: ________________

Stuart W. Haynes
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<tr>
<td>°C</td>
<td>Celsius</td>
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<tr>
<td>2-UP</td>
<td>2-undecylpyrrole</td>
</tr>
<tr>
<td>A (domain)</td>
<td>Adenylation (domain)</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
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<tr>
<td>ACP</td>
<td>Acyl carrier protein</td>
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<tr>
<td>AIBN</td>
<td>Azobis(isobutynitrile)</td>
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<tr>
<td>Apr</td>
<td>Apramycin</td>
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<td>Asp</td>
<td>Aspartic acid</td>
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<td>Acyl transferase</td>
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<tr>
<td>ATP</td>
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<tr>
<td>CD</td>
<td>Circular Dichroism</td>
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<td>CDA</td>
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<td>CoA</td>
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<td>DCM</td>
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<tr>
<td>DMAHB</td>
<td>3-dimethylallyl-4-hydroxybenzoic acid</td>
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</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
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<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMPU</td>
<td>N,N’-dimethyl-N,N’-propylene urea</td>
</tr>
<tr>
<td>DMS</td>
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</tr>
<tr>
<td>Et</td>
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<td>FAD</td>
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<td>Fatty acid synthase</td>
</tr>
<tr>
<td>FMN</td>
<td>Flavin mononucleotide</td>
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<td>HBM</td>
<td>4-hydroxy-2-2´-bipyrrole-4-methanol</td>
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<td>KAPA</td>
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<td>LC-MS</td>
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<td>LDA</td>
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</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
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<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
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<td>Ms</td>
<td>Methanesulfonyl (Mesyl)</td>
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<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NAD(P)</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate</td>
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<td>Description</td>
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<td>Triisopropylsilyl</td>
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<td>Ts</td>
<td>Tosyl</td>
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Abstract

Prodiginines are a large family of red-pigmented tripyrrole-based antibiotics. Their biosynthesis in *Streptomyces coelicolor* A3(2), by enzymes encoded in the red gene cluster, involves the condensation of 4-methoxy-2,2′-bipyrrrole-5-carboxaldehyde (MBC) and 2-undecylpyrrole, catalysed by the RedH enzyme to give undecylprodiginine. This is followed by the mechanistically interesting oxidative carbocyclisation, catalysed by RedG to give streptorubin B.

In this thesis prodiginine analogues have been generated by a mutasynthetic approach, in which chemically synthesised analogues of intermediates MBC and 2-undecylpyrrole were fed to mutant strains of *S. coelicolor* in which one of the genes required to biosynthesise MBC or 2-undecylpyrrole (redM or redL respectively) have been deleted. RedH and RedG have been shown to display relatively broad substrate tolerances and several analogues of both undecylprodiginine and streptorubin B have been generated by this approach. A variety of factors which are potentially limiting to substrate tolerances have been probed, including the steric size, alkyl chain hydrophobicity and introduction of π-electrons.

The absolute stereochemistry of streptorubin B has been investigated by a mutasynthetic approach in which 2-undecylpyrrole, stereospecifically labelled with deuterium, is fed to a mutant strain of *S. coelicolor* unable to biosynthesise 2-undecylpyrrole. During the course of the investigation streptorubin B was analysed on a homochiral stationary phase HPLC and evidence of both ent- and dia-streptorubin B was discovered in the natural product isolated from *S. coelicolor*. When the position of the deuterium label from the mutasynthesis experiment was investigated by ¹H-NMR and ²H-NMR the partial epimerization of the synthetic material became apparent. This made the definitive determination of the absolute stereochemistry of streptorubin B impossible. However, a tentative assignment of the absolute stereochemistry was possible. This was supported by comparison with the related natural products metacycloprodigiosin, the stereochemistry of which was established here by CD spectroscopy and chiral HPLC analyses, and roseophilin.
1.0 Introduction

1.1 Introduction to the prodiginine antibiotics

Prodiginines are a large family of red-pigmented antibiotics produced by actinomycetes and other eubacteria. Their structure is based around the tripyrrole nucleus observed in prodigiosin 1 (Figure 1), which is the major pigment observed in Serratia marcescens. Prodigiosin was first isolated in 1902 but not structurally resolved until 1960.\(^1\) Since its discovery, several prodigiosin-like pigments have been isolated. Gerber proposed prodiginine as a trivial name for the group of compounds which contain the common tripyrrole aromatic moiety, in which three pyrrole rings are linked in a highly conjugated system 2 (Figure 1).\(^2\)

![Figure 1: Prodigiosin 1 and the prodiginines general structure 2](image)

Among the known prodiginines most are produced by actinomycetes including the linear undecylprodiginine 3 (Figure 2), originally isolated from Streptomyces sp. Y-42, and several isomeric cyclic derivatives like butyl-meta-cycloheptylprodiginine (Streptorubin B) 4, meta-cyclo-prodigiosin (ethyl-meta-cyclononylprodigiosin, streptorubin A) 5,\(^3\) nonylprodigiosin 6, isolated from Actinomadura madurae,\(^4\) and roseophilin 8, isolated from Streptomyces griseoviridis which can be considered of related structure (Figure 2).\(^5\) Roseophilin actually contains a methoxyfuran in place of the methoxypyrrole ring but shares the conjugated core structure of the prodiginine antibiotics. These natural products all possess high levels of biological activity, and although they
have found no current clinical use due to their high non-specific cytotoxicity, (thought to result from their ability to facilitate double strand DNA cleavage in the presence of Cu$^{2+}$ ions and molecular oxygen)\textsuperscript{6} their pharmacological activity makes their study of interest. These structurally intriguing metabolites have already been shown to possess immunosuppressant properties at non-toxic doses, \textsuperscript{7, 6, 9, 10, 11} and the cyclic prodiginines, most notably metacycloprodigiosin 5 (streptorubin A), have been shown to display strong anti-malarial activity.\textsuperscript{12} Furthermore a synthetic prodiginine analogue, obatoclax 7 (Figure 2), is currently in phase 1/2 clinical trials for a variety of solid tumours and multiple myeloma.\textsuperscript{13} The activity of obatoclax appears to derive from its interaction with the Bcl-2 family of proteins, which are involved in mediating apoptotic processes within the cell. Anti-apoptotic Bcl-2 proteins (Bcl-xL, Mcl-1, and Bcl-2 itself) have been shown to be overproduced in numerous cancer cell lines, \textsuperscript{14, 15} making them promising targets for oncology treatments. \textsuperscript{16, 17} The Bcl-2 family of proteins are known to bind to pro-apoptotic proteins within the cell (including Bad, Bid and Bim), which are involved in initiating the signalling cascade that triggers cytochrome c release that in turn activates caspases responsible for dismantling the cell during apoptosis. \textsuperscript{18, 19} Obatoclax 7 has been shown to disrupt the binding of the anti-apoptotic Bcl-2 proteins with pro-apoptotic proteins and hence restore normal cell death processes within immortal cell lines overproducing Bcl-2. \textsuperscript{20, 21}
1.2 *Streptomyces coelicolor*

*Streptomyces coelicolor* A3(2) is a known producer of prodiginine antibiotics and is the subject of this research project. In 2002, the complete genome sequence was reported, making it an ideal strain to study prodiginine biosynthesis via the construction of designed mutants lacking one or more genes required for the biosynthesis of prodiginine antibiotics. *S. coelicolor* A3(2) is known to produce several structurally distinct antibiotics, including the calcium-dependent antibiotics (CDAs), actinorhodin, methylenomycins and the spore germination inhibitors, germicidins (Figure 3), all in addition to the compounds of interest for this study undecylprodiginine 3, and streptorubin B 4.
Figure 3: Some other known antibiotics produced by *S. coelicolor* A3(2)

### 1.3 Early studies of prodiginine biosynthesis

The first work on prodiginine biosynthesis focused on prodigiosin 1 production in *Serratia marcescens*, in which Fourier transform NMR (FT-NMR) spectroscopy studies were used to determine the incorporation of stable isotope labelled precursors. These studies first identified methionine as the source of the methoxy carbon and the incorporation of serine and proline into the 2-2′-bipyrrole ring system on the left hand side of prodigiosin, which, at this time was already suggested to be a common intermediate in both the prodigiosin and undecylprodiginine pathways due to their structural similarities. Later work in
Streptomyces longispororuber, a known producer of undecylprodiginine and streptorubin A, confirmed, again by FT-NMR studies, that the biosynthetic origin of the left hand side of undecylprodiginine is the same as observed for the left hand side of prodigiosin in S. marcescens. This investigation also established the biosynthetic origin of the right hand side monopyrrole fragment, which was determined to be derived from one unit of glycine and seven units of acetate. In addition this study was the first to confirm the common biosynthetic origin of undecylprodiginine and its cyclic derivatives, in this case streptorubin A, which was observed to display the same incorporation pattern of these stable isotope labels as undecylprodiginine (Figure 4).\(^{27}\)

Based on these stable isotope feeding experiments carried out in S. longispororuber, prodiginine biosynthesis in this organism was proposed to occur via a convergent method. The condensation of two late stage key intermediates, 4-methoxy-2,2′-bipyrrrole-5-carboxaldehyde 9 (MBC), and 2-undecylpyrrole 10 would yield undecylprodiginine and subsequently streptorubin A, in a matter consistent with the observed labelling pattern (Figure 4).\(^{27}\) Later studies confirmed the biosynthetic origin of streptorubin B as matching that previously established for streptorubin A and undecylprodiginine.\(^{28}\)

Some pioneering genetics studies identified several of the prodiginine biosynthetic genes in S. coelicolor by way of classic UV irradiation mutagenesis. Rudd and Hopwood identified mutants deficient in prodiginine biosynthesis and classified several genomic regions involved in directing prodiginine biosynthesis in S. coelicolor into five groups (A to E). The groups were classified on the basis of their co-synthesis behaviour of the red pigment, the structure of which was not known at this time.\(^{29}\) Subsequently, a mutant strain from class E was used as a host for screening DNA fragments from chromosomal digests of M145 (wild-type S. coelicolor known to produce the red pigment). This allowed the identification of a DNA fragment that efficiently complemented the class E
Feitelson et al. then identified a series of overlapping clones, which together complemented classes A, B and E, and introduced the new classification F as a result of a sub-division of the class E mutants. Later work by Hopwood et al. identified a final clone which complemented classes C and D. Together with the clones from the previous work this allowed the creation of a single clone capable of complementing all mutant classes and to result in production of the red pigment (now identified as a mixture of undecylprodiginine and streptorubin B) in a heterologous host *Streptomyces parvulus.*

![Diagram of proposed biosynthetic pathway to metacycloprodigiosin in *S. longispororuber* based on feeding of labelled precursors](image_url)
1.4 The red cluster

In 2002, the completion of the genome sequence of *S. coelicolor* A3(2), made the sequence of the red cluster available. It comprises twenty three genes arranged in four transcription units (Figure 5). The putative functions of the products of most of the genes were suggested on the basis of sequence comparisons. These putative functions led to a proposed biosynthetic pathway, which has been subsequently revised in light of subsequent experimental evidence.

![Figure 5: The red cluster](image)

By analogy with the proposed prodiginine biosynthetic pathway in *S. longispororuber* (Figure 4) and in light of the available genetic data, the biosynthetic origin of undecylprodiginine and streptorubin B in *S. coelicolor* was suggested to be analogous. Their biosynthesis was proposed to proceed via the condensation of two putative key late stage intermediates 4-methoxy-2, 2′-bipyrrole-5-carboxaldehyde 9 (MBC) and 2-undecylpyrrole 10 (2-UP). The presence and origin of MBC 9 and 2-undecylpyrrole 10 in the prodiginine biosynthetic pathway has subsequently been proven by a variety of experimental data.

Seven genes are known to be required for the biosynthesis of the key intermediate 4-methoxy-2, 2′-bipyrrole-5-carboxaldehyde 9 (MBC), and five genes are required for the biosynthesis of a second key intermediate 2-
undecylpyrrole 10 (2-UP). Two genes (redD and redZ) are known to encode pathway specific transcriptional regulators (Figure 5). One gene (redH) has been shown to encode the enzyme involved in the condensation of MBC 9 and 2-undecylpyrrole 10 to form undecylprodiginine 3. The protein encoded by redG shows similarity to Rieske non-heme, iron-dependent oxygenase enzymes and has been shown to be involved in the oxidative cyclisation of undecylprodiginine to form the carbocyclic derivative streptorubin B. The role of redJ and redT, is unclear however they appear to play a role in prodiginine biosynthesis because mutants in which they are knocked out show reduced prodiginine titres. Three genes (redE, redF and redY) do not appear to be required for prodiginine biosynthesis (Figure 5). Finally, redV is yet to be fully investigated and redS appears to encode for a truncated, non functional protein.

1.5 4-methoxy-2', 2'-bipyrrrole-5-carboxaldehyde (MBC) biosynthesis

4-methoxy-2', 2'-bipyrrrole-5-carboxaldehyde 9 (MBC) is a known intermediate common to both the prodiginine biosynthetic pathway in S. coelicolor and the related prodigiosin 1 biosynthetic pathway in Serratia species. The mechanism of biosynthesis proposed by Cerdeño et al. and Walsh et al. and then later revised by Williamson et al. and Stanley et al. appears to be common to both species with homologous enzymes in each pathway (Figure 6).

The MBC-derived portion of undecylprodiginine is generated from one unit each of acetate, L-proline and L-serine as shown by labelling experiments (Figure 4). L-proline is activated for loading onto the phosphopantetheine arm of the peptidyl carrier protein (PCP) RedO by adenylation catalysed by RedM (Figure 6). L-prolyl-RedO is then dehydrogenated by the FAD-dependent oxidase RedW to give pyrrole-2-carboxyl-RedO. The pyrrole-2-carboxyl group attached to
RedO is transferred to the C-terminal ketosynthase (KS) domain of RedX where concomitant decarboxylation and condensation with a malonyl group attached to one of the two tandem acyl carrier protein (ACP) domains in RedN gives a β-keto-ACP-thioester. Subsequently, the action of the C-terminal α-oxoamine synthase (OAS) domain of RedN cleaves the ACP-thioester by catalysing a decarboxylative condensation with L-serine. 4-hydroxy-2,2´-bipyrrole-4-methanol (HBM) results from subsequent, probably spontaneous, cyclisation and dehydration. Finally, oxidation of the hydroxymethyl group to the corresponding aldehyde by a currently undefined enzyme and SAM-dependent methylation of the pyrrole hydroxyl group catalysed by RedI gives 4-methoxy-2,2´-bipyrrole-5-carboxaldehyde (MBC). 42

Figure 6: Proposed biosynthetic pathway to MBC in S. coelicolor
1.6 2-undecylpyrrole biosynthesis

The 2-undecylpyrrole derived portion of undecylprodigine has been shown by labelling studies to be biosynthesised from seven units of acetate and one unit of glycine (Figure 4). RedP is thought to generate an acetoacyl thioester on the acyl carrier protein (ACP) RedQ, by decarboxylative condensation of an acetyl-CoA starter unit with a malonyl-CoA extender unit attached to RedQ (Figure 7). Subsequent keto-reduction, dehydration and enoyl-reduction of the resulting thioester intermediate gives butyryl-ACP, by the action of ketoreductase, dehydratase and enoylreductase enzymes from the type II fatty acid synthase family (FAS) in S. coelicolor. Iterative chain extension by the action of RedR and continued rounds of reduction and dehydration by the corresponding FAS enzymes after each elongation would give dodecanoyl-RedQ. This would then undergo one further chain extension after transfer to the N-terminal ACP domain of RedL via one of two mechanisms: direct transfer from RedQ, or via hydrolysis to the free acid which could subsequently be loaded onto the ACP domain after activation by the NRPS-like adenylation domain of RedL. The RedL ketosynthase (KS) domain then catalyses the reaction of the resulting dodecanoyl thioester with a malonyl thioester attached to the C-terminal ACP domain of RedL to form β-ketomyristoyl-ACP. The subsequent pyridoxal-5′-phosphate (PLP)-dependent decarboxylative condensation with glycine, catalysed by the C-terminal OAS domain of RedL, would result in chain release. Spontaneous cyclisation and elimination of water would lead to 4-keto-2-undecylpyrroline. Reduction of the keto group, catalysed by the NAD(P)H-dependent RedK, and elimination of water, yields 2-undecylpyrrole (Figure 7).
1.7 Condensation of MBC and 2-undecylpyrrole and subsequent carbocyclisation

As mentioned previously, after the formation of MBC and 2-undecylpyrrole, it was proposed that condensation of these two late stage intermediates is catalysed by RedH to give undecylprodiginine. RedH is predicted to encode for a protein with three functional domains; two of which show sequence similarity to the ATP-binding domains and the phosphotransfer domains respectively, of phosphoenol pyruvate synthase (PEPS) and pyruvate-phosphate dikinase (PPDK). However, the third proposed functional domain of RedH does not
show sequence similarity to any proteins or protein domains of known function.\(^3\) Using this knowledge it is hypothesised, by analogy with the known catalytic mechanism of PEPS and PPDK, that RedH activates the aldehyde carbonyl of MBC towards nucleophilic attack by phosphorylation of the aldehyde oxygen atom. Subsequent nucleophilic attack by C-5 of 2-undecylpyrrole at this activated position followed by elimination of phosphate would yield undecylprodiginine (Figure 8).

![Chemical structure](image)

**Figure 8: Action of RedH, catalysing the formation of undecylprodiginine**

The *redG* gene encodes a protein that is predicted to contain two domains and has been shown to catalyze the oxidative carbocyclisation of undecylprodiginine \(3\) to streptorubin B \(4\).\(^{42}\) CXH and CX\(_2\)H sequences found in the N-terminal domain of RedG match those known to bind iron within the Fe\(_2\)S\(_2\) cluster
characteristically found in N-terminal domains of the α-subunits of Rieske dioxygenases such as naphthalene dioxygenase (NDO) (Figure 9). The C-terminal domain of RedG contains an EX$_2$HX$_4$H sequence motif. This bears strong similarity to the known conserved sequence in the C-terminal domain of the α-subunit of NDO and related Rieske dioxygenases, DX$_2$HX$_4$H (Figure 9). This motif contains two His residues that bind the non-heme iron centre and an Asp residue, which is proposed to be involved in mediating electron transfer from the Rieske cluster to the non-heme iron centre via a hydrogen bond network. In RedG the mutation of the Asp residue of this motif to Glu is predicted to have no functional significance.

**Figure 9: Sequence comparison of RedG (S. coelicolor), homologue McpG (S. longispororuber) and archetypal Rieske dioxygenase, NDO (naphthalene dioxygenase)**

Prior to recent work in the Challis group on the red cluster, no previous examples of Rieske dioxygenases have been observed to carry out the chemically challenging C-H bond activation required for the proposed oxidative carbocyclisation of undecylprodiginine to streptorubin B. It has been shown that RedG catalyses the formation of the 10-membered carbocycle in streptorubin B, and that a RedG homologue (McpG) from *Streptomyces longispororuber* catalyses the analogous formation of the 12-membered carbocyclisation in metacycloprodigiosin (streptorubin A) (Figure 10). Oxidative cyclisations catalysed by non-heme iron dependent dioxygenase-like enzymes are generally assumed to proceed via a Fe(IV)=O intermediate, which...
is formed by the reductive cleavage of the O=O bond of an iron-bound molecular oxygen. This Fe(IV)=O intermediate is proposed to carry out a variety of stereo- and regio-specific cleavages of C-H bonds to generate carbon-centred radicals as the key intermediates in oxidative cyclisations.

![Figure 10: Different sites of cyclisation to form streptorubin B and metacycloprodigiosin (streptorubin A) from a common precursor.](image)

By analogy with this proposal, the oxidative carbocyclisation of undecylprodiginine to streptorubin B, in the case of RedG, or metacycloprodigiosin (streptorubin A), in the case of McpG, is predicted to occur via carbon-centred radicals at appropriate positions on the alkyl chain of undecylprodiginine. Transformation of a radical intermediate into streptorubin B can be envisaged by one of several pathways: either by (1) conversion to a hydroxylated intermediate, in which nucleophilic displacement of the hydroxyl group by C4 of the pyrrole followed by deprotonation and re-aromatization would yield streptorubin B (Figure 11, pathway A); or by (2) direct attack of the radical on C-4 of the pyrrole ring, to give a stabilised, conjugated radical intermediate.
from which abstraction of a second pyrrole-derived hydrogen atom by RedG would give streptorubin B (Figure 11, pathway B).

Figure 11: Outline of possible intermediates in the conversion of undecylprodiginine to streptorubin B, as catalysed by RedG.
1.8 Relative and absolute stereochemistry of streptorubin B

Examination of a molecular model of streptorubin B showed that free rotation about the bond linking the stereocentre of the alkyl chain to the pyrrole C-ring (C-4 of the pyrrole C-ring and C-7') is restricted, so that rotation of the alkyl chain across the plane of the tripyrrole ring system cannot occur freely. This forces the tripyrrole system and the hydrocarbon portion of the carbocycle to adopt a defined relative configuration. No signal broadening is observed in the $^1$H-NMR spectrum of the hydrochloride salt of streptorubin B at temperatures up to 120 °C, indicating that it is configurationally stable up to temperatures considerably above room temperature. NOESY experiments were used to define the relative stereochemistry in the hydrochloride salt of streptorubin B. Key long-range correlations in the NOESY spectrum allowed the relative configuration of the alkyl side chain of the carbocycle and the endocyclic alkyl carbons to be defined (Figure 12).

Figure 12: Key long-range correlations observed in the NOESY spectrum of streptorubin B hydrochloride, and the deduced relative stereochemical relationship of C8'-C11' to C2'-C6' in streptorubin B

This intriguing structural feature of streptorubin B is reflected in the highly diastereotopic nature of the alkyl protons of the carbocycle, especially the C-4'
pair of hydrogen atoms, one of which is heavily shielded by the ring current of the pyrrole π-electrons, resulting in a characteristic upfield-shift of its resonance to -1.54 ppm in the \(^1\)H-NMR spectrum. In comparison, the other C-4’ proton resonates at 1.16 ppm (Figure 13).

![Figure 13: Comparison of shielding effects on C-4’ protons of streptorubin B (left) and metacycloprodigiosin (streptorubin A, right)](image)

It is interesting to note that the -1.54 ppm signal in the \(^1\)H-NMR spectrum of streptorubin B is characteristic for the 10-membered carbocyclic ring system. The related compound metacycloprodigiosin (streptorubin A), isolated from *Streptomyces longispororuber*, has a twelve-membered carbocyclic ring instead of the 10-membered ring of streptorubin B. The comparable C-4’ proton in streptorubin A no longer resonates at -1.54 ppm, but at 0.28 ppm, seemingly because the larger ring allows the proton on C-4’ to be positioned further away from the pyrrole π-electrons. Thus the shielding effect is weaker, though still clearly significant, because it shifts the proton resonance substantially from the typical value of ~1.5 ppm for a CH\(_2\) group (Figure 13).

Also of note is that the circular dichroism spectra of methanol solutions of metacycloprodigiosin (streptorubin A) and streptorubin B are essentially
mirror-images (Figure 14). This may imply that metacycloprodigiosin (streptorubin A) and streptorubin B are pseudoenantiomeric (i.e. display opposite stereochemical configurations at their respective chiral centres).

![Comparison of CD spectra of streptorubin A and streptorubin B](image)

**Figure 14:** Circular dichroism spectra of metacycloprodigiosin (streptorubin A) and streptorubin B

### 1.9 Synthetic approaches to undecylprodiginine, metacycloprodigiosin and streptorubin B

Since the discovery of prodigiosin the total synthesis of prodiginine alkaloids has been of significant interest. In particular, the cyclic undecylic prodiginine derivatives and roseophilin have attracted considerable attention due to the synthetic challenge posed by the carbocyclic rings in their structures. Syntheses of streptorubin B and other carbocyclic prodiginines have been limited, primarily due to the strained ansa-bridged medium rings in these structures. Syntheses in which the stereochemistry of the carbocyclic ring systems has been controlled are particularly scarce. The first synthesis of
(racemic) metacycloprodigiosin (streptorubin A) involved elaboration of the ansa-bridged medium ring from cyclododecanone 11 via functionalisation and subsequent pyrrole formation. This reaction sequence consisted of twelve linear steps and proceeded with an overall yield of 0.02% from the cyclododecanone. Subsequent acid promoted coupling of the carbocyclic pyrrole 12 with MBC afforded the natural product (Scheme 1).

Fürstner et al. completed a formal total synthesis of the carbocyclic pyrrole fragment of both metacycloprodigiosin (streptorubin A) and streptorubin B with a much improved yield compared to Wasserman’s metacycloprodigiosin synthesis. A critical skeletal reorganisation catalysed by platinum (II) chloride is a key step in both cases. Overall yields of ≈ 16 % of 15 from cyclooctene 13 and ≈ 5 % of 18 from cyclodecene 16 over nine linear steps were reported (Scheme 2). However, the stereochemistry of the medium ring in the product was not controlled in either case, nor was the condensation of the bipyrrrole aldehyde (MBC) with 13 or 20 carried out to complete the total syntheses.
Scheme 2: Fürstner formal total syntheses of metacycloprodigiosin (streptorubin A) and streptorubin B via platinum catalysed rearrangement

The same N-tosyl dihydropyrrole 14 intermediate in Fürstner’s synthesis of 15 has also been synthesised by ring-closing metathesis using Grubbs’ second generation catalyst (Scheme 3). This pathway marginally increases the overall yield of 15 (to 19%) but consists of fifteen linear steps from trans-4-
hydroxyproline 19 and again the stereochemistry of the product was not controlled and the condensation with MBC to complete the total synthesis was not attempted.49

\[
\text{HO} \quad \underset{(i) - (iv)}{\text{OH}} \quad \underset{(v) - (xii)}{\text{OH}}
\]

(i) SOCl₂, MeOH (ii) pTsCl, Et₃N (iii) TBSCI, Imidazole (iv) NaBH₄, LiCl (v) Swern (vi) Ph₃P=CHCO₂Et (vii) H₂, Pd/C (viii) LiAlH₄ (ix) PCC (x) Ph₃P=CH₂ (xi) TBAF, THF then PCC DCM (one pot) (xii) H₂, Pd/C (xiii) Grubbs' 2nd gen. (cat.) (xiv) H₂, Pd/C (xv) BF₃·OEt₂

Scheme 3: Synthesis of tosyl dihydropyrrole intermediate 14 via Grubbs catalysis

Confusion regarding the structure of streptorubin B has resulted in several syntheses of the ortho-annelated isomer butylcycloheptylprodigiosin 22, in the belief that this is a natural product. Initially, it was synthesised by Fürstner et al. in eighteen linear steps from 20 (Scheme 4). Subsequently a synthesis via oxazole 24 using a combination of one-pot reactions was reported by Reeves (Scheme 5). Key steps in this synthesis were an one-pot conjugate addition/aldol reaction and a one-pot dehydration/oxazole hydrolysis/pyrrole.
formation reaction to afford the same formyl pyrrole 21 to that seen in Fürstner’s earlier synthesis, in only two steps from cyclononenone 23. The total synthesis was completed by the same method used by Fürstner to converted formyl pyrrole 21 to butylcycloheptylprodigiosin 22. In both these syntheses no control over the absolute stereochemistry of the product was attempted.

Scheme 4: Fürstner’s synthesis of butylcycloheptylprodigiosin
The first stereoselective synthesis of metacycloprodigiosin (streptorubin A) was recently reported by Thomson and coworkers, in which the absolute stereochemistry is installed by the enantioselective conjugate addition of an ethyl cuprate to enone. The stereochemistry of the reaction is controlled by using 6% (R,S)-JosiPhos to give a 93:7 mixture of enantiomers. The enolate product of this reaction is trapped using chlorosilyl enol ether, after which oxidative C-C bond formation yields the desired dialkenyl diketone in an enantioselective manner. Ring closing metathesis with Grubbs second generation catalyst followed by pyrrole formation upon exposure to NH₄OAc completed the formation of the key carbocyclic monopyrrole moiety (Scheme 6).
It is interesting to note that the synthesis of streptorubin B via a similar route has yet to be achieved, seemingly because of the increased strain in the 10-membered ring of streptorubin B compared to the 12-membered ring in metacycloprodigiosin (streptorubin A).\textsuperscript{54}

Finally, the total synthesis of the related natural product nonylprodigiosin 6 together with some synthetic analogues 29-32 has been reported by ring closing metathesis of various dialkene tripyrrole intermediates, formed by Suzuki coupling of dipyrrole intermediate with various heterocyclic boronic acids (Scheme 7).\textsuperscript{55} It should be noted that the tertiary carbon centres within the

Scheme 6: Thomson’s enantioselective synthesis of streptorubin A
carbocycles of metacycloprodigiosin (streptorubin A) and streptorubin B make them inaccessible by a similar synthetic strategy.

Scheme 7: Fürstner’s synthesis of nonylprodigiosin and synthetic analogues

1.10 Synthetic approaches to roseophilin

The total synthesis of the prodiginine-related natural product roseophilin has been reported by several groups as discussed below. In all the syntheses developed, the generation of the monopyrrole fragment 34 bearing the
carbocycle has proved to be a vital step. In all cases this fragment has been elaborated to N-protected roseophilin by base mediated condensation with furano-pyrrole 35 (Scheme 8).

![Scheme 8: Key late stage intermediates in roseophilin total syntheses](image)

(i) (a) nBuLi, THF, -50 °C (b) CeCl₃, THF, -78 °C (c) 34c, -78 °C to RT  
(ii) (a) TBAF (b) HCl (aq.)

The key carbocycle formation step en route to 34 has been performed by a variety of strategies. Fürstner et al. employed a palladium-catalysed carbanion addition to allylic epoxide 36 to furnish the racemic carbocycle 37 (Scheme 9, route 1). They later re-designed the early stages of their synthesis, to allow for easier adaptation to analogue synthesis, by replacing the palladium catalysed carbocyclisation with ring closing metathesis followed by hydrogenation to form the carbocycle 37 (Scheme 9, route 2). Compound 37 was then elaborated to 34 by the same method in both cases: Desilylation and oxidation provided the required precursor for palladium-catalysed pyrrole formation. Conversion of the resulting carboxylic acid to the corresponding acid chloride and treatment with tin chloride resulted in the formation of the required tricyclic ketone 38. The missing isopropyl substituent was then installed by base-mediated elimination of the adjacent sulfone followed by a 1,4-nucleophilic addition to give 34 (R = Bn). Debenzylation of this compound proved troublesome and required a reductive cleavage with calcium in liquid ammonia followed by re-oxidation of the adjacent carbonyl with pyridinium dichromate. The desired, base mediated coupling of the
potassium salt of 34b with 35 did not proceed; instead N-SEM 34c was required to observe the desired coupling in good yield.

Scheme 9: Fürstner's total syntheses of the roseophilin carbocyclic pyrrole fragment

(i) [Ru] cat. (10 mol %), DCM (ii) [RhCl(PPPh3)3] (cat.) H2, EtOH
(iii) [Pd(PPPh3)4] (10 mol %) and bis(diphenylphosphino)ethane (20 mol %), THF
(iv) TBAF, NH4F (v) Dess-Martin (vi) BnNH2, [Pd(PPPh3)3]
(vii) 1. NMe2 2. SnCl4

34a R = Bn
34b R = H
34c R = SEM

(viii) t-BuOK (excess)
(ix) Ca/NH3, then PDC
(x) KH, SEM-Cl
Robertson et al. later completed the formal total synthesis of roseophilin by developing a radical-mediated cyclisation of iodoalkene 39 using Bu$_3$SnH and AIBN as the key step in their synthesis of the roseophilin carbocyclic pyrrole fragment 34 (R = Bn, Scheme 10).\(^{58}\)

![Scheme 10: Robertson’s free radical cyclisation approach to roseophilin](image)

Tius and co-workers described the first enantioselective total synthesis of roseophilin and thus determined its absolute stereochemistry (Scheme 11).\(^{59}\)

The key step in their synthesis is an asymmetric cyclopentannelation reaction initiated by the addition of a lithioallene 41 bearing a chiral auxiliary to the required amide 40. Subsequent acid treatment results in the generation of the pentadienyl cation, the formation of which is followed by cyclisation via a 4π conrotation to yield the desired product 42 in 86 % ee. 42 is further elaborated to the desired precursor 34c through the Stetter reaction with 6-heptenal followed
by installation of the carbocyclic moiety via a Grubbs catalysed ring closing metathesis.

Scheme 11: Tius' enantioselective total synthesis of roseophilin monopyrrole fragment

(i) (a) nBuLi, -78 ºC to -30 ºC (b) HCl, HFIP/TFE (1:1)
(ii) Bz2O, Et3N, DMAP, DCM
(iii) 3-benzyl-5-(hydroxethyl)-4-methylthiazolidium chloride, Et3N, 1,4-dioxane, 6-heptanal, 70 ºC
(iv) Grubbs 1st gen. cat. (30 mol %), DCM, 40 ºC
(v) H2, 10 % Pd/C, THF
(vi) NH4OAc, Ti(O-iPr)4, 140 ºC (vii) KH, SEMCl, DMF

34c
In all of the roseophilin syntheses and synthetic approaches the 13-membered carbocycle is constructed before strain in the molecule is introduced by the formation of the fused five-membered rings.

In summary, the formation of the ansa-bridged medium ring has made the synthesis of carbocyclic prodiginines and related compounds difficult. The total syntheses of such compounds are typically long and challenging, making the preparation of analogues a daunting task.

1.11 Mutasynthesis as a strategy for preparation of natural product analogues

Natural products are often of such significant structural complexity that synthetic access to these compounds for their further investigation, including the structure-activity relationships necessary for drug lead development, is a common stumbling-block in their study. However, a variety of semi-synthetic or biotechnology approaches have been developed to ease their preparation.

(A) Precursor-directed biosynthesis

(B) Mutasynthesis

Figure 15: Schematic representation illustrating the differences between precursor-directed biosynthesis (A) and mutasynthesis (B)
One of the most widely used and successful strategies is mutasynthesis (or mutational biosynthesis) and the closely related technique, precursor-directed biosynthesis. Both of these systems are based upon the process of cellular uptake of relatively simple biosynthetic building blocks and their subsequent incorporation into complex antibiotics. These pathways differ by one crucial factor, in precursor-directed biosynthesis growth media of ‘wild-type’ producers of the metabolite of interest are supplemented with analogues of intermediates in the pathway. These analogues then compete with the natural building blocks for incorporation into the natural products. This can result in a range of natural product analogues being produced from a single incubation which require isolation or considerable optimisation of the incorporation of the desired precursor (Figure 15A). This is in contrast to mutasynthesis, which was first termed by Rinehart and proposed as an alternative method of obtaining a new diversity of compounds compared to those accessible through the existing precursor-directed biosynthesis. Mutasynthesis involves the feeding of analogues of crucial intermediates in antibiotic biosynthesis to mutants of the wild-type strain inactivated in an important step of the intermediate assembly (Figure 15B).

This process has been used elegantly to avoid lengthy total synthetic strategies by exploiting the biosynthetic machinery of a given microorganism to perform the required chemical transformation.

The first reported examples of mutasynthetic approaches relied on random mutagenesis. Cultures of the neomycin producer Streptomyces fradiae were treated with the mutagen nitrosoguanidine, which killed nearly all of the cells. The survivors were then grown and screened for a mutation which had resulted in the desired deoxystreptamine deficient phenotype. Colonies were grown with and without deoxystreptamine, an important subunit of neomycin, and checked for a zone of inhibition when grown with Bacillus subtilis. Colonies that showed
a zone of inhibition with deoxystreptamine and no zone of inhibition without deoxystreptamine were identified as mutants deficient in deoxystreptamine moiety biosynthesis. The selected mutant was then grown in the presence of other aminocyclitols closely related to deoxystreptamine. This study identified several mutasynthetic analogues of neomycin 44a-44c using streptamine, epistreptamine and 2,6-dideoxystreptamine (Scheme 12). In addition this technique was applied to other aminocyclitol containing antibiotics with some success including Kanamycin and Ribostamycin.62, 61

![Scheme 12: Feeding of aminocyclitol analogues to give neomycin and neomycin analogues](image)

Recent advances in sequencing technologies have resulted in a dramatic increase in the number of biosynthetic gene clusters being identified. This combined with the advent of new molecular biology techniques which have allowed for faster characterisation of the enzymes involved in antibiotic biosynthesis, has resulted in a deeper understanding of secondary metabolite biosynthesis at a genetic level. This has led to the generation of large numbers of designed biosynthetic mutants in which discrete steps in a biosynthetic pathway have been targeted and disrupted, and has resulted in a revitalisation in mutasynthetic techniques.
The aminocoumarin antibiotic, chlorobiocin (Scheme 13A), provides a good example of targeted genetic disruption and subsequent analogue generation by mutasynthesis. Chlorobiocin consists of three major components; an aminocoumarin moiety, a noviose sugar, and a 3-dimethylallyl-4-hydroxybenzoic acid (DMAHB) moiety. CloQ catalyses a key step in the biosynthesis of DMAHB, and when it was deleted from the producing strain *Streptomyces roseochromogenes*, the resulting CloQ<sup>−</sup> strain was unable to make chlorobiocin unless fed with synthetic DMAHB. This strain was then demonstrated to incorporate synthetic analogues of DMAHB (Scheme 13B) into new and biologically active chlorobiocin analogues.63, 64, 65

Scheme 13: (A) Chlorobiocin biosynthesis from DMAHB (B) a selection of DMAHB analogues successfully incorporated into chlorobiocin analogues

Polyketide antibiotics have been particularly well exploited by mutasynthesis, notably the starter unit of polyketides seem particularly amenable to substitution during mutasynthesis.66 Rapamycin (Scheme 14A), the immunosuppressant polyketide, incorporates a 4,5-dihydroxycyclohex-1-enecarboxylic acid (DHCHC) starter unit derived from shikimic acid. Researchers at Biotica generated a mutant strain of *Streptomyces hygroscopicus* (rapK<sup>−</sup>) which was unable to biosynthesise the DHCHC starter unit, and was subsequently unable to produce rapamycin unless supplemented with synthetic DHCHC. The rapK<sup>−</sup> mutant was
fed with a variety of analogues of DHCHC (Scheme 14B), resulting in the production of rapamycin analogues containing structural, regio- and stereo-chemical alterations in the starter unit derived portion (Scheme 14). Several of these “rapalogues” are currently being developed as potential drugs in collaboration with Wyeth.

![Scheme 14: (A) Rapamycin biosynthesis from DHCHC (B) a selection of DMAHB analogues successfully incorporated into chlorobiocin analogues](attachment:image.png)

Finally, the commercial viability of mutasynthesis strategies has been demonstrated in the manipulation of the biosynthetic pathway of avermectin (Figure 16A) in *Streptomyces avermitilis*. Generation of a mutant unable to biosynthesise the necessary branched chain fatty acid starter unit was achieved by inactivating the required branched-chain 2-oxo acid dehydrogenase. This strategy was used to generate 36 novel avermectin derivatives and demonstrated the remarkably broad substrate tolerances of this pathway, including analogues containing unsaturated elements and heterocyclic rings in place of the alkyl branched chain observed in avermectin. This work then led
the way for the mutasynthetic commercial synthesis of the antiparasitic veterinary drug, Doramectin (Figure 16B).\textsuperscript{70, 71}

![Figure 16: (A) Structure of avermectin A1 (B) Examples of avermectin analogues from mutasynthesis including Doramectin](image)

The design of a successful mutasynthetic strategy for streptorubin B analogues, which due to their unusual carbocyclic structure have proved synthetically challenging, would be particularly appealing. This would allow the demanding formation of this carbocyclic moiety to be carried out enzymatically. The extensive existing knowledge of the prodiginine biosynthetic pathway makes streptorubin B an ideal candidate for a mutasynthetic strategy. Concise syntheses towards intermediates proven to be key in prodiginine biosynthesis, for example 2-undecylprodiginine 10 and MBC 9, would make natural product analogue generation an achievable goal.

### 1.12 Total synthesis of undecylprodiginine

Undecylprodiginine, unlike its carbocyclic derivatives, has proved very amenable to total synthetic strategies. It has been synthesised by a convergent route involving acid-catalysed condensation of the two key intermediates 2-
undecylpyrrole 10 with 4-methoxy-2,2'-bipyrrrole-5-carboxaldehyde 9 (MBC), which have been synthesised by a variety of methods (Scheme 15). This convergent route mirrors Wasserman’s proposed biosynthetic pathway, which has since been substantiated in the Challis group.

![Scheme 15: Biomimetic total synthesis of undecylprodiginine](image)

1.13 Previous synthetic approaches to MBC

There are several reported syntheses of the bipyrrrole aldehyde intermediate in prodigiosin biosynthesis. MBC was first synthesised by Rapoport and co-workers via the reaction of ethyl 3-methoxypyrrole-2-carboxylate 45 with pyrroline (Scheme 16). This gave the 2,2'-pyrrolidinyl-pyrrole 46, which was subsequently dehydrogenated in refluxing xylene to give the desired 2,2'-bipyrrrole ester 47. However, at this stage the desired reduction of the ester proved troublesome. Lithium aluminium hydride failed to reduce the ester. However, reduction of the ester was achieved by MacFadyen-Stevens reduction via the corresponding hydrazide to give MBC 9. This chemistry provides a synthetic route to MBC. However, analogue generation by this approach is not feasible because of the protracted synthesis of the substituted pyrrole starting
material 45 and because of the troublesome and low-yielding MacFadyen-Stevens reduction and pyrrole-pyrroline coupling steps.

Scheme 16: First reported synthesis of MBC via pyrroline-pyrrole condensation

A later synthesis reported by Wasserman et al., uses a reactive intermediate generated by the reaction of singlet oxygen with 3-methoxy-2-pyrrole carboxylic acid tert-butyl ester 48 (Scheme 17). The transient imino hydroperoxide intermediate 49 has been shown to react with a number of nucleophiles, such as pyrrole, to give MBC 9 or 2-ethylpyrrole to give the corresponding A-ring substituted MBC analogue 50 (after subsequent MacFadyen-Stevens reduction of the resulting esters). Although this chemistry gives access to substituted MBC analogues, it is, like the related chemistry reported by Holden et al., limited by the low yields of both the singlet oxygen reaction and the subsequent MacFadyen-Stevens reduction.
Scheme 17: Singlet oxygen reaction to bipyrrrole fragment

A more concise and higher yielding route to MBC was recently reported by Tripathy and Lavallée, in follow up to a 2004 patent. This route is currently in use for the preparation of obatoclax (GX15-070), the first streptorubin B analogue to reach clinical trials in oncology. Treatment of commercially-available 4-methoxy-3-pyrrolin-2-one 51 (Scheme 18) with the Vilsmeier-Haack reagent, generated from diethylformamide and phosphorus oxybromide, yields 52 in one step. This reaction introduces in a single step both the bromide required for subsequent cross-coupling and the aldehyde ‘protected’ as an enamine. Importantly, this route has the potential to generate a large number of A-ring MBC analogues because of the broad substrate tolerance of the subsequent Suzuki cross-coupling reaction. This reaction, originally designed to couple the bromo pyrrole enamine 52 with BOC-pyrrole boronic acid 53 to give MBC (after enamine hydrolysis), can be envisaged to be applied with a variety of other boronic acids to give MBC analogues.
1.14 Previous synthetic approaches to 2-undecylpyrrole

Previous synthetic approaches to 2-undecylpyrrole have been limited in their scope. They are primarily based upon the addition of n-undecyl bromide to a solution of pyrrole magnesium bromide prepared in situ from ethyl magnesium bromide and freshly distilled pyrrole (Scheme 19). These reactions are typically low yielding, resulting in <30 % of monoalkylated pyrroles 10 and 54, contaminated with multiple insoluble polyalkylated pyrrole species 55. The mixture of 2-undecylpyrrole 10 and 3-undecylpyrrole 54 obtained by filtration of an acetone solution of the crude reaction mixture has to be separated by crystallisation (Scheme 19).⁷²
1.15 Aims and objectives

- To synthesise and isolate streptorubin B analogues via a mutasynthetic approach.

By making use of the existing knowledge of the prodiginine biosynthetic pathway in *S. coelicolor*, mutasynthesis of streptorubin B analogues can be envisaged by a combination of relatively straightforward synthetic methodologies and enzymatic processes that install the synthetically challenging carbocyclic moiety.

Initially, synthesis of key intermediates MBC and 2-undecylpyrrole, via routes amenable to analogue generation, would be used in order to optimise the incorporation of synthetic supplements into the biosynthetic process.

Finally, the mutasynthesis of streptorubin B analogues is foreseen by feeding of MBC and 2-undecylpyrrole analogues to *S. coelicolor* mutants deficient in intermediate biosynthesis (Figure 17).

Figure 17: Schematic representation of envisaged mutasynthesis approach
To elucidate the currently unknown absolute stereochemistry of streptorubin B

The relative stereochemistry of streptorubin B has been previously determined. However, the absolute stereochemistry of streptorubin B determined by the configuration at C-7′ is currently unknown (Figure 18).

Figure 18: Curly arrows indicate key correlations observed in the NOESY spectrum of streptorubin B hydrochloride, and the defined relative stereochemistry of streptorubin

Feeding of 2-undecylpyrrole containing a stereospecific deuterium label at C-4′, in a manner analogous to the 2-undecylpyrrole mutasynthesis strategy should allow the absolute stereochemistry of C-7′ to be defined. The protons at C-4′ which are diastereotopic and well-defined are an ideal choice for this mutasynthetic approach.
2.0 Results and discussion: Synthesis of 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde, 2-undecylpyrrole and analogues

Before the envisaged mutasynthetic approach towards prodiginine analogues could be undertaken a robust synthesis of the prodiginine precursors 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde 9 (MBC) and 2-undecylpyrrole 10 that is amenable to analogue generation had to be developed.

2.1 Synthesis of 4-methoxy-2,2'-bipyrrole-5-carboxaldehyde (MBC, 9)

At the outset of this work a Pd catalysed coupling approach to MBC was initially envisaged, and when researching the literature regarding possible procedures, a very similar approach to the envisaged route had recently been reported. This procedure was first described in a patent relating to a prodiginine analogue, obatoclax 6.\textsuperscript{76} This was subsequently published as a formal total synthesis of prodigiosin in which the preparation of MBC is described.\textsuperscript{75} Therefore the initial aim was to reproduce this literature method for MBC synthesis in good yield.

The N-BOC-pyrrole-2-boronic acid required for this synthesis was prepared by directed C-2 lithiation of N-BOC-pyrrole 56 using lithium tetramethylpiperazide (Scheme 20). Under these conditions, lithiation is known to be directed by the interaction of the BOC carbonyl oxygen with the lithiating species 57.\textsuperscript{78, 79} This allowed the regiospecific synthesis of N-BOC-pyrrole-2-boronic acid 56 in good yield.
4-methoxy-3-pyrrolin-2-one 51 was reported to be converted in good yield to bromo-enamine 52 in one step via the action of the Vilsmeier-Haack reagent obtained from reacting diethylformamide with phosphorus oxybromide (Scheme 21). This allowed C-acylation and bromination to be carried out in one step, without the need to protect the pyrrolidinone nitrogen. A subsequent Suzuki cross-coupling between the bromo-enamine 52 and N-BOC-pyrrole-2-boronic acid 53 furnished MBC in good yield after basic aqueous work-up. Both the BOC protecting group and the enamine were hydrolysed in the work-up to yield the doubly deprotected product as a crude solid. Washing of this largely insoluble solid with acetone removed residual triphenylphosphine and palladium-based impurities to give MBC 9 as a fine yellow solid.
2.2 Synthesis of MBC analogues with A-ring variation

We recognised that the synthesis of MBC described above could be readily adapted to prepare several A-ring analogues of MBC by simply varying the boronic acid used in the Suzuki-coupling. Phenyl 58, 2-thiophenyl 60 and 2-furanyl 61 boronic acid are all commercially available. However, N-BOC-indolyl-2-boronic acid 59, of particular interest because it could be used to prepare prodiginine analogues similar in structure to the clinical candidate, obatoclax 7, is not commercially available. Thus, N-BOC-indolyl-2-boronic acid 59 was synthesised via the same C-2-directed lithiation strategy used to synthesise N-BOC-pyrrole-2-boronic acid 53 (Scheme 20). The aromatic boronic acids 58-61 were used in Suzuki coupling reactions analogous to that used to synthesise MBC to synthesise the corresponding MBC analogues 62-65 (Scheme 22) in average to good yields.

![Scheme 22: Synthesis of MBC analogues by palladium-catalysed cross coupling of bromo enamine 52 with a variety of aromatic boronic acids](image-url)
The MBC analogues resulting from these reactions were sufficiently soluble in acetone to preclude their purification by washing with this solvent (as had been used to purify MBC). However, the indolyl analogue could be washed with a mixture of diethyl ether and chloroform to yield the desired product in high purity as a yellow solid. The remaining crude MBC analogues were purified on basic alumina.

### 2.3 Synthesis of MBC analogues with B-ring variation

In addition to MBC analogues that vary at the A-ring, analogues that differ at the B-ring would also be useful as probes of the substrate specificities of RedH and RedG. It was thought that the analogue with a furan B-ring might prove particularly interesting. By analogy with the known intermediates in the prodiginine biosynthetic pathway it was thought this compound could be an intermediate in the biosynthesis of the structurally-related natural product roseophilin. Therefore, it was reasoned that use of this compound in our mutasynthesis experiments in *S. coelicolor* might result in a roseophilin/prodiginine hybrid.

The initial proposed route to furanyl-pyrrole was from 4-methoxy-2(5H)-furanone (Scheme 23). Reduction of this compound with DIBAL-H or LiAlH₄ would produce 3-methoxyfuran, via the hemi-acetal intermediate. Subsequent reaction with the Vilsmeier-Haack reagent derived from DMF would furnish aldehyde. Finally, bromination of aldehyde with *N*-bromosuccinimide (NBS) followed by Suzuki coupling with N-BOC-pyrrole-2-boronic acid would yield the desired furanyl-pyrrole (Scheme 23).
Unfortunately, the reduction of compound 66 could not be achieved under a variety of conditions, probably because of the deactivation of the furanone carbonyl carbon towards nucleophilic attack by electron donation from the adjacent vinyl enol ether.

Commonly, Suzuki couplings are carried out with triflates in place of bromides. With this in mind, it was proposed that compound 66 could be deprotonated and triflated to afford the corresponding furan 72 in one step (Scheme 24). However, none of the desired product 72 could be obtained using a variety of bases such as LDA and Hüning’s base and triflating reagents such as triflic anhydride and N-(5-chloro-2-pyridyl) trilimide in different solvents e.g. DCM and THF.
Finally, synthesis of the desired bromo-aldehyde 70 was achieved via the furanone enamine 74 (Scheme 25). 4-methoxy-2(5H)-furanone 66 was reacted with excess dimethyl formamide dimethyl acetal. Continuous distillation of methanol from the reaction mixture drove the reaction to completion to give the furanone enamine 74 in high yield. Bromination of 74 was carried out with phosphorus oxybromide in chloroform to yield the desired bromo aldehyde 70 as an unstable solid, which decomposed rapidly to an intractable mixture of (presumably polymerisation) products. Suzuki coupling between the freshly prepared bromo aldehyde 70 and N-BOC-pyrrole-2-boronic acid 52 afforded the N-BOC-protected derivative 75 of the desired MBC analogue, which was deprotected with LiOH in MeOH to give the MBC analogue 71 in good yield.
2.4 Synthesis of 2-undecylpyrrole

With the synthesis of MBC and several analogues completed we shifted our focus to the other prodiginine biosynthetic intermediate, 2-undecylpyrrole. The synthesis of 2-undecylpyrrole was attempted by a number of methods. Initially, due to the success of the lithiation of N-BOC pyrrole with lithium tetramethylpiperazide during the preparation of N-BOC-pyrrole-2-boronic acid (Scheme 20), the same base was used to deprotonate N-BOC-pyrrole and reaction of the resulting anion with undecyl bromide and undecanal was examined (Scheme 26). Unfortunately, when the proposed reaction was attempted using undecyl bromide, only starting material was recovered from the reaction. Presumably this was due to insufficient $S_N2$ transition state stabilisation by the reaction solvent, THF. In retrospect, perhaps the use of a more polar co-solvent, such as DMPU, would have favoured the efficiency of this reaction. 2-acyl pyrroles are known to be reduced directly to the corresponding 2-alkyl pyrroles by treatment with sodium borohydride in alcohol solvents. Therefore, it was envisaged that reaction of 2-lithiated N-BOC-pyrrole with undecanal.
could provide a convenient method for the synthesis of 2-undecylpyrrole via the resulting alcohol 78.

Scheme 26: Proposed synthesis of 2-undecylpyrrole via lithiation of N-BOC pyrrole

The reaction of N-BOC-pyrrole with undecanal 77 afforded the expected alcohol 78, in low yield. However, the subsequent deprotection of the BOC group, which was required for hydroxyalkyl pyrrole reduction, did not yield the desired product 79 under the previously described nucleophilic deprotection conditions using LiOH/MeOH. Instead the removal of the BOC protecting group was accompanied by methylation of the free hydroxyl group to give the methyl ether 80, reduction of which gave none of the desired alkyl pyrrole 10. In fact, in the presence of sodium borohydride the methyl ether 80 afforded pyrrole and undecyl alcohol methyl ether, presumably via an initial retro-aldol reaction to
give pyrrole and O-methylated undecyl aldehyde, which is then reduced by sodium borohydride to give undecyl alcohol methyl ether.

BOC deprotection of 78 under acidic conditions, both with aqueous acid and trifluoroacetic acid, gave none of the desired product.\textsuperscript{85} Thermal decomposition of the BOC-protecting group resulted in the production of a complex mixture of compounds.\textsuperscript{86}

Consequently, an alternative synthesis of 2-undecylpyrrole was devised (Scheme 27), based upon the literature precedent of carboxylic acid activation via the corresponding pyridyl thioester.\textsuperscript{87, 88} Undecanoic acid 81 is activated as the corresponding 2-pyridylthioester 82 using 2,2′-dipyridyl-disulfide and triphenylphosphine. Reaction of the resulting thioester with pyrylmagnesium chloride, generated \textit{in situ}, resulted in regiospecific production of the 2-acyl pyrrole 83. Subsequent reduction with excess sodium borohydride in isopropanol proceeded cleanly to give 2-undecylpyrrole 10.

\begin{center}
\textbf{Scheme 27: Synthesis of 2-undecylpyrrole via pyridyl thioester}
\end{center}
The reduction of acyl pyrrole 83 to the corresponding alkyl pyrrole appears to be sensitive to the reaction conditions. A competing reduction-protonation-retro-aldol cleavage sequence, resulting in the formation of pyrrole and undecanal (which subsequently undergoes sodium borohydride mediated reduction to undecanol), appears to take place under certain conditions in addition to the desired reduction. The retro-aldol mediated process becomes the predominant pathway under anhydrous conditions and when sodium borohydride is added to a solution of 2-acylpyrrole in anhydrous isopropanol under argon. In contrast, when sodium borohydride is refluxed under atmospheric conditions in reagent grade isopropanol for a short period before addition of the 2-acylpyrrole, reduction to the alkyl pyrrole appears to be the only reaction that occurs. It is notable that, when refluxing sodium borohydride in reagent grade isopropanol, the basicity of the solution is markedly increased, presumably as a result of the reaction of sodium borohydride with a small amount of water present in the isopropanol. This observation suggests that activation of pyrrole as a leaving group by protonation at C-2 controls the rate of the undesired retro-aldol cleavage (Scheme 28). The retro-aldol side reaction appears to be completely shut down by increasing the basicity of the reaction mixture before addition of the acyl pyrrole.
2.5 Synthesis of 2-undecylpyrrole analogues

After the successful development of a synthetic route to 2-undecylpyrrole our attention turned to the synthesis of analogues of 2-undecylpyrrole, which could be used to probe the substrate specificity of RedH and RedG.

The robust synthetic route used to generate 2-undecylpyrrole is compatible with a variety of different functional groups, allowing for the rapid generation of several 2-undecylpyrrole analogues by the same route. Commercially available carboxylic acids were converted to their corresponding 2-alkyl pyrroles as described above, to afford 2-undecylpyrrole analogues which have C8 85, C10...
86, C12 87 or C18 88 alkyl chains. A 2-undecylpyrrole analogue with a C-10'-'C-11' double bond in the alkyl chain (Δ-10'-11') 89 was also prepared by this route (Scheme 29).

Scheme 29: 2-undecylpyrrole analogues synthesised from commercially available carboxylic acids using the methodology described in section 2.4

In addition to the 2-undecylpyrrole analogues prepared from commercially available carboxylic acids, it was decided to further probe the substrate specificity of RedH and RedG by synthesising some additional analogues from carboxylic acids that are not available commercially.

First, in order to establish the importance of steric effects on the reactions catalysed by RedH and RedG, carboxylic acids containing methyl-branches at C-9 or C-10 of the undecyl chain were synthesised (Scheme 30).
Scheme 30: Synthetic route to methyl-branched 2-undecylpyrrole analogues

Ethyl 7-bromoheptanoate 90 was converted to the corresponding phosphonium salt by reaction with excess triphenylphosphine in toluene. Subsequent treatment with n-butyl lithium generated the desired ylid 91. It should be noted that a side reaction resulting from the addition of the butyl anion to the carbonyl group lowered the yield of this reaction. To the ylid 91 was added either isovaleraldehyde 93 or 2-methyl butyraldehyde 92 and the resulting expected alkene products from each reaction 94 or 95 were isolated as inseparable mixtures of E and Z isomers. Hydrogenation of the double bonds in these compounds with hydrogen gas over Pd/C, followed by base promoted ester hydrolysis, in each case gave the desired methyl branched carboxylic acid 96 or 97. These two acids were converted to the corresponding 2-alkyl pyrroles 98.
and 99 via their pyridyl thioesters by the route described above for synthesis of 2-undecylpyrrole 10 (Scheme 27).

It was also decided to probe the specificity of RedH and RedG with substrates containing a polar ether linkage, either close to, or far from, the site of carbocyclisation to form streptorubin B. Therefore, carboxylic acids containing either a C2-C4 ether linkage, or a C5-C7 ether linkage were synthesised (Scheme 31). Thus, treatment of bromoacetic acid 100 in octanol with two equivalents of sodium metal resulted in the displacement of the bromide from the initially formed bromoacetate, via a modification of the Williamson ether synthesis (Scheme 31).

\[
\begin{align*}
\text{HO} & \begin{array}{c}
\text{Br} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{H} \\
\text{N}
\end{array} & \xrightarrow{\text{Octanol (excess)}} \text{HO} & \begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{H} \\
\text{N}
\end{array}
\end{align*}
\]

\[100 \rightarrow 101 \rightarrow 102 \]

\[
\begin{align*}
\text{HO} & \begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{H} \\
\text{N}
\end{array} & \xrightarrow{(i) \text{ Pentanol (excess)}} \text{HO} & \begin{array}{c}
\text{O} \\
\text{O} \\
\text{O} \\
\text{H} \\
\text{N}
\end{array}
\end{align*}
\]

\[103 \rightarrow 104 \rightarrow 105 \]

Scheme 31: Synthetic route to ether-containing 2-undecylpyrrole analogues

A subsequent acid/base extraction gave the expected C2-C4 ether linked acid 101 in good yield. The C5-C7 ether linked acid 104 was synthesised from methyl 5-bromovalerate 103, instead of the corresponding acid, to prevent cyclisation of
the carboxylate anion to form the δ-lactone. Treatment of methyl 5-bromovalerate 103 with one equivalent of sodium in pentanol gave the expected C5-C7 ether linked methyl ester, which proved difficult to separate from the residual pentanol. Therefore, the crude methyl ester was hydrolysed to give the corresponding acid 104, which could be purified by acid/base extraction. The ether-linked acids 101 and 104 were converted to the corresponding 2-alkyl pyrroles 102 or 105 using the route described above for the synthesis of 2-undecylpyrrole (Scheme 27).

2.6 Acid-catalysed coupling of MBC and analogues with 2-undecylpyrrole and analogues

Previously, bipyrrrole aldehydes analogous to those synthesised here have been condensed with alkyl pyrroles in a simple acid-catalysed reaction to complete the synthesis of prodiginines such as undecylprodiginine (Scheme 32).  

Scheme 32: Condensation of MBC and 2-undecylpyrrole to give undecylprodiginine

Therefore, it was clear that we could synthesise undecylprodiginine and several undecylprodiginine analogues using this approach. To this end, coupling of MBC 9 and each of the five A-ring analogues 62 to 65 with either synthetic 2-undecylpyrrole 10, or commercially available 2,4-dimethylpyrrole 106, in the presence of methanolic HCl, provided the predicted triaromatic prodiginine
analogues (Scheme 33). 2,4-dimethylpyrrole serves as a good choice for this condensation for several reasons. Firstly, to a first approximation, the condensation product can be considered similar to carbocyclic prodiginines like streptorubin B and meta-cycloprodigiosin because it has the same substitution pattern in the pyrrole C ring, bearing alkyl substituents on C-2 and C-4. Notably, this substitution pattern is shared by the prodiginine analogue, obatoclax 7, which is a current clinical candidate. The reaction was carried out in methanol with a catalytic amount of HCl, and gave the desired triaromatic compounds 107 to 116 in varying yields after purification on silica gel. These couplings, even though they are unoptimised and therefore not efficient, provided us with sufficient material for full characterisation and proved to be useful, not only for biological testing, but also as synthetic standards for the products of in vivo RedH-catalysed reactions.

Scheme 33: Synthesis of undecylprodiginine and streptorubin B like analogues with variation in the A-ring and C-rings
3.0 Results and discussion: Mutasynthetic production of undecylprodigine and streptorubin B analogues with MBC analogues

3.1 Mutasynthesis products of A-ring analogues of MBC

To probe the substrate specificity of RedH and RedG and to attempt to generate prodigine analogues, the MBC analogues varying in the prodigine A-ring 62 to 65 (Figure 19) were fed to a S. coelicolor mutant blocked in MBC biosynthesis.

The strain used was *Streptomyces coelicolor*, W39 (M511 redM::oriT-apr), in which the redM gene, involved in the biosynthesis of MBC and consequently of prodiginines, has been replaced by an apramycin resistance cassette. This strain is known to be deficient in MBC production and hence does not produce any prodigine antibiotics.

Figure 19: Structure of MBC and MBC A-ring analogues

Feeding of synthetic MBC 9 to *S. coelicolor* W39 was initially investigated in an attempt to optimise the production of prodiginines by mutasynthesis in this strain. Production levels of the products were monitored by LC-MS/MS analysis. Feeding of MBC to *S. coelicolor* W39 showed that good levels of production of
both undecylprodiginine (red trace) and streptorubin B (blue trace) can be achieved (Figure 21 top). This experiment later proved to be a vital positive control during analogue production. By comparing the $\lambda_{\text{max}}$ values, retention times and MS/MS fragment ions with authentic standards the compounds detected in this experiment were confirmed to be undecylprodiginine and streptorubin B respectively. An authentic standard of undecylprodiginine was synthesised via acid-mediated condensation between MBC and 2-undecylpyrrole (as described in chapter 2), and an authentic standard of streptorubin B was isolated from *S. coelicolor* M511. MBC was also fed to *S. coelicolor* W33, in which redH, the gene encoding the protein responsible for the condensation of MBC and 2-undecylpyrrole, has been replaced with an apramycin resistance cassette (Figure 21 bottom). This experiment proved useful as a control that revealed the background rate of non-enzymatic condensation (due to acid catalysis resulting from the acidified methanol extraction procedure used for prodiginine isolation).

![Figure 20: Mutasynthetic pathway to undecylprodiginine 3 and streptorubin B 4 when *S. coelicolor* W39 is fed with MBC 9.](image-url)
Feeding of MBC analogues 62 to 65 to *S. coelicolor* W39 resulted in the production of the corresponding undecylprodiginine analogues in low levels relative to the level of undecylprodiginine produced when feeding MBC to the W39 mutant. The structures of these undecylprodiginine analogues were...
confirmed by comparing the $\lambda_{\text{max}}$ values, retention times and MS/MS spectra with authentic standards synthesised via acid catalysed condensation of the MBC analogues 62 to 65 with 2-undecylypyrrole 10 (as described in section 2.6). The MBC analogues were also fed to S. coelicolor W33. These experiments were crucial to distinguish whether the prodiginine formation observed in the experiments with the W39 mutant was due to an enzymatic condensation, catalysed by RedH, or as a result of acid-mediated condensation during the extraction.

MBC analogues 62 to 65 were fed to the S. coelicolor W33 and W39 mutants in parallel, and the results of LC-MS/MS analyses were compared. Small amounts of undecylprodiginine analogues were observed in all cases in the experiments with the W33 (redH) mutant probably due to the isolation procedure in which acidified methanol is added to extract prodiginines from the mycelium pellet.
When feeding the thiophenyl analogue of MBC 64 to S. coelicolor W39 the level of corresponding undecylprodiginine analogue 114 produced was significantly higher (relative to the amount of residual 2-undecylpyrrole 10) than when this analogue was fed to the W33 mutant indicating that RedH is able to utilise the thiophenyl analogue of MBC (Figure 22 and Figure 23). However, no evidence for formation of the corresponding streptorubin B thiophenyl analogue 117 could be observed when 64 was fed to the W39 mutant (Figure 22 and Figure 23).

Figure 22: Mutasynthetic pathway to undecylprodiginine analogue 114 and proposed streptorubin B analogue 117 when S. coelicolor W39 is fed with thiophenyl analogue of MBC 64.
Figure 23: Extracted ion chromatograms (EICs) from LC-MS analyses of prodiginines produced by *S. coelicolor* W39 (top) and W33 (bottom) when fed with thiophenyl analogue of MBC 64.

- EIC at $m/z = 222$, corresponding to 2-undecylpyrrole
- EIC at $m/z = 411$, corresponding to undecylprodiginine analogue
- EIC at $m/z = 409$, corresponding to proposed streptorubin B analogue
When feeding the furanyl analogue of MBC 65 to *S. coelicolor* W39, the level of furanyl undecylprodiginine analogue 115 produced was significantly higher than in *S. coelicolor* W33 (again relative to the amount of residual 2-undecylypyrrole 10). There was no evidence for the production of the furanyl streptorubin B analogue 118 (Figure 24 and Figure 25).

![Figure 24: Mutasynthetic pathway to undecylprodiginine analogue 115 and proposed streptorubin B analogue 118 when *S. coelicolor* W39 is fed with furanyl analogue of MBC 65.](image-url)
Figure 25: Extracted ion chromatograms (EICs) from LC-MS analyses of prodiginines produced by *S. coelicolor* W39 (top) and W33 (bottom) when fed with furanyl analogue of MBC 65.
Unlike for the other analogues, the quantity of the phenyl undecylprodiginine analogue 112 (relative to residual 2-undecylpyrrole), was only modestly greater when the phenyl analogue of MBC 62 was fed to the W39 mutant compared to the W33 mutant. No evidence for the formation of a phenyl streptorubin B analogue 119 could be seen when feeding the phenyl analogue of MBC 62 to S. coelicolor W39 (Figure 26 and Figure 27).

![Chemical structures]

**Figure 26:** Mutasynthetic pathway to undecylprodiginine analogue 112 and proposed streptorubin B analogue 119 when S. coelicolor W39 is fed with phenyl analogue of MBC 62.
Figure 27: Extracted ion chromatograms (EICs) from LC-MS analyses of prodiginines produced by *S. coelicolor* W39 (top) and W33 (bottom) when fed with phenyl analogue of MBC 62.

- **EIC at m/z = 222**, corresponding to 2-undecylpyrrole
- **EIC at m/z = 405**, corresponding to undecylprodiginine analogue
- **EIC at m/z = 403**, corresponding to proposed streptorubin B analogue
The levels of undecylprodiginine analogues produced by feeding heterocyclic MBC analogues 64 and 65 to S. coelicolor W39 were significantly higher than when feeding the analogues to W33 (in which the condensing enzyme, RedH is not present). This unequivocally demonstrates that they are substrates of RedH. The reason for the smaller difference in observed prodiginine production between the W39 mutant and the W33 mutant when feeding the phenyl analogue of MBC 62 is not clear, it could be due to an increased in vivo stability of 62 resulting in a higher level of ‘background’ non-enzymatic condensation occurring as a result of the acidic extraction conditions used at the end of the incubation. Another plausible explanation could be the decreased bioavailability of the phenyl MBC analogue 62 compared to the thiophenyl and furanyl MBC analogues 64 and 65. Alternatively, the difference could be due to the absence in 62 of a heteroatom capable of donating a lone pair to the adjacent aromatic ring, which may be important for the enzymatic activity of RedH.

In all cases, when MBC analogues are fed the levels of undecylprodiginine analogues produced are considerably lower than when undecylprodiginine is produced by feeding with MBC (where levels of undecylprodiginine production are approaching that observed in wild type S. coelicolor).
No streptorubin B analogues were produced when feeding furanyl, thiophenyl and phenyl A-ring analogues of MBC to *S. coelicolor* W39 (Figure 22 to Figure 27). However, when the indolyl analogue of MBC 63 was fed to the W39 mutant there was evidence for the formation of an indolyl streptorubin B analogue 122. LC-MS analyses of extracts from the W39 mutant fed with the indolyl analogue of MBC 63 showed a low level of a compound with \( m/z \) and MS/MS fragment ions that would be expected for the indolyl streptorubin B analogue 122 (Figure 29).

![Figure 28: Mutasynthetic pathway to undecylprodiginine analogue 113 and proposed streptorubin B analogue 122 when *S. coelicolor* W39 is fed with indolyl analogue of MBC 63.](image-url)
Due to the small amount of the proposed indolyl analogue of streptorubin B generated in these experiments, insufficient material has been isolated to allow for the characterisation of this compound by NMR spectroscopy. Additionally,
because we have no amenable route for synthesis of the analogue 122, comparison with an authentic standard has not been possible. However, mass spectrometry has provided good evidence for the formation of the indolyl streptorubin B analogue 122 by mutasynthesis. High resolution mass spectrometry of the isolated compound gave a molecular formula corresponding to that of 122 (Calculated for C$_{29}$H$_{35}$N$_3$O: 442.2853 [M+H]$^+$ Found: 442.2856). In addition the fragmentation of the compound in MS/MS experiments (Figure 31) can be compared to the characteristic and known fragmentation pattern of streptorubin B (Figure 30). Several characteristic fragment ions of streptorubin B are mirrored in the fragmentation of 122. Initial loss of 15 mass units has been shown to be characteristic of heterocyclic methoxy groups, and is seen for both streptorubin B (m/z 392 to 377) and the indolyl analogue (m/z 442 to 425). The subsequent fragmentation of the alkyl chain can also be seen clearly, with the sequential loss of units with m/z = 14, until just the triheterocyclic core plus one or two -(CH$_2$)- units remain for both streptorubin B (m/z 252 and 238) and the indolyl analogue 122 (m/z 302 and 288).

The condensation of the indolyl analogue 122 of MBC with 2-undecylpyrrole occurs at levels much below that seen when undecylprodiginine is produced by feeding with MBC. Therefore suggesting that the potentially beneficial presence of the A-ring N-H, previously suggested as an important though non-vital H-bond donor (based on feeding of thiophenyl, phenyl and furanyl analogues of MBC), may be off-set by a degree of steric constraint in this case.
Figure 30: MS/MS fragmentation pattern of streptorubin B
Figure 31: MS/MS fragmentation pattern of indolyl streptorubin B analogue 122
3.2 Mutasynthesis products of B-ring analogues of MBC

Roseophilin contains a similar conjugated structure to that of the prodiginines. The main difference is that a furan replaces one of the pyrrole rings. Reasoning that roseophilin and the prodiginines could be biosynthesised via analogous pathways, a B-ring furanyl analogue of MBC 71 was synthesised, as described in section 2.3. This compound was fed to the W39 mutant to establish whether S. coelicolor could utilise this compound to biosynthesise a roseophilin–undecylprodiginine hybrid structure 120.

![Proposed mutasynthetic pathway to undecylprodiginine-roseophilin hybrid 120 and carbocyclic derivative 121 when S. coelicolor W39 is fed with B-ring furanyl analogue of MBC 71.](image)

In contrast to the MBC A-ring analogues, when the B-ring MBC analogue 71 was fed to either S. coelicolor W33 or S. coelicolor W39, no prodiginine-like compounds 120 or 121 were produced (Figure 33). It is unclear, at this stage,
whether this is because the furanyl B-ring analogue is not a substrate for RedH or simply because it cannot cross the membrane of *S. coelicolor* cells.

Figure 33: Extracted ion chromatograms (EICs) from LC-MS analyses of prodiginines produced by *S. coelicolor* W39 (top) and W33 (bottom) when fed with B-ring furanyl analogue of MBC 71.
The sequence of the putative roseophilin biosynthetic gene cluster of *Streptomyces griseoviridis* has recently become available. We have tentatively identified a gene which might be responsible for the conversion of a B-ring pyrrole in a prodiginine to the corresponding B-ring furan within this gene cluster. This would be consistent with the hypothesis that B-ring furanyl analogues of MBC appear to not be substrates for RedH.

### 3.3 Summary of conclusions of mutasynthesis with MBC analogues

Overall the mutasynthesis experiments with MBC analogues have proved a success, resulting in the generation of evidence for the formation of an indolyl streptorubin B analogue 122. It has also provided knowledge of the substrate specificity of both RedH and RedG with respect to MBC and the MBC-derived portion of undecylprodiginine.

These *in vivo* experiments do not allow a distinction to be made between factors affecting the bioavailability of these compounds, or enzyme specificity. However, RedH has been shown to catalyse the condensation of a variety of synthetic MBC analogues with 2-undecylpyrrole, although with varying levels of efficiency. The varying levels of production of these analogues suggests that perhaps the pyrrole N-H of the MBC A-ring is important, but not vital, for the binding to or the catalytic activity of RedH, and that the presence of a heteroatom in the MBC A-ring which is capable of donating a lone pair to the neighbouring aromatic ring is perhaps playing a key role in RedH substrate recognition or catalysis. The condensation of the indolyl analogue of MBC with 2-undecylpyrrole to form the corresponding undecylprodiginine analogue suggests that RedH does not impose a strong steric constraint on the A-ring of MBC, however some level of steric clash may account for the low level of conversion of indolyl MBC to indolyl
undecylprodiginine even though there is an A-ring N-H bond. In addition, these results perhaps suggest that the B-ring N-H of MBC may be required for substrate recognition or catalytic activity of RedH, and that the structurally related antibiotic roseophilin may be biosynthesised by conversion of prodiginine B-ring pyrrole to a furan.

Finally RedG seems to impose a high degree of structural constraint on the MBC-derived portion of undecylprodiginine in the oxidative carbocyclisation reaction to form streptorubin B. It appears as though the MBC A-ring N-H bond is vital for activity of RedG, and no other heteroatoms bearing lone pairs can act as a viable mimic. Although it is difficult to comment on the efficiency of the RedG cyclisation for the indolyl analogue because of the low activity of RedH it appears as though RedG functions well, suggesting that RedG imposes little steric constraint on the MBC derived portion of undecylprodiginine.
4.0 Result and discussion: Mutasynthesis of streptorubin B analogues using 2-undecylpyrrole analogues

4.1 Initial feeding studies of 2-undecylpyrrole analogues to a redL- mutant of S. coelicolor

We next investigated the mutasynthesis of streptorubin B (and undecylprodiginine) analogues using analogues of 2-undecylpyrrole 10. A variety of analogues were designed to further probe the substrate specificity of RedH and RedG and to generate streptorubin B analogues that are currently inaccessible by conventional synthesis. 2-undecylpyrrole analogues varying in the alkyl chain attached to the pyrrole ring were synthesised as described in chapter two.

The 2-undecylpyrrole analogues were fed to Streptomyces coelicolor W38, in which the redL gene, known to be involved in the biosynthesis of 2-undecylpyrrole, has been replaced by an apramycin resistance cassette. This strain cannot make 2-undecylpyrrole and hence does not produce any prodiginine antibiotics. Initial feeding studies with this strain were conducted with the natural substrate, 2-undecylpyrrole, in order to determine the level of undecylprodiginine and streptorubin B production. In addition, the 2-undecylpyrrole analogue with a terminal (Δ-10′-11′) alkene in the alkyl chain was used to probe the ability of RedH and RedG to tolerate changes in the alkyl chain of 2-undecylpyrrole.

Feeding 2-undecylpyrrole 10 to W38 (Figure 34) resulted in restoration of undecylprodiginine 3 production to levels approaching that observed in wild-type S. coelicolor M511. However, UV chromatograms monitoring absorbance at 533 nm from LC-MS analyses of extracts from this experiment showed that the ratio of streptorubin B 4 to undecylprodiginine 3 is substantially reduced (Figure 35)
compared with the ratio of streptorubin B/undecylprodiginine produced by *S. coelicolor* M511 (Figure 36).

Figure 34: Restoration of undecylprodiginine and streptorubin B production in *S. coelicolor* W38 when fed with 2-undecylpyrrole

Figure 35: UV chromatogram (533 nm) from LC-MS analysis of a mycelial extract of *S. coelicolor* W38 fed with 2-undecylpyrrole
When the Δ-10′-11′ 2-undecylpyrrole analogue 89 was fed to *S. coelicolor* W38 (Figure 37), production of the corresponding undecylprodiginine analogue 123 was observed at levels approaching the levels of undecylprodiginine production observed in wild-type *S. coelicolor* M511.

In addition, evidence for the formation of the corresponding streptorubin B analogue 124 was obtained from LC-MS analyses (Figure 38 and Figure 39).
The UV chromatogram monitoring absorbance at 533 nm showed a peak with a retention time similar to streptorubin B (Figure 39) and the extracted ion chromatogram at $m/z = 390$ confirmed this peak had the expected molecular mass for the analogue 124 (Figure 38).

**Figure 38:** Extracted ion chromatograms at $m/z = 390$ (blue trace) and $m/z = 392$ (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W38 fed with 2-undecylpyrrole Δ-10′-11′ analogue 89

**Figure 39:** UV chromatogram (533 nm) from LC-MS analysis of a mycelial extract of *S. coelicolor* W38 fed with 2-undecylpyrrole Δ-10′-11′ analogue 89
4.2 Use of S. coelicolor M511 redL::oriT-apr + redGH (W116) to boost production levels of streptorubin B analogues

The above experiments made it clear that isolation of the putative streptorubin B analogue produced when the Δ-10′-11′ 2-undecylpyrrole analogue 89 was fed to S. coelicolor W38 for characterisation by NMR spectroscopy would not be practically possible. Even though the levels of prodigamines produced when feeding 2-undecylpyrrole or Δ-10′-11′ 2-undecylpyrrole analogue 89 to S. coelicolor W38 were both approaching that observed in wild-type S. coelicolor, the ratio of streptorubin B to undecylprodigine was vastly reduced in both cases.

To overcome this problem, Paulina Sydor (another PhD student in the lab) investigated the effect of over-expressing either RedG and/or RedH. When redG is constitutively expressed in M511, RedG is expected to be over-produced in the resulting strain in comparison to M511 and hence was proposed to increase the ratio of streptorubin B in comparison to undecylprodigine in the new strain. However, she had previously shown that redG and redH integrated and constitutively expressed together restore production of streptorubin B more efficiently in a redG- mutant than when redG is expressed alone. Therefore she created both S. coelicolor M511 + redG (which contains an extra copy of redG) and S. coelicolor M511 + redGH (which contains an extra copy of both redG and redH). Indeed, when analysing the mycelial extract of these two strains she showed the ratio of streptorubin B to be increased more significantly in S. coelicolor M511 + redGH than in M511 + redG.42

Therefore to overcome the problem of the low ratio of streptorubin B analogues, Paulina Sydor created S. coelicolor M511 redL::oriT-apr + redGH (W116).42 This mutant is deficient in 2-undecylpyrrole biosynthesis, but contains extra copies of the redH and redG genes and is thus expected to over-produce both RedH and
RedG in comparison to the wild-type strain M511. Overproduction of these two proteins was expected to lead to an increase in the ratio of streptorubin B to undecylprodiginine produced when the mutant is fed with 2-undecylpyrrole, which could facilitate the isolation and purification of streptorubin B analogues. To test this hypothesis, 2-undecylpyrrole and the Δ-10'-11' 2-undecylpyrrole analogue 89 were fed to the *S. coelicolor redL::oriT-apr + redGH* mutant (W116). Overall levels of prodiginine production in these experiments did not significantly increase compared with earlier experiments, however, a striking increase in the ratio of streptorubin B to undecylprodiginine was observed. In both cases, when 2-undecylpyrrole 10 and the Δ-10'-11' 2-undecylpyrrole analogue 89 were independently fed to *S. coelicolor* W116 the UV chromatograms (Figure 41 and Figure 43) and the extracted ion chromatograms at the corresponding masses (Figure 40 and Figure 42) from LC-MS analyses showed higher levels of streptorubin B 4 and the streptorubin B analogue 124 than in previous experiments.

![Figure 40: Extracted ion chromatograms at m/z = 392 (blue trace) and m/z = 394 (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-undecylpyrrole](image-url)
Figure 41: UV chromatogram (533 nm) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-undecylpyrrole

![UV chromatogram](image1.png)

Figure 42: Extracted ion chromatograms at $m/z = 390$ (blue trace) and $m/z = 392$ (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with Δ-10′-11′ 2-undecylpyrrole analogue 89

![Extracted ion chromatograms](image2.png)

Figure 43: UV chromatogram (533 nm) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with Δ-10′-11′ 2-undecylpyrrole analogue 89

![UV chromatogram](image3.png)
It was not initially obvious why increasing the production of both RedH and RedG should affect the ratio of undecylprodiginine and streptorubin B. RedH catalyses the formation of undecylprodiginine which is then converted to streptorubin B by RedG. However, given the number of undecylprodiginine analogues observed in mutasynthesis experiments compared to the number of analogues of streptorubin B produced, RedH appears to display more relaxed substrate specificity than RedG. Perhaps condensation of 2-undecylpyrrole and MBC catalysed by RedH is so fast that multiple turnovers are occurring before one cyclisation reaction has been catalysed by RedG i.e. RedG is rate limiting in the formation of streptorubin B. Therefore undecylprodiginine can not be effectively passed from RedH to RedG and instead diffuses to and is trapped within the cell membrane by virtue of its undecyl alkyl chain. Undecylprodiginine is known to be membrane-bound in cells and its isolation involves mycelial extraction. This binding within the membrane would prevent RedG, which appears not to be membrane-bound, from accessing its substrate. The difference in catalytic efficiency between RedH and RedG, resulting in the trapping of undecylprodiginine in the membrane could explain the accumulation of undecylprodiginine in wild-type *S. coelicolor*. It could be suggested that the effect of this rate difference is amplified when using analogues of 2-undecylpyrrole or when the timescale of ‘production’ of 2-undecylpyrrole is altered from the constant level produced enzymatically in wild-type *S. coelicolor* to the sudden addition of a large concentration of 2-undecylpyrrole when it is fed to the *redL* mutant.

It is possible that decomposition of RedG has an influence on the undecylprodiginine/streptorubin B ratio. Many iron containing enzymes which activate molecular oxygen and hence generate very reactive intermediates are known to be susceptible to self-inactivation via proposed superoxide/peroxide mediated processes.\(^90, 91, 92, 93, 94\) If RedG does self-inactivate rapidly *in vivo*; the
decrease in ratio of RedG to RedH over time may be the reason for the accumulation of undecylprodiginine in S. coelicolor. As RedG inactivates, the rate of turnover of undecylprodiginine to streptorubin B would decrease. However, the production of undecylprodiginine would not (presuming RedH is stable). Therefore this ‘excess’ undecylprodiginine can not all be bound and turned-over by RedG but becomes imbedded within the cell membrane.

Furthermore, redH and redG are co-transcribed; therefore it is intriguing to speculate that perhaps RedH and RedG may form a catalytic complex. Perhaps RedG is more active in complex with RedH, or self-inactivates less quickly. It is also conceivable that undecylprodiginine could be channelled from RedH to RedG in a catalytic complex, thus reducing loss of undecylprodiginine to the membrane. If RedH and RedG are both overexpressed constitutively, in the redL::oriT-apr + redGH mutant (W116) a higher level of active RedG may be maintained within the cell. Thus increasing the rate of turnover of undecylprodiginine to streptorubin B.

Whatever the cause of the increased ratio of streptorubin B to undecylprodiginine when RedH and RedG are constitutively expressed in the RedL⁻ mutant, it allowed us to isolate the streptorubin B analogue produced when the Δ-10‘-11’ 2-undecylpyrrole analogue 89 is fed to S. coelicolor W116 in sufficient quantity for analysis by NMR and CD spectroscopies. Initial purification of the prodiginine mixture from the extract as the free bases was carried out by flash column chromatography on basic alumina. This was followed by HPLC separation of the hydrochloride salts of the streptorubin B analogue and the undecylprodiginine analogue.

NMR spectroscopic analysis of the isolated product provided strong evidence for the formation of a streptorubin B analogue by the mutasynthesis approach. Comparison of the ¹H-NMR spectrum of compound 124 (Figure 44) with that of
streptorubin B 4 (Figure 45) indicated that 124 contains the same carbocyclic ring as streptorubin B 4.

Figure 44: $^1$H-NMR spectrum of unsaturated streptorubin B analogue 124
A peak at -1.54 ppm, characteristic of the ansa-bridged 10-membered carbocycle of streptorubin B, resulting from unusually heavy shielding of one of the hydrogen attached to C-4' by the nearby pyrrole π electrons, is observed in the $^1$H-NMR spectrum of compound 124 (Figure 46a). Examination of the region between 7.5 and 6.0 ppm in the $^1$H-NMR spectrum of compound 124 shows the same pattern of peaks for the pyrrole protons as in streptorubin B, illustrating an equivalent pyrrole substitution pattern (Figure 46c). Signals corresponding to the monosubstituted alkene can also be seen with the expected intensities and multiplicities at 5.86 ppm (1H, multiplet), 5.07 ppm (1H, doublet, J = 18 Hz) and 5.01 ppm (1H, doublet, J = 10 Hz) (Figure 46c). In addition, the chemical shifts and multiplicities of the signals corresponding to the three protons alpha to the pyrrole C-ring system in streptorubin B are mirrored in the unsaturated analogue 124 (Figure 46b).
As discussed previously, streptorubin B 4 displays a very intense and distinctive circular dichroism spectrum due to the large optical activity of this compound, probably resulting from the ansa-bridged carbocycle. In order to establish if the level of stereocontrol RedG imparts on the carbocyclisation is affected with
undecylprodiginine analogues, the CD spectrum of compound 124 was recorded.

Figure 47: Circular dichroism spectrum of Δ-10’-11’ streptorubin B analogue 124

The CD spectrum of the streptorubin B Δ-10’-11’ analogue 124 (Figure 47) is very similar to that of natural streptorubin B (Figure 49). The fact that the peak for the analogue is slightly less intense might indicate that the same level of stereocontrol is not achieved with this analogue as with the natural compound. The peak height is normalised to prodiginine concentration as calculated by UV analysis with the known extinction coefficient 100500 M⁻¹ cm⁻¹. At this time the absolute stereochemistry of the carbocyclisation of these analogues has not been studied in detail. However, preliminary chiral HPLC analyses (which have been complicated by some decomposition of the cyclic material) show one major peak for compound 124 which has a poorly resolved shoulder and some other minor constituents, one of which ~ 39 minutes has a retention time similar to ent-streptorubin B (seen at ~34 minutes in analyses of streptorubin B, as
discussed in chapter 5) and this may account for the slightly reduced peak magnitude in the CD spectrum of 124. These experiments appear to indicate that the analogue 124 is enantiomerically enriched (if not enantiomerically pure) and that the major enantiomer has the same absolute stereochemistry as streptorubin B.

Figure 48: Comparison of the chiral HPLC analyses of streptorubin B (bottom trace) and streptorubin B Δ-10’-11’ analogue 124 (top trace)
4.3 Mutasynthesis of further streptorubin B analogues by feeding other 2-undecylpyrrole analogues to S. coelicolor W116

The isolation of the streptorubin B Δ-C10'-C11' analogue 124 from S. coelicolor W116 fed with the Δ-C10'-C11' analogue of 2-undecylpyrrole prompted us to investigate the feeding of the other synthetic 2-undecylpyrrole analogues described in chapter 2 to the W116 mutant. These experiments aimed to further probe the substrate specificity of both RedH and RedG and to generate further streptorubin B analogues which might facilitate exploration of the structure-activity relationship of these compounds.
4.3.1 Shortened alkyl chain analogues

4.3.1.1 2-decylpyrrole 86

Feeding of 2-decylpyrrole 86 to S. coelicolor W116 resulted in the production of decylprodiginine 125 and its carbocyclic derivative 126 as indicated by extracted ion chromatograms at $m/z = 378$ and 380 (Figure 51) and the UV chromatogram at 533 nm (Figure 52) from LC-MS analyses of mycelial extracts (Figure 50). About 30% of the 2-undecylpyrrole analogue 86 was consistently and reproducibly converted to prodiginine analogues in these experiments, a value that proved typical for feeding experiments with 2-undecylpyrrole analogues.

Figure 50: Mutasynthesis of decylprodiginine 125 and the corresponding streptorubin B analogue 126.
Figure 51: Extracted ion chromatograms at m/z = 378 (blue trace) and m/z = 380 (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-decylpyrrole 86

Figure 52: UV chromatogram (533 nm) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-decylpyrrole 86

The ratio of compounds 125 and 126 produced in this experiment was ~1:4 based on the UV chromatogram at 533 nm from LC-MS analysis. The streptorubin B analogue 126 was isolated in the same way as analogue 124 and characterised by $^1$H-NMR spectroscopy. This analysis indicated that compound 126 has a very similar structure to streptorubin B 4 (Figure 53). The signals for the pyrrole protons (Figure 54b), the protons alpha to the pyrrole C-ring (Figure 54c) and one of the protons attached to C-4’ (at -1.54 ppm) (Figure 54a) all matched those observed in the 1H-NMR spectrum of streptorubin B. The peak
at -1.54 ppm strongly indicates that the analogue contains a 10-membered carbocycle. A different ring size might have been obtained if the methyl terminus of the alkyl chain of undecylprodiginine is an important feature for substrate recognition and thus determines the site of cyclisation by the RedG enzyme.

Figure 53: $^1$H-NMR spectrum of streptorubin B analogue 126
CD spectroscopy was used to investigate the absolute stereochemistry of streptorubin B analogue 126. Comparison of the CD spectrum of compound 126 (Figure 55) with that of streptorubin B 4 (Figure 49) showed they are virtually identical, strongly indicating that the absolute stereochemistry of the two compounds are the same and that they are enantiomerically enriched to the same extent. This conclusion is confirmed by preliminary chiral HPLC analyses,
which identified only one major species (Figure 56). Taken together the CD and
$^1$H-NMR spectroscopic data indicate that a single streptorubin B analogue is
produced in this experiment, with an $n$-propyl side chain in place of the $n$-butyl
side chain on the carbocycle.

Figure 55: Circular dichroism spectrum of chain streptorubin B analogue 126

Figure 56: Comparison of the chiral HPLC analyses of streptorubin B (bottom
trace) and streptorubin B analogue 126 (top trace)
4.3.1.2 2-octylpyrrole 85

Our success in forming a shorter chain analogue of streptorubin B with 2-decylpyrrole led us to test the shortest extreme of chain length tolerated in the oxidative cyclisation by investigating the mutasynthesis of a streptorubin B analogue with 2-octylpyrrole. LC-MS/MS analyses of extracts from the incubation of 2-octylpyrrole 85 with S. coelicolor W116 identified a compound that generates an ion with $m/z$ corresponding to that predicted for octylprodiginine analogue 127 (Figure 58). However, no ion with $m/z$ corresponding to that predicted for streptorubin B analogue 128 could be detected (Figure 58). The UV chromatogram at 533 nm also showed only one peak, suggesting only octylprodiginine 127 was produced in this experiment (Figure 57).

Figure 57: Mutasynthesis of octylprodiginine 127 and the putative corresponding streptorubin B analogue 128.
Figure 58: Extracted ion chromatograms at $m/z = 350$ (blue trace) and $m/z = 352$ (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-octylpyrrole 85

Octylprodiginine 127 was produced in levels approaching the levels of undecylprodiginine in wild-type *S. coelicolor* as was the case for all 2-undecylpyrrole analogues investigated. High resolution mass spectrometry of the isolated compound gave a molecular formula corresponding to that of 127 (Calculated for $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}$: 352.2383 [M+H]$^+$ Found: 352.2379). Also when the fragmentation of the compound in MS/MS experiments (from LC-MS analyses) is compared to the known fragmentation pattern of undecylprodiginine it is clear that several characteristic fragment ions are common between both. Consequently, although the mutasynthesis experiments with 2-octylpyrrole 85 did not produce a streptorubin B analogue it did provide insight into the substrate specificities of RedH and RedG. RedH seems to tolerate the shorter alkyl chain in 2-octylpyrrole, seemingly efficiently catalysing the formation of octylprodiginine 127. In contrast, RedG appears to require a substrate with an alkyl chain that is longer than eight carbons in order to catalyse carbocycle formation.
4.3.2 Elongated alkyl chain analogues

4.3.2.1 2-dodecylpyrrole 87

The knowledge that RedH and RedG can tolerate shorter alkyl chains than those of their natural substrates stimulated us to investigate whether these enzymes can utilise substrates with longer alkyl chains than their natural substrates. To this end 2-dodecylpyrrole 87 was synthesised (as described in chapter two). When this compound was fed to S. coelicolor W116 peaks corresponding to dodecylprodiginine 129 and a carbocyclic derivative 130 were observed in both extracted ion chromatograms at \( m/z \) at 406 and 408 (Figure 60) and UV chromatograms at 533 nm (Figure 61) from LC-MS/MS analyses (Figure 59).

Figure 59: Mutasynthesis of dodecylprodiginine 129 and the corresponding streptorubin B analogue 130.
The putative streptorubin B analogue 130 was purified by flash column chromatography on alumina and reverse phase HPLC and analysed by $^1$H-NMR spectroscopy. The spectrum (Figure 62) clearly showed that the compound has a close structural relationship to streptorubin B (Figure 45), with a couple of key differences. The characteristic peak at -1.54 ppm indicative of a 10-membered ansa-bridged carbocycle was present (Figure 63a), however the intensity of this peak was lower than expected. Upon further investigation of the $^1$H-NMR
spectrum of **130** an additional signal, not seen in the spectrum of streptorubin B was observed at 0.23 ppm (Figure 63b). This signal has the same chemical shift as a distinctive signal in the $^1$H-NMR spectrum of metacycloprodigiosin (streptorubin A), which possesses a 12-membered ansa-bridged carbocycle. These two characteristic signals suggest that **130** is a mixture of compounds containing either a 10-membered (as in streptorubin B) or 12-membered (as in streptorubin A) carbocyclic ring in an approximate 60:40 ratio (from the intensities of the $^1$H-NMR integrations).

The signals for H-5, H-4 and H-3 of the A-ring and H-4 of the B-ring all had identical chemical shifts to the corresponding signals for streptorubin B (Figure 63d). However, two signals, only one of which had the same chemical shift as the corresponding proton in streptorubin B, were observed for H-1″ (7.12 and 7.09 ppm) and H-3 of the C-ring (6.51 and 6.33 ppm) (Figure 63d).

A similar picture emerged when analysing the signals due to H-1’ and H-7’, alpha to the pyrrole C-ring (Figure 63c). Two signals are observed for H-7’ in **130**, only one of which has the same chemical shift as the corresponding signal in streptorubin B. There also appears to be two overlapping signals for each of the protons attached to C-1’. These observations suggest that a second carbocyclic compound in addition to the analogue **130** is present. However, these data alone do not allow the structure of the second carbocyclic compound to be elucidated. The second carbocyclic compound could result from loss of ring size control by RedG due to the increase in alkyl chain length. Indeed, the additional signals observed for H-1″ and H-3 of the C-ring in the spectrum of **130** have similar chemical shifts to signals in the $^1$H-NMR spectrum of metacycloprodigiosin (streptorubin A), which contains a 12-membered ansa-bridged carbocycle. This combined with the presence of a signal at 0.23 ppm, suggests that the second carbocyclic compound present contains a
10-membered ansa-bridged carbocycle analogous to that observed in metacycloprodigiosin.

Figure 62: $^1$H-NMR spectrum of streptorubin B analogue 130
Figure 63: Comparison of diagnostic regions of the $^1$H-NMR spectrum of streptorubin B 4 (blue trace) and streptorubin B analogue 130 (red trace). Integration values are given below each peak.
To investigate the absolute stereochemistry of the streptorubin B analogue(s), a CD spectrum was recorded. The spectrum (Figure 64) differs significantly from that for streptorubin B 4 (Figure 49) and the other analogues reported in this thesis. The large absorbance at 533 nm is no longer present. However it is interesting to note that the baseline is not flat in this region. This CD-spectrum could result from a mixture of two constitutional isomers with opposite absolute stereochemistries (although a completely flat baseline would be expected for a 1:1 mixture of enantiomers).

Preliminary chiral HPLC analyses of the mixture of carbocyclic compounds obtained from the mutasynthesis experiment with 2-dodecylpyrrole gives three major peaks (Figure 65). One of which shows a similar retention time to streptorubin B (peak A), and the other two (peaks B and C) which together have an intensity approximately equal to that of peak A have a retention times similar to that of ent-streptorubin B (seen at ~34 mintues in analyses of streptorubin B as discussed in chapter 5). This suggests that the mixture of compounds (in addition to 130) that results from this experiment have opposite absolute stereochemistries. Together, analyses of 130 suggest that a mixture of compounds which contain either 10-membered or 12-membered ansa-bridged carbocycles and which have opposite absolute stereochemistry results from the mutasynthesis experiment with 2-dodecylpyrrole 87.
Figure 64: Circular dichroism spectrum of streptorubin B analogue 130

Figure 65: Comparison of the chiral HPLC analyses of streptorubin B (bottom trace) and streptorubin B analogue 130 (top trace).
4.3.2.2 2-octadecylpyrrole 88

The mutasynthesis experiments with 2-dodecylpyrrole show that both RedH and RedG can tolerate slight increases in alkyl chain length in their substrates, although the regioselectivity of the RedG-catalysed reaction appears to be affected. In order to probe the upper length limit of alkyl chains tolerated by RedH and RedG, 2-octadecylpyrrole 88 was fed to \textit{S. coelicolor} W116. However, no prodiginine-like compounds (e.g. 131 or 132) were produced in this experiment. This negative result might not reflect a limit to the substrate specificity of RedH or RedG, because the long alkyl chain in 2-octadecylpyrrole may adversely affect its cellular uptake.

\[ \text{Figure 66: Structures of the mutasynthesis products expected to result from the feeding of 2-octadecylpyrrole to } S. \text{ coelicolor W116.} \]
4.3.3 Analogues containing methyl branches in the C-ring alkyl chain

4.3.3.1 2-(10′-methylundecyl)pyrrole 99

To probe the effect of steric changes near the site of carbocyclisation on the reaction catalysed by RedG an analogue of 2-undecylpyrrole with a methyl group at C-10′ 99 was synthesised (as described in chapter 2) and fed to S. coelicolor W116. Compounds with the predicted chromatographic properties for undecylprodigine analogue 133 and streptorubin B analogue 134 were detected in extracted ion chromatograms (at m/z 406 and 408) (Figure 68) and in UV chromatograms (at 533 nm) (Figure 69) in LC-MS/MS analyses of culture extracts.

Figure 67: Mutasynthesis of undecylprodigine analogue 133 and the corresponding streptorubin B analogue 134.
The putative streptorubin B analogue was isolated from the culture extracts by liquid chromatography on alumina and reverse phase and analysed by $^1$H-NMR spectroscopy (Figure 70). The $^1$H-NMR spectrum of the isolated carbocyclic compound very closely matched that of streptorubin B (Figure 45) suggesting the compound had structure 134. The signal at -1.54 ppm, characteristic of the 10-membered ansa-bridged ring of streptorubin B is present in the spectrum of 134 (Figure 71a). Examination of the region between 7.5 and 6.0 ppm shows
that the pyrrole protons give identical signals to those observed for streptorubin B (Figure 71b). Finally the signals for the protons attached to C-1' and C-7' of 134 are very similar to the signals for the corresponding protons in streptorubin B (Figure 71c). The extra signal resulting from the additional methyl group in 134 is not clearly discernible in the \(^1\)H-NMR spectrum, but the increased complexity in the signals at around 1 ppm indicates its presence. Also, correlations between protons attached to C-12' and C-11' can be seen through to the protons attached to C-7'.

Figure 70: \(^1\)H-NMR spectrum of streptorubin B analogue 134
To investigate the absolute stereochemistry of streptorubin B analogue 134 its CD spectrum was recorded (Figure 72). It is similar to the spectrum for streptorubin B (Figure 49). However the magnitude of the absorbance at 533 nm is reduced relative to streptorubin B. It is not clear whether this reduction in absorbance is due to partial loss of stereocontrol in the RedG-catalysed
carbocyclisation reaction or whether it results directly from the change in structure. Preliminary chiral HPLC analyses of 134 are very similar to those for streptorubin B and show only one major peak for this compound, indicating that the absolute stereochemistry of these two compounds is the same and that they are enantiomerically enriched to a very similar extent. This suggests that 134 has the same level of enantiopurity as natural streptorubin B. Therefore indicating that the methyl branched substituent on the carbocycle may alter the magnitude of the Cotton effect displayed by this compound.

Figure 72: Circular dichroism spectrum of streptorubin B analogue 134
Figure 73: Comparison of the chiral HPLC analyses of streptorubin B (bottom trace) and streptorubin B analogue 134 (top trace)

The results of the NMR and CD spectroscopic analyses together with the results of the preliminary chiral HPLC analyses suggest strongly that a single streptorubin B analogue with the same absolute stereochemistry and an additional methyl group at C-10’ is the sole carbocyclic product of this mutasynthesis experiment.

4.3.3.2 2-(9’-methylundecyl)pyrrole 98

In order to further probe the substrate tolerances of RedG, 2-(9’-methylundecyl)pyrrole 98 was fed to S. coelicolor W116 (Figure 74). The compound was synthesised (as described in chapter 2) and fed as a racemate to investigate whether RedG shows any enantioselectivity. LC-MS/MS analyses of the resulting mycelial extract showed the presence of a compound with the \( m/z \) and UV absorbance predicted for the undecylprodiginine analogue 135 and two compounds with the \( m/z \) and UV absorbances predicted for the streptorubin B analogue 136 (Figure 75 and Figure 76).
Figure 74: Mutasynthesis of undecylprodiginine analogue 135 and the corresponding streptorubin B analogue 136.

Figure 75: Extracted ion chromatograms at m/z = 406 (blue trace) and m/z = 408 (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-(9′-methylundecyl)pyrrole 98.
These two peaks corresponding to putative streptorubin B analogues were initially proposed to arise from the diastereomeric nature of these analogues, resulting from the racemic mixture of 98 that was fed. Both of these putative streptorubin B analogues were isolated and analysed by $^1$H-NMR spectroscopy.

136a (retention time 11 minutes) displayed a very similar $^1$H-NMR spectrum (Figure 77) to the spectrum of streptorubin B (Figure 45). It possesses the characteristic signal at -1.54 ppm indicative of the presence of a 10-membered ansa-bridged carbocycle in 136a (Figure 78a). Signals in the aromatic region of the spectrum of 136a are very similar to the signals in the corresponding region of the spectrum of streptorubin B (Figure 78c). The protons alpha to the pyrrole C-ring resonate at similar frequencies in both spectra (Figure 78b). However closer inspection of the aromatic and “benzylic” regions of the spectrum of 136a allows identification of clear peak doubling at 6.50-6.55 ppm and 3.20-3.30 ppm. Similar signal doubling was observed in the $^1$H-NMR spectrum of dodecylprodiginine analogue 130 in which a mixture of compounds is present (Figure 63), suggesting that 136a is a mixture of two closely related compounds.
Unlike with dodecylprodiginine analogue 130, the spectrum of 136a does not show several significantly different signals for the pyrrole protons nor does it show the doubling of all the signals corresponding to protons alpha to the pyrrole C-ring. Only the signal at 6.52 ppm, which corresponds to H-3 of the C-ring and the signal at 3.20 ppm, which corresponds to H-7’ are duplicated. Both of these protons are proximal to the carbocycle and the duplicated signals are shifted to a lesser extent than seen for the duplicated signals in dodecylprodiginine analogue 130. It is reasonable to propose that the signal doubling in the spectrum of 136a results from the presence of a mixture of diastereoisomers due to a mixture at C9’ (arising from the racemic 2-(9’-methylundecyl)pyrrole used in this experiment).

Figure 77: $^1$H-NMR spectrum of streptorubin B analogue 136a
Figure 78: Comparison of diagnostic regions of the $^1$H-NMR of streptorubin B 4 (blue trace) and streptorubin B analogue 136a (red trace). Integrals are indicated below each signal.

Preliminary chiral HPLC analysis of the apparent mixture of streptorubin B analogues 136a results in the separation of the two compounds, (which are
inseparable by normal reverse-phase HPLC) and indicated that they are present in an approximately 50:50 ratio (Figure 79). This confirms that the mixture of two compounds observed in the $^1$H-NMR spectrum corresponds to two diastereoisomers of the anticipated streptorubin B analogue 136. This raises two questions: (1) Is the CD spectrum of the mixture different from the spectrum of streptorubin B; and (2) if both diastereoisomers are accounted for by the peak 136a at ~11 min in the LC-MS analysis, what is the structure of the other compound with $m/z = 408$ (compound 136b) and a retention time of 15 minutes?

![Figure 79: Comparison of the chiral HPLC analyses of streptorubin B (bottom trace) and streptorubin B analogue 136a (top trace)](image)

A CD spectrum of the diastereomeric mixture 136a was recorded (Figure 80). It closely resembles the spectra of streptorubin B analogues 124 and 134 described previously. It shows an intense positive absorbance at 533 nm as seen for streptorubin B (Figure 49). The intensity of the absorbance is lower than that seen for streptorubin B. However, it is approximately equal to the intensity of the absorbance at 533 nm for the other analogues, suggesting that the diastereomeric mixture in 136a has little, if any, effect on the CD spectrum.
Presumably the ‘axial’ chirality associated with the carbocycle is the major reason for the intense absorbance at 533 nm in the CD spectrum and other chiral elements in the structure have little effect. However, the above results suggest that more detailed studies of the stereochemical outcome of the carbocyclisation of undecylprodiginine analogues are required.

![CD spectrum of 136a (in MeOH c = 0.167 mM)](chart)

**Figure 80: Circular dichroism spectrum of streptorubin B analogue 136a**

We next sought to address the question: what is the second compound with \( m/z = 406 \) and retention time ~15 min (136b). The compound proved difficult to separate from the corresponding undecylprodiginine analogue 135 on a preparative scale. However, some initial \(^1\)H-NMR studies were carried out on the material obtained.

The \(^1\)H-NMR spectrum of 136b bears a close resemblance to that of undecylprodiginine 3. The pattern of the signals corresponding to the pyrrole protons in 136b is essentially identical to that seen for undecylprodiginine 3. The large triplet at 2.91 ppm corresponding to the two non-diastereotopic protons
alpha to the pyrrole C-ring system is also seen in the spectrum of undecylprodiginine 3 (Figure 81). These features of the $^1$H-NMR spectrum both indicate that 136b is not a carbocyclic derivative of undecylprodiginine. A complex multiplet appears in the alkenyl region of the $^1$H-NMR spectrum of 136b at ~5.2 ppm, which (when analysed in deuterated chloroform to avoid the overlap of this signal with the residual solvent peak of deuterated methylene chloride) from the integration corresponds to two protons (Figure 82). This multiplet shows connectivity in 2D COSY spectra to the alkyl chain protons of 136b. Therefore it appears that the reduction in mass of this compound relative to the undecylprodiginine analogue results from a desaturation of the alkyl chain rather than oxidative carbocyclisation. This presumably arises via a second hydrogen radical abstraction catalysed by RedG (Figure 83). It appears that the RedG catalysed carbocyclisation is perturbed by the methyl group beta to the site of cyclisation. Thus, initial alkyl radical generation can be followed by either: (a) cyclisation analogous to that seen in streptorubin B formation and a subsequent abstraction of a pyrrole-derived proton by RedG; or (b) abstraction of a second alkyl hydrogen atom by RedG on the carbon atom adjacent to the site of initial radical formation to form the observed double bond(s). Further experiments will be required to establish the regio/stereochemistry of the double bond(s) in 136b. Therefore the mutasynthesis experiment with 2-(9'-methylundecyl)pyrrole 98 results in the formation of a mixture of unsaturated compounds 136b, in addition to the predicted analogues of undecylprodiginine 135 and streptorubin B 136a (Figure 84).
Figure 81: Comparison of the $^1$H-NMR spectra (CD$_2$Cl$_2$, 400 MHz) of 136b (red trace) and undecylprodiginine (blue trace). Note that the alkene protons are partially obscured by the CH$_2$Cl$_2$ signal.
Figure 82: Aromatic/alkene C-H region of the $^1$H-NMR spectrum (CDCl$_3$, 400MHz) of compound 136b (note that the seventh aromatic C-H signal is obscured by the chloroform signal).

Figure 83: Divergence of pathways from a putative radical intermediate in RedG-catalysed oxidation of methylated undecylprodiginine 135
Figure 84: Summary of compounds obtained from mutasynthesis experiments with 2-(9’-methylundecyl)pyrrole

4.3.4 2-undecylpyrrole analogues containing ether linkages in the alkyl chain

4.3.4.1 2-(3’-oxa-undecyl)pyrrole 102

The incorporation of heteroatoms such as nitrogen, oxygen or sulphur into the streptorubin B carbocycle could lead to a significant change in physico-chemical properties due to the electronegativity and lone pairs of the heteroatoms. To test whether RedH and RedG can tolerate a heteroatom in the alkyl chains of their substrates an analogue containing an oxygen atom at C-3’ was fed to S. coelicolor W116 (Figure 85). Extracted ion chromatograms at m/z = 394 and 396 (Figure 86) and UV chromatograms at 533 nm (Figure 87) from LC-MS/MS
analyses indicated that the predicted undecylprodiginine analogue 137 and streptorubin B analogue 138 were both produced. It should be noted in this case that the undecylprodiginine analogue 137 is eluted as a mixture of interconverting configurational isomers probably resulting from rotation about the C1”-C2 of the B-ring carbon-carbon double bond. The compounds associated with each peak were separately collected from the HPLC separation. When re-analysed by HPLC, each compound gave the same mixture of two peaks as when first analysed. LC-MS analyses of this experiment were performed with methanol in place of acetonitrile because of severe problems with acetonitrile supply. This is the likely origin of the two peaks for 137 (similar peak duplication is observed for undecylprodiginine under certain HPLC conditions).

Figure 85: Mutasynthesis of undecylprodiginine analogue 137 and the corresponding streptorubin B analogue 138.
Isolation of the putative streptorubin B analogue 138 proved to be difficult due to decomposition during purification. It is not clear what the mechanism of decomposition is. It is possible that 2-(3′-oxa-undecyl)pyrrole 102 is largely decomposing \textit{in vivo} before incorporation into the corresponding prodiginine analogues. Alternatively, the prodiginine analogues themselves might be decomposing. Indeed, both of these decomposition routes could be occurring simultaneously. It is interesting to note that (3′-oxa-2-undecyl)pyrrole 102
decomposes significantly faster than all the other 2-undecylpyrrole analogues synthesised in this study. Even at reduced temperatures, it quickly degrades to a black oil, via a presumed polymerisation pathway that is similar to the acid-catalysed polymerisation of pyrrole.

Due to the insufficient amount of material isolated, a satisfactory $^1$H-NMR spectrum of 138 could not be obtained. However, the compound was investigated by high resolution mass spectrometry to confirm the molecular formula of the isolated compound (Calculated for C$_{24}$H$_{31}$N$_3$O$_2$: 394.2489 [M+H]$^+$ Found: 394.2490), and analyses of this compound in MS/MS experiments showed several ions corresponding to analogous characteristic fragments in the MS/MS spectrum of streptorubin B.

In addition, the CD spectrum of 138 was recorded (Figure 88) and is very similar to the CD spectrum of streptorubin B (Figure 49). The large positive absorbance at 533 nm results from the hydrochloride salt of the conjugated tripyrrole ring system and implies that the analogue contains an ansa-bridged carbocycle with a stereocentre. This illustrates well the sensitivity of CD spectroscopy particularly for the identification of compounds which display large Cotton effects like the prodiginines. It allows detection of carbocyclic prodiginines at levels well below the detection limit of high-field NMR spectroscopy.
The evidence from CD spectroscopy combined with the LC-MS/MS data allows a tentative identification of the expected oxygen-containing streptorubin B analogue 138 as a product of the mutasynthesis experiment with 2-(3′-oxa-undecyl)pyrrole 102. However the lack of NMR data precludes the conclusive structural assignment of this compound at the present time.

4.3.4.2 2-(6′-oxa-undecyl)pyrrole 105

In light of the above results it was thought useful to probe whether other polar linkages could be tolerated elsewhere within the alkyl chain of the substrates of RedG and RedH. Therefore, a 2-undecylpyrrole analogue with an oxygen atom directly adjacent to the site of carbocyclisation, 2-(6′-oxa-undecyl)pyrrole 105, was fed to S. coelicolor W116 (Figure 89). LC-MS/MS analyses (Figure 90 and Figure 91) revealed that only one compound with a \( m/z \) corresponding to the
undecylprodiginine analogue 139 was produced. No compound with the $m/z$ corresponding to carbocyclic analogue 140 could be detected.

Figure 89: Mutasynthesis of undecylprodiginine analogue 139 and the proposed corresponding streptorubin B analogue 140.

Figure 90: Extracted ion chromatograms at $m/z = 394$ (blue trace) and $m/z = 396$ (red trace) from LC-MS analysis of a mycelial extract of *S. coelicolor* W116 fed with 2-(6'-oxa-undecyl)pyrrole 105.
High resolution mass spectrometry was used to confirm the molecular formula of the isolated compound 139, (Calculated for C$_{24}$H$_{33}$N$_3$O$_2$: 396.2646 [M+H]$^+$ Found: 396.2649). In addition, the fragmentation of the compound in MS/MS experiments was compared to the known fragmentation pattern of undecylprodiginine (as with the other undecylprodiginine analogues observed) and showed several common characteristic ions.

Interestingly, the UV chromatogram showed that another compound with a retention time of approximately 5 minutes (m/z = 264) had been produced with a $\lambda_{\text{max}}$ of 533 nm, which is very characteristic of prodiginine hydrochloride salts. The structure of this compound has tentatively been assigned as 144 (Figure 92) in which the carbon chain from C-7’ to C-11’ has been cleaved from the corresponding undecylprodiginine analogue 139. Unfortunately 144 has at this stage not been isolated from the cell culture due to the relatively small amount of material being produced precluding the isolation of this compound within the time-restraints at the end of this research. However, the MS/MS fragmentation pattern of this compound does support the structural assignment as 144. It is proposed that this cleavage occurs as a result of the action of RedG. The C-7’
radical could rebound onto the Fe(III)-OH species to generate a C-7′ hydroxyl group 142. The resulting hemiacetal 142 would be unstable and could decompose to give \( n \)-pentanal and 144.

![Proposed mechanism for the de-alkylation of undecylprodiginine analogue 139 catalysed by RedG](image)

Figure 92: Proposed mechanism for the de-alkylation of undecylprodiginine analogue 139 catalysed by RedG

### 4.3.4 Summary of conclusions of mutasynthesis with 2-undecylpyrrole analogues

Several analogues of streptorubin B have been isolated via the proposed mutasynthetic approach during this research project. Generation of the compounds has informed us about the degree of substrate tolerances displayed by both RedH and RedG and has also given us rapid access to a variety of analogues of streptorubin B for potential structure-activity relationship analyses. RedH shows a quite broad substrate tolerance, catalysing the condensation of nearly all of the 2-undecylpyrrole analogues tested with MBC to generate the corresponding undecylprodiginine analogues. Of the analogues tested only 2-octadecylpyrrole was not accepted by RedH, suggesting that very long C-ring alkyl chains are not tolerated, although the unknown cell membrane permeability
properties of this compound may account for the lack of condensation by RedH. In addition to the corresponding undecylprodiginine analogues of the streptorubin B analogues generated (Figure 94), RedH accepted and catalysed the condensation of 2-octylpyrrole 85 and 2-(6'-oxa-undecyl)pyrrole 105 with MBC to give the predicted undecylprodiginine analogues 128 or 139 (Figure 93).

![Figure 93: Undecylprodiginine analogues from mutasynthesis experiments with 2-undecylpyrrole analogues where no streptorubin B analogues were observed](image)

RedG also shows a notable degree of substrate tolerance, catalysing the carbocyclisation of a variety of structurally diverse undecylprodiginine analogues (produced in vivo by the action of RedH) to give the predicted streptorubin B analogues in most cases (Figure 94).
At this stage the stereochemistry of the carbocyclisation catalysed by RedG needs further study, however, the analogues synthesised here appear to nearly all show the same absolute stereochemistry as streptorubin B and a similar degree of enantiomeric enrichment. The only exception is analogue 130 which was isolated from the experiment with 2-dodecylpyrrole, in which there appears to be a mixture of compounds with either 10-membered (as in streptorubin B) or 12-membered (as in streptorubin A) carbocyclic rings (in an approximate 60:40 ratio respectively from $^1$H-NMR integrations) which have opposite stereochemistry.
5.0 Results and discussion: Stereochemical elucidation of Streptorubin B

5.1 Introduction

As described previously, the relative stereochemistry of the major configurational/conformational isomer of streptorubin B hydrochloride (resulting from restricted rotation in the carbocycle) has been elucidated by NOESY experiments. However, the absolute stereochemistry of streptorubin B (i.e. the configuration of the C-7′ stereocentre was unknown at the outset of this project) (Figure 95).

Figure 95: Selected spectroscopic properties of streptorubin B. Red arrows indicate key correlations observed in the NOESY spectrum of streptorubin B hydrochloride salt (left). The signals for the diastereotopic C-4′ hydrogens are separated by 2.7 ppm in the $^1$H-NMR spectrum of streptorubin B hydrochloride salt (right).

At the beginning of this project knowledge of the absolute stereochemistry of chiral prodiginines was very limited. Roseophilin was the only member of the family with known absolute configuration. This was determined by enantioselective total synthesis by Tius and co-workers. Subsequent comparison of the CD spectra of their synthetic material with the natural product allowed the absolute stereochemistry of roseophilin to be assigned as $R$, $R$ (Figure 96).
Figure 96: Enantioselective total synthesis of roseophilin reported by Tius and co-workers.

Recently, the enantioselective total synthesis of metacycloprodigiosin (streptorubin A) was reported (Figure 97).53

![Chemical structure of metacycloprodigiosin](image)

Figure 97: Enantioselective total synthesis of streptorubin A via key diketone intermediate 32
However, the absolute stereochemistry of the natural compound was not reported due to the lack of an authentic sample for comparison purposes. In collaboration with the authors of this report, we have determined the absolute stereochemistry of the major enantiomer of metacycloprodigiosin (streptorubin A) as \( R \) by CD-spectroscopic comparisons with an authentic standard isolated from \( S. longispororuber \) (Figure 98).

![Graph comparing CD spectra of synthetic \((R)\)-metacycloprodigiosin and metacycloprodigiosin from \( S. longispororuber \)](image)

*Figure 98: Comparison of circular dichroism spectrum of synthetic \((R)\)-metacycloprodigiosin and metacycloprodigiosin from \( S. longispororuber \)*

Both metacycloprodigiosin (streptorubin A) and roseophilin have the same absolute configuration and large negative Cotton effects in their CD spectra. In contrast, streptorubin B displays a large positive Cotton effect in its CD spectrum. Thus, from this comparison we propose that streptorubin B is pseudoenantiomeric to metacycloprodigiosin/roseophilin and, as a consequence has a \( 4'\)-\( S \) configuration (Figure 99).
5.2 Stereochemical elucidation of streptorubin B by mutasynthesis with stereospecifically deuterium labelled 2-undecylpyrrole

Making use of the knowledge of the relative stereochemistry of streptorubin B and its biosynthetic pathway, we decided to use a mutasynthetic approach to determine the absolute stereochemistry of streptorubin B. Feeding of 2-undecylpyrrole with a stereospecific deuterium label at C-4' to a mutant of *S. coelicolor* blocked in 2-undecylpyrrole biosynthesis (e.g. W116), should result in production of streptorubin B, labelled stereospecifically on C-4' of the carbocycle (Figure 100). It is known that the signals for the two protons attached to C-4' of streptorubin B are well resolved in the $^1$H-NMR spectrum due to the shielding influence of the pyrrole π-electrons on one of the protons (Figure 95). Thus it should then be straightforward to determine which of the C-4' hydrogen atoms of streptorubin B has been labelled with deuterium in this experiment using $^1$H and $^2$H-NMR spectroscopy. From these data we should then be able to assign the absolute stereochemistry of streptorubin B.
Figure 100: Possible stereochemical outcomes from feeding (R)-[C-4′-D₁]-2-undecylpyrrole to S. coelicolor W116. The proton/deuteron expected to have a chemical shift of -1.54 ppm in each case is highlighted in red (note that the absolute stereochemical descriptor for C-4′ in [C-4′-D₁]-2-undecylpyrrole and [C-4′-D₁]-streptorubin B changes due to a change in the substituent priorities).

5.3 Synthesis of (R)- and (S)-[C-4′-D₁]-2-undecylpyrrole

The stereoselective syntheses of (R)- and (S)-[C-4′-D₁]-2-undecylpyrrole employed L-glutamic acid 146 as the starting material. This was converted to the lactone 147 by diazotisation of the amino group with nitrous acid generated in situ from sodium nitrite and hydrochloric acid (Scheme 34). Cyclisation of the resulting diazo compound proceeds via a double inversion mechanism (due to neighbouring group participation by the carboxylic acid alpha to the azide), resulting in retention of stereochemistry. Subsequent chemoselective reduction of the carboxyl group in 147 to the corresponding hydroxyl group without reducing the lactone was effected with BH₃·SMe₂ to yield the desired alcohol 148. Tosylation of 148 afforded 149 in excellent yield.⁹⁶ This intermediate was reacted with sodium methoxide, generated in situ by the addition of one equivalent of sodium metal to dry methanol, to open the lactone. The resulting alkoxide displaced the tosylate to give the epoxide 150. Opening of the epoxide 150 at the least-hindered carbon with the cuprate derived from n-hexyl lithium and copper cyanide yielded lactone (R)-151.⁹⁶ This lactone is a known natural product, which has been identified as a flavour compound from fruits and as a sex pheromone in insects, and consequently has a known optical rotation.⁹⁷ ⁹⁸
Comparison of the optical rotation of the synthetic lactone with literature values for the natural product confirmed the absolute configuration of this compound.

Scheme 34: Synthesis of (R)-4-heptylbutyrolactone 151

With gram quantities of (R)-151 in hand, the opening of the lactone to give either the desired deuterated acid (S)-152 or a precursor of this acid was explored. As expected, no displacement of the carboxylate was observed when the lactone was reacted with lithium aluminium deuteride. Only the doubly-deuterated diol 153 was recovered from this reaction (Scheme 35).
Scheme 35: Attempted direct ring opening of lactone (R)-151

A step-wise route to deuterated acid 152 via the reduction of lactone (R)-151 with lithium aluminium hydride to the diol 154, followed by selective protection of the primary alcohol, tosylation of the secondary alcohol, displacement of the tosylate with LiAlD₄, deprotection and oxidation of the primary alcohol to the carboxylic acid (Scheme 36) was also considered as an alternative to the route outlined in Scheme 35. Initial reduction of (R)-151 to the desired diol 155 was successful. However this route was not pursued further because a more concise route was devised.
Scheme 36: Synthesis of the desired deuterated acid 152 via reduction of lactone (R)-151 to the corresponding diol.

During the course of exploratory chemistry aimed at the synthesis of \((S)-152\) from \((R)-151\), the lactone was opened with trimethyl aluminium and dimethyl amine to give the corresponding hydroxy amide 159 (Scheme 37).\(^9\) However, the tosylation of the hydroxyl group (required for subsequent deuteride displacement) did not give the expected tosyl amide 160. Instead this tosylation reaction resulted in reformation of the lactone, presumably via the imine 161 formed by intramolecular displacement of tosylate by the oxygen of the amido group. This sequence of reactions occurs with inversion and was subsequently applied to the synthesis of \((S)-151\) (the enantiomer of the initial lactone).
Scheme 37: Unanticipated conversion of (R)-151 to (S)-151 via tosyl amide 160

The lactone could also be opened by reaction of (R)-151 with excess iodotrimethylsilane, a reagent known to convert esters to the corresponding acids.\textsuperscript{100, 101} Seemingly, initial silylation of the oxygen atom of the carbonyl group activates the lactone towards subsequent S\textsubscript{N}2 ring-opening by iodide (Scheme 38).\textsuperscript{102} The resulting iodo silyl ester 162 can be hydrolysed to the corresponding iodo acid 163. However, this compound proved to be somewhat unstable due to the propensity for recyclisation to the lactone via intramolecular displacement of the iodide by the carbonyl group (Scheme 38).
Scheme 38: Synthesis of iodo acid 163 via iodo trimethylsilyl ester 162 by the reaction of lactone (R)-151 with iodotrimethylsilane. The iodo acid was prone to recyclisation to the lactone via elimination of HI.

To overcome the problem of the instability of 163, silyl ester 162 was converted to the methyl ester 164 by the addition of excess methanol to the reaction mixture after complete ring-opening had occurred (Scheme 39). When using exactly one equivalent of iodotrimethylsilane to open the lactone, subsequent ester formation with methanol does not occur, instead an excess of iodotrimethylsilane is required to effect ester formation. Therefore, it seems that the formation of catalytic hydriodic acid, as a result of the reaction between the remaining iodotrimethylsilane and the added methanol catalyses the formation of the methyl ester. The iodo methyl ester (S)-164 is stable to column chromatography on silica gel and shows no propensity towards instability.
Chemoselective displacement of iodide in (S)-164, without reduction of the ester, was achieved with sodium borodeuteride in dimethyl sulphoxide (this polar aprotic solvent is known to dramatically increase the rate of S_N2 displacements)\textsuperscript{103, 104} affording the deuterated ester (R)-165. Base-mediated ester hydrolysis was followed by conversion of the resulting acid (R)-152 to the desired labelled 2-undecylpyrrole (R)-145 (Scheme 40), by the route described previously for synthesis of 2-undecylpyrrole from pyrrole and undecanoic acid.
Conversion of \((S)-151\), obtained from \((R)-151\) via the inversion procedure described above (Scheme 37), to \((S)-[\text{C-4'-D},]_1\)-2-undecylpyrrole \((S)-145\) was accomplished via the route used to convert \((R)-151\) to \((R)-145\) (Scheme 41).
Scheme 41: Synthesis of (S)-[C-4′-D1]-2-undecylpyrrole (S)-145 from lactone (S)-151 via initial lactone inversion
5.4 Feeding of stereoselectively labelled 2-undecylpyrroles to S. coelicolor W116

Both enantiomers of labelled 2-undecylpyrrole were fed separately to the S. coelicolor W116 mutant, which is unable to biosynthesise 2-undecylpyrrole. Analysis of the labelled streptorubin B purified from these experiments gave somewhat unexpected results. Although LC-MS analyses indicated that C-4′ singly labelled streptorubin B was produced in both experiments, a signal at -1.54 ppm was observed in the $^1$H-NMR spectrum of both labelled compounds (Figure 101 and Figure 102). The multiplicity of this signal was the same in both cases, but markedly different to that observed for unlabelled streptorubin B. However, the integral value for this signal was very different for the two compounds.

![Figure 101: Comparison of $^1$H-NMR spectra of labelled streptorubin B isolated from S. coelicolor W116 fed with (R)-[C-4′-D$_1$]-2-undecylpyrrole (bottom trace) and streptorubin B isolated from S. coelicolor M511 (top trace)]
Feeding of (R)-[C-4′-D₁]-2-undecylpyrrole (R)-145 resulted in streptorubin B with an integral value of 0.7 for the signal at -1.54 ppm relative to a value of ~1 for each of the pyrrole protons (Figure 101). In contrast, feeding of (S)-[C-4′-D₁]-2-undecylpyrrole (S)-145 yielded labelled streptorubin B in which the signal at -1.54 ppm had an integral value of approximately 0.3 relative to a value of ~1 for each of the pyrrole protons (Figure 102).

³H-NMR spectra of each labelled form of streptorubin B were recorded to confirm that a mixture of streptorubin B diastereomers was produced in both experiments. These experiment showed two peaks for each compound, one at 1.16 and one at -1.54 ppm (Figure 103 and Figure 104), confirming that a mixture of diastereomers results from both experiments (Figure 105).
Figure 103: $^2\text{H}$-NMR spectrum of labelled streptorubin B isolated from *S. coelicolor* fed with (R)-[C-4'-D$_1$]-2-undecylpyrrole

Figure 104: $^2\text{H}$-NMR spectrum of labelled streptorubin B isolated from *S. coelicolor* fed with (S)-[C-4'-D$_1$]-2-undecylpyrrole
5.5 Investigation of why streptorubin B diastereoisomers are produced and absolute stereochemical assignment of streptorubin B

The above results indicated that either (a) the RedG-catalysed carbocyclisation was not enantiospecific, but only enantioselective, giving a 70:30 ratio of enantiomers, or (b) partial epimerisation of the labelled 2-undecylpyrroles had occurred during the final steps of the synthesis.

To establish the enantiopurity of streptorubin B, a sample isolated from *S. coelicolor* was analysed by HPLC on a homochiral stationary phase. The free
base of streptorubin B was analysed on a ChiralPak IA column (Chiral Technologies Europe). Diethylamine (0.1 %) was added to the mobile phase of hexane/isopropanol to ensure formation of the free base. Recently, the synthesis of racemic streptorubin B has been completed by Thomson and co-workers by an analogous route to that previously described for their total synthesis of metacycloprodigiosin (streptorubin A).\textsuperscript{54, 53} Racemic streptorubin B was also analysed under the same conditions.

A major species, eluting at 9 minutes (peak A) was observed in the chromatogram of natural streptorubin B isolated from \textit{S. coelicolor}; together with two minor species, eluting at 12 minutes (peak B), and 40 minutes (peak C) respectively (Figure 106b). The areas under the peaks corresponding to the minor species were both \textasciitilde 5 \% of the area under the peak for the major species. Comparison of the chromatograms for natural (enantioenriched) and synthetic (racemic) streptorubin B identified peaks A and C as the streptorubin B enantiomers (Figure 106a and Figure 106b). However, the identity of the species corresponding to peak B was unclear. It was thought that perhaps it may correspond to a minor diastereomer.
To test this hypothesis, peak A was collected and left to stand for seven days at room temperature. Reanalysis of the sample after this time showed that ~3% of peak A had been converted to peak B (Figure 107), this reequilibration of peak A seems to confirm the hypothesis that peak B corresponds to a minor diastereomer of the major enantiomer of streptorubin B. It therefore appears that dia-streptorubin B resulting from the slow rotation of the carbocyclic ring across the plane of the tri-pyrrole ring system of streptorubin B occurs as a minor component of the natural product. The rate of the equilibration to a ~5% portion of dia-streptorubin B appears to be quite slow (as illustrated by the reanalysis of freshly isolated peak A Figure 107b). The ratio of dia-streptorubin B to streptorubin B presumably is governed by the influence of a favourable steric interaction observed in dia-streptorubin that is not seen in streptorubin B between the butyl side chain and the proton attached to C-1″ (Figure 108). The identity of the species corresponding to the peak with retention time of 10 minutes in analyses of racemic streptorubin B (Figure 106a) is yet to be
confirmed, due to time constraints at the end of this research. However, it is possible that this may prove to be ent-dia-streptorubin B or perhaps simply a small impurity from the synthetic preparation of racemic streptorubin B by Thomson and co-workers.

![UV chromatograms from HPLC analyses](image)

Figure 107: UV chromatograms from HPLC analyses on a homochiral stationary phase monitoring absorbance at 470 nm ($\lambda_{\text{max}}$ of prodigine free base) of (a) streptorubin B isolated from *S. coelicolor* (b) reanalysis of freshly isolated streptorubin B peak A (c) reanalysis of streptorubin B peak A after seven days at room temperature
Figure 108: Structures of different isomeric forms of streptorubin B

Figure 109: 3D-model of streptorubin B (left) and dia-streptorubin B (right), showing the different steric constraints of each.

The related carbocyclic prodiginine metacycloprodigiosin (streptorubin A) was also investigated using the same chiral HPLC method. We have shown that metacycloprodigiosin (streptorubin A) and streptorubin B are inseparable by HPLC on a conventional reverse phase column. Therefore it can be reasoned that any differences in the retention times of metacycloprodigiosin (streptorubin A) and streptorubin B on a homochiral HPLC column are due to absolute stereochemical differences, rather than structural differences. In addition, both enantiomers of metacycloprodigiosin (streptorubin A) have been recently synthesised by Thomson and co-workers via a previously described route.\textsuperscript{53}
Samples of both enantiomers of metacycloprodigiosin (streptorubin A) provided by Thomson and co-workers were analysed independently.

Analysis of synthetic (9′-R)-metacycloprodigiosin (streptorubin A) showed a major species with a retention time of 19 minutes (peak E) and a minor species with a retention time of 9 minutes (peak D). The area under the minor peak was ~ 5 % of that under the major peak (Figure 110c). In contrast, analysis of (9′-S)-metacycloprodigiosin afforded a major species with a retention time of 9 minutes and a minor species with a retention time of 19 minutes (Figure 110b). Analysis of (±)-metacycloprodigiosin gave two species in a 1:1 ratio with retention times of 9 and 19 minutes (Figure 110a). Comparison of these chromatograms with chromatogram of natural metacycloprodigiosin isolated from *S. longispororuber* showed that the major enantiomer of the natural product has the 9′-R-configuration (Figure 110d), as we had previously deduced from CD spectroscopic analyses. The presence of the opposite enantiomer in the samples of synthetic 9′-S- and 9′-R-metacycloprodigiosin indicates that the syntheses are only highly enantioselective rather than enantiospecific. Interestingly, the natural metacycloprodigiosin appears to consist of a mixture of both 9′ configurations in an approximate ratio of 85:15, indicating that the configuration previously identified by CD spectroscopic analyses corresponds only to the major enantiomer present in the natural product.
Finally, the samples of deuterium-labelled streptorubin B from the feeding experiments with (S)-[C-4′-D₁]-2-undecylpyrrole and (R)-[C-4′-D₁]-2-undecylpyrrole were analysed using the homochiral HPLC method previously described, to rule out differences in the ratio of the enantiomers and diastereomers resulting from the presence of the label. Both analyses gave results comparable with the results for natural streptorubin B isolated from *S. coelicolor*, with a major peak at 9 minutes, and minor peaks 12 and 34 minutes (Figure 111).
The above results indicate that a ~90:10 ratio of labelled products would be expected from the mutasynthesis experiments with stereospecifically deuterium labelled 2-undecylpyrrole. Thus, the 70:30 mixture observed in the mutasynthesis experiments must result from partial epimerisation during the synthesis. Chiral HPLC analyses of key intermediates in the synthesis of the deuterium-labelled 2-undecylpyrroles should allow the source of this racemisation to be discovered. Thus, analysis of the lactones (S)- and (R)-, the iodo esters (S)- and (R)- and/or the deuterated acids (S)- and (R)- (Figure 112) should pin down in which step the epimerisation is occurring. The
deuterated acids, as suspected, proved inseparable by chiral HPLC using a variety of eluents. The iodo esters also proved inseparable using the chiral HPLC system.

Figure 112: Key intermediates in synthetic pathway to labelled 2-undecylpyrrole

However, indirect analysis of the stereochemical purity of the iodo esters (S)- and (R)-164 was possible by exposing them to aqueous sodium hydroxide, which resulted in ester hydrolysis and iodide displacement by the resulting carboxylate, thus reforming the lactones (R)- and (S)-151 respectively (Scheme 42).

Scheme 42: Base mediated reformation of lactone (R)-151 from iodo ester (S)-164
The reformed lactones \( (R) \)- and \( (S) \)-151 were isolated in good yield from these reactions. When a 1:1 mixture of the \( (R) \)- and \( (S) \)- lactones was analysed by HPLC on a homochiral stationary phase, good separation of the enantiomers was observed (Figure 113b). Independent analyses of the lactones derived from the cyclisation of \( (S) \)- and \( (R) \)-164 showed each to be enantiomerically pure (Figure 113a and Figure 113c). Further support for this conclusion came from optical rotation measurements of each recyclised lactone, which were identical to the optical rotations measured for the original lactones from which the iodo esters \( (S) \)- and \( (R) \)-164 were derived.

Figure 113: UV chromatogram from HPLC analyses on a homochiral stationary phase monitoring absorbance at 225 nm for (a) \( (R) \)-151 (b) \( (\pm) \)-151 (c) \( (S) \)-151
The above results indicate that partial epimerisation is occurring during the reaction of the iodo esters 164 with sodium borodeuteride. We assumed that this reaction would occur with clean inversion by a $S_N2$ mechanism. However, on close inspection of the literature there is some evidence that iodide displacement with sodium borohydride does not proceed with inversion, but instead can result in racemisation.\textsuperscript{105} It is important to note that in those studies the substrates were all tertiary alkyl iodides, which are likely to substitute via $S_N1$ mechanisms. The conversion of (R)- and (S)-164 to (S)- and (R)-165, respectively does not result in complete racemisation, but in an 80:20 mixture of enantiomers. The most likely explanation is that the reaction proceeds via a combination of $S_N1$ (40 \%) and $S_N2$ (60 \%) mechanisms.

An alternative explanation is that intramolecular displacement of iodide by the lone pair of the methyl ester, followed by attack of deuteride on the resulting methylated lactone (which would result in overall double inversion of the stereocentre) competes with the predicted $S_N2$ mechanism (Scheme 43 and Scheme 44). Equally, this double inversion pathway can be thought of as the same as a $S_N1$ mechanism, except that co-ordination of the oxygen lone pair is shielding one face of the cation, thus resulting in overall retention of stereochemistry (Scheme 45).

\textbf{Scheme 43: Anticipated iodide displacement mechanism}
Another possibility is that the small amount of iodide generated initially in the reaction causes rapid racemisation of the remaining alkyl iodide by displacement of iodide with iodide, prior to displacement by deuteride. If this is the case then the iodide displacement with deuteride may proceed exclusively via a $S_N^2$ process but the alkyl iodide would only remain enantiopure until the concentration of iodide in the reaction mixture was low. If any of the proposed double displacement processes predominate over the $S_N^2$ process, \((R)-165\) would be the major product from \((R)-164\). However, if the $S_N^2$ mechanism is predominant then \((S)-165\) would be the major product from...
(R)-164. This uncertainty regarding the absolute configuration of the major enantiomer in samples of 164, makes it impossible to assign the absolute stereochemistry of streptorubin B based solely on the results of the mutasynthesis experiments with [C-4′-D1]-2-undecylpyrrole. Based on all the above analyses, including the comparison of CD spectra of carbocycle prodiginines, we tentatively conclude that the formation of compound 145 proceeds ~60 % via the S\textsubscript{N}2 pathway and ~40 % via the S\textsubscript{N}1 pathway to give the desired deuterium labelled intermediates in ~60 % ee, which are incorporated into streptorubin B, ent-streptorubin B and dia-streptorubin B as shown in Figure 114. Thus, allowing the tentative assignment (based on this conclusion) of the absolute configuration of streptorubin B as 4′-S.

![Figure 114: Tentative stereochemical assignment of streptorubin B from mutasynthesis experiments as illustrated with (R)-[C-4′-D1]-2-undecylpyrrole (note that assignment of absolute stereochemistry of alkyl chain C4 reverses due to a change in the substituent priorities after cyclisation).](image-url)
6.0 Conclusions and perspectives

6.1 Mutasynthesis of streptorubin B analogues – probing substrate tolerances of RedH and RedG

A proof-of-principle of the mutasynthetic approach for production of streptorubin B analogues has been achieved. The experiments carried out during the course of this research have probed the substrate specificity of the enzymes required for the condensation and subsequent carbocyclisation of the late stage intermediates 2-undecylpyrrole and MBC. They have also revealed varying degrees of substrate tolerance of RedH and RedG. RedH appears to show relatively relaxed substrate specificity. Analogues of undecylprodiginine in which either the A-ring or the C-ring alkyl chain have been altered can be produced. Although the condensation of MBC analogues with 2-undecylpyrrole does occur, such analogues appear to be poorly tolerated by RedH and the levels of undecylprodiginine analogues produced are vastly reduced compared to the levels of undecylprodiginine produced by feeding MBC. In contrast, RedH appears not to discriminate against 2-undecylpyrrole alkyl chain analogues. Even quite dramatic reductions in length and changes in structure such as the inclusion of heteroatoms, increases in steric bulk and units of unsaturation are tolerated. It seems only quite extreme alterations to 2-undecylpyrrole are not tolerated by RedH. However, due to the in vivo nature of the mutasynthesis experiments, the question of whether it is enzyme substrate tolerance or cell membrane permeability that determines whether these compounds are incorporated arises.

The substrate tolerance of RedG appears somewhat more limited. It seems that an N-H group is required on the undecylprodiginine A-ring for RedG to catalyse carbocyclisation (as illustrated by the feeding of MBC analogues), perhaps because this indicates a crucial hydrogen bond between substrate and enzyme.
However, RedG has been demonstrated to carbocyclise a variety of analogues with alterations in the C-ring alkyl chain. A selection of potentially limiting factors including the steric size, alkyl chain hydrophobicity and introduction of π-electrons have been probed.

6.2 Mutasynthesis of streptorubin B analogues – access to new compounds versus total synthesis

Synthetic access to streptorubin B has proved challenging, particularly in an enantioselective fashion. Therefore, the total synthesis of a large array of streptorubin B analogues for the development of a structural-activity relationship for potential therapeutic applications is a daunting prospect. However, the work carried out during this investigation has shown the potential of a mutasynthetic strategy for providing rapid access to a large number of streptorubin B analogues. A variety of different functionalities have been incorporated into analogues by this approach and these should influence the interactions of the characteristic 10-membered carbocycle of streptorubin B with molecular targets. It could therefore prove interesting to investigate the effect of these structural changes on the biological properties of the compounds.

The synthetic routes to both 2-undecylpyrrole and MBC that have been developed should be applicable to the production of a multitude of further analogues in addition to those that have already been synthesised. The optimisation of culture conditions and purification techniques throughout this work led to the rapid production of several streptorubin B analogues in milligram quantities towards the end of the investigation. Therefore, with both synthetic methodologies and culture and purification conditions established, a new method of routine production of streptorubin B analogues can be envisaged by:

(1) synthesis of new MBC and 2-undecylpyrrole analogues using the simple
chemistry developed; and (2) subsequent feeding of the MBC/2-undecylpyrrole analogues to appropriate *S. coelicolor* mutants, followed by isolation of streptorubin B analogues using established protocols. This methodology should allow access to streptorubin B analogues at a rate comparable to the most efficient total synthesis routes.

### 6.3 *In vitro* application of RedH and RedG

Future overproduction of soluble and active RedH and RedG proteins may provide a useful method for accessing further analogues of streptorubin B via chemoenzymatic synthesis. The mutasynthetic approach established has been necessitated by the lack of availability of functional recombinant RedH and RedG. The large size of RedH has hindered its overproduction in soluble form, whereas RedG, like many other Rieske non-heme iron-dependent oxygenases has proved to be unstable under standard aerobic protein purification conditions. The vast majority of the protein after purification has lost the iron-sulphur cluster, which is required for activity, and any attempts to reconstitute this cluster have thus far proved unsuccessful.

An *in vitro* chemoenzymatic synthesis strategy for the production of streptorubin B analogues can be envisaged to be advantageous in several ways. Currently all probing of substrate specificity of RedH and RedG is hampered by the requirement for the compounds used to permeate the membranes of *S. coelicolor* cells. This problem would be negated by an *in vitro* approach and negative results could be taken as true reflections of enzyme specificity. The poor turnover of undecylprodiginine to streptorubin B when fed to *S. coelicolor* mutants (presumably because its hydrophobic nature causes it to become embedded in the cell membrane) has also presented an obstacle to efficiently probing the substrate specificity of RedG. This has necessitated feeding of MBC
and 2-undecylpyrrole analogues rather than undecylprodiginine analogues. Thus the preferences of RedH can not be deconvoluted from those of RedG. In addition an in vitro approach could be used to optimise the production of carbocyclised streptorubin B derivatives either by increasing the ratio of RedG to RedH, or by recycling isolated undecylprodiginine analogues by re-exposure to RedG. Alternatively, an in vitro approach should allow streptorubin B analogues to be produced directly by synthesising and feeding undecylprodiginine analogues. It is known that the synthesis of undecylprodiginine (or an analogue) is feasible by an acid-mediated condensation of 2-undecylpyrrole (or an analogue) and MBC (or an analogue), and similar condensations have been carried out successfully during this research.

6.4 Probing the mechanism of carbocyclisation by RedG

This thesis has shown that RedG can be applied to generate analogues of streptorubin B by mutasynthesis. However, the mechanism of the remarkable carbocyclisation catalysed by RedG is unknown. The carbocyclisation is proposed to proceed via formation of an alkyl radical at C-7’ before either: (1) subsequent hydroxylation via radical rebound followed by nucleophilic displacement of the resulting hydroxyl group by C-4 of the pyrrole C-ring; or (2) by attack of the C-7’ radical on C-4 of the pyrrole C-ring followed by abstraction of a second pyrrole-derived hydrogen radical. Mechanistic studies targeting radical intermediates often employ mechanistic probes, typically using substrates that when the radical is formed, suffer a characteristic skeletal rearrangement. In many cases radical intermediates undergo further reaction very rapidly and the probe used is therefore required to undergo a very fast rearrangement in order to trap the radical. In this case the involvement of an alkyl radical intermediate at C-7’ could be probed by
synthesising a 2-undecylpyrrole analogue with a cyclopropane appended to C5'-C6' or C8'-C9' via a Simmons-Smith type cyclopropanation of an unsaturated 2-undecylpyrrole intermediate.\textsuperscript{106, 107} Analyses of the mycelial extract of a \textit{S. coelicolor} mutant deficient in 2-undecylpyrrole production (e.g. W116) fed with cyclopropanated 2-undecylpyrrole analogues, would reveal whether a skeletal rearrangement consistent with a radical intermediate has occurred (Figure 115).

![Figure 115: Mechanism of the proposed skeletal rearrangement of cyclopropanated undecylprodiginine analogue](image)

\textbf{6.5 Further experiments to confirm the absolute stereochemistry of streptorubin B}

The investigation of the absolute stereochemistry of streptorubin B was hampered by partial epimerisation during the displacement of iodide with sodium borodeuteride in the preparation of 2-undecylpyrrole stereospecifically labelled at C-4'. This unfortunately gave an unclear result in the mutasynthesis experiment and allowed only a tentative assignment of the absolute stereochemistry of streptorubin B to be proposed. An alternative synthesis of
labelled 2-undecylpyrrole should allow definitive determination of the absolute stereochemistry. The synthetic route that was considered, but not pursued in favour of a more concise route, should prove successful. Complete reduction of the key lactone intermediate \((R)-151\) (used in the original synthesis) to the diol followed by selective protection of the primary hydroxyl group, and activation of the remaining hydroxyl group by tosylation, should allow \(S_N2\) displacement of the tosylate with deuteride to be carried out without any competing \(S_N1\) displacement. Specifically, this revised route should favour the suppression of any epimerisation because (1) tosylate is a poorer leaving group and a poorer nucleophile than iodide (pKa ArSO\(_3\)H = -6.4, pKa HI = -10); (2) LiAlD\(_4\) is a more reactive nucleophile than NaBD\(_4\).

![Scheme 46](image)

Scheme 46: Proposed alternative synthetic route to labelled 2-undecylpyrrole avoiding partial epimerisation due to competing \(S_N1\) mechanism in displacement of a leaving group with deuteride.
7.0 Experimental Section

7.1 General Experimental

Dry toluene, dichloromethane, methanol and acetonitrile were produced by distillation from calcium hydride under argon, and stored over 4 Å molecular sieves. THF was dried in a still over potassium for 3 days before use. Dry triethylamine, diisopropylamine and 2,2,6,6-tetramethylpiperidine were obtained by distillation from sodium hydroxide pellets under argon and stored over sodium hydroxide pellets. All other reagents and solvents were used as supplied. Petroleum ether refers to the fraction of light petroleum boiling between 40 °C and 60 °C. Solvents were evaporated using a Buchi Rotavapor R-200 equipped with a Buchi Vacuubrand pump.

Flash column chromatography was conducted on Fluka Silica Gel (40-63 μm, 60 Å), or Basic aluminum oxide (activated basic Brockman 1 standard grade 150 mesh). TLC was performed on aluminium backed plates pre-coated with Merck silica gel 60 F$_{254}$, visualised by UV radiation. Phosphomolybdic acid, potassium permanganate, bromocresol green and vanillin were also used for visualisation of TLC plates.

IR spectra were recorded using a Perkin-Elmer Avatar 320 Fourier Transformation spectrometer. Only selected absorptions are reported, in units of wavenumbers ($\nu_{\text{max}}$/cm$^{-1}$).

Melting points were recorded on a Stuart Scientific SMP10 instrument.

$^1$H-NMR spectra were recorded at 300, 400 or 700 MHz using Bruker DPX300, DPX400 or AV700 spectrometers respectively. Chemical shifts ($\delta$$_H$) are quoted in ppm with reference to the residual solvent peak. The data in parentheses follow the order (i) multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, (ii) number of equivalent protons, (iii) coupling constant (J): in Hz to the
nearest 0.5 Hz, (iv) assignment. COSY was used in selected cases to aid assignment.  
$^{13}$C-NMR spectra were recorded on Bruker DPX300, DPX400 and AV700 spectrometers at 75, 100 or 175 MHz. HMBC and HMQC spectra were recorded to aid assignments. Chemical shifts ($\delta_c$) are quoted in ppm with reference to the residual solvent peak. The assignment is given in parentheses.  
$^2$H-NMR spectra were recorded on Bruker AV500 and AV700 spectrometers at 76.7 MHz or 107.45 MHz.  
CD spectra were recorded on a Jasco J-815 CD spectrometer.  
CHN analysis was performed by Warwick Analytical Services using an Exeter Analytical CE 440.  
Low resolution ESI mass spectra were recorded using a Bruker Esquire 2000 spectrometer. High resolution mass spectra were recorded on a Bruker microTOF spectrometer equipped with an ESI source.  
LC-MS was carried out on an Agilent 1100 HPLC instrument with the outflow connected via a splitter (10% to mass spectrometer, 90% to waste) to a Bruker Daltonics esquire HCT plus mass spectrometer equipped with an ESI source.  
Semi-preparative HPLC was carried out on an Agilent 1200 instrument.  
HPLC analyses on a homochiral stationary phase were carried out on an Agilent 1100 instrument.  
Optical rotation were recorded on an Optical Activity Ltd. AA-1000, using a cell of pathlength 10 cm, according to the equation $[\alpha]_D^{25} = (100 \alpha)/lc$. Where l = path length and c = concentration in g/100 ml.  
S. coelicolor strains W33, W38, W39 and W116 were all created and provided by Anna Stanley from work during her PhD research at the University of Warwick.  
S. coelicolor strain W116 was created and provided by Paulina Sydor from work during her PhD research at the University of Warwick.
To a mixture of diethylformamide (1.50 ml, 13.25 mmol, 3.0 equiv) and chloroform (5 ml) at 0 °C a solution of phosphorus oxybromide (3.17 ml, 11.06 mmol, 2.5 equiv) in chloroform (15 ml) was added dropwise under argon and the resulting yellowish suspension was stirred at 0 °C for 30 min under argon.

A solution of 4-methoxy-3-pyrrrol-2-one (0.50 g, 4.42 mmol, 1.0 equiv) was added dropwise to the previous solution at 0 °C. The resulting mixture was warmed to room temperature then heated to 65 °C for 5 hours.

After pouring the mixture onto ice, the aqueous layer was adjusted to pH 7-8 with NaOH (aq., 2 N). Ethyl acetate (20 ml) was added and the mixture was filtered over Celite to remove phosphorus salts. The aqueous layer was separated and extracted with ethyl acetate (2 x 20 ml). The combined organic layers were washed with brine (2 x 20 ml), dried over magnesium sulphate and evaporated in vacuo to obtain a brown oil. The compound was purified though a pad of silica using 20% ethyl acetate/hexane as eluent, to leave a yellow oil, which solidified on standing.

Yield: 0.9564 g (83 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 1.22 (t, 3H, 7.1 Hz, H-7 or H-9), 1.23 (t, 3H, 7.1, H-7 or H-9), 3.30 (q, 2H, 7.1, H-6 or H-8), 3.67 (s, 3H, H-10), 4.05 (q, 2H, 7.1, H-6 or H-8), 5.52 (s, 1H, H-2), 6.91 (s, 1H, H-5).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 12.55 (C7 or C9), 14.82 (C7 or C9), 44.55 (C6 or C8), 51.18 (C6 or C8), 58.01 (C10), 96.39 (C2), 120.77 (C1), 133.53 (C4), 138.67 (C5), 165.34 (C3).

IR (ν$_{max}$/cm$^{-1}$): 2973, 2936, 1629, 1516
m.p.: 38-39°C
HRMS: m/z calculated for C\textsubscript{10}H\textsubscript{15}Br\textsuperscript{79}N\textsubscript{2}O: 259.0446 [M+H]⁺. Found: 259.0448

4-Methoxy-1H,1'H-[2,2']bipyrolyl-5-carbaldehyde 9\textsuperscript{73}

\[ \text{Pd(OAc)}_2 (0.30 \text{ mmol, 0.1 equiv) and triphenylphosphine (1.35 mmol, 0.45 equiv) were added to toluene (1 ml) under argon. The resulting bright yellow solution was stirred for 30 min at 70 °C. A degassed solution of (5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine (3.00 mmol, 1.0 equiv) and N-BOC-pyrrole boronic acid (3.30 mmol, 1.1 equiv) in 10 % water/dioxane (15 ml) was added to the suspension of Pd(PPh}_3\textsubscript{4}. Sodium carbonate (9.00 mmol, 3.0 equiv) was added and the mixture was stirred for 3.5 hrs at 100 °C. The solution was treated with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 15 min at 100 °C, then treated again with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 10 min more at 100 °C. The mixture was poured onto water (50 ml) and the pH was lowered to pH 7 with HCl (aq., 2 N) and stirred for 15 min. The brown precipitate was recovered by filtration over a fritted disc funnel and washed with water (2 x 15 ml), and acetone (2 x 10 ml) to leave a yellow solid. 0.433 g (75 %)

\textsuperscript{1}H-NMR (DMSO-\textit{d}_6, 400MHz) δ: 3.82 (s, 3H, H-10), 6.11 (d, 1H, 4.4Hz, H-7), 6.26 (s, 1H, H-4), 6.76 (d, 1H, 4.4 Hz, H-9), 6.90 (t, 1H, 4.4Hz, H-8), 9.32 (s, 1H, H-1), 11.21 (br s, 1H, N-H), 11.41 (br s, 1H, N-H)
$^{13}$C-NMR (DMSO-$d_6$, 175MHz) δ: 57.70 (C-10), 90.82 (C-4), 108.26 (C-9), 109.24 (C-7), 117.87 (C-2), 120.64 (C-8), 123.66 (C-6), 133.63 (C-5), 159.05 (C-3), 171.54 (C-1).

IR (ν$_{max}$/cm$^{-1}$): 3243, 3188, 3038, 2950, 2840, 1593, 1544

m.p.: 284 °C [lit. 275 °C]$^{73}$

HRMS: m/z calculated for C$_{10}$H$_{10}$N$_{2}$O$_{2}$: 191.0821 [M+H]$^+$. Found: 191.0824

5-(1H-Indol-2-yl)-3-methoxy-1H-pyrrole-2-carbaldehyde 63$^{77}$

Pd(OAc)$_2$ (0.30 mmol, 0.1 equiv) and triphenylphosphine (1.35 mmol, 0.45 equiv) were added to toluene (1 ml) under argon. The resulting bright yellow solution was stirred for 30 min at 70 °C. A degassed solution of (5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine (3.00 mmol, 1.0 equiv) and N-BOC-indole boronic acid (3.30 mmol, 1.1 equiv) in 10 % water/dioxane (15 ml) was added to the suspension of Pd(PPh$_3$)$_4$. Sodium carbonate (9.00 mmol, 3.0 equiv) was added and the mixture was stirred for 3.5 hrs at 100 °C. The solution was treated with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 15 min at 100 °C, then treated again with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 10 min more at 100 °C.

The mixture was poured onto water (50ml) and the pH lowered to 7 with HCl(aq., 2 N) and stirred for 15 minutes. The resulting brown precipitate was recovered by filtration and washed with water (2 x 15 ml) and then dissolved in acetone. The solvent was then removed in vacuo and the resulting solid was treated with chloroform and diethyl ether (1:2, 5 ml) and the solution let stand for
10 minutes until a solid as obtained. The desired product was then obtained as a yellow solid by filtration and washed with diethyl ether (2 x 5 ml).

0.562 g (78 %)

$^1$H-NMR (DMSO-$d_6$, 400MHz) $\delta$: 3.92 (s, 3H, H-14), 6.59 (s, 1H, H-7), 7.04 (t, 1H, 5.0 Hz, H-11), 7.10 (s, 1H, H-4), 7.15 (t, 1H, 5.0 Hz, H-10), 7.41 (d, 1H, 5.0 Hz, H-12), 7.53 (d, 1H, 5.0 Hz, H-9), 9.49 (s, 1H, H-1), 11.51 (br s, 1H, N-H), 11.92 (br s, 1H, N-H)

$^{13}$C-NMR (DMSO-$d_6$, 100MHz) $\delta$: 57.92 (C-14), 93.24 (C-4), 100.76 (C-7), 111.23 (C-12), 118.67 (C-2), 119.75 (C-11), 120.32 (C-9), 122.31 (C-10), 128.12 (C-8), 129.41 (C-6), 131.78 (C-13), 136.72 (C-5), 157.91 (C-3), 172.98 (C-1).

IR ($\nu_{\text{max}}$/cm$^{-1}$): 3254, 3234, 3215, 2943, 1588, 1525.

m.p.: $>300$ °C

HRMS: $m/z$ calculated for C$_{14}$H$_{12}$N$_2$O$_2$: 241.0977 [M+H]$^+$. Found: 241.0981

**General procedure for other bicyclic aldehydes**

Pd(OAc)$_2$ (0.30 mmol, 0.1 equiv) and triphenylphosphine (1.35 mmol, 0.45 equiv) were added to toluene (1 ml) under argon. The resulting bright yellow solution was stirred for 30 min at 70 °C. A degassed solution of (5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine (3.00 mmol, 1.0 equiv) and the required boronic acid (3.30 mmol, 1.1 equiv) in 10 % water/dioxane (15 ml) was added to the suspension of Pd(PPh$_3$)$_4$. Sodium carbonate (9.00 mmol, 3.0 equiv) was added and the mixture was stirred for 3.5 hrs at 100 °C. The solution was treated with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 15 min
at 100 °C, then treated again with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 10 min more at 100 °C.

The mixture was poured onto water (50 ml) and the pH lowered to 7 with HCl (aq., 2 N) and stirred for 15 min. The resulting mixture was extracted with ethyl acetate (3 x 75 ml). The combined organic fractions were washed with brine, dried over magnesium sulphate and evaporated to leave an oily residue which was purified by chromatography through basic alumina.

3-methoxy-5-phenyl-1H-pyrrole-2-carbaldehyde 62

Product was purified by flash column chromatography (basic alumina, 10% ethyl acetate in hexane) to leave a red solid.

0.1411g (70%)

$^1$H-NMR (Acetone-$d_6$, 400MHz) $\delta$:  3.89 (s, 3H, H-10), 6.52 (s, 1H, H-4), 7.30 (t, 1H, 7.5 Hz, H-9), 7.38 (t, 2H, 7.5 Hz, H-8), 7.84 (d, 2H, 7.5 Hz, H-7), 9.51 (s, 1H, H-1), 10.52 (br s, 1H, N-H)

$^{13}$C-NMR (Acetone-$d_6$, 175MHz) $\delta$: 58.29 (C-10), 94.10 (C-4), 120.25 (C-2), 126.09 (C-7), 129.13 (C-9), 129.57 (C-8), 131.80 (C-6), 138.36 (C-5), 159.06 (C-3), 174.19 (C-1)

IR ($\nu_{\max}$/cm$^{-1}$): 3246, 2821, 1617, 1561

m.p.: 137-138 °C

HRMS: $m/z$ calculated for C$_{12}$H$_{11}$NO$_2$: 202.0868 [M+H]$^+$. Found: 202.0872

3-methoxy-5-(thiophen-2-yl)-1H-pyrrole-2-carbaldehyde 64

Product was purified by flash column chromatography (basic alumina, 10% ethyl acetate in hexane) to leave a red solid.

0.1834 g (56 %)
$^1$H-NMR (Acetone-d$_6$, 400MHz) δ: 3.88 (s, 3H, H-10), 6.29 (s, 1H, H-4), 7.16 (dd, 1H, 4.0, 3.0 Hz, H-8), 7.51 (d, 1H, 4.0 Hz, H-7), 7.67 (d, 1H, 3.0 Hz, H-9), 9.52 (s, 1H, H-1), 10.49 (br s, 1H, N-H)

$^{13}$C-NMR (Acetone-d$_6$, 175MHz) δ: 58.27 (C-10), 94.24 (C-4), 119.45 (C-2), 125.43 (C-9), 126.67 (C-7), 128.73 (C-8), 133.32 (C-6), 134.26 (C-5), 158.52 (C-3), 173.94 (C-1)

IR (ν$_{\text{max}}$/cm$^{-1}$): 3205, 3081, 2951, 1614, 1577, 1530

m.p.: 160-161 °C

HRMS: m/z calculated for C$_{10}$H$_9$NO$_2$: 208.0432 [M+H]$^+$. Found: 208.0436

5-(furan-2-yl)-3-methoxy-1H-pyrrole-2-carbaldehyde 65

Product was purified by flash column chromatography (basic alumina, 10% ethyl acetate in hexane) to leave a red solid.

0.1861 g (60 %)

$^1$H-NMR (Acetone-d$_6$, 400MHz) δ: 3.93 (s, 3H, H-10), 6.32 (s, 1H, H-4), 6.57 (dd, 1H, 4.0, 3.0 Hz, H-8), 6.97 (d, 1H, 4.0 Hz, H-9), 7.57 (d, 1H, 3.0 Hz, H-7), 9.53 (s, 1H, H-1), 10.55 (br s, 1H, N-H)

$^{13}$C-NMR (Acetone-d$_6$, 175MHz) δ: 58.38 (C-10), 93.32 (C-4), 108.0 (C-9), 112.66 (C-8), 119.88 (C-2), 130.24 (C-6), 144.14 (C-7), 147.71 (C-5), 158.95 (C-3), 174.24 (C-1)

IR (ν$_{\text{max}}$/cm$^{-1}$): 3207, 2924, 1601, 1546

m.p.: 185-186 °C

HRMS: m/z calculated for C$_{10}$H$_9$NO$_3$: 192.0661 [M+H]$^+$. Found: 192.0665
General procedure for N-BOC heterocycle boronic acids

\[
\begin{align*}
\text{nBuLi (1.5 M in hexanes) (1.10 equiv) was added slowly to a solution of} \\
\text{2,2,6,6-tetramethylpiperidine (1.10 equiv) in THF at -78 °C under argon. After} \\
\text{stirring for 10 min the mixture was allowed to warm to 0 °C over 30 min and then} \\
\text{cooled again to -78 °C. A solution of the corresponding N-BOC heterocycle (1.0} \\
\text{equiv) in THF was added slowly so as the temperature remained below -65 °C.} \\
\text{After the reaction mixture was stirred for 2 hrs at -78 °C, trimethyl borate (3.0} \\
\text{equiv) in THF was added and the mixture was allowed to warm to room} \\
\text{temperature overnight. Subsequently HCl (aq., 0.3 N) was added, the volatile} \\
\text{solvent was removed \textit{in vacuo} and the residue extracted with diethyl ether. Combined} \\
\text{organic phases were washed with water and dried over magnesium sulphate. The solution was} \\
\text{slowly concentrated until a solid began to precipitate and then cooled to 0 °C} \\
\text{before filtering off solid and washing with cold diethyl ether. Thorough drying of} \\
\text{the resulting solid afforded the boronic acid as an off-white solid.}
\end{align*}
\]

N-BOC pyrrole-2-boronic acid 53

0.6879g (72%)

\(^1\text{H-NMR (CDCl}_3, 400MHz) \delta: 1.56 (s, 9H, H-1), 6.14 (t, 1H, 4.0 Hz, H-6), 7.03 (d,} \\
1H, 4.0 Hz, H-5), 7.37 (d, 1H, 4.0 Hz, H-7), 7.55 (br s, 2H, variable with} \\
\text{concentration, B-OH}_2 \)

\(^1\text{C-NMR (CDCl}_3, 100MHz) \delta: 27.95 (C-1), 85.57 (C-2), 112.06 (C-6), 127.04 (C-} \\
5), 128.75 (C-7), 132.12 (C-4), 152.31 (C-3)
IR ($\nu_{\text{max}}$/cm$^{-1}$): 3336, 3171, 2984, 1704, 1554

HRMS: $m/z$ calculated for C$_9$H$_{14}$BNO$_4$: 212.1094 [M+H]$^+$. Found: 212.1099

**N-BOC indol-2-boronic acid 59**

1.7054 g (88 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 1.66 (s, 9H, H-1), 7.15 (t, 1H, 5.5 Hz, H-8), 7.21 (s, 2H, variable with concentration, B-OH$_2$), 7.27 (t, 1H, 5.5 Hz, H-9), 7.42 (s, 1H, H-5), 7.49 (d, 1H, 5.5 Hz, H-7), 7.91 (d, 1H, 5.5 Hz, H-10)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 29.50 (C-1), 85.67 (C-2), 115.17 (C-9), 116.44 (C-8), 120.94 (C-10), 122.64 (C-7), 125.90 (C-5), 127.60 (C-6), 132.89 (C-4), 136.43 (C-11), 154.11 (C-3).

IR ($\nu_{\text{max}}$/cm$^{-1}$): 3337, 3146, 2972, 1692

HRMS: $m/z$ calculated for C$_{13}$H$_{16}$BNO$_4$: 262.1251 [M+H]$^+$. Found: 262.1253

**5-Dimethylaminomethylene-4-methoxy-5H-furan-2-one 74**

![Structure](image)

A solution of 4-methoxy-2(5H)-furanone (2.00 g, 17.53 mmol, 1.0 equiv) in dimethyl formamide dimethyl acetal (20 ml) was made up in a round bottom flask equipped for distillation. The mixture was heated in an oil bath at 110 °C with slow, continuous distillation of methanol. After 3 hours the mixture was cooled to room temperature and the excess of solvent-reagent was removed *in vacuo* to leave a yellow/brown solid. Product was purified by flash column chromatography (silica, ethyl acetate) to leave a yellow solid.

2.750 g (92 %)
$^1$H-NMR (CDCl$_3$, 400MHz) δ: 3.06 (s, 6H, H-6), 3.87 (s, 3H, H-7), 4.89 (s, 1H, H-2), 6.06 (s, 1H, H-5)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 42.45 (C-6), 58.43 (C-7), 80.59 (C-2), 120.19 (C-4), 122.78 (C-5), 169.98 (C-3), 171.38 (C-1)

IR (ν$_{max}$/cm$^{-1}$): 2990, 2901, 2822, 1704, 1655, 1555

m.p.: 57 – 58 °C [lit. 57 – 58 °C]$^{82}$

HRMS: m/z calculated for C$_6$H$_{11}$NO$_3$: 170.0817 [M+H]$^+$. Found: 170.0820

5-Bromo-3-methoxy-furan-2-carbaldehyde 70

To 5-Dimethylaminomethylene-4-methoxy-5H-furan-2-one (1.00 g, 5.90 mmol, 1.0 equiv) in chloroform (15 ml) at 0 °C was added a solution of phosphorus oxybromide in chloroform (15 ml) dropwise. The resulting blue/green mixture was then stirred under reflux over night. The reaction was quenched with water and the aqueous layer adjusted to pH 14 (NaOH aq. 2 N). The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 50 ml). The combined organic fractions were washed with Na$_2$CO$_3$ (aq., 20 ml, sat.) and brine (20 ml). The organic layer was then dried over magnesium sulphate and evaporated to dryness, to yield an unstable white solid.

0.2854 g (24 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 4.06 (s, 3H, H-6), 6.92 (s, 1H, H-2), 9.43 (s, 1H, H-5)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 63.19 (C-1), 95.66 (C-4), 119.55 (C-1), 131.44 (C-2), 168.73 (C-3), 182.10 (C-5).

m.p.: 58- 60 °C

HRMS: m/z calculated for C$_6$H$_5$Br$_{79}$O$_3$: 204.9500 [M+H]$^+$. Found: 204.9509
2-(5-Formyl-4-methoxy-furan-2-yl)-pyrrole-1-carboxylic acid tert-butyl ester

Pd(OAc)$_2$ (0.30 mmol, 0.1 equiv) and triphenylphosphine (1.35 mmol, 0.45 equiv) were added to toluene (1 ml) under argon. The resulting bright yellow solution was stirred for 30 min at 70 °C. A degassed solution of 5-Bromo-3-methoxy-furan-2-carbaldehyde (1.00 mmol, 1.0 equiv) and N-BOC-pyrrole boronic acid (1.10 mmol, 1.1 equiv) in 10 % water/dioxane (15 ml) was added to the suspension of Pd(PPh$_3$)$_3$. Sodium carbonate (3.00 mmol, 3.0 equiv) was added and the mixture was stirred for 3.5 hrs at 100 °C. The solution was treated with sodium methoxide (1.00 mmol, 1.0 equiv) and stirred for 15 min at 100 °C, then treated again with sodium methoxide (1.00 mmol, 1.0 equiv) and stirred for 10 min more at 100 °C.

The mixture was poured onto water (50 ml) and the pH was lowered to 7 with HCl (aq., 2 N) and stirred for 15 min. The resulting mixture was extracted with ethyl acetate (3 x 75 ml). The combined organic fractions were washed with brine, dried over magnesium sulphate and evaporated to leave an oily residue. Purification of the residue by column chromatography through basic alumina (20 % ethyl acetate/hexane) yielded a green vicious oil.

0.2423 g (83 %)

$^1$H-NMR (Acetone-$d_6$, 400MHz) δ: 1.44 (s, 9H, H-12), 4.01 (s, 3H, H-10), 6.34 (t, 1H, 3.5 Hz, H-8), 6.70 (dd, 1H, 1.5, 3.5 Hz, H-9), 6.85 (s, 1H, H-4), 6.91 (dd, 1H, 1.5, 3.5 Hz, H-7).

HRMS: $m/z$ calculated for C$_{15}$H$_{18}$NO$_5$: 292.1185 [M+H]$^+$. Found: 292.1192
3-Methoxy-5-(1H-pyrrol-2-yl)-furan-2-carbaldehyde 71

To a solution of 2-(5-Formyl-4-methoxy-furan-2-yl)-pyrrole-1-carboxylic acid tert-butyl ester (0.199 g, 0.684 mmol) in THF (5 ml) was added lithium hydroxide (0.164 g, 6.84 mmol, 10 equiv) in methanol (5 ml) under argon and the resulting solution was stirred at room temperature and monitored by TLC. On completion of the reaction the solvent was removed and the resulting yellow solid was washed with water to yield the desired product as a yellow/green solid.

0.113 g (87 %)

\[^{1}\text{H-NMR (Acetone-}d_6, 400MHz) \delta:\ 4.03 \text{ (s, 3H, H-10), 6.23 (dd, 1H, 2.5 Hz, 1.0 Hz, H-8), 6.75 (d, 1H, 2.5 Hz, H-9), 6.76 (s, 1H, H-4), 7.02 (d, 1H, 2.5 Hz, H-7), 9.47 (s, 1H, H-1), 11.11 (br s, 1H N-H)}\]

\[^{13}\text{C-NMR (Acetone-}d_6, 175MHz) \delta:\ 59.72 \text{ (C-10), 95.02 (C-4), 109.84 (C-5), 111.28 (C-8), 111.38 (C-9), 121.85 (C-6), 123.16 (C-7), 136.54 (C-1), 153.31 (C-2), 161.27 (C-3).}\]

m.p.: 154 – 155 °C

IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3255, 3179, 3121, 2931, 2834, 1620, 1589, 1563

HRMS: \text{m/z calculated for } C_{10}H_9NO_3: 192.0661 \text{ [M+H]}^+. \text{ Found: } 192.0667
**2-(1-Hydroxy-undecyl)-pyrrole-1-carboxylic acid tert-butyl ester 78**

To 2,2,6,6-tetramethylpiperidine (0.676 ml, 4.00 mmol, 1.0 equiv) in THF (5 ml) under argon, was added nBuLi (1.6M, 2.50 ml, 4.00 mmol, 1.0 equiv) dropwise at -78 °C. The resulting mixture was stirred at -78 °C for 10 min, warmed to 0 °C over 30 min and then cooled to -78 °C before addition of N-BOC pyrrole (0.669 ml, 4.00 mmol, 1.0 equiv) in THF (10ml). After stirring for 1 hour, undecanal (0.823 ml, 4.00 mmol, 1.0 equiv) was added dropwise. The reaction was stirred for 3 hrs at -78 °C before quenching at -78 °C by addition of NaOH (aq, 1 N, 10 ml), the solution was immediately allowed to warm to room temperature, diethyl ether (20 ml) was added and the organic layer separated. The organic layer was then washed with water (2 x 10 ml), NaOH (aq, 1 N, 5 ml) and brine (2 x 10 ml), before drying over magnesium sulphate. Removal of the solvent *in vacuo* left a reddish oil, which was purified by flash column chromatography (silica, 5% ethyl acetate in hexane) to leave a colourless oil.

0.3202 g (23 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.82 (t, 3H, 7.0 Hz, H-15), 1.21 (m, 16H, H-7-H-14), 1.49 (s, 9H, H-17), 1.76 (m, 2H, H-6), 3.92 (t, 1H, 7.5 Hz, H-5), 4.77 (quartet, 1H, 7.5 Hz, H-16), 6.02 (t, 1H, 6.5 Hz, H-2), 6.11 (d, 1H, 6.5 Hz, H-3), 7.12 (d, 1H, 6.5 Hz, H-1)

HRMS: m/z calculated for C$_{20}$H$_{35}$NO$_3$: 338.2617 [M+H]$^+$. Found: 338.2612
To a solution of 2-(1-Hydroxy-undecyl)-pyrrole-1-carboxylic acid tert-butyl ester (0.300 g, 0.89 mmol, 1.0 equiv) in THF (10 ml) was added lithium hydroxide (0.164 g, 6.84 mmol, 7.5 equiv) in methanol (5 ml) under argon and the resulting solution was stirred overnight at room temperature. Volatiles were then removed in vacuo and the residue was partitioned between HCl (aq, 0.1 N, 20 ml) and diethyl ether (20 ml). The resulting aqueous layer was separated and extracted with diethyl ether (2 x 20 ml). The combined organic fractions were washed with brine (20 ml), dried over magnesium sulphate and evaporated to dryness in vacuo to leave the title compound. 0.078 g (35 %)

\[ \text{HRMS: } m/z \text{ calculated for } C_{20}H_{35}NO_3: 338.2617 [M+H]^+. \text{ Found: 338.2612} \]

Undecanoic acid (1.00g, 5.37mmol, 1.0 equiv), 2,2-dipyridyldisulfide (2.37 g, 10.74 mmol, 2.0 equiv) and triphenyl phosphine (2.82 g, 10.74 mmol, 2.0 equiv) were stirred in dry toluene (9 ml) at room temperature under argon for 24 hours. This reaction mixture was then cooled to -78 °C and treated dropwise with
pyrrolylmagnesium chloride (0.35 M, 92.0 ml, 32.2 mmol, 6.0 equiv; prepared from 16.1 ml, 2.0 M ethylmagnesium chloride in dry THF diluted with 75 ml toluene and 2.23 ml pyrrole, -40°C to -10°C, 10 min). TLC indicated the reaction was complete after 45 min at which time the reaction was quenched at -78 °C with NH₄Cl (sat. aq., 10 ml) and the products were extracted with diethyl ether (3 x 100 ml). The combined organic phases were washed with 5% potassium carbonate (3 x 20 ml), water (20 ml) and brine (20 ml), then dried over magnesium sulphate and evaporated to dryness to leave off-white solid. The product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

1.02 g (81%)

¹H-NMR (CDCl₃, 400MHz) δ: 0.81 (t, 3H, 7.5 Hz, H-15), 1.22 (m, 14H, H-8-H-14), 1.66 (quintet, 2H, H-7, 7.0 Hz), 2.69 (t, 2H, 7.0 Hz, H-6), 6.22 (m, 1H, H-2), 6.81 (m, 1H, H-3), 6.92 (m, 1H, H-1), 9.81 (br s, 1H, N-H).

¹³C-NMR (CDCl₃, 100MHz) δ: 15.05 (C-15), 22.70, 25.36, 29.33, 29.40, 29.52, 29.55, 29.60 and 31.91 (C-7-C-14), 38.07 (C-6), 110.49 (C-2), 116.06 (C-3), 124.48 (C-1), 132.11 (C-4), 191.32 (C-5)

IR (νmax/cm⁻¹): 3280, 2915, 2850, 1642, 1546

m.p.: 48 - 49 °C


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**2-Undecyl-1H-pyrrole 10**

![Structure of 2-Undecyl-1H-pyrrole 10](image)

To sodium borohydride (0.457 g, 12.10 mmol, 6.0 equiv) in refluxing isopropanol (20 ml) was added 1-(1H-Pyrrol-2-yl)-undecan-1-one (0.474 g, 2.01 mmol, 1.0 equiv) and the resultant mixture left to reflux overnight. The mixture was diluted
with water and stirred until completely dissolution, then volatiles were removed in vacuo, and the residue was extracted with ethyl acetate (3 x 20 ml). Combined organic fractions were then dried over magnesium sulphate and evaporated to dryness to leave a pale yellow oil that solidifies on standing. The product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

0.4173 g (94%)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.81 (t, 3H, 7.5 Hz, H-15), 1.22 (m, 16H, H-7-H-14), 1.56 (quintet, 2H, H-6, 7.0 Hz), 2.52 (t, 2H, 7.0 Hz, H-5), 5.81 (m, 1H, H-3), 6.03 (m, 1H, H-2), 6.61 (m, 1H, H-1)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 14.14 (C-15), 22.72, 27.76, 29.37, 29.42, 29.48, 29.61, 29.65, 29.68, 29.71 and 31.94 (C-5-C-14), 104.86 (C-3), 108.26 (C-2), 115.96 (C-1), 132.91 (C-4).

IR ($\nu_{max}$/cm$^{-1}$): 3350, 2915, 2846

m.p.: 43 – 44 °C [lit: 42 – 43 °C]

HRMS: $m/z$ calculated for $C_{15}$H$_{27}$N: 222.2222 [M+H]$^+$. Found: 222.2219

Octyloxy-acetic acid 101

To dry octanol (20 ml) was added sodium metal (0.29 g, 12.5 mmol, 2.5 equiv) under argon and the resulting solution was stirred overnight until all the sodium had dissolved. To the resulting pale yellow solution was added bromoacetic acid (0.69 g, 5.0 mmol, 1 equiv). The reaction was stirred overnight before removal of the solvent by reduced pressure distillation. The residue was then partitioned between ethyl acetate (50 ml) and NaHCO$_3$ (1M, 50 ml). The organic layer was separated and extracted with further portions of NaHCO$_3$ (1M, 2 x 50 ml). The
combined aqueous fractions were then acidified with HCl (conc.) and extracted
with diethyl ether (3 x 50 ml). The combined organic fractions were then dried
over magnesium sulphate and evaporated to dryness to leave colourless oil.

Yield: 0.823 g (87 %)

\[ ^1 \text{H-NMR (CDCl}_3, 400MHz) \delta: 0.87 \text{ (t, 3H, 7.0 Hz, H-10), 1.21 – 1.35 (m, 10H, H-5-H-9), 1.60 \text{ (quintet, 2H, 7.0 Hz, H-4), 3.57 \text{ (t, 2H, 7.0 Hz, H-3), 4.12 \text{ (s, 2H, H-2) }}] 

\[ ^{13} \text{C-NMR (CDCl}_3, 100MHz) \delta: 14.18 \text{ (C-10), 22.60, 25.87, 29.18, 29.33, 29.37 and 31.77 \text{ (C-4-C-9), 67.65 \text{ (C-3), 72.09 \text{ (C-2), 175.38 \text{ (C-1) }}] 

IR (\nu_{\text{max}}/\text{cm}^{-1}): 3072 \text{ (broad), 2953, 2923, 2854, 1727}

HRMS: m/z calculated for C_{16}H_{20}O_{3}: 189.1485 [M+H]^+. Found: 189.1487

5-Pentyloxy-pentanoic acid 104

To dry pentanol (15 ml) was added sodium metal (0.25 g, 11.0 mmol, 1.0 equiv)
der under argon and the resulting solution stirred overnight until all the sodium had
dissolved. To the resulting pale yellow solution was added 5-bromovaleric acid
methyl ester (2.15 g, 11.0 mmol, 1 equiv). The reaction was stirred overnight
before removal of the solvent by reduced pressure distillation. The residue was
refluxed in a vigorously stirred solution of THF (75 ml) and NaOH (aq, 2M, 75
ml) overnight. The aqueous layer was then separated and acidified with HCl
(conc.). The acidified aqueous layer was then extracted with diethyl ether (3 x 50
ml) and the combined organic fractions dried over magnesium sulphate and
evaporated to leave the desired product as crude yellow oil. The product was
purified by acid/base extraction according to the following. The crude product
was dissolved in diethyl ether (50 ml) and extracted with NaHCO₃ (1M, 3 x 50 ml). The combined aqueous fractions were then acidified with HCl (conc.) and extracted with diethyl ether (3 x 50 ml). The combined organic fractions were then dried and evaporated to leave a pale yellow oil.

Yield: 0.878 g, (44 %)

¹H-NMR (CDCl₃, 400MHz) δ: 0.86 (t, 3H, 7.0 Hz, H-10), 1.32 (m, 4H, H-8, H-9), 1.51 – 1.77 (m, 6H, H-3, H-4, H-7), 2.36 (t, 2H, 7.5 Hz, H-2), 3.33 (m, 4H, H-6 and H-5).

¹³C-NMR (CDCl₃, 100MHz) δ: 14.58 (C-10), 21.47, 22.48, 28.27, 28.92 and 29.27 (C-3-C-4 and C-7-C-9), 33.74 (C-2), 70.19 (C-5 or C-6), 70.99 (C-5 or C-6), 179.44 (C-1)

IR (νₓₓmax/cm⁻¹): 3075 (broad), 2931, 2858, 1707

HRMS: m/z calculated for C₁₀H₂₀O₃: 189.1485 [M+H]⁺. Found: 189.1483

10-Methyl-undec-7-enoic acid ethyl ester 95

To ethyl 7-bromoheptanoate (2.0 ml, 10.28 mmol, 1.0 equiv) in toluene (20 ml) was added triphenyl phosphine (3.24 g, 12.34 mmol, 1.2 equiv) and the resulting mixture refluxed for 48 hours. The toluene was then removed in vacuo with vigorous stirring and the resulting viscous oil was suspended in THF (80 ml). The resulting suspension was cooled to 0 °C and nBuLi (1.6 M, 6.4 ml, 10.28 mmol, 1.0 equiv) was added dropwise to give a pale orange solution to which isovaleraldehyde (1.07 ml, 10.28 mmol, 1.0 equiv) was added at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred
overnight. The reaction was quenched by the addition of NH₄Cl (sat., 40 ml) and the resulting aqueous layer was extracted with diethyl ether (3 x 50 ml). Combined organic fractions were washed with brine (50 ml), dried over magnesium sulphate and concentrated in vacuo. The residue was purified by flash column chromatography (silica, 2 % ethyl acetate/hexane) to leave a colourless oil
Yield: 1.63 g (70 %)

¹H-NMR (CDCl₃, 400MHz) δ: 0.82 (d, 6H, 6.5 Hz, H-13, H-14), 1.19 (t, 3H, 7.5 Hz, H-1), 1.29 (m, 4H, H-6, H-7), 1.49 (doublet of quartets, 1H, 6.0 Hz, 7.0 Hz, H-12), 1.55 (m, 2H, H-5), 1.82 (t, 2H, 5.0 Hz, H-11), 1.95 (t, 2H, 5.0 Hz, H-8), 2.21 (t, 2H, 7.0 Hz, H-4), 4.05 (quartet, 2H, 7.5 Hz, H-2), 5.31 (m, 2H, H-9, H-10)

¹³C-NMR (CDCl₃, 100MHz) δ: 14.23 (C-1), 22.34 (2C, C-13 and C-14), 24.89, 27.07, 28.80 and 29.33 (C-5-C-7 and C-12), 30.30 (C-4), 34.32 (C-8), 36.36 (C-11), 60.11 (C-2), 129.25 (C-9 or C-10), 130.18 (C-9 or C-10), 173.78 (C-3).

IR (νmax/cm⁻¹): 2953, 2854, 1755, 1625, 1095


9-Methyl-undec-7-enoic acid ethyl ester 94

To ethyl 7-bromoheptanoate (2.0 ml, 10.28 mmol, 1.0 equiv) in toluene (20 ml) was added triphenyl phosphine (3.24 g, 12.34 mmol, 1.2 equiv) and the resulting mixture refluxed for 48 hours. The toluene was then removed in vacuo with vigorous stirring and the resulting viscous oil was suspended in THF (80 ml). The resulting suspension was cooled to 0 °C and nBuLi (1.6 M, 6.4 ml, 10.28 mmol, 1.0 equiv) was added dropwise to give a pale orange solution to which 2-methylbutyraldehyde (1.07 ml, 10.28 mmol, 1.0 equiv) was added at 0 °C. The
resulting mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched by the addition of NH₄Cl (sat., 40 ml) and the resulting aqueous layer was extracted with diethyl ether (3 x 50 ml). Combined organic fractions were washed with brine (50 ml), dried over magnesium sulphate and concentrated in vacuo. The residue was purified by flash column chromatography (silica, 2 % ethyl acetate/hexane) to leave a colourless oil.

Yield: 1.75 g (75 %)

¹H-NMR (CDCl₃, 400MHz) δ: 0.77 (t, 3H, 7.0 Hz, H-14,), 0.85 (d, 3H, 7.0 Hz, H-13,), 1.18 (t, 3H, 7.5 Hz, H-1), 1.29 (m, 6H, H-12, H-6, H-7), 1.56 (m, 2H, H-5), 1.96 (m, 2H, H-8), 2.22 (overlapping m and t, 3H, H-4, H-11), 4.05 (quartet, 2H, 7.5 Hz, H-2), 5.06 (dd, 1H, 5.5 Hz, 7.0 Hz, H-10), 5.24 (m, 1H, H-9)

¹³C-NMR (CDCl₃, 100MHz) δ: 11.94 (C-14), 14.23 (C-1), 21.04 (C-13), 24.88, 27.27, 28.80 and 29.52 (C-5-C-7 and C-12), 30.25 (C-4), 33.38 (C-11), 34.32 (C-8), 60.11 (C-2), 128.20 (C-9), 136.40 (C-10), 173.77 (C-3).

IR (νmax/cm⁻¹): 2964, 2821, 1765, 1612, 1082, 905


**10-Methyl-undecanoic acid ethyl ester**

![10-Methyl-undecanoic acid ethyl ester](image)

To a solution of 10-Methyl-undec-7-enoic acid ethyl ester (0.922 g, 4.07 mmol, 1.0 equiv) in ethanol was added Pd/C (10 %, 1.0 g) and the stirred mixture was evacuated in vacuo and flushed with hydrogen gas 5 times. The resulting suspension was stirred and maintained under hydrogen atmosphere overnight. The reaction was poured over Celite which was then washed with ethanol (2 x
50 ml). The combined washings were then evaporated in vacuo and the resulting residue was purified by flash column chromatography (silica, 2 % ethyl acetate/hexane) to leave the desired compound as a colourless oil.

Yield: 0.922 g (99 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.85 (d, 6H, 6.5 Hz, H-13, H-14), 1.15 (dt, 3H, 7.0 Hz, 7.5 Hz, H-11), 1.25 (m, 12H, H-6-H-10 and H-1), 1.50 (doublet of quartets, 1H, 6.0 Hz, 7.0 Hz, H-12), 1.61 (m, 2H, H-5), 2.28 (t, 2H, 7.0 Hz, H-4), 4.11 (quartet, 2H, 7.5 Hz, H-2)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 15.45 (C-1), 22.75 (2C, C-13 and C-14), 24.73 (C-5), 27.45, 29.18, 29.29, 29.44 and 29.75 (C-6-C-10), 27.87 (C-12), 33.84 (C-4), 38.15 (C-11), 60.54 (C-2), 174.47 (C-3)

IR ($\nu_{\text{max}}$/cm$^{-1}$): 2953, 2854, 1755, 1095

HRMS: $m/z$ calculated for C$_{14}$H$_{26}$O$_2$: 229.2162 [M+H]$^+$. Found: 229.2168

9-Methyl-undecanoic acid ethyl ester

To solution of 9-Methyl-undec-7-enoic acid ethyl ester (0.658 g, 4.07 mmol, 1.0 equiv) in ethanol was added Pd/C (10 %, 1.0 g) and the stirred mixture was evacuated in vacuo and flushed with hydrogen gas 5 times. The resulting suspension was stirred and maintained under hydrogen atmosphere overnight. The reaction was poured over Celite which was then washed with ethanol (2 x 50 ml). The combined washings were then evaporated in vacuo and the resulting residue was purified by flash column chromatography (silica, 2 % ethyl acetate/hexane) to leave the desired compound as a colourless oil.

Yield: 0.658 g (99 %)
\( ^1\text{H-NMR (CDCl}_3, 400\text{MHz}) \delta: 0.82 - 0.84 \) (overlapping m, 6H, H-13 and H-14),
1.09 (m, 2H, H-12), 1.18 (t, 3H, 7.5 Hz, H-1), 1.27 (m, 11H, H-6-H11), 1.57 (quintet, 2H, 6.5 Hz, H-5), 2.28 (t, 2H, H-4), 4.05 (quartet, 2H, 7.5 Hz).

\( ^{13}\text{C-NMR (CDCl}_3, 100\text{MHz}) \delta: 11.56 \) (C-13 or C-14), 14.86 (C-1), 19.25 (C-13 or C-14), 24.56 (C-5), 29.12, 29.25, 29.35 and 29.87 (C-6-C-10 and C-12), 34.26 (C-4), 34.78 (C-12), 61.23 (C-2), 173.75 (C-3).

IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 2955, 2853, 1752, 1093

HRMS: \( m/z \) calculated for C_{14}H_{26}O_{2}: 229.2162 [M+H]^+. Found: 229.2165

10-Methyl-undecanoic acid 97

To a solution of 10-Methyl-undecanoic acid ethyl ester (0.922g, 4.07 mmol) in THF (50 ml) was added NaOH (aq, 2N, 50 ml) and the resulting immiscible liquids stirred vigorously under reflux (75 °C) overnight. The aqueous layer was then separated and acidified (HCl. aq, 10 N). Acidified aqueous layer then extracted with diethyl ether (3 x 50 ml) and the combined organic extracts were washed with brine (40 ml), dried over magnesium sulphate and evaporated in vacuo to leave the desired compound as a white solid.

Yield: 0.644 g (80 %)

\( ^1\text{H-NMR (CDCl}_3, 400\text{MHz}) \delta: 0.86 \) (d, 6H, 7.0 Hz, H-11, H12), 1.15 (m, 2H, H-9), 1.26 (m, 10H, H-8-H-4), 1.50 (doublet of quartets, 1H, 6.0 Hz, 7.0 Hz, H-10), 1.62 (quintet, 2H, 6.5 Hz, H-3), 2.34 (t, 2H, 6.5 Hz, H-2)

\( ^{13}\text{C-NMR (CDCl}_3, 100\text{MHz}) \delta: 22.63 \) (2C, C-11 and C-12), 24.67 (C-3), 27.38, 29.08, 29.26, 29.47, 29.86 (C-4-C-8), 27.97 (C-10), 34.04 (C-2), 39.04 (C-9), 180.67 (C-1)
IR (ν_{max}/cm^{-1}): 2987, 2951, 2887, 1712

HRMS: m/z calculated for C_{14}H_{26}O_{2}: 201.1849 [M+H]^+. Found: 201.1853

9-Methyl-undecanoic acid 96

![Structural formula of 9-Methyl-undecanoic acid]

To a solution of 9-Methyl-undecanoic acid ethyl ester (0.658 g, 4.07 mmol) in THF (50 ml) was added NaOH (aq, 2N, 50 ml) and the resulting immiscible liquids stirred vigorously under reflux (75 °C) overnight. The aqueous layer was then separated and acidified (HCl, aq, 10 N). Acidified aqueous layer then extracted with diethyl ether (3 x 50 ml) and the combined organic extracts were washed with brine (40 ml), dried over magnesium sulphate and evaporated in vacuo to leave the desired compound as a white solid.

Yield: 0.422 g (77 %)

{H-NMR (CDCl_{3}, 400MHz) δ: 0.82 - 0.84 (overlapping m, 6H, H-11 and H-12), 1.09 (m, 2H, H-10), 1.26 (m, 11H, H-4-H9), 1.63 (quintet, 2H, 6.5 Hz, H-3), 2.33 (t, 2H, H-2)

{C-NMR (CDCl_{3}, 100MHz) δ: 11.36 (C-11 or C-12), 19.18 (C-11 or C-12), 24.68 (C-3), 27.02, 29.07, 29.29, 29.49 and 29.78 (C-4-C-8), 34.13 (C-2), 34.39 (C-9), 36.59 (C-10), 180.57 (C-1)

IR (ν_{max}/cm^{-1}): 2979, 2953, 2888, 1713

HRMS: m/z calculated for C_{14}H_{26}O_{2}: 201.1849 [M+H]^+. Found: 201.1842

**General Method for formation of acyl pyrrole from carboxylic acids**

Carboxylic acid (1.0 equiv), 2,2-dipyridylisulfide (2.0 equiv) and triphenyl phosphine (2.0 equiv) were stirred in dry toluene at room temperature under argon for 24 hours. This reaction mixture was then cooled to -78°C and treated
dropwise with pyrrolylmagnesium chloride (0.35M, 3.0 equiv; prepared from 2.0 M ethylmagnesium chloride in dry THF diluted with toluene and pyrrole, -40°C to -10°C, 10 min). After 45 minutes the reaction was quenched at -78 °C with NH₄Cl (sat. aq., 10 ml) and the products extracted with diethyl ether. Combined organic phases were washed with 5% potassium carbonate, water and brine, then dried over magnesium sulphate and evaporated to dryness to leave the desired 2-acyl pyrrole. The product was typically purified by passage through a plug of silica (40% ethyl acetate/hexane) before purification by flash column chromatography as described below.

1-(1H-Pyrrol-2-yl)-undec-10-en-1-one

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 1.65 g (87 %)

¹H-NMR (CDCl₃, 400MHz) δ: 1.20-1.40 (m, 10H, H-7-H-11), 1.70 (quintet, 2H, 7.5 Hz, H-12), 2.05 (q, 2H, 7.5 Hz, H-13), 2.75 (t, 2H, 7.5 Hz, H-6), 4.90 (dd, 2H, 8.0 Hz, 10.5 Hz, H-15), 5.81 (m, 1H, H-14), 6.30 (m, 1H, H-2), 6.90 (m, 1H, H-3), 7.05 (m, 1H, H-1), 9.05 (br s, 1H)

¹³C-NMR (CDCl₃, 100MHz) δ: 25.36 (C-12), 28.92, 29.10, 29.25, 29.35 and 29.41 (C-7-C-11), 33.81 (C-13), 38.05 (C-6), 110.48 (C-2), 114.15 (C-15), 116.16 (C-3), 124.60 (C-1), 132.10 (C-4), 139.22 (C-14), 191.33 (C-5).

IR (νmax/cm⁻¹): 3280, 2920, 2848, 1639, 1547

m.p.: 33- 34 °C

HRMS: m/z calculated for C₁₅H₂₃NO: 234.1852 [M+H]+. Found: 234.1856
**1-(1H-Pyrrol-2-yl)-octan-1-one**

![Chemical structure of 1-(1H-Pyrrol-2-yl)-octan-1-one]

Purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 1.49 g, 96%

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.85 (t, 3H, 7.0 Hz, H-12), 1.15 – 1.35 (m, 8H, H-8-H-11), 1.70 (quintet, 2H, 7.5 Hz, H-7), 2.75 (t, 2H, 7.5 Hz, H-6), 6.25 (m, 1H, H-2), 6.90 (m, 1H, H-3), 7.00 (m, 1H, H-1), 10.0 (br s, 1H)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.25 (C-12), 22.65, 25.41, 29.14, 29.46 and 31.73 (C-7-C-11), 38.08 (C-6), 110.45 (C-2), 116.24 (C-3), 124.71 (C-1), 132.10 (C-4), 191.42 (C-5)

IR (ν$_{max}$/cm$^{-1}$): 3276, 2955, 2917, 2851, 1640, 1546

m.p.: 31-33 °C

HRMS: m/z calculated for C$_{12}$H$_{19}$NO: 194.1539 [M+H]$^+$. Found: 194.1543

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**1-(1H-Pyrrol-2-yl)-octadecan-1-one**

![Chemical structure of 1-(1H-Pyrrol-2-yl)-octadecan-1-one]

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.7652 g (29%)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.90 (t, 3H, 7.0 Hz, H-22), 1.25 – 1.40 (m, 28H, H-8-H-21), 1.75 (quintet, 2H, 7.0 Hz, H-7), 2.80 (t, 2H, 7.0 Hz, H-6), 6.30 (m, 1H, H-2), 6.95 (m, 1H, H-3), 7.00 (m, 1H, H-1), 9.40 (br s, 1H).
2-Octyloxy-1-(1H-pyrrol-2-yl)-ethanone

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a low-melting point solid.

Yield: 0.747 (76 %)

\(^1\)H-NMR (CDCl\(_3\), 400MHz) \(\delta: 0.87\) (t, 3H, 7.0 Hz, H-14), 1.27 - 1.38 (m, 10H, H-9-H-13), 1.65 (quintet, 2H, 7.5 Hz, H-8), 3.52 (t, 2H, 7.5 Hz, H-7), 4.49 (s, 2H, H-6), 6.27 (m, 1H, H-2), 7.06 (m, 2H, H-3 and H-1), 10.09 (br s, 1H).

\(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \(\delta: 14.10\) (C-14), 22.66, 26.06, 29.25, 29.41 and 29.65 (C-9-C-13), 31.82 (C-8), 72.11 (C-7), 73.70 (C-6), 110.78 (C-2), 116.90 (C-3), 125.06 (C-1), 129.98 (C-4), 187.35 (C-5)

IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3287, 2928, 2913, 2849, 1740, 1633

m.p.: 34 - 35 °C

HRMS: \(m/z\) calculated for C\(_{14}\)H\(_{29}\)NO\(_2\): 238.1802 [M+H]\(^+\). Found: 238.1806
5-Pentyloxy-1-(1H-pyrrol-2-yl)-pentan-1-one

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a low-melting point solid.

Yield: 0.6114 (78 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.89 (t, 7.0 Hz, H-14), 1.31 (m, 4H, H-12-H-13), 1.58 (m, 2H, H-11), 1.64 (m, 2H, H-7), 1.81 (m, 2H, H-8), 2.81 (t, 2H, 7.0 Hz, H-6), 3.40 (t, 2H, 7.0 Hz, H-9), 3.44 (t, 2H, 7.0 Hz, H-10), 6.26 (m, 1H, H-2), 6.92 (m, 1H, H-3), 7.04 (m, 1H, H-1), 10.12 (br s, 1H).

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 14.05 (C-14), 22.07, 22.56, 28.39, 29.44, 29.46 (C-7, C-8 and C-11-C-13), 37.69 (C-6), 70.51 (C-10), 71.04 (C-9), 110.44 (C-2), 116.35 (C-3), 124.83 (C-1), 132.03 (C-4), 191.03 (C-5).

IR ($\nu_{\text{max}}$/cm$^{-1}$): 3275, 2930, 2856, 1739, 1633

m.p.: 25 – 26 °C

HRMS: $m/z$ calculated for C$_{14}$H$_{23}$NO$_2$: 238.1802 [M+H]$^+$. Found: 238.1801

1-(1H-Pyrrol-2-yl)-decan-1-one

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 1.55 g (87 %)
1H-NMR (CDCl₃, 400MHz) δ: 0.88 (t, 3H, 7.5 Hz, H-14), 1.27 (m, 12H, H-8-H-13), 1.73 (quintet, 2H, 7.0 Hz, H-7), 2.77 (t, 2H, 7.0 Hz, H-6), 6.27 (m, 1H, H-2), 6.93 (m, 1H, H-3), 7.04 (m, 1H, H-1)

13C-NMR (CDCl₃, 100MHz) δ: 14.13 (C-14), 22.69, 25.44, 29.31, 29.49 and 31.90 (1 signal missing due to overlapping signals, C-7-C-13), 38.08 (C-6), 110.42 (C-2), 116.37 (C-3), 124.87 (C-1), 132.09 (C-4), 191.49 (C-5)

IR (νmax/cm⁻¹): 3279, 2953, 2863, 1631, 1547

m.p.: 32 – 34 °C

HRMS: m/z calculated for C₁₄H₂₃NO: 222.1852 [M+H]+. Found: 222.1856

1-(1H-Pyrrol-2-yl)-dodecan-1-one

![Structural diagram of 1-(1H-Pyrrol-2-yl)-dodecan-1-one]

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 1.78 (89 %)

1H-NMR (CDCl₃, 400MHz) δ: 0.88 (t, 3H, 7.5 Hz, H-16), 1.26 (m, 16H, H-8-H-15), 1.71 (quintet, 2H, 7.0 Hz, H-7), 2.73 (t, 2H, 7.0 Hz, H-6), 6.27 (m, 1H, H-2), 6.90 (m, 1H, H-3), 7.01 (m, 1H, H-1)

13C-NMR (CDCl₃, 100MHz) δ: 14.14 (C-16), 22.73, 25.49, 29.39, 29.51, 29.55, 29.67, 31.96 (2 signals missing due to overlapping C-7-C-15), 38.08 (C-6), 110.37 (C-2), 116.59 (C-3), 125.15 (C-1), 132.07 (C-4), 191.58 (C-5)

IR (νmax/cm⁻¹): 3285, 2955, 2859, 1635, 1539

m.p.: 56 – 57 °C

HRMS: m/z calculated for C₁₆H₂₇NO: 250.2165 [M+H]+. Found: 250.2168
10-Methyl-1-(1H-pyrrol-2-yl)-undecan-1-one

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.609 g (76 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.86 (d, 6H, 7.0 Hz, H-15, H-16), 1.15 (m, 2H, H-13), 1.25 (m, 10H, H-12-H-8), 1.51 (doublet of quartets, 1H, 6.0 Hz, 7.0 Hz, H-14), 1.71 (quintet, 2H, 6.5 Hz, H-7), 2.75 (t, 2H, 6.5 Hz, H-6), 6.27 (m, 1H), 6.90 (m, 1H), 7.01 (m, 1H)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 23.18 (2C), 25.95, 28.48, 30.06, 30.23, 30.41 and 30.52 (C7-C-12), 27.91 (C14), 38.57 (C-6), 39.55 (C-13), 110.85 (C-2), 116.98 (C-3), 125.54 (C-1), 132.72 (C-4), 192.01 (C-5)

IR (ν$_{\text{max}}$/cm$^{-1}$): 3279, 2983, 2865, 1652, 1548

m.p.: 42- 45 °C

HRMS: m/z calculated for C$_{16}$H$_{27}$NO: 250.6165 [M+H]$^+$. Found: 250.2162

9-Methyl-1-(1H-pyrrol-2-yl)-undecan-1-one

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a colourless oil.

Yield: 0.453 g (82 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.84 – 0.85 (overlapping 2 x m, 6H, H-15 and H-16), 1.09 (m, 2H, H-14), 1.27 (m, 11H, H-8-H-13), 1.71 (quintet, 2H, 7.0 Hz, H-
$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.81 (t, 3H, 7.5 Hz, H-15), 1.22 (m, 13H, H-8-H-14), 1.68 (q, 2H, H-7, 7.0 Hz), 2.69 (t, 2H, 7.0 Hz, H-6), 6.22 (m, 1H, H-2), 6.81 (m, 1H, H-3), 6.92 (m, 1H, H-1), 9.81 (br s, 1H, N-H).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.01 (C-15), 22.59, 25.26, 29.28, 29.39, 29.50 and 31.80 (1 signal missing due to overlapping, C7 and C9-C14), 28.98 (3 signals, C-8), 37.92 (C-6), 110.22 (C-2), 116.41 (C-3), 124.99 (C-1), 131.95 (C-4), 191.44 (C-5)

IR (ν$_{\text{max}}$/cm$^{-1}$): 3279, 2935, 2865, 1615, 1542

m.p.: 49 – 50 °C

HRMS: $m/z$ calculated for C$_{15}$H$_{24}$DNO: 237.2072 [M+H]$^+$. Found: 237.2075
**S-(4-D)-1-(1H-Pyrrol-2-yl)-undecan-1-one**

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.354 g (88 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.81 (t, 3H, 7.5 Hz, H-15), 1.22 (m, 13H, H-8-H-14), 1.68 (q, 2H, H-7, 7.0 Hz), 2.69 (t, 2H, 7.0 Hz, H-6), 6.22 (t, 1H, H-2), 6.81 (m, 1H, H-1), 9.81 (br s, 1H, N-H).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.26 (C-15), 22.14, 25.24, 29.29, 29.37, 29.52 and 31.82 (1 signal missing due to overlapping, C7 and C9-C14), 28.96 (3 signals, C-8), 37.93 (C-6), 110.22 (C-2), 116.43 (C-3), 124.99 (C-1), 131.96 (C-4), 191.44 (C-5)

IR (ν$_{max}$/cm$^{-1}$): 3279, 2935, 2865, 1665, 1542

m.p.: 48-49 °C

HRMS: m/z calculated for C$_{15}$H$_{24}$DNO: 237.2072 [M+H]$^+$. Found: 237.2070

**General procedure for reduction of acyl pyrrole to alkyl pyrroles**

To sodium borohydride (6.0 equiv) in refluxing isopropanol was added the corresponding 2-acyl pyrrole (1.0 equiv) and the resultant mixture left to reflux overnight. The mixture was diluted with water and stirred until complete dissolution, the volatiles were then removed in vacuo, and the residue was extracted with ethyl acetate. The combined organic fractions were then dried over magnesium sulphate and evaporated to dryness to leave the title compound as a yellow oil that typically solidifies on standing. The product was then purified by flash column chromatography as described below.
2-Undec-10-ethyl-1H-pyrrole 89

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 1.00 g (82 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 1.25-1.42 (m, 12H, H-6-H-11), 1.61 (quintet, 2H, 7.5 Hz, H-12), 2.07 (q, 2H, 7.5 Hz, H-13), 2.60 (t, 2H, 7.0 Hz, H-5), 4.98 (dd, 2H, 8.0 Hz, 10.5 Hz, H-15), 5.72 (m, 1H, H-14), 5.95 (m, 1H, H-3), 6.16 (m, 1H, H-2), 6.63 (m, 1H, H-1), 7.90 (br s, 1H, N-H)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 27.79 (C-5), 29.00, 29.19, 29.25, 29.37, 29.46, 29.58 and 29.74 (C-6-C-12), 33.88 (C-13), 104.90 (C-3), 108.27 (C-2), 114.19 (C-15), 116.01 (C-1), 132.91 (C-4), 139.29 (C-14).

IR (ν$_{max}$/cm$^{-1}$): 3352, 2916, 2846

m.p.: 28 – 29 °C

HRMS: m/z calculated for C$_{15}$H$_{25}$N: 220.2060 [M+H]$^+$. Found: 220.2064

2-Decyl-1H-pyrrole 86

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.173 (93 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.88 (t, 3H, 7.5 Hz, H-14), 1.26 (m, 14H, H-7-H-13), 1.56 (quintet, 2H, 7.0 Hz, H-6), 2.59 (t, 2H, 7.0 Hz, H-5), 5.91 (m, 1H, H-3), 6.13 (m, H-2), 6.66 (m, H-1)
\(^{13}\text{C-NMR (CDCl}_3, 100\text{MHz})\): 14.14 (C-14), 22.70, 27.76, 29.36, 29.42, 29.61, 29.63, 29.70 and 31.93 (1 signal missing due to overlapping, C-5-C-13), 104.86 (C-3), 108.25 (C-2), 115.96 (C-1), 132.92 (C-4)

IR \((\nu_{\text{max}}/\text{cm}^{-1})\): 3349, 2935, 2842

m.p.: 38 – 40 °C

HRMS: \(m/z\) calculated for \(\text{C}_{16}\text{H}_{25}\text{N}\): 208.2060 \([\text{M+H}]^+\). Found: 208.2065

2-Dodecyl-1H-pyrrole 87

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.159 g (85 %)

\(^1\text{H-NMR (CDCl}_3, 400\text{MHz})\): 0.88 (t, 3H, 7.0 Hz, H-16), 1.26 (m, 18H, H-7-H-15), 1.62 (quintet, 2H, 7.0 Hz, H-6), 2.59 (t, 2H, 7.0 Hz, H-5), 5.91 (m, 1H, H-3), 6.13 (m, 1H, H-2), 6.65 (m, 1H, H-1).

\(^{13}\text{C-NMR (CDCl}_3, 100\text{MHz})\): 14.09 (C-16), 22.67, 27.71, 29.33, 29.38, 29.44, 29.57, 29.65 and 31.90 (3 signals missing due to overlapping, C-5-C-15), 104.80 (C-3), 108.18 (C-2), 115.91 (C-1), 132.86 (C-4).

IR \((\nu_{\text{max}}/\text{cm}^{-1})\): 3351, 2931, 2854

m.p.: 51 – 53 °C

HRMS: \(m/z\) calculated for \(\text{C}_{16}\text{H}_{29}\text{N}\): 236.2373 \([\text{M+H}]^+\). Found: 236.2378
2-(2-Octyloxy-ethyl)-1H-pyrrole 102

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a colourless oil.

Yield: 0.279 g (79 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.94 (t, 3H, 7.0 Hz, H-14), 1.35 (m, 10H, H-9-H-13), 1.67 (quintet, 2H, 7.0 Hz, H-8), 2.90 (t, 2H, 7.0 Hz, H-5), 3.50 (t, 2H, 7.0 Hz, H-7), 3.68 (t, 2H, 7.0 Hz, H-6), 5.96 (m, 1H, H-3), 6.15 (m, 1H, H-2), 6.71 (m, 1H, H-1), 8.67 (br s, 1H)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 14.16 (C-14), 22.73, 26.33, 27.99, 29.34, 29.49, 29.84, 31.89 (C-5 and C-8-C-13), 70.86 (C-6), 71.32 (C-7), 105.37 (C-3), 107.75 (C-2), 116.58 (C-1), 130.77 (C-4).

IR ($\nu_{\max}$/cm$^{-1}$): 3389, 2922, 2852, 1735

HRMS: m/z calculated for C$_{14}$H$_{25}$NO: 224.2009 [M+H]$^+$. Found: 224.2007

2-(5-Pentyloxy-pentyl)-1H-pyrrole 105

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a colourless oil.

Yield: 0.2286 g (79 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.96 (t, 3H, 7.0 Hz, H-14), 1.37 (m, 4H, H-12-H-13), 1.44 (m, 2H, H-6), 1.61 -1.70 (m, 6H, H-7-H-8 and H-11), 2.63 (t, 2H, 7.0 Hz, H-5), 3.45 (m, 4H, H-9 and H-10), 5.95 (m, 1H, H-3), 6.16 (m, 1H, H-2), 6.67 (m, 1H, H-1), 8.09 (br s, 1H)
$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.11 (C-14), 22.61, 25.93, 27.63, 28.43, 29.49, 29.52, 29.56 (C-5-C-8 and C-11-C-13), 70.82 (C-10), 71.08 (C-9), 104.92 (C-3), 108.18 (C-2), 116.09 (C-1), 132.67 (C-4).

IR (ν$_{max}$/cm$^{-1}$): 3391, 2932, 2832, 1741

HRMS: m/z calculated for C$_{14}$H$_{25}$NO: 224.2009 [M+H]$^+$. Found: 224.2013

2-Octyl-1H-pyrrole 85

Product was purified by flash column chromatography (silica, 5% ethyl acetate in hexane) to leave a low melting point white solid.

Yield: 0.820 g, 75 %

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.95 (t, 3H, 7.0 Hz, H-12), 1.30 – 1.47 (m, 10H, H-7-H-11), 1.72 (quintet, 2H, 7.5 Hz, H-6), 2.64 (t, 2H, 7.5 Hz, H-5), 5.93 (m, 1H, H-3), 6.22 (m, 1H, H-2), 6.71 (m, 1H, H-1) 7.85 (br s, 1H)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 13.12 (C-12), 22.75, 29.34, 29.49, 29.50, 29.63, 29.76, 31.96 (C-5-C-11), 104.89 (C3), 108.26 (C-2), 116.03 (C-1), 132.96 (C-4).

IR (ν$_{max}$/cm$^{-1}$): 3378, 2922, 2852

m.p.: 25 - 26 °C

HRMS: m/z calculated for C$_{12}$H$_{21}$N: 180.1747 [M+H]$^+$. Found: 180.1744

2-Octadecyl-1H-pyrrole 88

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.
Yield: 0.665 g (91 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.89 (t, 3H, 7.0 Hz, H-22), 1.25 – 1.40 (m, 30H, H-7-H-21), 1.60 (quintet, 2H, 7.0 Hz, H-6), 2.56 (t, 2H, 7.0 Hz, H-5), 5.83 (m, 1H, H-3), 6.12 (m, 1H, H-2), 6.61 (m, 1H, H-1), 7.82 (br s, 1H).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.27 (C-22), 22.82, 27.86, 29.50, 29.53, 29.59, 29.72, 29.80, 29.81, 29.83 (7 carbon missing due to overlapping signals, C-6-C-21), 32.06 (C-5), 104.92 (C-3), 105.14 (C-2), 116.56 (C-1), 132.96 (C-4)

IR (ν$_{\text{max}}$/cm$^{-1}$): 3348, 2955, 2913, 2846

m.p.: 71 – 71 °C

HRMS: m/z calculated for C$_{22}$H$_{41}$N: 320.3312 [M+H]$^+$. Found: 320.3316

2-(10-Methyl-undecyl)-1H-pyrrole 99

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a colourless oil.

Yield: 0.086 g (91 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.86 (d, 6H, 7.0 Hz, H-15, H-16), 1.15 (m, 2H, H-13), 1.25 (m, 10H, H-7-H-12), 1.51 (doublet of quartets, 1H, 6.0 Hz, 7.0 Hz, H-14), 1.54 (quintet, 2H, 6.5 Hz, H-6), 2.56 (t, 2H, 6.5 Hz, H-5), 5.86 (m, 1H, H-3), 6.13 (m, 1H, H-2), 6.69 (m, 1H, H-1)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 23.32 (2C, C-15 and C-16), 25.41, 28.98, 30.09, 30.19, 30.27, 30.35, 30.41 and 30.59 (C5-C-12), 27.96 (C14), 39.57 (C-13), 104.87 (C-3), 108.99 (C-2), 115.55 (C-1), 132.72 (C-4)

IR (ν$_{\text{max}}$/cm$^{-1}$): 3365, 2984, 2945, 2819

HRMS: m/z calculated for C$_{16}$H$_{29}$N: 236.2373 [M+H]$^+$. Found: 236.2375
2-(9-Methyl-undecyl)-1H-pyrrole 98

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a colourless oil.

Yield: 0.084 g (90 %)

\[^1\text{H-}\text{NMR (CDCl}_3, 400MHz) \delta: 0.84 \text{– 0.85 (overlapping 2 x m, 6H, H-15 and H-16), 1.09 (m, 2H, H-14), 1.27 (m, 11H, H-8-H-13), 1.61 (quintet, 2H, 7.0 Hz, H-6), 2.56 (t, 2H, 7.0 Hz, H-5), 5.86 (m, 1H, H-3), 6.04 (m, 1H, H-2), 6.67 (m, 1H, H-1).} \]

\[^{13}\text{C-}\text{NMR (CDCl}_3, 100MHz) \delta: 11.32 \text{ (C-15 or C-16), 19.13 (C15 or C-16), 25.36, 26.98, 28.13, 29.42, 29.44 and 29.78 (2 signal missing due to overlapping, C-5-C-12), 34.30 (C-13), 36.53 (C-14), 104.25 (C-3), 108.31 (C-2), 115.98 (C-1), 132.11 (C-4) IR (v\text{max/cm}^{-1}): 3395, 2995, 2951, 2819} \]

HRMS: m/z calculated for C\text{16}H\text{29}N: 236.2373 [M+H\text{]+}] Found: 236.2371

\(\text{R-(4-D}_1\text{-2-Undecyl-1H-pyrrole 145} \)

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.070 g (78 %)
H-NMR (CDCl$_3$, 400MHz) δ: 0.82 (t, 3H, 7.5 Hz, H-15), 1.22 (m, 15H, H-7-H-14), 1.56 (quintet, 2H, H-6, 7.0 Hz), 2.53 (t, 2H, 7.0 Hz, H-5), 5.81 (m, 1H, H-3), 6.03 (m, 1H, H-2), 6.63 (m, 1H, H-1)

C-NMR (CDCl$_3$, 100MHz) δ: 14.51 (C-15), 22.41, 27.84, 29.38, 29.51, 29.62, 29.68, 29.72 and 31.84 (1 signal missing due to overlap, C-5-C-7 and C-9-C-14), 29.43 (3 signals, C-8) 104.87 (C-3), 108.31 (C-2), 115.91 (C-1), 132.92 (C-4).

IR ($\nu_{max}$/cm$^{-1}$): 3351, 2914, 2831

m.p.: 44 – 45 °C

HRMS: $m/z$ calculated for C$_{15}$H$_{26}$DN: 223.2279 [M+H]$^+$. Found: 223.2280

S-(4-D$_1$)-2-Undecyl-1H-pyrrole 145

Product was purified by flash column chromatography (silica, 10% ethyl acetate in hexane) to leave a white solid.

Yield: 0.088 g (95 %)

H-NMR (CDCl$_3$, 400MHz) δ: 0.81 (t, 3H, 7.5 Hz, H-15), 1.22 (m, 15H, H-7-H-14), 1.56 (quintet, 2H, H-6, 7.0 Hz), 2.53 (t, 2H, 7.0 Hz, H-5), 5.82 (m, 1H, H-3), 6.03 (m, 1H, H-2), 6.63 (m, 1H, H-1)

C-NMR (CDCl$_3$, 100MHz) δ: 14.61 (C-15), 22.84, 27.98, 29.37, 29.49, 29.61, 29.69, 29.72 and 31.87 (1 signal missing due to overlap, C-5-C-7 and C-9-C-14), 29.44 (3 signals, C-8) 104.87 (C-3), 108.21 (C-2), 115.84 (C-1), 132.96 (C-4).

IR ($\nu_{max}$/cm$^{-1}$): 3349, 2916, 2834

HRMS: $m/z$ calculated for C$_{15}$H$_{26}$DN: 223.2279 [M+H]$^+$. Found: 223.2280
A solution of NaNO$_2$ (31.57 g, 457.5 mmol, 1.5 equiv) in water (70 ml) was added dropwise over approximately 6 hours to a vigorously stirred mixture of L-glutamic acid (44.87 g, 305.0 mmol, 1.0 equiv) in water (120 ml) and HCl. (conc., 65 ml) at 0-5 °C. The resulting pale yellow solution was left to stir overnight at room temperature. Evaporation of solvent under reduced pressure left a pale yellow oil together with white solid. Ethyl acetate (150 ml) was added and the insoluble material was removed by vacuum filtration. The organic filtrate was then dried over magnesium sulphate and evaporated to leave a pale yellow oil which partially crystallised under vacuum. The resulting sticky solid was triturated with chloroform twice to leave the desired product as a crude white solid. Purification by vacuum distillation (b.p. 158 °C, 0.5 mmHg) gave the desired product as a pure white solid.

Yield: 13.28 g (53 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 2.41 (m, 1H, H-3a), 2.65 (m, 3H, H-4 and H-3b), 5.03 (m, 1H, H-2), 10.15 (br s, 1H, -COOH)

$^{13}$C-NMR (DMSO-$d_6$, 100MHz) $\delta$: 25.33 (C-3), 26.67 (C-4), 75.35 (C-2), 171.56 (C-5), 176.73 (C-1).

IR ($\nu_{\text{max}}$/cm$^{-1}$): 1711, 1778, 2939, 2975, 3034

m.p.: 65 – 68 °C [lit. 71 – 73 °C$^{108}$]

$[\alpha]_D^{25}$: +14.9 (c = 1.0, EtOH) [lit. +15.6 (c = 2.0, EtOH)]$^{108}$

In a 3-neck flask fitted with a reflux condenser borane methyl sulphide (10 M, 11.7 ml, 116.3 mmol, 1.15 equiv) was added dropwise over 1 hour at room temperature to a stirred solution of S-5-Oxo-tetrahydro-furan-2-carboxylic acid (13.14g, 101.03 mmol, 1.00 equiv) in THF (70 ml) under argon. The reaction was then stirred for a further 3 hours before quenching by the cautious addition of methanol (60 ml). The solvent was then removed by distillation at atmospheric pressure and the resulting residue was distilled under vacuum to give the desired product as a colourless oil (134 – 135 °C, 1.0 mmHg).
Yield: 8.13 g (69 %)

\[ ^1H-NMR (CDCl_3, 400MHz) \delta: 2.21 (m, 2H, H-3), 2.60 (m, 2H, H-4), 3.02 (br s, 1H, -OH), 3.65 (m, 1H, H-1a), 3.92 (m, 1H, H-1b), 4.66 (m, 1H, H-2) \]

\[ ^13C-NMR (CDCl_3, 100MHz) \delta: 23.16 (C-3), 28.71 (C-4), 64.08 (C-1), 80.71 (C-2), 177.89 (C-5). \]

IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3404 (broad), 2937, 3873, 1752.

\[ [\alpha]_D^{25} +28.3 \text{ (c = 1.0, EtOH) [lit. +29.6 (c = 0.4, EtOH)]}^{108} \]

HRMS: \(m/z\) calculated for \(C_5H_8O_3\): 117.0546 [M+H]^+. Found: 117.0549
To \textit{S}-5-Hydroxymethyl-dihydro-furan-2-one (7.87g, 67.77 mmol, 1.0 equiv) in chloroform (70 ml) at 0 °C was added pyridine (10.91 ml, 135.54 mmol, 2.0 equiv). To the resulting mixture was added \textit{p}-toluenesulfonyl chloride (19.38 g, 101.66, 1.5 equiv) in small portions. The reaction was stirred for 7 hours (until complete by TLC), and was then quenched by the dropwise addition of water (60 ml) and the resulting cloudy solution was stirred for 30 minutes. The reaction was then diluted with DCM (50 ml), and the organic layer was separated and washed successively with HCl (aq. 0.5 N, 50 ml), water (50 ml), NaHCO$_3$ (sat. 50 ml) and water (50 ml). The organic layer was then dried over magnesium sulphate and the solvent evaporated \textit{in vacuo}. The resulting solid was recrystallised from ethanol to yield the desired product as a white solid.

Yield: 15.60 (86 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 2.15 (m, 1H, H-3a), 2.36 (m, 1H, H-3b), 2.41 (s, 3H, H-10), 2.51 (m, 2H, H-2), 4.16 (dd, 1H, 11.0 Hz, 3.5 Hz, H-5a), 4.20 (dd, 1H, 11.0 Hz, 3.5 Hz, H-5b), 4.69 (m, 1H, H-4), 7.38 (d, 2H, 8.0 Hz, H-8), 7.80 (d, 2H, 8.0 Hz, H-9)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 21.68 (C-10), 23.46 (C-3), 27.92 (C-2), 70.11 (C-5), 76.52 (C-4), 127.94 (C-7), 130.10 (C-8), 132.17 (C-6), 145.48 (C-9), 176.22 (C-1)

IR ($\nu_{\text{max}}$/cm$^{-1}$): 1419, 1597, 1767, 2961

$[\alpha]_D^{25}$: +46.5 (c = 1.0, CHCl$_3$) [lit. +46.2 (c = 1.63, CHCl$_3$)]

m.p.: 82 - 83 °C [lit. 84 – 85 °C]

HRMS: $m/z$ calculated for C$_{12}$H$_{14}$O$_5$S: 271.0635 [M+H]$^+$. Found: 271.0637
S-3-Oxiranyl-propionic acid methyl ester 150

S-Toluene-4-sulfonic acid 5-oxo-tetrahydro-furan-2-ylmethyl ester (13.92 g, 51.50 mmol, 1.0 equiv) was added to a solution prepared by dissolving sodium (1.20 g, 54.07 mmol, 1.01 equiv) in anhydrous methanol (125 ml). The mixture was left for 2 hours (until complete by TLC). The solvent was then removed from the reaction in vacuo and the resulting residue was partitioned between water (75 ml) and diethyl ether (75 ml). The aqueous layer was then further extracted with diethyl ether (2 x 50 ml), and the combined organic fractions were then washed with brine (2 x 40 ml), dried over magnesium sulphate and the solvent removed in vacuo to leave the desired product as a volatile colourless oil.

Yield: 4.88 g (76 %)

\(^1\)H-NMR (CDCl\(_3\), 400MHz) \(\delta\): 1.78 (m, 1H, H-3a), 1.97 (m, 1H, H-3b), 2.47 (t, 2H, 7.5 Hz, H-4), 2.51 (m, 1H, H-1a), 2.77 (t, 1H, 4.5 Hz, H-1b), 2.98 (m, 1H, H-2), 3.69 (s, 3H, H-6)

\(^1^3\)C-NMR (CDCl\(_3\), 100MHz) \(\delta\): 27.51 (C-3), 30.13 (C-4), 46.83 (C-1), 51.02 (C-2), 51.51 (C-6), 173.11 (C-5)

IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 1437, 1732, 2929, 2954, 2995

\([\alpha]_D^{25}\): -23.3 (c = 1.0, CHCl\(_3\)) [lit. -18.0 (c = 1.1, CHCl\(_3\))]\(^{109}\)

HRMS: m/z calculated for C\(_6\)H\(_{10}\)O\(_3\): 131.0703 [M+H]. Found 131.0702
To a slowed stirred slurry of copper(I) cyanide (3.01 g, 33.64 mmol, 1.0 equiv) in THF (35 ml) at -78 °C was added hexyl lithium (2.3 M in hexanes, 29.25 ml, 67.28 mmol, 2.0 equiv). The heterogeneous mixture was then allowed to gradually warm until complete dissolution (-10 °C). The resulting mixture was added dropwise to a solution of S-Toluene-4-sulfonic acid 5-oxo-tetrahydro-furan-2-ylmethyl ester (4.37 g, 33.64 mmol, 1.0 equiv) in THF (50 ml) at -78 °C over approximately 45 minutes. The reaction was monitored by TLC. On completion, the reaction was quenched with 10% NH₄OH/sat. NH₄Cl (aq., 50ml), and diluted with diethyl ether (50 ml). The aqueous layer was then extracted with diethyl ether (2 x 50 ml). The combined organic fractions were washed with water (50 ml), brine (50 ml), dried over magnesium sulphate and the solvent removed in vacuo to leave the desired product as a colourless oil, which was purified by flash column chromatography on silica (40% diethyl ether/petroleum ether).

Yield: 5.83 g (94 %)

¹H-NMR (CDCl₃, 400MHz) δ: 0.88 (t, 3H, 7.0 Hz, H-1), 1.29 -1.44 (m, 10H, H-2-H-6), 1.58 (m, 1H, H-7a), 1.73 (m, 1H, H-7b), 1.85 (m, 1H, H-9a), 2.32 (dt, 1H, 19.0 Hz, 8.0 Hz, H-9b), 2.53 (t, 2H, 8.0 Hz, H-10), 4.48 (quintet, 1H, 7.0 Hz, H-8).

¹³C-NMR (CDCl₃, 100MHz) δ: 14.15 (C-1), 22.69, 25.31, 28.09, 28.95, 29.20, 29.37, and 31.80 (C-2-C-6 and C-9-C-10), 35.67 (C-7), 81.15 (C-8), 177.39 (C-11).

IR (ν max/cm⁻¹): 1459, 1771, 2855, 2924

[α]D²⁵⁺: + 47.6 (c = 1.0, CHCl₃) [lit. +45.3 (c = 1.5 - 2.5, MeOH)]⁹⁷
HRMS: m/z calculated for C$_{11}$H$_{20}$O$_2$: 185.1536 [M+H]$^+$. Found 185.1533

**R-4-Hydroxy-undecanoic acid dimethylamide 159**

To a stirred suspension of dimethylamine hydrochloride (1.17 g, 14.34 mmol, 2.0 equiv) in DCM (30 ml) at 0 °C was added trimethyl aluminium (2.0 M in hexanes, 7.46 ml, 14.92 mmol, 2.08 equiv). The resulting mixture was stirred for approximately 2 hours to result in a clear colourless solution. This solution was then added in a dropwise manner to a solution of R-5-Heptyl-dihydro-furan-2-one (1.32 g, 7.17 mmol, 1.0 equiv) in DCM (30 ml) at room temperature. The reaction mixture was left overnight at room temperature after which the reaction was judged to have reach completion by TLC and was quenched by the cautious dropwise addition of HCl (aq., 1 N, 20ml) at 0 °C. The organic layer was separated and the aqueous layer further extracted with DCM (3 x 30 ml). The combined organic fractions were dried over magnesium sulphate and evaporated in vacuo to leave the desired compound as a yellow oil. The product was purified by passage through a small plug of silica eluted with diethyl ether to yield a pale yellow oil.

Yield: 1.32 g (80 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.86 (t, 3H, 7.0 Hz, H-1), 1.26 (m, 10H, H-2-H-6), 1.43 (m, 2H, H-7), 1.72 (m, 1H, H-9a), 1.83 (m, 1H, H-9b), 2.50 (m, 2H, H-10), 2.71 (br s, 1H, -OH), 2.95 (s, 3H, H-12), 3.0 (s, 3H, H-13), 3.60 (m, 1H, H-8)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.50 (C-1), 23.06, 26.17, 29.72, 30.12, 30.53, 32.25 and 32.32 (C-9, C-10 and C-2-C-6), 35.97 (C13), 37.79 (C12), 38.25 (C-7), 71.83 (C-8), 174.31 (C-11)

$[^\alpha]_D^{25}$: + 4.0 (c = 1.0, CHCl$_3$)

HRMS: m/z calculated for C$_{13}$H$_{27}$NO$_2$: 230.2115 [M+H]$^+$. Found: 230.2117
To $R$-4-Hydroxy-undecanoic acid dimethylamide (0.354 g, 1.54 mmol, 1.0 equiv) in chloroform (2.0 ml) was added pyridine (0.25 ml, 3.08 mmol, 2.0 equiv) at 0 °C. $p$-toluenesulfonyl chloride (0.441 g, 2.31 mmol, 1.5 equiv) was then added in portions to the solution still held at 0 °C. The mixture was then allowed to warm to room temperature overnight. After quenching with the addition of HCl (aq., 2 N, 10 ml) the chloroform was removed *in vacuo* and the resulting aqueous residue was extracted with diethyl ether (3 x 15 ml) to remove the tosylated amine by-product otherwise inseparable from the desired product. The aqueous layer was then basified to hydrolyse the intermediate imine to the desired lactone. After re-acidification with HCl (conc.), the aqueous layer was extracted with diethyl ether (3 x 15 ml). The combined organic fractions were then dried over magnesium sulphate and evaporated *in vacuo* to leave the desired lactone as a pale yellow oil. The crude oil was purified by flash column chromatography (silica, 30% diethyl ether/petroleum ether).

Yield: 0.229 g (81 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.88 (t, 3H, 7.0 Hz, H-1), 1.29 -1.44 (m, 10H, H-2-H-6), 1.58 (m, 1H, H-7a), 1.73 (m, 1H, H-7b), 1.85 (m, 1H, H-9a), 2.32 (dt, 1H, 19.0 Hz, 8.0 Hz, H-9b), 2.53 (t, 2H, 8.0 Hz, H-10), 4.48 (quintet, 1H, 7.0 Hz, H-8).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.15 (C-1), 22.54, 25.56, 28.12, 28.96, 29.21, 29.37, and 31.80 (C-2-C-6 and C-9-C-10), 35.68 (C-7), 81.15 (C-8), 177.40 (C-11).

IR ($\nu_{\text{max}}$/cm$^{-1}$): 1459, 1771, 2855, 2924

$[\alpha]_D^{25}$: - 47.4 (c = 1.0, CHCl$_3$)
HRMS: \( m/z \) calculated for \( \text{C}_{11}\text{H}_{20}\text{O}_2 \): 185.1536 \([\text{M+H}]^+\). Found: 185.1532

**S-4-Iodo-undecanoic acid methyl ester 164**

To a stirred solution of \( R \)-5-Heptyl-dihydro-furan-2-one (0.815 g, 4.43 mmol, 1.0 equiv) under argon in DCM (15 ml) that was protected from light was added iodosiltrimethylsilane (2.4 ml, 17.72 mmol, 4.0 equiv) dropwise. The resulting pale yellow solution was left to stir at room temperature until the lactone ring-opening was complete as judged by \(^1\text{H}-\text{NMR}\). Upon completion anhydrous methanol (0.90 ml, 22.15 mmol, 5.0 equiv) was added. The reaction was monitored by \(^1\text{H}-\text{NMR}\) until complete ester formation (as judged by comparison of integral values of signals corresponding to H-8 and H-12). The reaction was then diluted with DCM (10 ml) and \( \text{Na}_2\text{S}_2\text{O}_3 \) (10 %, 30 ml) added. The aqueous layer was then extracted with diethyl ether (2 x 20 ml) and all organic fractions were combined, dried over magnesium sulphate and evaporated *in vacuo*. The residual pale yellow oil was purified by flash column chromatography (silica, 1 % diethyl ether/petroleum ether) to leave the desired compound as a colourless oil.

Yield: 1.38 g (96 %)

\(^1\text{H}-\text{NMR} \) (CDCl\(_3\), 400MHz) \( \delta \): 0.81 (t, 3H, H-1), 1.22 (m, 8H, H-2-H-5), 1.35 (m, 1H, H-6a), 1.45 (m, 1H, H-6b), 1.65 (m, 1H, H-7a), 1.81 (m, 1H, H-7b), 2.00 (q, 2H, 8.0 Hz, H-9), 2.46 (m, 2H, H-10), 3.61 (s, 3H, H-12), 4.05 (m, 1H, H-8)

\(^{13}\text{C}-\text{NMR} \) (CDCl\(_3\), 100MHz) \( \delta \): 13.99 (C-1), 22.53, 28.65, 29.01, 29.36 and 31.67 (C2-C-6), 34.06 (C-10), 35.35 (C-9), 38.18 (C-8), 40.64 (C-7), 51.57 (C-12), 172.95 (C-11)

IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 2953, 2854, 1755, 1095, 586

\([\alpha]_D^{25}\): -3.52 (c = 1.0, CHCl\(_3\))
**R-4-lodo-undecanoic acid methyl ester 164**

Method as for S-4-lodo-undecanoic acid methyl ester above.

Yield: 1.26 g (92 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.81 (t, 3H, H-1), 1.22 (m, 8H, H-2-H-5), 1.35 (m, 1H, H-6a), 1.45 (m, 1H, H-6b), 1.65 (m, 1H, H-7a), 1.81 (m, 1H, H-7b), 2.00 (q, 2H, 8.0 Hz, H-9), 2.46 (m, 2H, H-10), 3.61 (s, 3H, H-12), 4.05 (m, 1H, H-8)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 13.99 (C-1), 22.53, 28.65, 29.01, 29.36 and 31.67 (C2-C-6), 34.06 (C-10), 35.35 (C-9), 38.18 (C-8), 40.64 (C-7), 51.57 (C-12), 172.95 (C-11)

IR ($\nu_{max}$/cm$^{-1}$): 2952, 2858, 1761, 1111, 582

$[\alpha]_D^{25}$: + 3.50 (c = 1.0, CHCl$_3$)

HRMS: m/z calculated for C$_{12}$H$_{23}$IO$_2$: 327.0815 [M+H]$^+$. Found: 327.0812

**R-4-D$_{1}$-Undecanoic acid methyl ester 165**

To a solution of S-4-lodo-undecanoic acid methyl ester (1.38 g, 4.23 mmol, 1.0 equiv) in dimethylsulphoxide (20 ml) was added sodium borodeuteride (0.355 g, 8.46 mmol, 2.0 equiv). The resulting suspension was heated to 85 °C for 4 hours (complete by TLC). The reaction was quenched by the addition of HCl (aq., 2 N, 20 ml) after which the mixture was diluted with water (30 ml) and extracted with petroleum ether (3 x 40 ml). The combined organic fractions were dried over magnesium sulphate and evaporated in vacuo to leave the desired
compound as a pale pink oil. The product was purified by flash column chromatography (silica, 2 % diethyl ether/petroleum ether) to leave the desired compound as a colourless oil.

Yield: 0.733 g (86 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.85 (t, 3H, H-1), 1.23 (m, 13H, H-2-H-8), 1.58 (m, 2H, H-9), 2.27 (t, 2H, 7.5 Hz, H-10), 3.63 (s, 3H, H-12).

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 14.63 (C-1), 25.42 (C-9), 29.30 (3 signals, C-8), 23.23, 29.72, 29.86, 29.98, 30.10 and 32.44 (C-2-C-7), 34.63 (C-10), 51.92 (C-12), 174.84 (C-11).

IR ($v_{\text{max}}$/cm$^{-1}$): 3012, 2963, 2878, 1743

HRMS: $m/z$ calculated for C$_{12}$H$_{23}$DO$_2$: 202.1912 [M+H]$^+$. Found: 202.1916

*S-4-D$_1$-Undecanoic acid methyl ester 165*

Method as for *R-4-D$_1$-Undecanoic acid methyl ester above.

Yield: 0.453 g (93 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.85 (t, 3H, H-1), 1.23 (m, 13H, H-2-H-8), 1.58 (m, 2H, H-9), 2.27 (t, 2H, 7.5 Hz, H-10), 3.63 (s, 3H, H-12).

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 14.61 (C-1), 25.84 (C-9), 29.32 (3 signals, C-8), 23.41, 29.77, 29.81, 29.94, 30.20 and 32.51 (C-2-C-7), 34.41 (C-10), 51.98 (C-12), 174.89 (C-11).

IR ($v_{\text{max}}$/cm$^{-1}$): 3012, 2963, 2878, 1742

HRMS: $m/z$ calculated for C$_{12}$H$_{23}$DO$_2$: 202.1912 [M+H]$^+$. Found: 202.1914
R-4-D₃-Undecanoic acid 152

![R-4-D₃-Undecanoic acid structure](image)

To a solution of R-4-D₃-Undecanoic acid methyl ester (0.668 g, 3.33 mmol) in THF (50 ml) was added NaOH (aq, 2N, 50 ml) and the resulting immiscible liquids stirred vigorously under reflux (75 °C) overnight. The aqueous layer was then separated and acidified (HCl. aq, 10 N). The acidified aqueous layer was then extracted with diethyl ether (3 x 50 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml), and the combined organic extracts were washed with brine (40 ml). The organic layer was washed with brine (40 ml), and the combined organic extracts were dried over magnesium sulphate and evaporated in vacuo leave the desired compound as a white solid.

Yield: 0.558 g (90 %)

¹H-NMR (CDCl₃, 400MHz) δ: 0.85 (t, 3H, H-1), 1.23 (m, 13H, H-2-H-8), 1.58 (m, 2H, H-9), 2.37 (t, 2H, 7.5 Hz, H-10).

¹³C-NMR (CDCl₃, 100MHz) δ: 14.08 (C-1), 24.58 (C-9), 28.66 (3 signals, C-8), 22.68, 29.15, 29.31, 29.42, 29.56 and 31.90 (C-2-C-8), 34.13 (C-10), 180.68 (C-11)

IR (νmax/cm⁻¹): 3005, 2963, 1705

m.p.: 28 – 31 °C [undecanoic acid lit. 28.5 °C]

HRMS: m/z calculated for C₁₁H₂₁DO₂: 188.1755 [M+H]+. Found: 188.1756

S-4-D₃-Undecanoic acid 152

![S-4-D₃-Undecanoic acid structure](image)

Method as for R-4-D₃-Undecanoic acid above.

Yield: 0.318 g (75 %)
$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.85 (t, 3H, H-1), 1.23 (m, 13H, H-2-H-8), 1.58 (m, 2H, H-9), 2.37 (t, 2H, 7.5 Hz, H-10).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.12 (C-1), 24.56 (C-9), 28.65 (3 signals, C-8), 22.63, 29.18, 29.33, 29.48, 29.55 and 31.95 (C-2-C-8), 34.18 (C-10), 180.67 (C-11)

IR (ν$_{max}$/cm$^{-1}$): 2998, 2965, 1707

m.p.: 29 – 31 °C [undecanoic acid lit. 28.5 °C]

HRMS: m/z calculated for C$_{11}$H$_{21}$DO$_2$: 188.1755 [M+H]$^+$. Found: 188.1757

**General procedure for acid catalysed coupling of bicyclic and monocyclic compounds**

To a suspension of the corresponding bicyclic aldehyde (0.25 mmol, 1.0 equiv) and corresponding substituted pyrrole (0.30 mmol, 1.2 equiv) in methanol (5 ml) was added methanolic HCl (2 N, 100 μl). The resulting brightly coloured solution was stirred for 3 hours at room temperature. The solvent was then removed *in vacuo* and the resulting solid dissolved in ethyl acetate (20 ml), and washed with saturated aqueous sodium bicarbonate (2 x 25 ml) and brine (30 ml). Resulting organic layer was dried over magnesium sulphate and the solvent removed *in vacuo* to leave a brightly coloured crude solid, which was purified by flash column chromatography.

**Undecylprodiginine**

Crude residue was not washed with sodium carbonate prior to purification. Residue was purified using flash column chromatography over silica gel with gradient elution of 0-70% diethyl ether/petroleum ether.
51 mg (52 %)

$^1$H-NMR (CDCl$_3$) δ: 0.80 (t, 3H, 7.0 Hz, H-24), 1.20 (m, 18H, H-15-H-23), 2.10 (m, 2H, H-14), 3.90 (s, 3H, H-25), 5.75 (d, 1H, 3.5 Hz, H-11), 5.95 (s, 1H, H-6), 6.05 (t, 1H, 3.5 Hz, H-3), 6.40 (d, 1H, 3.5 Hz, H-12), 6.55 (d, 1H, 3.5 Hz, H-4), 6.60 (d, 1H, 4.0 Hz, H-2), 6.75 (s, 1H, H-9)

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.28, 22.85, 27.49, 29.34, 29.53, 29.54, 29.74, 29.78, 29.83, 29.89, 32.09, 58.51, 95.80, 108.71, 110.19, 112.88, 116.14, 116.17, 122.98, 128.48, 128.54, 138.83, 144.62, 160.22, 169.16.

IR (ν$_{max}$/cm$^{-1}$): 3267, 3102, 2919, 2849, 1613, 1575, 1561, 1547

m.p.: 86-87 °C

HRMS: m/z calculated for C$_{25}$H$_{35}$N$_3$O: 394.2853 [M+H]$^+$. Found: 394.2855

**Pyrrolyl-2,4-dimethylprodiginine**

![Pyrrolyl-2,4-dimethylprodiginine](image)

Crude residue was not washed with sodium carbonate prior to purification.

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-70% ethyl acetate/hexane. Compound appears as two rotational conformers on $^1$H-NMR timescale. Peaks unresolved at 298 K.

41 mg (62 %)

Conformer 1: $^1$H-NMR (CDCl$_3$ at 233 K, 500MHz) δ: 1.50 (s, 3H, H-14), 2.05 (s, 3H, H-15), 3.90 (s, 3H, H-16), 5.55 (s, 1H, H-12), 6.00 (d, 1H, 3.0 Hz, H-2), 6.10 (s, 1H, H-6), 6.50 (d, 1H, 3.0 Hz, H-4), 6.55 (t, 1H, 3.0 Hz, H-3), 6.80 (s, 1H, H-9), 11.16 (s, 1H, NH), 12.30 (s, 1H, NH).
Conformer 2: $^1$H-NMR (CDCl$_3$ at 233 K, 500MHz) $\delta$: 2.20 (s, 3H, H-14), 2.50 (s, 3H, H-15), 3.95 (s, 3H, H-16), 5.95 (d, 1H, 3.5 Hz, H-2), 6.05 (s, 1H, H-12), 6.30 (d, 1H, 3.5 Hz, H-4), 6.85 (t, 1H, 3.5 Hz, H-3), 6.90 (s, 1H, H-6), 7.15 (s, 1H, H-9), 12.25 (s, 1H, NH), 12.45 (s, 1H, NH)

IR ($\nu_{\text{max}}$/cm$^{-1}$): 3215, 2936, 2856, 1645, 1598, 1564

HRMS: $m/z$ calculated for C$_{16}$H$_{17}$N$_3$O: 268.1444 [M+H]$^+$. Found: 268.1449

**Indolyl undecylprodiginine analogue**

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-20 % diethyl ether/petroleum ether.

16 mg (18 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 0.80 (t, 3H, 7.0 Hz, H-28), 1.20 (m, 16H, H-27-H-20), 1.60 (m, 2H, H-19), 2.60 (t, 2H, 7.5 Hz, H-18), 3.80 (s, 3H, H-29), 5.75 (s, 1H, H-10), 5.95 (d, 1H, 3.5 Hz, H-15), 6.50 (d, 1H, 3.5 Hz, H-16), 6.85 (s, 1H, H-8), 6.90 (s, 1H, H-13), 7.15 (t, 1H, 6.5 Hz, H-4), 7.25 (t, 1H, 7.5 Hz, H-5), 7.50 (d, 1H, 7.5 Hz, H-6), 8.05 (d, 1H, 7.5 Hz, H-3)

$^{13}$C-NMR (CDCl$_3$, 100MHz) $\delta$: 14.12, 28.41, 28.93, 29.34, 29.36, 29.41, 29.54, 29.60, 29.64, 31.90, 58.27, 83.78, 97.91, 109.47, 111.53, 114.68, 118.75, 121.15, 121.54, 122.95, 125.19, 128.93, 129.70, 136.59, 138.26, 150.34, 159.57, 166.85.

IR ($\nu_{\text{max}}$/cm$^{-1}$): 3250, 3118, 2923, 2852, 1732, 1623, 1571, 1547

m.p.: 58-59 °C

HRMS: $m/z$ calculated for C$_{29}$H$_{37}$N$_3$O: 444.3009 [M+H]$^+$. Found: 444.3008
Indolyl-2,4-dimethylprodiginine

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-20% ethyl acetate/hexane.

43 mg (50 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 1.65 (s, 3H, H-18), 2.05 (s, 3H, H-19), 4.00 (s, 3H, H-20), 5.60 (s, 1H, H-16), 6.30 (s, 1H, H-10), 6.65 (d, 1H, 6.0 Hz, H-6), 6.80 (s, 1H, H-8), 6.85 (t, 1H, 6.0 Hz, H-4), 6.90 (t, 1H, 6.0 Hz, H-5), 7.00 (s, 1H, H-13), 7.40 (d, 1H, 6.0 Hz, H-3)

IR ($\nu_{\text{max}}$/cm$^{-1}$): 3242, 2945, 1735, 1635, 1575, 1542

HRMS: $m/z$ calculated for C$_{20}$H$_{19}$N$_3$O: 318.1601 [M+H]$^+$. Found: 318.1606

Thiophenylundecylprodiginine analogue

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-30% diethyl ether/petroleum ether.

54 mg (52 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.80 (t, 3H, 7.0 Hz, H-24), 1.20 (m, 16H, H16-H-23), 1.70 (m, 2H, H-15), 2.70 (t, 2H, 7.0 Hz, H-14), 3.80 (s, 3H, H-25), 5.85 (s, 1H, H-6), 5.95 (d, 1H, 3.5 Hz, H-11), 6.50 (d, 1H, 3.5 Hz, H-12), 6.75 (s, 1H, H-9), 7.05 (dd, 1H, 4.0, 3.0 Hz, H-3), 7.30 (d, 1H, 4.0 Hz, H-4), 7.40 (d, 1H, 3.0 Hz, H-2).
\[ \text{13C-NMR (CDCl}_3, \text{ 100MHz) } \delta: 14.27, 22.83, 28.55, 28.94, 29.50, 29.59, 29.65, 29.78, 29.83, 29.87, 32.06, 58.46, 94.81, 109.50, 117.72, 120.37, 127.02, 127.89, 128.05, 130.04, 140.81, 141.15, 143.38, 160.14, 168.41. \]

IR (\( \nu_{\text{max}} / \text{cm}^{-1} \)): 3254, 2917, 2848, 1603, 1542

m.p.: 87-88 °C

HRMS: \( m/z \) calculated for \( \text{C}_{25}\text{H}_{34}\text{N}_2\text{OS} \): 411.2465 [M+H]+. Found: 411.2464

**Thiophenyl-2,4-dimethylprodiginine**

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-30% diethyl ether/petroleum ether.

37 mg (52 %)

\[ \text{1H-NMR (CDCl}_3, \text{ 400MHz) } \delta: 2.05 \text{ (s, 3H, H-14), 2.30 \text{ (s, 3H, H-15), 3.85 \text{ (s, 3H, H-16), 5.80 \text{ (s, 1H, H-12), 5.90 \text{ (s, 1H, H-6), 6.80 \text{ (s, 1H, H-9), 7.00 \text{ (t, 1H, 4.0 Hz, H-3), 7.30 \text{ (d, 1H, 4.0 Hz, H-2), 7.40 \text{ (d, 1H, 4.0 Hz, H-4).}}} \}

IR (\( \nu_{\text{max}} / \text{cm}^{-1} \)): 3231, 2951, 2865, 1689, 1515

HRMS: \( m/z \) calculated for \( \text{C}_{16}\text{H}_{10}\text{N}_2\text{OS} \): 285.1056 [M+H]+. Found: 285.1056
**Furanyl undecylprodiginine analogue**

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-30% diethyl ether/petroleum ether.

15 mg (15 %)

\(^1\)H-NMR (CDCl\(_3\), 400MHz) \(\delta: 0.80\) (t, 3H, 7.0 Hz, H-24), \(1.20\) (m, 16H, H-16-H-23), \(1.70\) (m, 2H, H-15), \(2.65\) (t, 2H, 7.0 Hz, H-14), \(3.80\) (s, 3H, H-25), \(5.90\) (d, 1H, 3.5 Hz, H-11), \(5.95\) (d, 1H, 3.5 Hz, H-12), \(6.45\) (dd, 1H, 4.0, 3.0 Hz, H-3), \(6.50\) (d, 1H, 4.0 Hz, H-2), \(6.80\) (s, 1H, H-6), \(6.90\) (d, 1H, 3.0 Hz, H-4), \(7.50\) (s, 1H, H-9)

\(^{13}\)C-NMR (CDCl\(_3\), 100MHz) \(\delta: 14.58, 23.68, 28.25, 28.68, 29.11, 29.31, 29.42, 29.45, 29.68, 29.88, 32.98, 58.15, 96.84, 110.15, 115.84, 121.21, 126.31, 127.96, 128.35, 132.65, 143.65, 145.84, 146.84, 163.84, 170.32.

IR (\(\nu_{max}/cm^{-1}\)): 3261, 2951, 2922, 2848, 1612, 1579, 1541

m.p.: 70-71 °C

HRMS: \(m/z\) calculated for C\(_{25}\)H\(_{34}\)N\(_2\)O\(_2\): 395.2693 [M+H]\(^+\). Found: 395.2694

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**Furanyl-2,4-dimethylprodiginine**

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-40% ethyl acetate/hexane.

23 mg (34 %)
$^1$H-NMR (CDCl$_3$, 400MHz) δ: 2.20 (s, 3H, H-14), 2.35 (s, 3H, H-15), 3.85 (s, 3H, H-16), 5.80 (s, 1H, H-12), 5.90 (s, 1H, H-6), 6.45 (t, 1H, 3.0 Hz, H-3), 6.85 (s, 1H, H-9), 6.90 (d, 1H, 3.0 Hz, H-2), 7.45 (d, 1H, 3.0 Hz, H-4)

IR (ν$_{max}$/cm$^{-1}$): 3294, 2913, 2865, 1635, 1568, 1512

HRMS: m/z calculated for C$_{16}$H$_{16}$N$_2$O$_2$: 269.1285 [M+H]$^+$. Found: 269.1287

Phenyl undecylprodiginine analogue

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-20% ethyl acetate/hexane.

79 mg (97 %)

$^1$H-NMR (CDCl$_3$, 400MHz) δ: 0.80 (t, 3H, 6.5 Hz, H-26), 1.20 (m, 16H, H-18-H-25), 1.65 (m, 2H, H-17), 2.65 (t, 1H, 7.0 Hz, H-16), 3.80 (s, 1H, H-27), 5.90 (d, 1H, 3.5 Hz, H-13), 6.00 (s, 1H, H-8), 6.50 (d, 1H, 3.5 Hz, H-14), 6.80 (s, 1H, H-11), 7.35 (m, 3H, H-3-H-5), 7.90 (d, 2H, 7.0 Hz, H-2 and H-6).

$^{13}$C-NMR (CDCl$_3$, 100MHz) δ: 14.26, 22.82, 28.59, 29.04, 29.48, 29.51, 29.64, 29.65, 29.75, 29.80, 32.04, 58.39, 94.73, 109.63, 118.45, 120.68, 126.87, 128.63, 129.69, 130.11, 135.23, 140.94, 143.67, 165.56, 168.53.

IR (ν$_{max}$/cm$^{-1}$): 3251, 2960, 2917, 2848, 1613, 1594, 1579, 1542

m.p.: 100-101 °C

HRMS: m/z calculated for C$_{27}$H$_{36}$N$_2$O: 405.2900 [M+H]$^+$. Found: 405.2897
Phenyl-2,4-dimethylprodiginine

Crude residue purified using flash column chromatography over silica gel with gradient elution of 0-20% diethyl ether/hexane.

44 mg (79 %)

$^1$H-NMR (CDCl$_3$, 400MHz) $\delta$: 2.10 (s, 3H, H-16), 2.30 (s, 3H, H-17), 3.80 (s, 3H, H-18), 5.75 (s, 1H, H-14), 6.00 (s, 1H, H-8), 6.85 (s, 1H, H-11), 7.30 (m, 3H, H-3-H-5), 7.90 (d, 2H, 7.0 Hz, H-2 and H-6).

IR (\nu_{max}/cm^{-1}): 3261, 2953, 2945, 2831, 1648, 1589, 1519

HRMS: m/z calculated for C$_{18}$H$_{18}$N$_2$O: 279.1492 [M+H]$^+$. Found: 279.1493
### 7.2 Summary of selected characteristic resonances from $^1$H-NMR spectroscopy analyses of streptorubin B analogues from mutasynthesis experiments

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<td>(a) 3.34</td>
<td>(a) 3.34</td>
<td>(a) 3.30-3.34</td>
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<tr>
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<td>(b) 2.56</td>
<td>(b) 2.54</td>
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</tr>
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<td>(b) -1.54</td>
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<td>(b) -1.54 or 0.26</td>
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<td>3.12</td>
<td>3.10 or 3.02</td>
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<td>6.51</td>
<td>6.52 or 6.50</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1’</td>
<td>(a) 3.34</td>
<td>(a) 3.34</td>
<td>(a) 3.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) 2.54</td>
<td>(b) 2.54</td>
<td>(b) 2.53</td>
<td></td>
</tr>
<tr>
<td>4’</td>
<td>(a) 1.11-1.89</td>
<td>(a) 1.12 - 1.91</td>
<td>(a) 1.11- 1.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) -1.54</td>
<td>(b) -1.54</td>
<td>(b) -1.54</td>
<td></td>
</tr>
<tr>
<td>7’</td>
<td>3.10</td>
<td>3.06</td>
<td>3.26 or 3.21</td>
<td></td>
</tr>
<tr>
<td>10’</td>
<td>1.32-1.42</td>
<td>1.51-1.67</td>
<td>1.31-1.43</td>
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</tr>
<tr>
<td>11’</td>
<td>0.90-0.97</td>
<td>~ 0.84 – 0.89</td>
<td>0.89-0.91</td>
<td></td>
</tr>
<tr>
<td>12’</td>
<td>n/a</td>
<td>~ 0.84 – 0.89</td>
<td>0.81-0.85</td>
<td></td>
</tr>
<tr>
<td>1”</td>
<td>7.12</td>
<td>7.12</td>
<td>7.12</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proton number</th>
<th>Undecylprodiginine (DCM)</th>
<th>136b (DCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5</td>
<td>7.27</td>
<td>7.26</td>
</tr>
<tr>
<td>1”</td>
<td>7.06</td>
<td>7.06</td>
</tr>
<tr>
<td>C3</td>
<td>7.02</td>
<td>7.03</td>
</tr>
<tr>
<td>A3</td>
<td>6.89</td>
<td>6.89</td>
</tr>
<tr>
<td>A4</td>
<td>6.39</td>
<td>6.40</td>
</tr>
<tr>
<td>C2</td>
<td>6.24</td>
<td>6.24</td>
</tr>
<tr>
<td>B4</td>
<td>6.17</td>
<td>6.17</td>
</tr>
<tr>
<td>C6′-C8′</td>
<td>n/a</td>
<td>5.36-5.06 (multiplet)</td>
</tr>
<tr>
<td>-OCH$_3$</td>
<td>4.03</td>
<td>4.03</td>
</tr>
<tr>
<td>1’</td>
<td>2.92</td>
<td>2.91</td>
</tr>
<tr>
<td>11’</td>
<td>0.87</td>
<td>0.83       (triplet)</td>
</tr>
</tbody>
</table>

1′ (a) 3.34  
(b) 2.54  
1″ 7.12
7.3 General procedure for Streptomyces sp. spore stock creation

To mannitol-soy flour agar plates was added 100 μl of sterile water, followed by 10 μl of spore suspension and the spores were spread across the surface of the plate. After incubation for 3 days at 30 °C sterile water (5 ml) was added to the plate, and an inoculation loop was used to loosen the spores on the surface. The spores suspended in the water were transferred to a syringe with a glass wool filter, and filtered into a 15 ml sterile tube to remove mycelial fragments. The water was then centrifuged for 10 min at 4000 rpm to pellet the spores, the supernatant was discarded and the spores then re-suspended in the remaining water. The spore suspension was then diluted with water (300 μl) and transferred to a cryotube, to which 50 % glycerol solution (300 μl) was added. The resulting spore suspension in 25 % glycerol was stored at -20 °C for future use.

7.4 Feeding of synthetic MBC analogues for mutasynthesis and restoration experiments

R5 agar plates were overlaid with sterile permeable membranes (12 – 14 kDa molecular weight cut off). Sterile water (100 μl) was added to the plates, followed by the spore suspension (10 μl) and the spores were spread across the surface of the plate. After 3 days of incubation at 30 °C, the desired MBC (or MBC analogue) in DMSO (200 μl, 5 mg/ml) was diluted with water (2 ml) and the resulting suspension was added to the plate in small droplets across the surface. The plate was incubated at 30 °C for a further 2 days. The membrane was then peeled off and the mycelia were placed into a 15 ml centrifuge tube.
Acetonitrile:Methanol (1:1, 10 ml) + 0.1%(v/v) HCl (aq, 2 N) was added followed by sonication (approximately 5 minutes) to break up the mycelia. The resulting suspension was then centrifuged at 4000 rpm for 10 min and the organic supernatants were analysed by LC-MS.

7.5 **Feeding of synthetic 2-undecylpyrrole analogues for large scale mutasynthesis and isolation**

25 square 50 ml R5 agar plates were overlaid with sterile permeable membranes (12 - 14 KDa molecular weight cut-off). Sterile water (200 μl) was added to the plates, followed by the spore suspension (20 μl) and the spores were spread across the surface of the plate. After 3 days of incubation at 30 °C, the desired 2-undecylpyrrole analogue in methanol (200 μl, 5 mg/ml) was diluted with water (2 ml) and the resulting suspension was added to each plate in small droplets across the surface. The plates were incubated at 30 °C for a further 2 days. The membranes were then peeled off and the mycelia combined into four 50 ml centrifuge tubes. Methanol (20 ml) + 0.1%(v/v) HCl (aq, 2 N) was added followed by sonication (approximately 5 minutes) to break up the mycelia. The resulting suspension was then centrifuged at 4000 rpm for 10 min and the organic supernatants were combined. The mycelia were then further extracted with two further sequences of methanol (20 ml) + 0.1%(v/v) HCl (aq, 2 N) and centrifugation (4000 rpm for 10 min). The combined bright red organic extracts were then concentrated in vacuo, and the resulting residue was then partitioned between water (pH 3, 100 ml) and chloroform (100 ml) and filtered under vacuum to remove residual mycelial fragments. The resulting biphasic mixture was then diluted with water (pH 3, 100 ml), and the deep red organic layer was separated. The resulting
aqueous layer was then further extracted with chloroform (2 x 100 ml) and the resulting organic extracts were combined, dried over magnesium sulphate and concentrated in vacuo to leave a dark red viscous oil.

7.6 LC-MS analysis

Liquid chromatography – mass spectrometry was used to analyse the extracts from feeding studies. An Agilent 1100 HPLC instrument connected to a Bruker HCT+ mass spectrometer was used. Samples were analysed on an Agilent Eclipse XDB-C8 column (4.6 mm x 150 mm, 5 μm, column temperature 25 °C) using the elution profile in Table 1 below.

Table 1: Elution profile for LC-MS analysis of prodiginines

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Water (pH 3 with HCl)</th>
<th>Acetonitrile (or Methanol)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>50</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>75</td>
<td>1.3</td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>80</td>
<td>1.4</td>
</tr>
<tr>
<td>23</td>
<td>50</td>
<td>50</td>
<td>1.0</td>
</tr>
</tbody>
</table>

7.7 Semi-preparative liquid chromatography purification of streptorubin B and analogues

The crude mixture isolated from the mycelial extraction procedure was purified through basic alumina, eluting with hexane/ethyl acetate (0-15 %) as follows. Initial washing of the column with hexane yielded a black pigmented mixture of compounds which was discarded. Subsequent washing with 5-15 % hexane/ethyl acetate yielded one small fraction of a yellow pigment fraction
which was also discarded followed by a large brightly coloured yellow pigmented fraction. Elution with 15% ethyl acetate/hexane was then continued until the pigmentation of the fractions faded. The later yellow pigmented fraction was evaporated to dryness in vacuo to leave a bright yellowish red semi-crude mixture of undecylprodiginine (or an analogue) and streptorubin B (or an analogue) as their free bases. The mixture was then dissolved in chloroform and washed with water (pH 3 with HCl.). The aqueous layer was then separated and extracted with two further portions of chloroform. The combined organic fractions were then dried over magnesium sulphate and evaporated to dryness in vacuo to leave a bright red semi-crude mixture of undecylprodiginine (or an analogue) and streptorubin B (or an analogue).

An Agilent 1200 HPLC instrument was used to purify the desired streptorubin B (or an analogue) from the semi-crude mixture of undecylprodiginine (or an analogue) and streptorubin B (or an analogue) obtained after purification through basic alumina. Streptorubin B (or an analogue) was purified first on an Agilent Zorbax XDB-C18 column (21.2 x 150 mm, 5 μm, column temperature 25 °C) using the elution profiles detailed below in either Table 2, Table 3 or Table 4.

Table 2: 1st purification for streptorubin B and for all analogues (with the exception of C9'-methyl branched analogue and the C3'-oxa analogue).

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Water (pH 3 with HCl)</th>
<th>MeOH (+ 0.1 % 2N HCl)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>30</td>
<td>70</td>
<td>20.0</td>
</tr>
<tr>
<td>5.00</td>
<td>25</td>
<td>75</td>
<td>20.0</td>
</tr>
<tr>
<td>7.50</td>
<td>20</td>
<td>80</td>
<td>20.0</td>
</tr>
<tr>
<td>10.00</td>
<td>10</td>
<td>90</td>
<td>20.0</td>
</tr>
<tr>
<td>11.25</td>
<td>10</td>
<td>90</td>
<td>20.0</td>
</tr>
<tr>
<td>11.50</td>
<td>30</td>
<td>70</td>
<td>20.0</td>
</tr>
<tr>
<td>12.50</td>
<td>30</td>
<td>70</td>
<td>20.0</td>
</tr>
</tbody>
</table>
Table 3: 1st purification for C9'-methyl branched analogue

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Water (pH 3 with HCl)</th>
<th>MeOH (+0.1 % 2N HCl)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>28</td>
<td>72</td>
<td>20.0</td>
</tr>
<tr>
<td>5.00</td>
<td>25</td>
<td>75</td>
<td>20.0</td>
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<tr>
<td>7.50</td>
<td>24</td>
<td>76</td>
<td>20.0</td>
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<tr>
<td>13.00</td>
<td>24</td>
<td>76</td>
<td>20.0</td>
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<td>14.00</td>
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<td>90</td>
<td>20.0</td>
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<td>16.00</td>
<td>10</td>
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</tr>
<tr>
<td>16.25</td>
<td>28</td>
<td>72</td>
<td>20.0</td>
</tr>
<tr>
<td>17.00</td>
<td>28</td>
<td>72</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Table 4: 1st purification for C3'-oxa analogue

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Water (pH 3 with HCl)</th>
<th>MeOH (+0.1 % 2N HCl)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>32</td>
<td>68</td>
<td>20.0</td>
</tr>
<tr>
<td>5.00</td>
<td>29</td>
<td>71</td>
<td>20.0</td>
</tr>
<tr>
<td>7.50</td>
<td>20</td>
<td>80</td>
<td>20.0</td>
</tr>
<tr>
<td>10.00</td>
<td>10</td>
<td>90</td>
<td>20.0</td>
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<tr>
<td>11.25</td>
<td>10</td>
<td>90</td>
<td>20.0</td>
</tr>
<tr>
<td>11.50</td>
<td>32</td>
<td>68</td>
<td>20.0</td>
</tr>
<tr>
<td>12.50</td>
<td>32</td>
<td>68</td>
<td>20.0</td>
</tr>
</tbody>
</table>

The products with retention times as listed below (Table 5) were collected by an automated fraction collector system triggered by either time or peak gradient. The combined fractions were then partitioned between chloroform and water (pH 3, with HCl). The aqueous layer was separated and extracted with two further portions of chloroform. The combined organic extracts were then washed with water, dried over magnesium sulphate and evaporated *in vacuo* to leave the streptorubin B (or an analogue) as a bright red solid.
Table 5: Retention time of carbocyclic prodiginines during first purification on C18 column

<table>
<thead>
<tr>
<th>Prodiginine</th>
<th>Retention time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptorubin B</td>
<td>6.5 – 7.8</td>
</tr>
<tr>
<td>C10 streptorubin B analogue</td>
<td>5.5 – 6.5</td>
</tr>
<tr>
<td>C12 streptorubin B analogue</td>
<td>8.2 – 9.3</td>
</tr>
<tr>
<td>Alkenyl streptorubin B analogue</td>
<td>4.0 – 5.0</td>
</tr>
<tr>
<td>C10 methyl branched streptorubin B analogue</td>
<td>5.7 – 6.2</td>
</tr>
<tr>
<td>C9 methyl branched streptorubin B analogue</td>
<td>6.2 – 7.5</td>
</tr>
<tr>
<td>C2-C4 ether linked streptorubin B analogue</td>
<td>3.5 – 4.8</td>
</tr>
</tbody>
</table>

The resulting solid was re-dissolved in methanol for subsequent additional purification on the same Agilent 1200 HPLC system. An Agilent Zorbax SB-Phenyl column (21.2 x 250 mm, 7 μm, column temperature 25 °C) was used with the elution profile detailed below in Table 6.

Table 6: 2nd Purification for streptorubin B and all analogues

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Water (pH 3 with HCl)</th>
<th>MeOH (+0.1 % 2N HCl)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>24</td>
<td>76</td>
<td>20.0</td>
</tr>
<tr>
<td>10.00</td>
<td>17</td>
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<td>20.0</td>
</tr>
<tr>
<td>16.50</td>
<td>24</td>
<td>76</td>
<td>20.0</td>
</tr>
</tbody>
</table>
7.8 Stereochemical analyses of streptorubin B and analogues by CD spectroscopy

A Jasco J-815 CD spectrometer was used to record the circular dichroism spectra of streptorubin B (or an analogue) and metacycloprodigiosin. Samples were dissolved in spectroscopic grade methanol (Sigma-Aldrich) and the CD spectrum from 700 to 200 nm of each solution was measured in a 1 mm path length cuvette. Instrument settings were as follows: resolution 0.4 nm, bandwidth 2.0 nm, sensitivity 200 mdeg, response 1 s, speed 200 nm/min. The concentration of each sample was subsequently determined from the intensities of the signal at 533 nm in the UV-vis chromatogram (recorded on a Perkin-Elmer Lambda 35 UV/VIS spectrometer, 1 cm path length) of each compound with the known extinction coefficient (100 500 M⁻¹ cm⁻¹) after an appropriate dilution.

7.9 Stereochemical analyses of streptorubin B and analogues by HPLC on a homochiral stationary phase

Liquid chromatography on a homochiral stationary phase was used to analyse streptorubin B (or an analogue) and metacycloprodigiosin. Samples were prepared as the corresponding free base before analyses by dissolving in hexane/isopropanol (95:5) + 0.1 % diethylamine. An Agilent 1100 HPLC instrument was used. Samples were analysed on a Chiral Technologies Europe ChiralPak IA column (4.6 mm x 250 mm, column temperature 20 °C) using the elution profile in Table 7 below.
Table 7: Elution profile for HPLC analyses of prodiginines on a homochiral stationary phase.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Hexane (+0.1 % diethylamine)</th>
<th>Isopropanol (+0.1 % diethylamine)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>95</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>20.00</td>
<td>95</td>
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<td>95</td>
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<tr>
<td>60.00</td>
<td>95</td>
<td>5</td>
<td>1.0</td>
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</table>

7.10 Stereochemical analyses of lactone intermediates (R)- and (S)-151 by HPLC on a homochiral stationary phase

Liquid chromatography on a homochiral stationary phase was used to analyse the lactone intermediates (R)- and (S)-151. Samples were prepared by dissolving in hexane/isopropanol. An Agilent 1100 HPLC instrument was used. Samples were analysed on a Chiral Technologies Europe ChiralPak IA column (4.6 mm x 250 mm, column temperature 20 °C) using the elution profile in Table 8 below.

Table 8: Elution profile for HPLC analyses of lactones (R)- and (S)-151 on a homochiral stationary phase.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Hexane</th>
<th>Isopropanol</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>96</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>10.00</td>
<td>95</td>
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<td>1.0</td>
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<td>25.00</td>
<td>95</td>
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<tr>
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</tr>
<tr>
<td>30.00</td>
<td>96</td>
<td>4</td>
<td>1.0</td>
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8.0 References


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