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Myxovirescin Analogues Via Macrocyclic Ring–Closing Metathesis

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Abstract—A short, efficient route has been developed to analogues of myxovirescin using ring–closing metathesis whereby the antibacterial activity has been retained.

Myxovirescin A_1 (Antibiotic TA) **1** is a broad spectrum, antibiotic produced by gliding bacteria of the *Myxococcus* species.[1,2] It represents a new class of antibiotic, unrelated to existing agents and having a unique mode of action.[3] Thus it was an attractive starting point for a semi-synthetic program aimed at finding an analogue with improved antibacterial potency and physicochemical properties (Fig. 1).

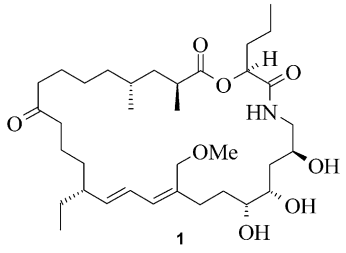

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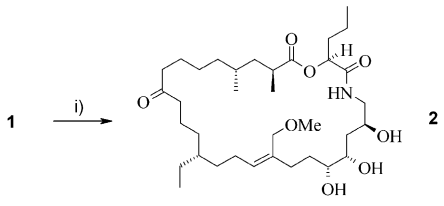
Figure 1. Myxovirescin A1.

Such a program would require bulk supply of Myxovirescin but the latter was available only in very limited quantities by fermentation. Its structural complexity precludes a full synthetic approach, since the two different total syntheses which have been reported require a large number of steps (>40).[4] Based on very limited SAR,[5] published studies show that removal of some substitution on the left-hand side does not affect activity. In addition, we prepared the novel analogue, dihydro-myxovirescin A1 (**2**) (Scheme 1) and showed it to be at least equipotent to myxovirescin A1 against a range of bacteria in vitro (Table 1).

We predicted that **3** would be expected to retain antibacterial activity. Total synthesis of this compound, however, would still require a large number of steps. Extending this by removing all of the left side chiral groups in the final target would mean that a synthetic approach to analogues such as **4** was feasible (Fig. 2).

Key challenges in the synthesis of **4** include the generation of four chiral centres and closure to form the 28-membered ring. Macrolactamisation or lactonisation as the final connecting step seemed unattractive as the previous two reported total syntheses of myxovirescins required functional group manipulations that significantly lengthened the synthesis. A more efficient disconnection is of the double bond and we opted to form the olefin via a ring–closing metathesis as the key step. This had advantages over prior methods and a more conventional Wittig or Julia approach for double bond formation because of the mildness of the reaction and the wide variety of functional groups tolerated. The double bonds would serve as ideal masked functionality.

At the time of this work, ring-closing metathesis to form tri-substituted olefins had received little attention in the literature because of its inherent difficulty. Precedence



Scheme 1. Reagents and conditions: (i) Pd/C, H_2, MeOH.

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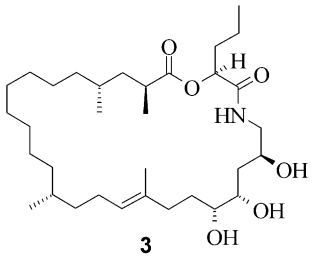
Table 1. Antibacterial data for Myxovirescin and the synthetic analogues

Species	ATCC[a] number	Myxovirescin A1	Dihydro-myxovirescin A1	4	15	16
		MIC[b] μg/mL		Active at 200 μg/mL		
Corynebacterium sp.	68984	> 50	12.5	Y	N	Y
Pasteurella sp.	67535	12.5	0.78	Y	N	Y
Pasteurella sp.	67777	12.5	1.56	N	N	N
Pasteurella sp.	69527	25	3.12	N	N	N
Pasteurella sp.	71886	12.5	0.78	N	N	N
Pasteurella multocida	61096	> 50	1.56	N	N	N
Pasteurella multocida	63560	> 50	0.78	N	N	N
Actinomyces meyeri	61789	> 50	12.5	Y	Y	Y
Actinomyces sp.	70710	> 50		Y	N	Y
Bacteroides bivus	64362	> 50	6.25	N	N	N
Bacteroides buccae	67792	12.5	> 50	N	N	N
Bacteroides capillosus	60802	25	12.5	N	N	N
Bacteroides oralis	72708	> 50	6.25	N	N	Y
Bacteroides ovatus	66640	> 50	12.5	N	N	Y
Bacteroides uniformis	62723	> 50	12.5	N	N	Y
Fusobacterium mortiferum	63425	12.5	> 50	N	N	N
Fusobacterium sp.	74714	> 50		N	N	N
Propionibacterium sp.	72661	> 50		Y	N	Y
Porphyromonas gingivalis	D23C	25	1.56	N	N	Y
Porphyromonas canoris	NCTC 12835	25		Y	Y	Y
Porphyromonas canoris	NCTC 12836	25		Y	Y	Y
Escherichia coli	NCTC 10418	6.25	3.12	N	N	N
Staphylococcus aureus	NCTC 6571	> 50	> 50	N	N	Y

[a]ATCC number is the reference number in the American Type Culture Collection.
[b]MIC is the minimum inhibitory concentration and was determined using the agar method.

came from Hoveyda's pioneering approach to the 14-membered macrocycle, Fluvirucin B_1, using Schrock's molybdenum catalyst.[6] A key emerging area of thought for successful macrocyclic metathesis is the evidence for 'relay' functionality that assists the reaction. Coordination of this polar group onto the merging carbene unit helps to assemble the reacting sites within a coordination sphere of the metal and thus favours cyclisation over competing oligomerisation pathways.[7] In our case we had an ester and amide bond directly opposite the site of ring-closure and at a large enough distance from the nearest oxygen and the alkene to avoid making the chelate too stable. The key disconnections and fragments we employed are shown in Scheme 2.



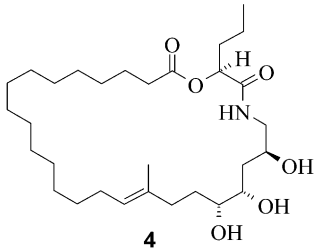
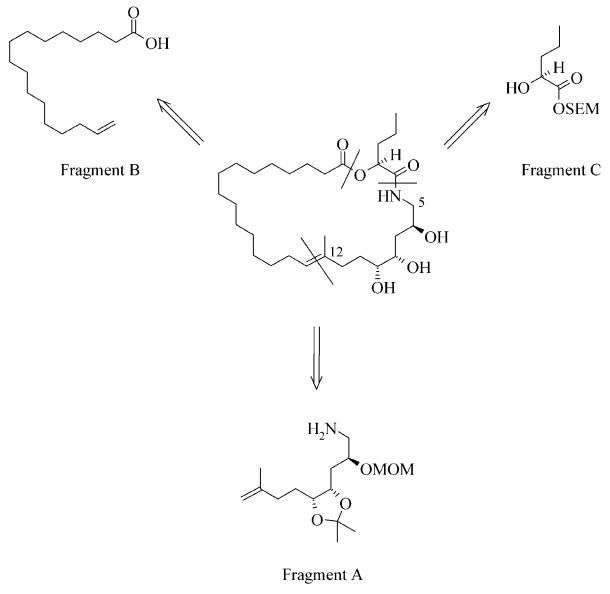


Figure 2. Minimum pharmacophore **3** and our simplified analogue **4**.

Our initial studies focused on the preparation of the C_5-C_{12} segment or fragment A (myxovirescin numbering) with particular attention paid to the generation of the asymmetric triol as illustrated in Scheme 3. We used the protocol from William's published work in the synthesis of this fragment.[4e] Use of a readily available carbohydrate precursor would establish the triol with the correct absolute stereochemistry. Thus, commercially available α-D-mannopyranoside was converted into the known hemiacetal **5** using a reported protocol.[8] This served as a substrate for Wittig elongation of the carbon chain. Catalytic propionic acid was added to limit formation



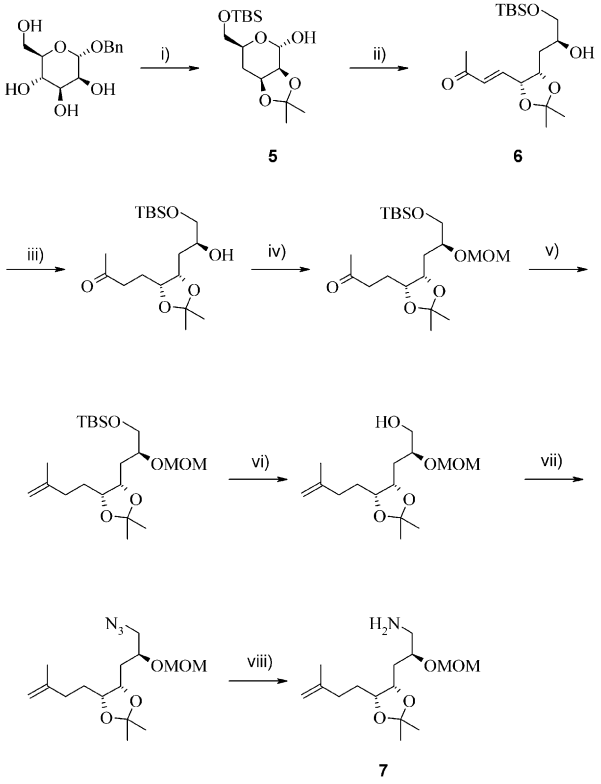
Scheme 2. Disconnection of **4**.

of tetrahydropyrans, derived from intramolecular Michael addition of the α,β-unsaturated product **6**, by protonation of the initial formed alkoxide.[9] Application of this modification led to the enone in a 70% yield with isolation of about 15% of Michael products. The enone was selectively reduced with H_2 and Rh/Al_2O_3 and the free hydroxyl protected as a MOM ether. The ketone was converted into an *exo*-methylene, and the silyl group removed with TBAF. The primary amine was obtained via mesylation of the alcohol followed by displacement with lithium azide and reduction with triphenylphosphine in refluxing THF to give **7**.

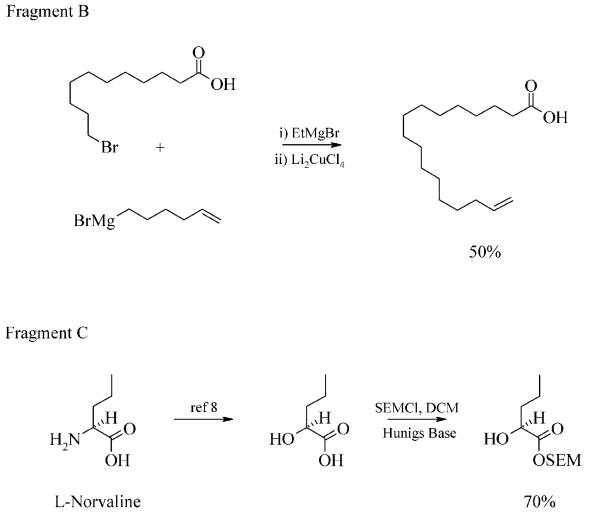
Heptadec-16-enoic acid (fragment B) was readily prepared from a copper catalysed coupling of 11-bromoundecanoic acid and the Grignard derived from bromohexene.[10] 2-(*S*)-hydroxyvaleric acid (fragment C) was obtained from L-Norvaline by double inversion of an intermediate nitronium species.[11] The acid was then protected as its SEM ester ready for coupling (Scheme 4).

Fragments B and C were coupled together using 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide (CMC) as the coupling agent. The SEM group was removed under mild conditions using $MgBr_2$ at −20 °C avoiding epimerisation. **8** was then coupled with fragment A, again, using the CMC coupling reagent to give the metathesis precursor **9** (Scheme 5).

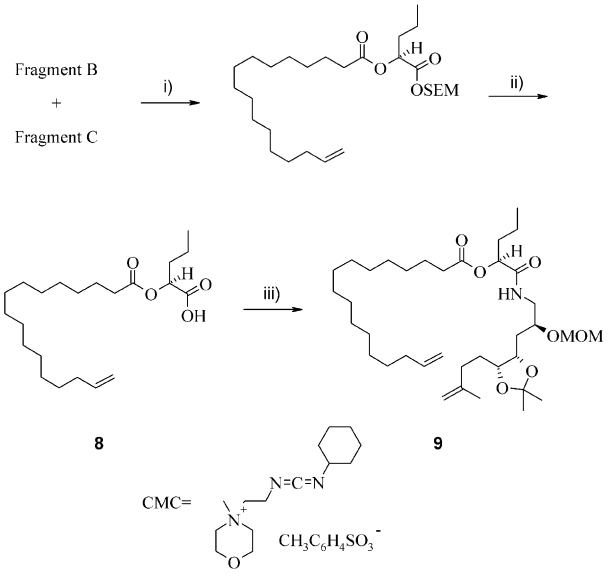
Based on previous reported studies on medium and large ring synthesis, elevated temperatures would be necessary for effective macrocyclisation. Of the commercial catalyst available at the time, the reactive Schrock molybdenum catalyst had been shown to more easily undergo metathesis to form tri-substituted double bonds than the Grubbs' ruthenium equivalent.[12] The biggest disadvantage of the former, however, is its instability to both air and moisture.[13] Novel reactive second generation ruthenium catalysts have since been reported to readily affect the transformation to form tri-substituted double bonds using the Arduengo/Wanzlick carbene type ligands.[14] In any case, when the diene was treated with a high loading of Mo catalyst **10** (50%) in benzene (0.01M) and heated to 60 °C for 15 h the macrocycle was obtained in 55% yield as a 2:1 mixture of inseparable isomers. The remaining mass balance was attributed to oligomerisation, forming predominantly the dimer. With lower catalyst loadings the yield was greatly reduced, adding higher amounts seemed to have no improved effect (Scheme 6). Acid promoted deprotection



Scheme 3. Reagents and conditions: (i) ref 8; (ii) acetonyltriphenylphosphorane, propionic acid, 70%; (iii) H_2, Rh/Al_2O_3 90%; (iv) MOMCl, iPr_2EtN, 90%; (v) Ph_3PCH_2Br, *t*-BuOK, 98%; (vi) TBAF, THF, 99%; (vii) (a) MsCl, Et_3N; (b) LiN_3, DMF 99% over two steps; (viii) Ph_3P, aq THF, 99%.



Scheme 4.



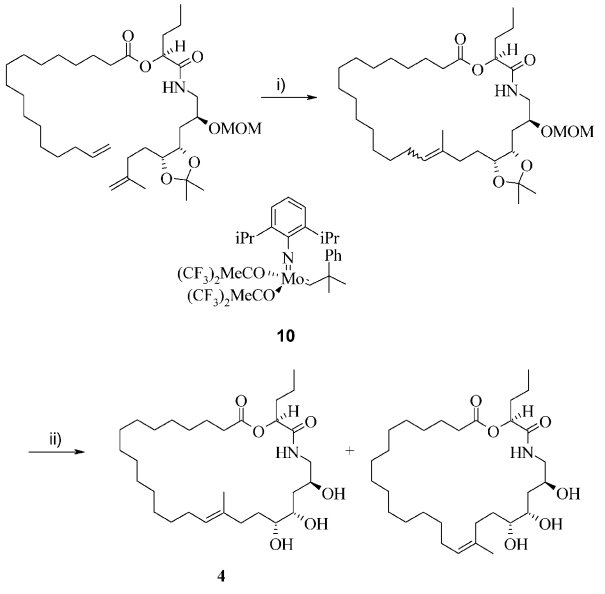
Scheme 5. Reagents and conditions: (i) CMC, DCM, DMAP, 95%; (ii) $MgBr_2{\cdot}Et_2O$, 90%; (iii) CMC, DCM, fragment A, 95%.

of the acetal groups was achieved by stirring in aqueous methanol/THF to afford the triol, **4**. The two isomers were now readily separable. The desired *E* isomer was assigned as the major component by nmr analysis. The antibacterial activity is shown in Table 1.

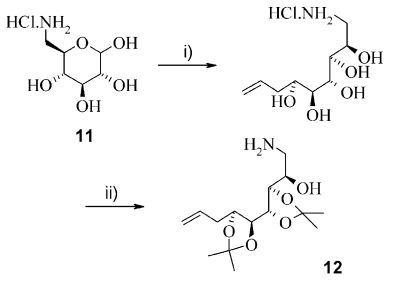
As a follow-up to this exciting result we wanted to produce further analogues in which the triol unit of the molecule was varied. The aim of this was two-fold:

1. To return the lipophilicity to a similar value to myxovirescin by introducing extra hydroxyl groups.
2. To shorten the synthesis by starting from a commercially available amino-sugar.

The most suitable amino-sugar was **11**, which is commercially available from Lancaster. We needed to introduce a terminal olefin, which is required for the ring-closing metathesis, and add three carbons to the sugar to give the right chain–length. This was attempted using indium chemistry since organometallic reagents derived from indium can be used in the protic solvents necessary to solubilise unprotected amino-sugars. However, previous attempts to allylate amino-sugars using



Scheme 6. Reagents and conditions: (i) **10**, benzene, 60 °C, 55% 2:1 mixture of isomers; (ii) $HClO_4$, THF, MeOH, H_2O, 70%.



Scheme 7. Reagents and conditions: (i) In, allyl bromide, EtOH, H_2O; (ii) acetone, TsOH, dimethoxypropane, 20 °C, 18 h, 20% over 2 steps.

either tin or indium had failed.[15] Thus it was unsurprising when the reaction using methallyl bromide gave a very low yield, especially since the latter is known to be a poor substrate for the indium reaction.[15]

However, on repeating the reaction using the more reactive allyl bromide, followed by protection of the product as a bis-acetonide,[16] a workable overall yield of 20% was obtained as shown in Scheme 7.

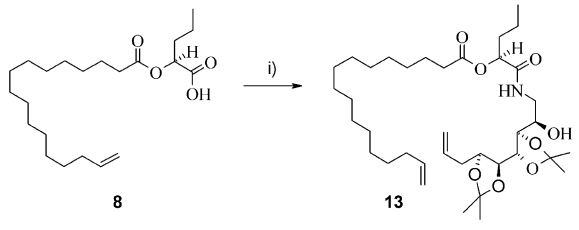
Thus our second objective had been met, since the synthesis of the corresponding protected triol required for **4**, although high-yielding, was 12 steps long compared to just two for this bis-acetonide **12**. The stereochemistry of the new chiral centre formed in this reaction was assigned by analogy to the corresponding reaction with glucose.[15] The next step was to couple the amine, **12** with the chiral acid **6**, this was achieved using CMC again as the coupling agent to furnish the metathesis precursor (Scheme 8).

The corresponding metathesis reaction towards **4** had required the use of the highly unstable, molybdenum-based, Schrock's catalyst, but this is not compatible with unprotected hydroxyl groups. However, **13** does not possess a disubstituted olefin so it should be possible to use the more robust, ruthenium-based, Grubbs' catalyst. Thus the reaction was attempted using this catalyst in methylene chloride under argon. This worked cleanly at the first attempt to give the desired macrocycle, **14** in 90% yield. The methylene chloride was first degassed by sparging with argon, then addition of the catalyst was performed in a glove box under argon. **14** was then deprotected to yield the desired myxovirescin analogue **15** by treatment with aqueous acetic acid. The postulated geometry of the double bond of the major product was assigned by analogy with **4** (Scheme 9).

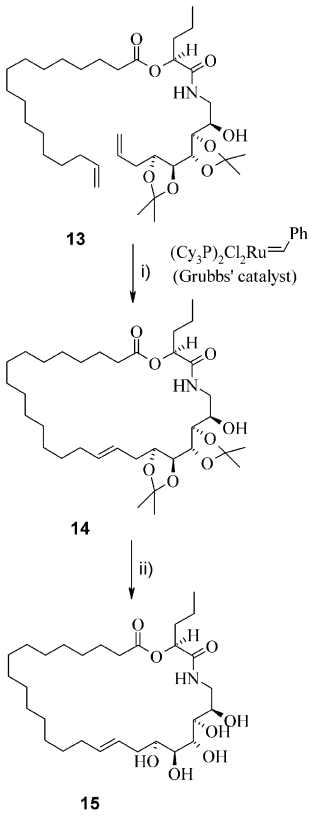
Prior to completing this sequence with the chiral acid **8** the route was pioneered using the racemic acid. Yields were comparable and the final product **16** was a mixture of epimers at the α position to the amide.

Gratifyingly both **15** and the corresponding mixture of epimers **16** showed similar antibacterial activity to **4** indicating that there is scope to modify the triol unit and retain activity. The antibacterial data is shown in Table 1.

In summary, we have developed a novel synthesis of the myxovirescin template and shown that ring-closing metathesis is a viable method for the formation of trisubstituted double bonds in very large rings. In addition,



Scheme 8. Reagents and conditions: (i) CMC, **12**, CH_2Cl_2, 20 °C, 1 h.



Scheme 9. Reagents and conditions: (i) Grubbs' catalyst, CH_2Cl_2 under Ar, 20 °C, 18 h, 90%; (ii) AcOH, H_2O, 60 °C, 64%.

starting from a commercially available amino-sugar, a short efficient route has been developed to analogues of myxovirescin in which the triol unit has been varied and antibacterial activity has been retained. The results indicate that there is considerable scope to vary both the number and stereochemistry of the hydroxyls in myxovirescin and work is underway to develop short routes to other amino-sugars that could be used as starting materials.

Acknowledgements

Thanks to Neil Cheesman for the supply of 16-heptadecenoic acid, to Susana Torrente for the provision of **8** and to Tony Cardno for determining the antibacterial activity of the compounds, data is listed in Table 1.

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