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Short Research Article

Methods for the synthesis of carbon-13 labelled acids and esters†

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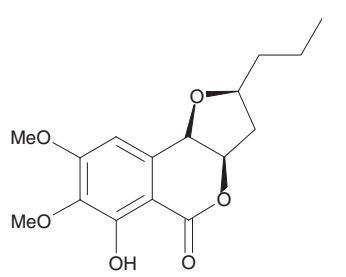
Abstract: Syntheses of isotopically labelled putative biosynthetic intermediates to the natural products monocerin **1**, hectochlorin **2** and strobilurin A **3** are described. For the preparation of [9,10-$^{13}C_2$]dihydroisocoumarin **10**, a stereoselective aldol condensation of $^{13}C_2$-acetylated chiral auxiliary **5** was used to assemble the labelled C9-C14 fragment. The preferred approaches to the syntheses of [1,2-$^{13}C_2$]5,5-dichlorohexanoic acid **15** and the *N*-acetylcysteamine derivative of [1,2-$^{13}C_2$]cinnamic acid **19** involved a Horner-Wadsworth-Emmons chain extension and Knoevenagel reaction, respectively. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: aldol reaction; carbon-13 labelling; Horner–Wadsworth–Emmons; Knoevenagel

Introduction

Substrates incorporating stable isotopic labels have proved valuable for a range of studies in bio-organic chemistry and in particular for the elucidation of biosynthetic pathways. However, feeding studies to intact organisms with labelled putative biosynthetic intermediates often lead to a variable level of incorporation of isotopic label into the final metabolite. An effective method to detect a low level of incorporation of carbon-13 into a metabolite is to use precursors with two carbon-13 labels located at vicinal sites and to detect the ^{13}C–^{13}C coupling by ^{13}C-NMR spectroscopy.[1] Thus efficient and flexible methods are required to introduce the vicinal carbon-13 labels. Herein methods are described for the synthesis of carbon-13 labelled putative biosynthetic intermediates to the natural products monocerin **1**, hectochlorin **2** and strobilurin A **3** using three different approaches: a stereoselective aldol reaction, Horner-Wadsworth-Emmons chain extension and the Knoevenagel reaction.



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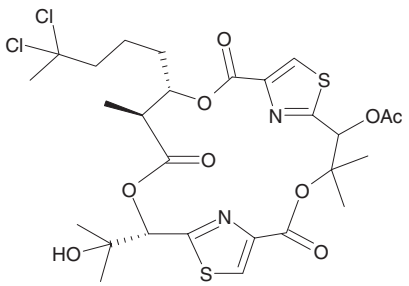

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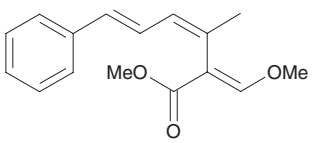

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Results and discussion

Synthesis and incubation studies with (9,10-$^{13}C_2$) dihydroisocoumarin 10

Monocerin **1** is a polyketide derived natural product which has been isolated from a number of fungi

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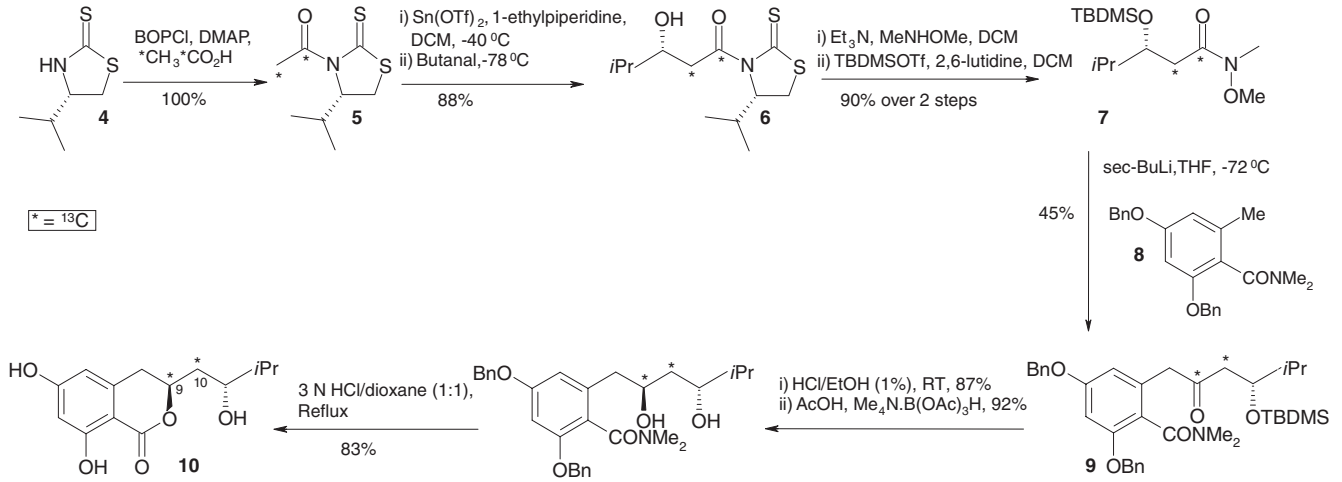

Scheme 1 Synthesis of [9,10-$^{13}C_2$]dihydroisocoumarin 10.

including *Dreschlera monoceras* and *Dreschlera ravenelii*.[2] Results from feeding studies using isotopically labelled sodium acetate with *D. ravenelii*[3] led to the proposal that dihydroisocoumarin **10** is the first polyketide synthase (PKS) free intermediate in the biosynthesis of monocerin. Hence **10** was required in isotopically labelled form for incorporation studies. A stereoselective aldol reaction was used to prepare the labelled C9-C14 fragment using the sulfur containing thiazolidinethione auxiliary **4** (Scheme 1).

Originally the mixed anhydride generated from sodium $^{13}C_2$-acetate and pivaloyl chloride was used to acylate the sodium salt of thiazolidinethione **4** giving **5** in 35% yield.[4] Recently we have found that a more efficient approach to **5** involves treatment of auxiliary **4** with $^{13}C_2$-acetic acid, BOPCl and catalytic DMAP giving the product in quantitative yield. A stereoselective aldol reaction of acetylated auxiliary **5** with butanal in the presence of tin triflate and 1-ethylpiperidine[5] gave the required alcohol **6** as a single diastereomer.[4] Following cleavage of the auxiliary and formation of the silyl ether, the pivotal step was coupling the resultant Weinreb amide **7** with the anion of benzamide **8** (prepared using *sec*-butyl lithium) to give **9** with the required carbon skeleton of our target **10**. Following further functional group modifications including deprotection of the silyl ether under mild conditions, a stereoselective reduction and finally treatment of the resultant dihydroxyamide with acid, the target [9,10-$^{13}C_2$]dihydroisocoumarin **10** was isolated in good yield. Incubation studies of **10** with *D. ravenelii* showed that an exceptionally high incorporation (60%) of intact isotopic label into monocerin had occurred.[4]

Synthesis of (1,2-$^{13}C_2$)5,5-dichlorohexanoic acid 15

Hectochlorin **2** was isolated from the marine cyanobacterium *Lyngbya majuscula* and possesses a structure in accord with a mixed PKS-NRPS biosynthetic origin.[6] Of particular interest is the unusual dichlorohexanoic acid side chain and a sample of [1,2-$^{13}C_2$]5,5-dichlorohexanoic acid **15** was required to investigate the biosynthesis of hectochlorin. Acetylated chiral thiazolidinethiones, as illustrated above, and other acetylated auxiliaries including oxazolidinones and sultams have been used widely to introduce vicinal $^{13}C_2$ labels. Whilst these starting materials could be considered for the synthesis of **15**, with no asymmetric centre present in the target, a more efficient strategy proved to be assembly of the required C_6-framework via a Horner–Wadsworth–Emmons chain extension of protected 4-oxobutanal **11** which in turn was prepared from ethyl acetoacetate (Scheme 2).

The ketonic carbonyl of ethyl acetoacetate was protected as a cyclic acetal prior to reduction of the ester with diisobutylaluminium hydride (DIBAL) giving aldehyde **11** in 65% yield after purification by column chromatography. Treatment of **11** with commercially available [1,2-$^{13}C_2$]triethylphosphonoacetate gave unsaturated ester **12**. Reduction of **12** with Pd/C under a hydrogen atmosphere, followed by deprotection of the acetal using cerium(III) chloride gave ethyl [1,2-$^{13}C_2$]5-oxohexanoate **13** in 73% yield over the two steps. To complete the synthesis of the target compound **15**, it was necessary to convert ketone **13** to dichloride **14**. Initially geminal dichlorination proved to be problematic e.g. using either PCl_5/DCM/H_2O

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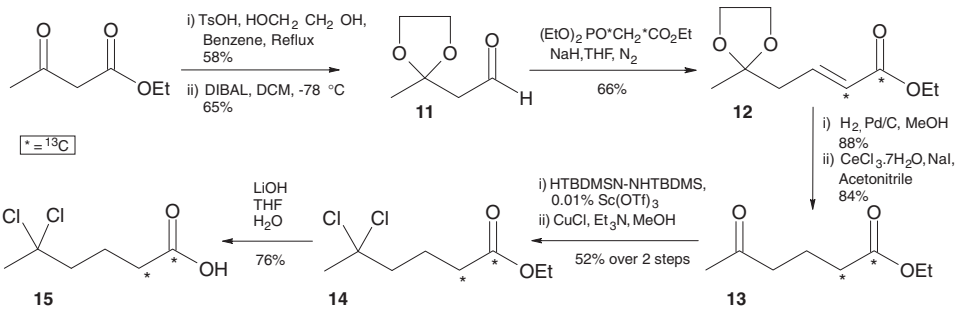
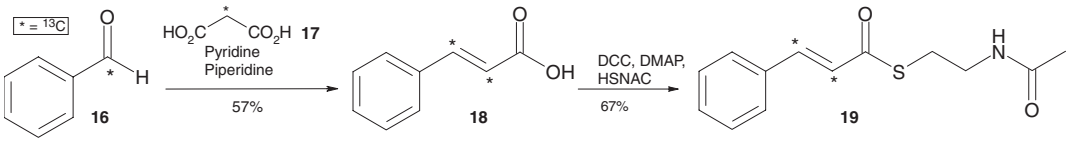


Scheme 2 Synthesis of [1,2-$^{13}C_2$]5,5-dichlorohexanoic acid 15.



Scheme 3 Synthesis of the SNAC derivative of [2,3-$^{13}C_2$]cinnamic acid 19.

followed by alumina/$SOCl_2$[7] or $H_2NNH_2 \cdot H_2O$/molecular sieves/MeOH followed by $CuCl/Et_3N$/MeOH,[8] none of the required product **14** was isolated. Recently Myers and Furrow[9] have reported a valuable modification of the Takeda conditions[8] for the synthesis of *gem*-dihalides from ketones. Using their reagent, prepared by treatment of anhydrous hydrazine (care, potentially explosive) with TBDMSCl, ketone **13** was converted to a hydrazone which on reaction with $CuCl/Et_3N$/MeOH gave dichloro ester **14** in 52% yield. Finally hydrolysis of the ester using lithium hydroxide completed the synthesis of the target [1,2-$^{13}C_2$]5,5-dichlorohexanoic acid **15**.

Synthesis of *N*-acetylcysteamine thiol ester of (2,3-$^{13}C_2$)cinnamic acid 19

Strobilurin A **3** is produced by the fungi *Strobililurus tenacellus* and *Oudemansilla mucida* and is of mixed biosynthetic origin.[10] Feeding studies with labelled precursors have shown that the strobilurins are polyketide in origin with a benzoate starter extended by acetate derived malonate units. The benzoate starter unit itself appears to originate from the shikimate pathway by degradation of phenylalanine to benzoyl-CoA via cinnamic acid prior to assembly. To confirm this proposed pathway the S-*N*-acetyl cysteamine (SNAC) derivative of [2,3-$^{13}C_2$]cinnamic acid **19** was required. It has been shown that often SNAC derivatives are more readily incorporated into a biosynthetic pathway than the parent carboxylic acid–this has been attributed in part to SNAC acting as a mimic of coenzyme A.[11]

A very simple 2-step procedure was used for the synthesis of the required [2,3-$^{13}C_2$]thiol ester **19** involving a Knoevenagel reaction followed by thioesterification. First treatment of commercially available [1-^{13}C]benzaldehyde **16** and [2-^{13}C]malonic acid **17** with a mixture of piperidine and pyridine at reflux gave [2,3-$^{13}C_2$]cinnamic acid **18**. This was followed by a DCC, DMAP mediated coupling of the acid **18** with *N*-acetylcysteamine giving the target compound **19** in 67% yield (Scheme 3).

Conclusion

Three methods have been used for the introduction of vicinal carbon-13 labels into substrates required for biosynthetic studies: an aldol reaction, Horner-Wadsworth-Emmons chain extension and a Knoevenagel reaction. Each is efficient and lends flexibility for the incorporation of vicinal carbon-13 labels into a substrate. The labelled dihydroisocoumarin **10** was incorporated into the fungal metabolite monocerin **1** at an exceptionally high level. Feeding studies with the labelled 5,5-dichlorohexanoic acid **15** and SNAC cinnamic ester **19** are in progress.

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studies on chlorinated marine natural products including hectochlorin.

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