

# An Asymmetric Total Synthesis of (–)-(3*R*)-Inthomycin C

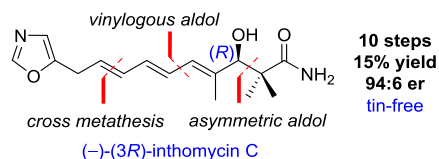
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**ABSTRACT:** A short (ten step) and efficient (15% overall yield) synthesis of the natural product (–)-(3*R*)-inthomycin C is reported. The key steps comprise three C–C bond forming reactions: (i) a vinylogous Mukaiyama aldol, (ii) an olefin cross metathesis and (iii) an asymmetric Mukaiyama-Kiyooka aldol. This route is notable for its brevity and has the advantage of lacking stoichiometric tin-promoted cross-coupling reactions present in previous approaches. Initial investigations on the biological activity of (–)-(3*R*)-inthomycin C and structural analogues on human cancer cell lines are also described for the first time.



The inthomycins are a small family of polyene natural products isolated from *Streptomyces* sp. containing a methylene-interrupted oxazolyl-triene motif common to a broader class of antibiotic natural products known as the oxazolomycins. Differing in the geometry of their respective triene motifs, the inthomycins have attracted considerable attention from synthetic chemists due to their interesting biological activity and closely related structures.

Isolated by Omura in 1990 from *Streptomyces* sp. OM-5714, inthomycin A (4*Z*,6*Z*,8*E*) was the first member of the inthomycin family to be discovered.<sup>1</sup> In 1991, Henkel and Zeeck isolated its geometrical isomers, inthomycin B (4*Z*,6*E*,8*E*) and inthomycin C (4*E*,6*E*,8*E*), Scheme 1, from *Streptomyces* sp. Gö 2 and also re-isolated inthomycin A.<sup>2</sup>

Inthomycin A displays moderate antifungal activity against cellulose-containing *Phytophthora parasitica* and *Phytophthora capsici*.<sup>3</sup> Potent herbicidal activity has also been demonstrated against radish seedlings<sup>3</sup> and velvet leaf<sup>4</sup> in addition to the inhibition of cellulose biosynthesis in *Acetobacter xylinum*.<sup>1</sup> Inthomycins A and B also show *in vitro* inhibitory activity against human prostate cancer growth by suppressing tumor-stromal cell interactions.<sup>5,6</sup> To our knowledge, there have been no reported studies on the biological activity of inthomycin C.

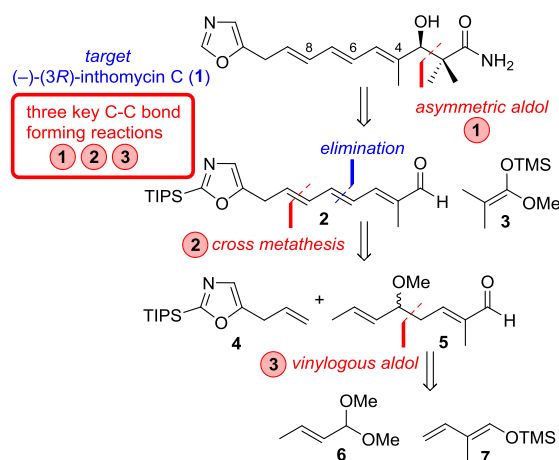
To date, there have been four reported asymmetric total syntheses of inthomycin C by Taylor,<sup>7</sup> Ryu,<sup>8</sup> Hatakeyama<sup>9</sup> and Hale.<sup>10</sup> Additionally, a formal synthesis of racemic material has been reported by Maulide,<sup>11</sup> and an asymmetric formal synthesis has been reported by Reddy.<sup>12</sup> Recently, Hale and Hatakeyama jointly confirmed the absolute configuration of (–)-(3*R*)-inthomycin C by collaboration.<sup>13</sup> It is noteworthy that all published syntheses of (–)-(3*R*)-inthomycin C (ranging between 11–16 steps) rely on a Stille cross-coupling reaction

to form the C7–C8 bond and differ in their approach toward the synthesis of the β-hydroxyl carbonyl moiety.<sup>14</sup>

As part of our ongoing efforts to prepare oxazolomycin B,<sup>15,16</sup> we required an efficient synthesis of (–)-(3*R*)-inthomycin C and sought to differentiate our approach by avoiding the use of the stoichiometric organotin reagents on which all previous syntheses rely. Initial investigations on the biological activity of synthetic (–)-(3*R*)-inthomycin C and structural analogues on human cancer cell lines are also described for the first time.

Our retrosynthetic analysis of (–)-(3*R*)-inthomycin C was based around three key C–C bond forming reactions (Scheme 1). In common with previous work, we planned to use an asymmetric aldol reaction between **2** and **3** to set the C-3 stereocentre. As an alternative disconnection to the triene motif, we envisaged that aldehyde **2** could then be prepared via the cross metathesis (CM) of alkenes **4** and **5**, followed by an elimination reaction, which should afford the thermodynamically most stable (4*E*,6*E*,8*E*) isomer. The final C–C bond forming reaction to investigate would be a (γ-selective) vinylogous aldol reaction between a crotonaldehyde acetal derivative (**6**) and dienyl silyl ether **7** to form alkene **5**.

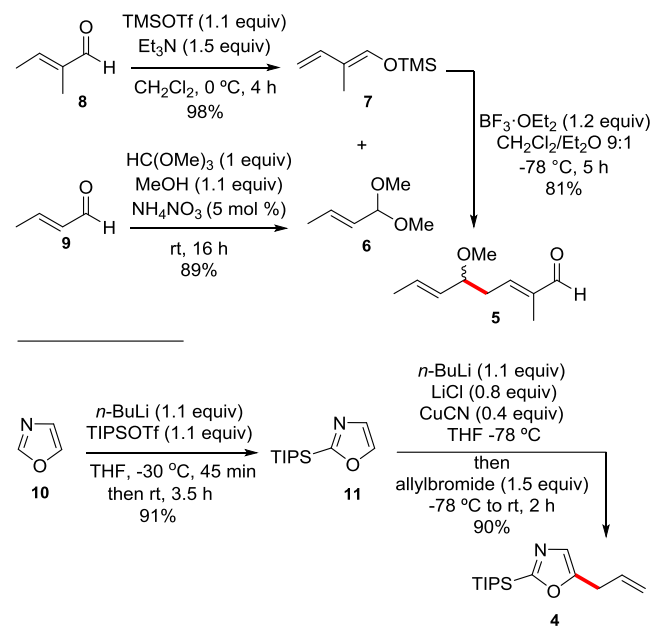
## Scheme 1. Retrosynthesis of (–)-(3*R*)-inthomycin C



We began by preparing the two cross metathesis alkene precursors **4** and **5** (Scheme 2). Tiglic aldehyde **8** was converted into its silyl enol ether **7** (geometry proven by nOe) and this was reacted with acetal **6** (itself prepared in one step from crotonaldehyde **9**). As expected, the vinylogous Mukaiyama aldol reaction of acetal **6** and enol ether **7** took place with high selectivity for the  $\gamma$ -position of the enol ether.<sup>17</sup> Pleasingly, **5** was isolated as the desired *E*-alkene isomer, as proven by extensive nOe measurements.

Separately, and following precedent from Merck, oxazole **10** was lithiated and then protected at the *C*-2 position with a TIPS group (**11**).<sup>18,19</sup> Subsequent lithiation of **11** then took place at the *C*-5 position to furnish allyl TIPS oxazole **4** after transmetalation and quenching *in situ* with allyl bromide.

## Scheme 2. Synthesis of the Cross Metathesis Precursors



With **4** and **5** in hand, we turned to the second of our key C–C bond forming reactions: the cross metathesis to form **12**

(Table 1). This summary of the optimisation results using Hoveyda-Grubbs II (HG-II) catalyst reveals that an excess of the less reactive alkene **5** relative to **4** was necessary in order to achieve good yields of **12** (compare entries 1, 2 and 4).<sup>20,21</sup> When an excess of **4** was used in the CM, significant quantities of the homodimerization product derived from **4** were observed (this product was shown to be almost inactive in a subsequent CM reaction). Screening showed dichloromethane at a temperature of 40 °C to be optimal, with dichloroethane at 80 °C giving no improvement in yield (entry 3). The use of a syringe pump to allow the slow addition of **4**, and in so doing reduced its concentration even further, resulted in an additional increase in yield to 48% (entry 5). Finally, the highest yield of **12** (57%) was obtained by the use of Grubbs II (GII) catalyst under the previously optimised conditions (entry 7). Note that the *trans* stereochemistry of **12** was confirmed by the olefinic coupling constant ( $^3J_{\text{HH}} = 15.4$  Hz).

**Table 1. Optimization of the Cross Metathesis Reaction to form 12**

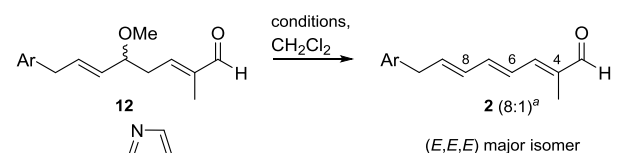
entry	<b>4</b> (equiv)	<b>5</b> (equiv)	catalyst (10 mol %)	solvent	temperature (°C)	time (h)	yield of <b>12</b> <sup>a</sup> (%)
1	3	1	HG-II	CH <sub>2</sub> Cl <sub>2</sub>	40	28	17
2	1	1.5	HG-II	CH <sub>2</sub> Cl <sub>2</sub>	40	26	32
3	1	2	HG-II	DCE	80	23	26
4	1	3.3	HG-II	CH <sub>2</sub> Cl <sub>2</sub>	40	25	41
5 <sup>b</sup>	1	5	HG-II	CH <sub>2</sub> Cl <sub>2</sub>	40	23	48
6 <sup>b</sup>	1	10	HG-II	CH <sub>2</sub> Cl <sub>2</sub>	40	48	41
7 <sup>b</sup>	1	5	G-II	CH <sub>2</sub> Cl <sub>2</sub>	40	51	57


<sup>a</sup>Isolated yields. <sup>b</sup>Syringe pump addition of **4** over 7 h.

With compound **12** in hand, we then looked toward the development of conditions for the elimination of methanol, and formation of the triene motif (Table 2). As shown below, preliminary attempts at elimination employing Brønsted base or acid were unsuccessful (entries 1 and 2).<sup>22</sup> However, we were pleased to observe the formation of **2** in 25% yield when TBSOTf and Et<sub>3</sub>N were employed (entry 3). Only trace amounts of product were observed when Brønsted acids were added after silyl enol ether formation using TBSOTf and Et<sub>3</sub>N (entries 4 and 5). Crucially, the addition of the oxophilic Lewis acid Sc(OTf)<sub>3</sub> to the TBSOTf/Et<sub>3</sub>N mixture afforded **2** in 57% yield, by successfully promoting the elimination of methanol from the silyl enol ether intermediate (entry 6). This reaction was found to be particularly sensitive to water, and the use of freshly distilled Et<sub>3</sub>N and a greater excess of the reagents further increased the yield to 74% (entry 7). The all *E* geometry of **2** was confirmed by olefinic  $^3J_{\text{HH}}$  coupling constant values. In each instance **2** was formed as a mixture of diastereoisomers, typically 8:1 in favour of the desired all *E* isomer. It was possible to enrich the purity of **2** up to 22:1 dr by silica column chromatography, although large quantities could not be isolated with this ratio. However, **2** could be

reliably enriched to ratios of around 14.3:1 dr on scale, and it was this material that we used to continue the synthesis.

**Table 2. Optimization of the Elimination Reaction**



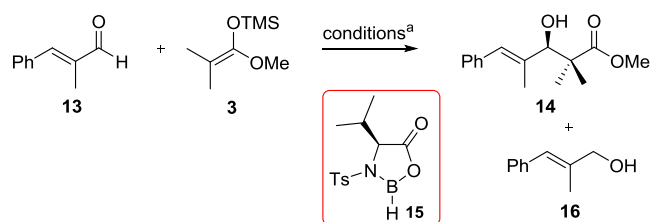
Ar = 

entry	reagents (equiv)	temperature (°C)	time (h)	yield <b>2</b> <sup>b</sup> (%)
1	DBU (2)	rt	6	0
2	AcOH (12)	rt-100	18	0
3	TBSOTf (1.1), Et <sub>3</sub> N (1.5)	0-rt	22	25
4	TBSOTf (1.5), Et <sub>3</sub> N (2); then CSA (1.5)	0-rt	20	trace
5	TBSOTf (2), Et <sub>3</sub> N (3); then Pyr·HBr (2)	0-rt	55	6
6	TBSOTf (1.1), Et <sub>3</sub> N (1.5); then Sc(OTf) <sub>3</sub> (2)	0-rt	29	57
7 <sup>c</sup>	TBSOTf (1.8), Et <sub>3</sub> N (2.8); then Sc(OTf) <sub>3</sub> (2.3)	0-rt	21	74

<sup>a</sup>Although the identity of the minor isomer could not be proven unequivocally, <sup>3</sup>J<sub>HH</sub> values indicate a probable (4*E*,6*E*,8*Z*) geometry. <sup>b</sup>Isolated product obtained as a mixture of isomers, typically 8:1. <sup>c</sup>Freshly distilled Et<sub>3</sub>N.

We next turned our attention toward the asymmetric induction of the (3*R*) stereocentre. A wide range of asymmetric aldol reaction catalysts and conditions were investigated using **13** as a model aldehyde substrate. Of those conditions tested, the most promising were those of Mukaiyama-Kiyooka, as employed previously by Taylor.<sup>7</sup> Under these reaction conditions, chiral oxazaborolidinone **15** is formed *in situ* which promotes the asymmetric reaction of a silyl enol ether (Table 3). Application of these reaction conditions afforded **14** in 73% yield with 94:6 er in addition to a 19% yield of the reduction by-product **16**, (entry 1). Alternative amino acids were also screened at this stage. However *N*-Ts-L-valine was shown to be optimal. Changes in timings designed to combat possible incomplete catalyst formation (entry 2) or incomplete complexation of **13** and **15** (entry 3) both led to decreased yields of **14**. In an attempt to minimise the unwanted reduction process leading to **16**, we investigated reducing the equivalents of borane (entry 4), and alternative borane sources (entry 5). However these did not provide an increase in either the yield or stereoselectivity of the reaction. However, addition of **13** by syringe pump over a 3 h period was found to result in the optimal yield of **14** while maintaining the high enantioselectivity of the reaction (entry 7). Treatment of the crude reaction mixture with 1 M aqueous HCl was found to be necessary to deprotect any TMS protected **14** formed during the reaction. The (*R*) stereochemistry of **14** was confirmed unambiguously via the preparation and analysis of both (*R*)- and (*S*)-MTPA esters.<sup>23</sup>

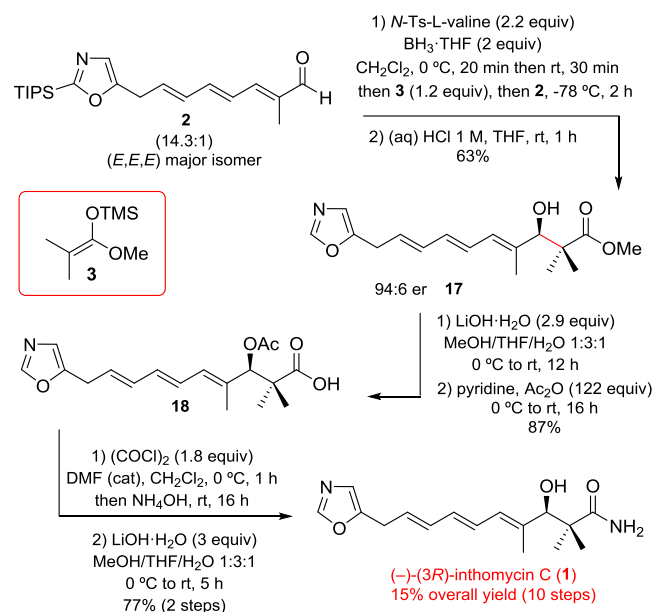
**Table 3. Optimization of the Asymmetric Aldol Conditions**



entry	procedure/ variation	yield <b>14</b> <sup>b</sup> (%)	er <sup>c</sup>	yield <b>16</b> <sup>b</sup> (%)
1	standard. see conditions below <sup>a</sup>	73	94:6	19
2	3 h before addition of <b>13</b>	62	92:8	29
3	addition of <b>3</b> 1 h after <b>13</b>	53	93:7	45
4	BH <sub>3</sub> ·THF (1 equiv)	51	91:9	6
5	BH <sub>3</sub> ·DMS (2 equiv)	91	65:35	0
6	syringe pump addition of <b>13</b> over 3 h	81	93:7	14

<sup>a</sup> *N*-Ts-L-valine (2.2 equiv), BH<sub>3</sub>·THF (2 equiv), CH<sub>2</sub>Cl<sub>2</sub>, 0-rt, 30 min, -78 °C, **13**, then 1.2 equiv **3**, 2 h; ii) 1 M (aq) HCl THF, rt, 1 h. <sup>b</sup> Isolated yields. <sup>c</sup> Determined by HPLC (chiral stationary phase) with reference to a racemic standard.

**Scheme 3. Completion of the Synthesis**



Pleasingly, subjection of **2** to the optimised Mukaiyama-Kiyooka conditions, followed by TIPS deprotection, afforded adduct **17** in 63% yield and with 94:6 er (Scheme 3). We had now completed a formal synthesis of (-)-(3*R*)-inthomycin C and intersected a common intermediate with previous workers in the field. Analytical and spectroscopic data of compound **17** were in close agreement with those previously reported.<sup>7-11</sup> Given the recent debate concerning the absolute stereochemistry of (-)-(3*R*)-inthomycin C, the (*R*)- and (*S*)-MTPA esters of **17** were then prepared and analysis proved unambiguously the (*R*) configuration (see Supporting Information for details).<sup>23</sup>

Ester hydrolysis and acetylation of **17** afforded acid **18** in 87% yield. Finally, amidation via *in situ* acid chloride formation, followed by treatment with lithium hydroxide afforded a 11.1:1 mixture of isomers of (–)-(3*R*)-inthomycin C in 77% yield over 2 steps.<sup>8</sup> Pleasingly, measurement of the specific rotation for synthetic (–)-(3*R*)-inthomycin C obtained in this work  $[\alpha]_D -8.2$  (c 1.0, CHCl<sub>3</sub>) was in close agreement with all values reported previously, with the exception of one.<sup>7</sup> However, thorough collaborative work by the groups of Hale and Hatakeyama has concluded that the previous positive  $[\alpha]_D$  value obtained was erroneous due to the presence of a urea contaminant.<sup>13</sup>

In collaboration with a preclinical validation facility at the Cancer Research UK Oxford Centre, several (–)-(3*R*)-inthomycin C precursors, including the natural product itself, were assayed for viability/cytotoxicity using human cancer cell lines (chosen from HeLa, H460, MCF-7, KMS-12BM and SKOV-3). Despite IC<sub>50</sub> values >50 μM being obtained in each case, further investigation revealed a surprising proteasome inhibition activity for ester **17**. IC<sub>50</sub> values for proteasome inhibition in H460 and KMS-12BM cells were determined as 11 and 32 μM respectively. Subsequent experiments demonstrated that the inhibitory effect of **17** was reversible in nature. To the best of our knowledge there have been no previous reports of similar compounds acting as proteasome inhibitors and given the importance of proteasome inhibition in the field of oncology, we anticipate that this will prove an exciting area of investigation in the future.<sup>24</sup>

In conclusion we have reported the total synthesis of (–)-(3*R*)-inthomycin C in 15% yield (94:6 er) from oxazole over 10 steps from commercially available oxazole. The (3*R*) stereochemistry was confirmed unambiguously by MTPA ester derivatisation, further supporting recent work by Hale and Hatakeyama.<sup>13</sup> A key advantage of this route is its concise nature and scalability (in total ca. 250 mg of **18** was prepared). This study was the first to investigate the biological activity of (–)-(3*R*)-inthomycin C and preceding intermediates in human cancer cell lines, and a surprising proteasome inhibition response was discovered for ester **17**. Work is currently underway in our laboratories toward the total synthesis of oxazolomycin B and will be disclosed in due course.

## ASSOCIATED CONTENT

**Supporting Information** The Supporting Information is available free of charge on the ACS Publications website.

Full experimental details, copies of spectral data (PDF).

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**Notes** The authors declare no competing financial interest.

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