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The Absolute Configuration for Inthomycin C: Revision of Previously Published Work with a Reinstatement of the (3*R*)-Configuration for (−)-Inthomycin C

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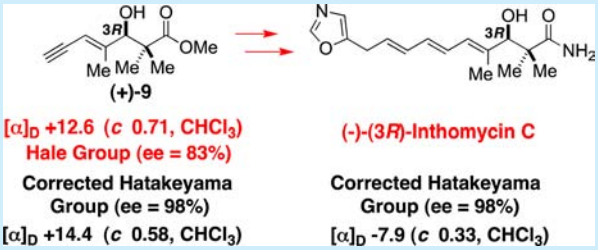
Supporting Information

ABSTRACT: Stereochemical evidence is presented to demonstrate that (−)-inthomycin C has (3*R*)- and not (3*S*)-stereochemistry. Careful reappraisal of the previously published work[2−5] now indicates that the Hatakeyama, Hale, Ryu, and Taylor teams *all* have synthesized (−)-(3*R*)-inthomycin C. The newly measured $[\alpha]_D$ of pure (−)-(3*R*)-inthomycin C (98% ee) is −7.9 (*c* 0.33, $CHCl_3$) and not −41.5 (*c* 0.1, $CHCl_3$) as was previously reported in 2012.

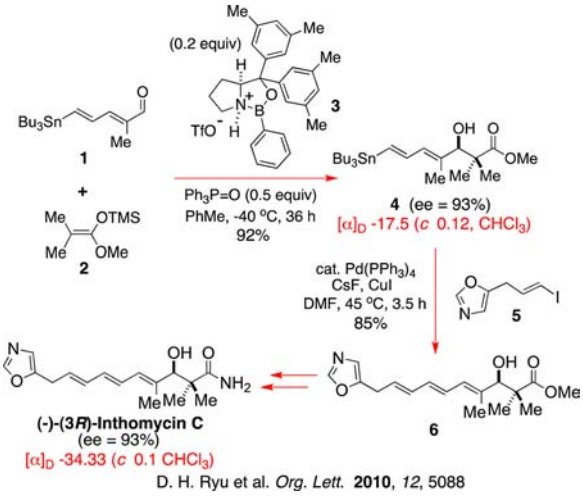


Molecules of the inthomycin/phthoxazolin class[1] continue to excite the organic chemistry community due to their novel oxazolo-triene structures and their diverse biological actions which, in some instances, are associated with significant anticancer, insecticidal, and herbicidal effects. So far, the greater bulk of synthetic effort on this class[2−6] has focused on the total synthesis of (3*R*)-inthomycin C,[2−5] which, despite the small size and deceptively simple structure, has proven a most challenging and troublesome molecule for synthesis due to the diverse collection of unusually interposed functional groups.

The molecular constitution of inthomycin C was first disclosed by Henkel and Zeeck in 1991,[1a] who suggested that inthomycin C possessed (3*R*)-stereochemistry at the hydroxyl stereocenter. This followed derivatization as a 3-O-(*S*)-2-phenylbutanoate ester and careful 2D-NMR comparisons with other family members. Unfortunately, the Zeeck group was never able to measure the $[\alpha]_D$ for natural (3*R*)-inthomycin C, due to their inability to obtain pure material. Subsequently, a successful total synthesis of (3*R*)-inthomycin C by Taylor et al.[2] led to an $[\alpha]_D$ of +25.9 (*c* 0.27, $CHCl_3$) for material of 76% ee, contaminated with 20% tetramethylurea. Thereafter, the natural product was given a (+)-designation.

That status persisted until 2010, when the Ryu group reported a new total synthesis of (−)-(3*R*)-inthomycin C[3] that exploited an asymmetric aldol reaction with the chiral oxazaborolidinium triflate catalyst **3** to provide the C(3)-hydroxyl stereocenter in the target (Scheme 1). Significantly, the Ryu team thereafter recorded an $[\alpha]_D$ of −34.33 (*c* 0.1, $CHCl_3$) for pure (−)-(3*R*)-inthomycin C of 93% ee.[3] Although this $[\alpha]_D$ measurement was similar in magnitude to the value recorded by Taylor et al. (given the ee),[2] the sign was opposite. Despite the discrepancy, the Ryu team never went on to confirm their new (3*R*)-assignment for

Scheme 1. 2010 (−)-(3*R*)-Inthomycin C Synthesis of Ryu et al.



(−)-inthomycin C, basing the assignment solely on analogy with other asymmetric aldol reactions that were described in their *Org. Lett.* paper.[3]

The issue of inthomycin C stereochemistry took a further turn in 2012, when the Hatakeyama laboratory reported a second total synthesis of (−)-(3*R*)-inthomycin C[4] that proceeded by way of the chiral *β*-lactone **8** and the (3*R*)-enynol **9** (Scheme 2). On this occasion, an $[\alpha]_D$ of −41.5 (*c* 0.1, $CHCl_3$) was recorded

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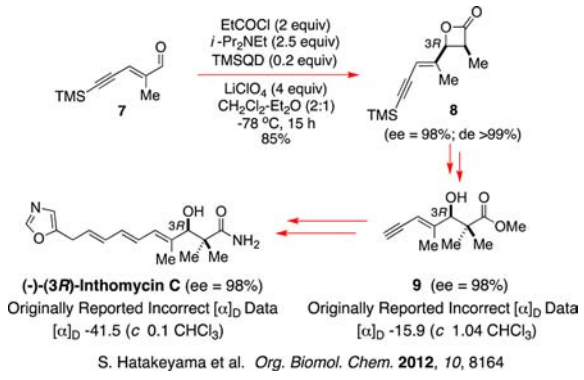
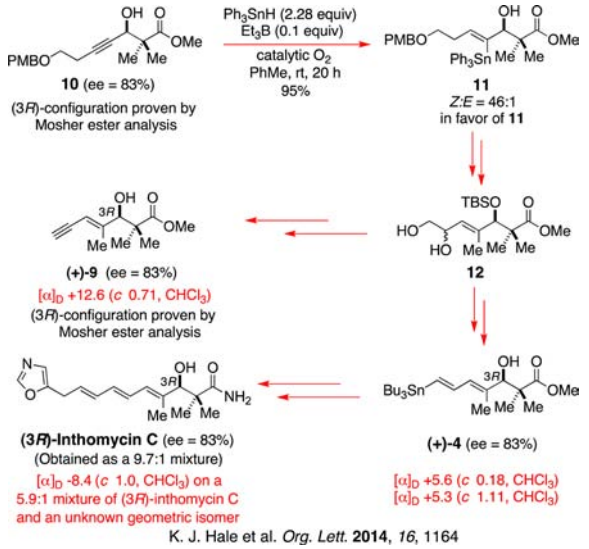
Scheme 2. A Summary of the Stereochemically Correct Total Synthesis of (−)-(3*R*)-Inthomycin C By the Hatakeyama Group[4] with Its Two Incorrect $[\alpha]_D$ Values



S. Hatakeyama et al. *Org. Biomol. Chem.* **2012**, *10*, 8164

Scheme 3. A Summary of the Correct Hale Group Total Synthesis of (3*R*)-Inthomycin C With Its $[\alpha]_D$ Values[5]



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for (−)-(3*R*)-inthomycin C, which possessed 98% ee. The Hatakeyama group's stereochemical assignment was based upon the successful total synthesis of (+)-(3*R*)-inthomycins A and B, which demonstrated that the (3*R*)-configuration emerged from the key TMSQD-catalyzed asymmetric [2 + 2]-ketene aldehyde cycloaddition that was being used to control C(3)-stereochemistry. Given that only the geometry of the enal was changed in this total synthesis of (3*R*)-inthomycin C, the Hatakeyama team saw no reason why such a change would dramatically alter the stereochemical outcome of the new cycloaddition to obtain **8**. Thus, after securing a full total synthesis of (−)-(3*R*)-inthomycin C via the route summarized in Scheme 2, the Hatakeyama group did not question the (3*R*)-assignment that had been made, since Ryu and co-workers had also obtained an $[\alpha]_D$ of −34.33 (*c* 0.1, $CHCl_3$) for a product of apparently identical (3*R*)-configuration and slightly lower ee (93%).[3] The Hatakeyama results were published, with the belief that the (+) $[\alpha]_D$ measurement recorded by Taylor et al.[2] for (3*R*)-inthomycin C might have arisen from the 20% tetramethylurea contaminent that was also present in their sample.

Not long after the Hatakeyama report, another paper appeared in *Org. Lett.* from the Hale laboratory,[5] where a new total synthesis of (3*R*)-inthomycin C was described that intersected directly with the Ryu and Hatakeyama group syntheses. The Hale synthesis used a Carreira asymmetric alkynylation to forge the C(3)-hydroxyl stereochemistry of **10** (Scheme 3) and an O-directed free radical alkyne hydrostannation reaction with Ph_3SnH/catalytic Et_3B to set the target's trisubstituted olefin geometry. Significantly, the Hale team provided the first incontrovertible evidence for the (3*R*)-hydroxyl configuration of dienylstannane **4**, an intermediate that was ultimately used by them to complete their total synthesis. In this regard, Hale et al. reported that **4** had an $[\alpha]_D$ of +5.3 (*c* 1.11, $CHCl_3$).[5] The same (3*R*)-intermediate was also featured in the synthesis of Ryu et al. (see Scheme 1), but strikingly, these workers reported an $[\alpha]_D$ of −17.5 (*c* 0.12, $CHCl_3$) for material of 93% ee.[3]

Unlike the Ryu team, however, the Hale group had definitively assigned their absolute stereochemistry for (+)-**4** by performing a Mosher ester analytical study on the Carreira alkynol precursor **10**. As a result, their assignment was completely secure. The Hale data clearly suggested that the Ryu group had synthesized the enantiomeric (*S*)-dienylstannane, and consequentially (3*S*)-(−)-inthomycin C, a conclusion that became even more credible when considered alongside the report of Taylor et al. that (3*R*)-inthomycin C had an $[\alpha]_D$ of +25.9 (*c* 0.27, $CHCl_3$) for material of 76% ee contaminated with 20% tetramethylurea.[2]

In light of these discrepant findings, the Hale group undertook a synthesis of the (3*R*)-enynol **9** of the Hatakeyama pathway,[5] starting from diol **12** which had previously been featured in the Hale synthesis of (+)-**4**. This led Hale et al. to complete a second formal total synthesis of (3*R*)-inthomycin C.[5] Significantly, the Hale team now recorded an $[\alpha]_D$ of +12.6 (*c* 0.71, $CHCl_3$) for their (3*R*)-enynol **9** of 83% ee. Yet, in the earlier 2012 *Org. Biomol. Chem.* report of Hatakeyama et al.,[4] the (3*R*)-alkynol **9** had been indicated to have an $[\alpha]_D$ of −15.9 (*c* 1.04, $CHCl_3$) for material of 98% ee.

On the basis of the Hatakeyama group's *Org. Biomol. Chem.* paper,[4] and the Hale team's definitive assignment of (3*R*)-stereochemistry to the alcohol in (+)-**9** (via a second Mosher ester analysis study), Hale et al. concluded[5] that the Hatakeyama group must have prepared the (3*S*)-enantiomer of enynol **9**. Moreover, since the Hatakeyama team had also recorded an $[\alpha]_D$ of −41.5 (*c* 0.1, $CHCl_3$) for inthomycin C,[4] which was opposite in sign to the value of Taylor et al.,[2] the Hale team further concluded[5] that the Hatakeyama group must have prepared (3*S*)-inthomycin C, analogously to Ryu et al.,[3] since the Taylor team[2] had apparently confirmed their (3*R*)-stereochemistry for synthetic (+)-inthomycin C by Mosher ester analysis.

Given the Hatakeyama laboratory's concern that a potential flaw had been uncovered in their published *Org. Biomol. Chem.* work, they immediately decided to recheck their laboratory records to see if an inadvertent error had been made in their synthesis, or if there had been a problem in the reporting of their experimental data. As part of that review the Hatakeyama team reexamined their $[\alpha]_D$ data for the (3*R*)-enynol **9**, and to their dismay, they found that a serious error had somehow crept into their published 2012 *Org. Biomol. Chem.* manuscript.[4] Rather than the Hatakeyama group (3*R*)-enynol **9** having an $[\alpha]_D$ of −15.9 (*c* 1.04, $CHCl_3$), as was originally stated (see Scheme 2), the Hatakeyama laboratory records actually revealed an $[\alpha]_D$ of +15.6 (*c* 1.04, $CHCl_3$) for **9** (see the Supporting Information (SI) for this actual measurement). The correct +15.6 $[\alpha]_D$ measurement now suggested that the Hale and Hatakeyama teams had intersected after all and that both teams had indeed completed total syntheses of (−)-(3*R*)-inthomycin C!

The Hatakeyama team subsequently searched through their compound collection and found that they had a sample of

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authentic (3*R*)-(+)-enynol **9** still available from their 2012 total synthesis of (−)-(3*R*)-inthomycin C. They then employed the Hale et al.[5] experimental procedures to convert this sample of **9** into the (*R*)- and (*S*)-MTPA Mosher esters. When the NMR spectra of the resulting esters were compared with those in the 2014 *Org. Lett.* report of Hale et al.,[5] an excellent (almost perfect) match was found (see the SI). The Hatakeyama team then measured the $[\alpha]_D$ of their original sample of (3*R*)-**9**, following a further purification by SiO_2 flash chromatography, and found that (3*R*)-**9** had an $[\alpha]_D$ of +12.2 (*c* 0.95, $CHCl_3$) which correlated nicely with the +12.6 value reported by Hale et al. earlier this year.

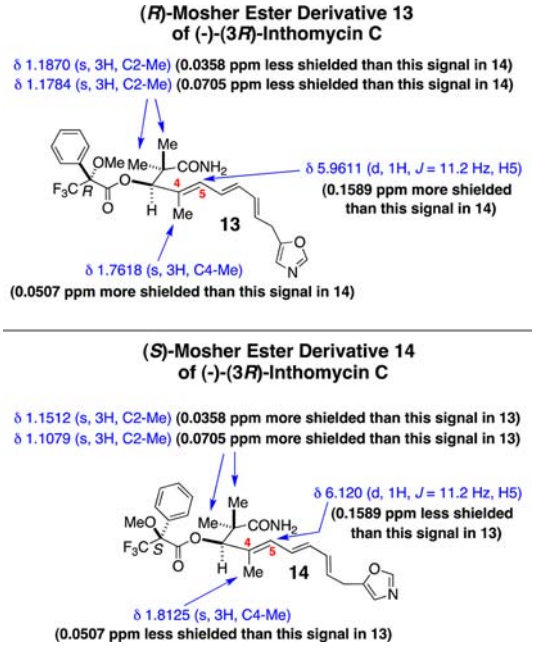
Hatakeyama next contacted the Hale team to alert them to the error that had appeared in the 2012 *Org. Biomol. Chem.* paper.[4] The two groups then worked together to resolve the discordant $[\alpha]_D$ values that had been published. Hatakeyama agreed to send Hale their purified authentic sample of the (3*R*)-enynol **9** for independent NMR comparison and verification of its $[\alpha]_D$. When the Hale group ran the 400 MHz ^1H NMR spectra of the Hatakeyama sample of **9** in $CDCl_3$, they found that it matched theirs in every respect and was highly pure. **The Hale group also recorded the $[\alpha]_D$ of the sample of (3*R*)-9 obtained from the Hatakeyama team and independently found it to be +14.4 (*c* 0.58, $CHCl_3$), which now correlated very well with the +12.6 report for authentic (3*R*)-9 of 83% ee published by the Hale group.** The two teams now agreed that both had synthesized the same (3*R*)-compound, and moreover, they accepted that the Hatakeyama team had most definitely prepared (3*R*)-inthomycin C and not the (3*S*)-enantiomeric counterpart.

To put this correlation beyond any future doubt, Hale and Hatakeyama decided that they would both try to prepare the (*R*)- and (*S*)-MTPA esters of their respective (3*R*)-inthomycin C samples, to unambiguously establish their absolute configurations by Mosher ester NMR analysis, and provide further corroboration that both groups had synthesized the same product. We also wished to make these spectra available to other groups currently working in the field to assist their future assignments.

When the Hatakeyama team reanalyzed their original 2012 sample of (−)-(3*R*)-inthomycin C, they found that it had decomposed. It was therefore agreed that the Hatakeyama group would convert their remaining sample of the (+)-(3*R*)-enynol **9** into pure, 98% ee, (3*R*)-inthomycin C, and that the $[\alpha]_D$ of this material would be measured, to put the issue of the $[\alpha]_D$ for (3*R*)-inthomycin C beyond all question.

Fortunately, the Hale group still possessed a sample of chemically synthesized (3*R*)-inthomycin C in their compound collection that had never been exposed to the ravages of $CDCl_3$, and which had withstood storage in the freezer at −28 °C for seven months under N_2. Proton analysis at 400 MHz of that sample in CD_3OD confirmed the pure condition, consisting of a 5.6:1 mixture of (3*R*)-inthomycin C and the previously described[5] unknown geometric stereoisomer. The Hale team therefore converted this (3*R*)-inthomycin C sample into the (*R*)- and (*S*)-MTPA ester derivatives **13** and **14** by treatment with the (*R*)- and (*S*)-MTPA acids, DCC, and DMAP in CH_2Cl_2. The key data that they gathered are presented in Scheme 4. The analysis unambiguously confirmed that the originally assigned (3*R*)-configuration of the Hale sample of inthomycin C was correct. Concurrent with this, the Hatakeyama group did the same and, once more, a perfect match was obtained with the Hale data, confirming that the Hale and Hatakeyama laboratories had both synthesized (3*R*)-inthomycin C (see the SI).

Scheme 4. (3*R*)-Inthomycin C Mosher Ester Analyses Undertaken by the Hale and Hatakeyama Teams



The aforementioned NMR studies on the (3*R*)-inthomycin C Mosher ester samples **13** and **14** in $CDCl_3$ revealed some quite spectacular chemical shift differences for the H(5)-proton of the trisubstituted alkene that lay adjacent to the C(3)-O-stereocenter. In the (*R*)-MTPA ester **13**, this proton resonated at δ 5.9611 ppm, which was ∼0.1589 ppm more shielded than the corresponding resonance for the (*S*)-MTPA ester **14**, which appeared at δ 6.1200 ppm. The C(4)-Me group for the (*R*)-MTPA ester **13** likewise appeared at δ 1.7618 ppm, which is 0.0507 ppm more shielded than that for the (*S*)-MTPA ester **14**, which resonated at δ 1.8125 ppm. This is what one would anticipate. Likewise the geminal dimethyl groups of **13** and **14** showed all of the expected shielding−deshielding trends.

Since the Hatakeyama team was now the only one to have a totally pure, 98% ee, sample of (3*R*)-inthomycin C in hand, the $[\alpha]_D$ of their freshly resynthesized material was measured and found to be −7.9 (*c* 0.33, $CHCl_3$) (Figure 1). Thus, (3*R*)-

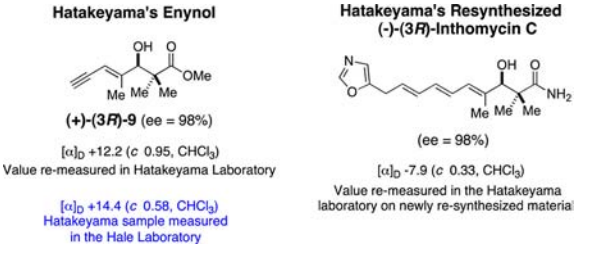


Figure 1. Corrected Hatakeyama Team $[\alpha]_D$ Data.

inthomycin C does indeed have a negative $[\alpha]_D$ when pure in $CHCl_3$. This value however is much lower in magnitude than the original $[\alpha]_D$ measurement of the 2012 *Org. Biomol. Chem.* paper.[4] The latter value, which was −41.5 (*c* 0.1, $CHCl_3$), now appears to be erroneous. Moreover, the newly recorded $[\alpha]_D$ of −7.9 lies much closer to the Hale group[5] $[\alpha]_D$ value of −8.4 (*c* 1.0, $CHCl_3$) reported for the 5.9:1 mixture that they obtained using the Ryu team's Stille cross-coupling.[3]

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The newly measured $[\alpha]_D$ for (−)-(3*R*)-inthomycin C is also significantly lower than the $[\alpha]_D$ value of −34.33 (*c* 0.1, $CHCl_3$) of Ryu et al.,[3] which our two teams now believe to be in error, certainly as regards the magnitude. However, the fact that our two groups and the Ryu team each recorded a negative $[\alpha]_D$ for (3*R*)-inthomycin C very strongly suggests that the original positive $[\alpha]_D$ measured by Taylor et al.[2] needs to be reevaluated. Consequentially, the Hale group now no longer claims a total synthesis of (+)-(3*R*)-inthomycin C, as was published in our 2014 *Org. Lett.* communication,[5] but rather a total synthesis (−)-(3*R*)-inthomycin C, due to our intersection with the Hatakeyama laboratory (+)-(3*R*)-enynol **9**[4] whose $[\alpha]_D$ value has now been corrected here.

Of course, there is still the outstanding issue of the Ryu team $[\alpha]_D$ measurement for **4**, which was reported to be −17.5 (*c* 0.12, $CHCl_3$),[3] and which is different from the Hale group measurement of $[\alpha]_D$ +5.3 (*c* 1.1, $CHCl_3$)[5] for a molecule of proven (3*R*)-stereochemistry. Again, we believe that the $[\alpha]_D$ measured for **4** by Ryu et al. needs to be carefully reevaluated, since there are significant discrepancies between a number of the $[\alpha]_D$ measurements in the Ryu group paper and those reported by the Hale and Hatakeyama teams, including the trienyl methyl ester **6**. While there is an acceptable agreement between the Hatakeyama[4] and Hale[5] team $[\alpha]_D$ values for **6** [Hale et al. $[\alpha]_D$ −0.43 (*c* 0.7, $CHCl_3$) for a 17:1 mixture (83% ee); Hatakeyama et al. $[\alpha]_D$ +0.78 (*c* 1.39, $CHCl_3$) for pure material of 98% ee], the values of Taylor et al.[2] for **6** [$[\alpha]_D$ +5.2 (*c* 1.55, $CHCl_3$) 76% ee] and Ryu et al.[3] [$[\alpha]_D$ +8.48 (*c* 0.9, $CHCl_3$) 93% ee] both deviate significantly from ours.

So where exactly does this leave the community with regard to the status of the various total syntheses that have so far been accomplished of (3*R*)-inthomycin C? After carefully reviewing all of the available published evidence, and our own newly acquired (*R*)- and (*S*)-MTPA ester data on (−)-(3*R*)-(−)-inthomycin C, we believe that the Taylor, Ryu, Hatakeyama, and Hale groups have each prepared (−)-(3*R*)-inthomycin C.

A fresh reappraisal of the syntheses of Taylor et al.[2] and Ryu et al.[3] now very strongly suggests that each of them have synthesized (−)-(3*R*)-inthomycin C, despite their discrepant $[\alpha]_D$ values. In the case of Taylor et al., we believe that their team has achieved this target based on their correct total synthesis of (+)-(3*R*)-inthomycin B and the Mosher ester data that they have reported for their methyl ester precursor of (+)-inthomycin B. The latter very clearly shows the same sort of chemical shift trends that we observed for the (*R*)- and (*S*)-MTPA esters of (−)-(3*R*)-inthomycin C, which is precisely what one would expect. Also, the Kiyooka asymmetric aldol process used by Taylor et al. would be expected to give rise to the (3*R*)-product, and it did successfully provide (+)-(3*R*)-inthomycin B, whose structure is not in doubt. Taken together, the combined weight of evidence suggests that the Taylor team indeed synthesized (3*R*)-inthomycin C, but there is an anomaly in their $[\alpha]_D$ measurement in $CHCl_3$, possibly due to the 20% tetramethylurea contaminent that was present.[2,7]

As for the Ryu group, like the Hatakeyama group, they report a negative $[\alpha]_D$ for their synthetic (3*R*)-inthomycin C.[3] Their chiral oxazaborolidinium triflate catalyst **3** also gave rise to the very same stereochemical outcome in other aldol reactions reported in their paper, reactions where the aldol adduct configuration was independently proven. Mechanistically, as well, the Kiyooka asymmetric aldol process used by the Taylor team very likely proceeds by a similar transition state to that mediated by the Ryu catalyst **3**. So, on balance, we now believe that Ryu et al. have indeed prepared (−)-(3*R*)-inthomycin C, as was originally claimed,[3] notwithstanding the discrepant $[\alpha]_D$ data that they report for **4**, **6**, and (−)-(3*R*)-inthomycin C itself.

The combined new stereochemical and $[\alpha]_D$ evidence that we have gathered with the Hatakeyama group confirms that the Hale and Hatakeyama teams have both synthesized (−)-(3*R*)-inthomycin C by their respective routes.[4,5] In the case of the Hale team, while the depictions of the absolute stereochemistry in our 2014 *Org. Lett.* paper[5] still remain the same, our previous assertion that a synthesis of (+)-(3*R*)-inthomycin C had been achieved must now be corrected to a claim of a synthesis of (−)-(3*R*)-inthomycin C instead.[5]

We trust that other workers in the field will feel much greater confidence in venturing toward (−)-(3*R*)-inthomycin C now that the absolute configuration of this molecule has been securely assigned.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, NMR spectra, and the Hatakeyama group $[\alpha]_D$ measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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