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A total synthesis of the marine macrolide biselide A is described that relies on an enantiomerically enriched  $\alpha$ -chloroaldehyde as the sole chiral building block. Several strategies to construct the macrocycle are presented including a macrocyclic Reformatsky reaction that ultimately provides access to the natural product in a longest linear sequence of 18 steps. Biological testing of synthetic biselide A suggests this macrolide disrupts cell division through a mechanism related to the regulation of microtubule cytoskeleton organization. Overall, this concise synthesis and insight gained into the mechanism of action should inspire medicinal chemistry efforts directed at structurally related anticancer marine macrolides.

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(MIC = 0.03  $\mu\text{g mL}^{-1}$ ).<sup>4</sup> Biselide A (7) was found to be  $\sim 5$  to 10 fold less active than haterumalide NA against a panel of 10 human cancer cell lines. However, unlike the haterumalides, biselide A showed no toxicity at concentrations as high as 50  $\mu\text{g mL}^{-1}$  in a brine shrimp assay. This later result led to speculation that C20 oxidation in the biselides makes these compounds generally less toxic and thus better drug leads.<sup>9</sup> Several simplified synthetic analogues of haterumalide NA have also been reported, and these studies found that both the macrolide and side chain are critical for biological activity.<sup>12</sup>

The image displays eight chemical structures of haterumalides and biselides, arranged in two columns. Each structure is a complex macrocyclic molecule with a chlorine atom at C7, an acetate group (OAc) at C3, and a side chain at C15. The side chain at C15 is a 3-methylbut-3-en-2-yl ester, where the ester group is represented as OR. The structures are numbered 1 through 8, with their corresponding names and substituents listed below them.

**1: haterumalide B**

**2: haterumalide NA (R = H)**

**3: haterumalide NB (R = *n*-Bu)**

**4: haterumalide NA methyl ester (R = Me)**

**5: haterumalide NC (R = *n*-Bu)**

**6: haterumalide ND (R = H)**

**7: biselide A (R = H)**

**8: biselide B (R = ...)**

Broad interest in both the haterumalides and biselides has been stimulated by their potentially useful biological activity.<sup>4</sup> For example, haterumalide NA (2) is cytotoxic to P388 cells ( $IC_{50} = 0.32 \mu g mL^{-1}$ )<sup>3</sup> and a potent antimycotic

Fig. 1 Representative examples of haterumalide (1–3, 5 and 6) and biselide (6 and 7) natural products.

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Structurally, the haterumalides and biselides possess a 14-membered macrolide that incorporates a *trans*-substituted tetrahydrofuran (THF) ring in addition to an unusual (*Z,Z*)-1,4-chlorodiene fragment (C4–C8). In 2003, Kigoshi reported the first total synthesis of *ent*-haterumalide NA methyl ester (**4**) and reassignment of the stereochemical relationship between the C14 and C15 centers as *erythro* (Fig. 2).<sup>13</sup> Since the absolute stereochemical assignment of **2** was based on modified Mosher's ester analysis at the C15 alcohol,<sup>3</sup> this synthetic work also resulted in a reassignment of the absolute stereochemistry of the haterumalides and biselides. Key to the success of this first total synthesis (26 steps in longest linear sequence (LLS)) was the development of an intramolecular Reformatsky reaction to construct both the macrocycle and the C2–C3 bond.<sup>13</sup> In the same year, Snider reported the second total synthesis of *ent*-haterumalide NA methyl ester that involved a Yamaguchi

macrolactonization as a key step (29 steps in LLS). Both of these syntheses relied on an aluminum hydride reduction of a propargylic alcohol to construct a *Z*-configured C7–C8 olefin and a Nozaki–Hiyama–Kishi (NHK)<sup>14–16</sup> reaction to append the side chain to the fully functionalized macrolide core.

The first total synthesis of the natural product haterumalide NA (**2**) was reported by Hoyer in 2005 (20 steps in LLS),<sup>17</sup> who demonstrated the 14-membered macrolide could be formed through a Pd-catalyzed chloroallylation that also introduced the *Z*-vinyl chloride function. In 2008 Roulland disclosed a synthesis of haterumalide NA *via* a process involving a Suzuki–Miyaura cross-coupling of a 1,1-dichloroalkene to form the C8–C9 bond, followed by macrolactonization (19 steps in LLS).<sup>18</sup> Kigoshi also reported a second generation synthesis of haterumalide NA in 2008 that involved a similar coupling strategy to construct the C8–C9 bond but exploited a macrolactonization reaction (33 steps in LLS).<sup>12,19</sup> In the same year Borhan reported the first synthesis of haterumalide NC (**5**) (18 steps in LLS) using a chlorovinylidene chromium carbenoid to construct the C8–C9 bond.<sup>20</sup> More recently, the first synthesis of biselide A (**7**) was reported by Hayakawa and Kigoshi in 2017 (34 steps in LLS).<sup>21</sup> Here, again, a Suzuki–Miyaura coupling was used to construct the C8–C9 bond and a macrolactonization reaction was employed. Notably, the C3 stereogenic center was introduced using an auxiliary controlled asymmetric aldol reaction.

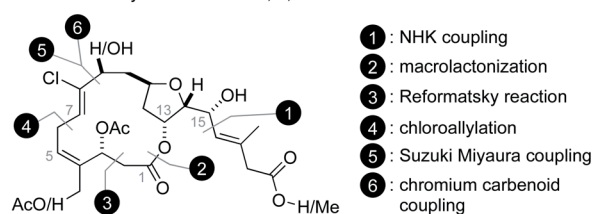
Based on the intriguing structure and biological activity of biselide A (**7**), and our longstanding interest in the synthesis of THF-containing marine natural products,<sup>22–31</sup> we became intent on developing a total synthesis of **7** that would also support additional biological testing. We have previously reported<sup>22</sup> straightforward synthetic routes to hydroxytetrahydrofurans that exploit diastereoselective aldol reactions between lithium enolates and enantiomerically enriched  $\alpha$ -chloroaldehydes<sup>32</sup> (Fig. 2, 9). These later materials can be prepared in excellent enantiomeric excess *via* the organocatalytic  $\alpha$ -chlorination of aldehydes using processes developed by Jørgensen,<sup>33</sup> MacMillan<sup>34,35</sup> or Christmann.<sup>36,37</sup> As depicted in Fig. 2, we planned to exploit this strategy using the chloroketone **17**<sup>38</sup> to rapidly access the THF **13**. From here, a sequence involving a metathesis reaction,<sup>17</sup> esterification,<sup>21</sup> or Reformatsky<sup>13</sup> reaction would expectedly provide the 14-membered ring. Our efforts to explore each of these individual reactions as macrocyclization strategies and the ultimate realization of a total synthesis of biselide A (**7**) and biological testing of our synthetic material is described below.

## Results and discussion

### Enantioselective synthesis of $\alpha$ -chloroaldehyde **24**

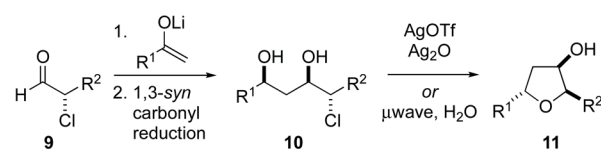
We have previously reported the aldol reaction of **17** and ( $\pm$ )-**18** (Fig. 2, PG = TBS) provides a route to the tetrahydrofuran **13** in racemic form.<sup>38</sup> To support an enantioselective synthesis of biselide A (**7**), we examined the enantioselective  $\alpha$ -chlorination of protected  $\beta$ -hydroxyaldehydes **19** and **20**. Unfortunately, in both cases  $\alpha$ -chlorination resulted predominantly in elimination, affording acrolein as the major product (Table 1, entries 1 and 2). Using *D*-proline catalysis, the TBS-protected alcohol **21**

Previous total syntheses of *ent*-**2**, **2**, **5** and **7**



year	target	group	macrocyclization reaction	steps in LLS
2003	<i>ent</i> - <b>2</b>	Kigoshi	3	26
2003	<i>ent</i> - <b>2</b>	Snider	2	29
2005	<b>2</b>	Hoyer	4	20
2008	<b>2</b>	Roulland	5	19
2008	<b>5</b>	Borhan	6	18
2008	<b>2</b>	Kigoshi	2	33
2017	<b>7</b>	Hayakawa/ Kigoshi	2	34

Streamlined synthesis of 3-hydroxy tetrahydrofurans



Retrosynthetic analysis for biselide A (**7**)

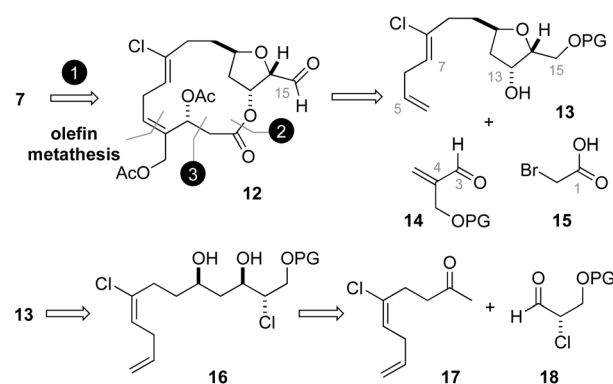


Fig. 2 Previous syntheses of haterumalide and biselide natural products and a chlorohydrin-based strategy for the synthesis of biselide A (**7**). LLS = longest linear sequence.

Table 1 Enantioselective  $\alpha$ -chlorination of aldehyde **21**

19: R = Bn	22: R = Bn				
20: R = TIPS	23: R = TIPS				
21: R = TBS	24: R = TBS				
Entry	Aldehyde	Method <sup>a</sup>	Time (h)	Yield (%)	ee (%)
1	19	A	4	<10 <sup>b</sup>	na
2	20	A	4	<10 <sup>b</sup>	na
3	21	A	4	90	15
4	21	B	18	30 <sup>d</sup>	80
5	21	C	16	35 <sup>d</sup>	94
6	21	C <sup>c</sup>	16	40 <sup>d</sup>	90

<sup>a</sup> A: **25** (10 mol%), NCS (1 equiv.), CH<sub>2</sub>Cl<sub>2</sub>, rt; B: **26** (10 mol%), NCS (1 equiv.), CH<sub>2</sub>Cl<sub>2</sub>; C: **27** (15 mol%), LiCl (1.5 equiv.), Cu(TFA)<sub>2</sub> (0.5 equiv.), Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (1 equiv.), H<sub>2</sub>O (2.1 equiv.), rt. <sup>b</sup> The major product was acrolein. <sup>c</sup> Reaction at 10 °C. <sup>d</sup> Product accompanied by formation of acrolein.

was cleanly converted into the  $\alpha$ -chloroaldehyde **24** in good yield albeit expectedly<sup>33</sup> low ee. We next examined the use of *D*-prolinamide and found that the  $\alpha$ -chloroaldehyde **24** could be prepared in much improved enantioselectivity (entry 4), but was again accompanied by predominant formation of acrolein. Finally, we explored the  $\alpha$ -chlorination process reported by MacMillan<sup>35</sup> (entries 5 and 6). Here, the  $\alpha$ -chloroaldehyde **24** was produced in up to 94% ee and modest yield (40%). Efforts focused on further optimizing these conditions by reducing the reaction temperature (e.g., entry 6), adding pH 8 buffer, proton scavengers (e.g., 2,6-di-*tert*-butyl-4-methylpyridine) or varying the amount of water, failed to improve the result summarized in entry 5.

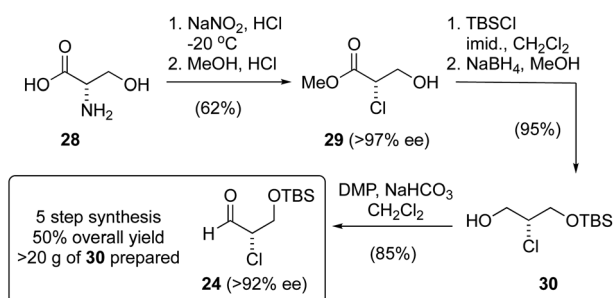
Considering these challenges, we investigated the conversion of *L*-serine into **24** using a process reported by De Kimpe for the amino acids Ile, Phe and Val (Scheme 1).<sup>39</sup> For our purpose, *L*-Ser (**28**) was first converted into the chloroester **29** via double Waldon inversion<sup>39</sup> followed by esterification. It was critical that this chlorination reaction was executed at temperatures below –15 °C to avoid racemization. For example, at 0 °C the chloroester was produced in 60% ee, while reaction at –20 °C reliably

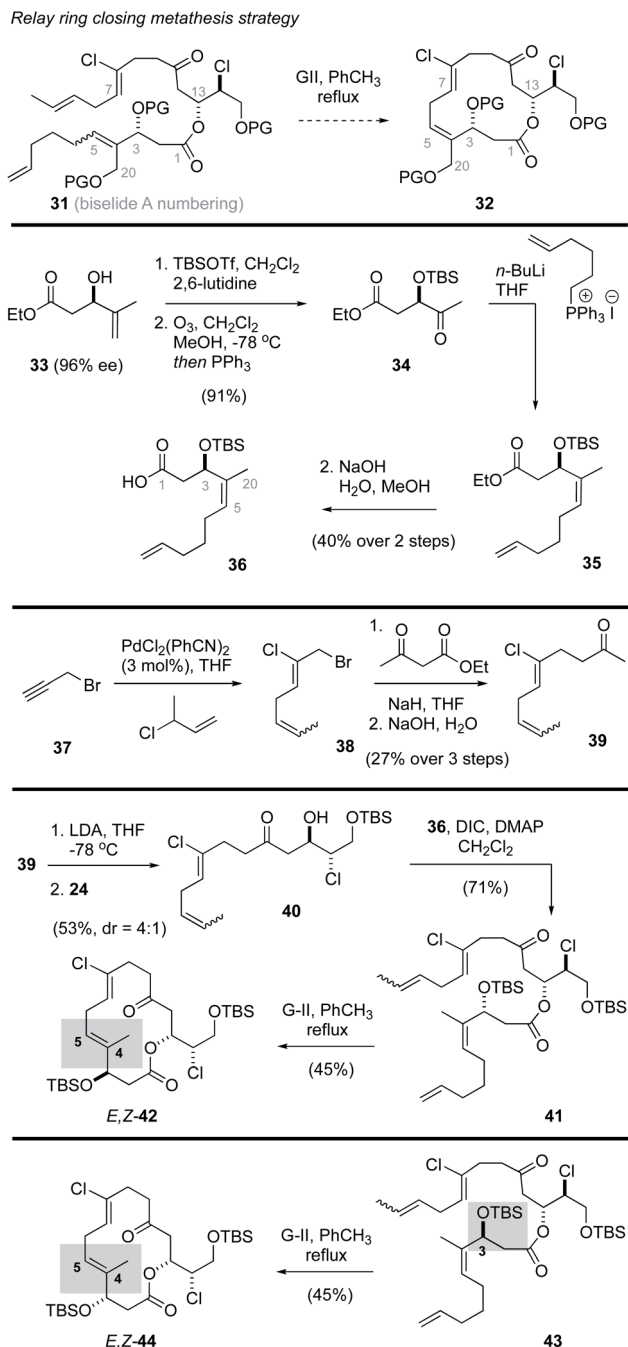
provided the chloroester in >97% ee. Protection of the alcohol as a TBS ether and reduction then gave the chlorohydrin **30**, which could be routinely prepared on >10 g scale via this 4-step process. Oxidation of **30** using PCC gave the  $\alpha$ -chloroaldehyde **24** in excellent yield (95%), though again we noted an erosion in enantiomeric purity. As such, we examined other oxidation protocols and found that using Dess–Martin periodinane<sup>40</sup> with the addition of solid NaHCO<sub>3</sub>, the  $\alpha$ -chloroaldehyde **24** could be prepared on up to 15 g scale in good yield and enantiomeric purity (>92% ee). While this sequence is longer (5 steps vs. 3 steps) than that involving a direct enantioselective  $\alpha$ -chlorination (Table 1), it provided a reliable alternative when large amounts of  $\alpha$ -chloroaldehyde **24** were required.

### Ring closing metathesis approach to biselide A

Prior studies on the haterumalides by Hoyer and co-workers<sup>17</sup> involved use of a relay ring closing metathesis (RRCM)<sup>41</sup> approach to form both the C4–C5 alkene and the 14-membered macrocycle. From these studies it was found that substrates with an intact THF ring failed to undergo RRCM, likely due to strain in the resulting bridged bicycle. However, in “relaxed” model systems that lacked the THF ring, RRCM reactions were successful.<sup>41</sup> Inspired by this work, we first explored a strategy in which the THF would be assembled after macrocyclization, and the macrocycle itself would be produced through a process involving a “relaxed” RRCM reaction (e.g., **31** → **32**, Scheme 2). The synthesis of the RRCM precursor followed a straightforward sequence of reactions that initiated with the known ester **33**,<sup>42</sup> prepared in 97% ee via kinetic resolution using a Sharpless asymmetric epoxidation reaction.<sup>43</sup> Protection of the alcohol as a TBS ether, oxidative cleavage of the alkene function and subsequent Wittig reaction and hydrolysis gave the *Z*-alkene **36** in good overall yield. While this material lacks oxygenation at the position corresponding to C20 in biselide A, it was viewed as a good model substrate for exploring the RRCM reaction and would yield access to the haterumalide family of marine macrolides (e.g., **2**, Fig. 1). Synthesis of the required chloroalkene **38** exploited a palladium catalysed chloroallylation reaction developed by Kaneda.<sup>44,45</sup> Alkylation of ethylacetoacetate followed by decarboxylation then gave the methyl ketone **39** as a 1 : 1 mixture of *E*- and *Z*-isomers as indicated.

With the required fragments in hand, a lithium aldol reaction between the enolate derived from methyl ketone **39** and the  $\alpha$ -chloroaldehyde **24** gave the keto-chlorohydrin **40**<sup>22,31,38</sup> in modest yield (53%, d.r. = 4 : 1). Esterification of the chlorohydrin using the carboxylic acid **36** then gave the ketoester **41**. A subsequent RRCM reaction using conditions reported by Hoyer<sup>41</sup> gave truncation products (~40% yield) along with the *E*,*Z*-macrocycle **42** (45% yield). The *E*-configuration of the C4–C5 alkene function in **42** was assigned by nOe analysis. In an effort to access the desired *Z*,*Z*-isomer, several reaction parameters were evaluated including temperature (40 °C to 110 °C), solvent (toluene, CH<sub>2</sub>Cl<sub>2</sub>, hexanes), catalyst (Grubbs II,<sup>46</sup> Hoveyda–Grubbs II<sup>47</sup>), concentration, addition rate and use of various additives known to prevent isomerization<sup>48</sup> (e.g., benzoquinone). In no instance was the desired *Z*,*Z*-macrocycle formed.

Scheme 1 Multigram synthesis of  $\alpha$ -chloroaldehyde **24**.



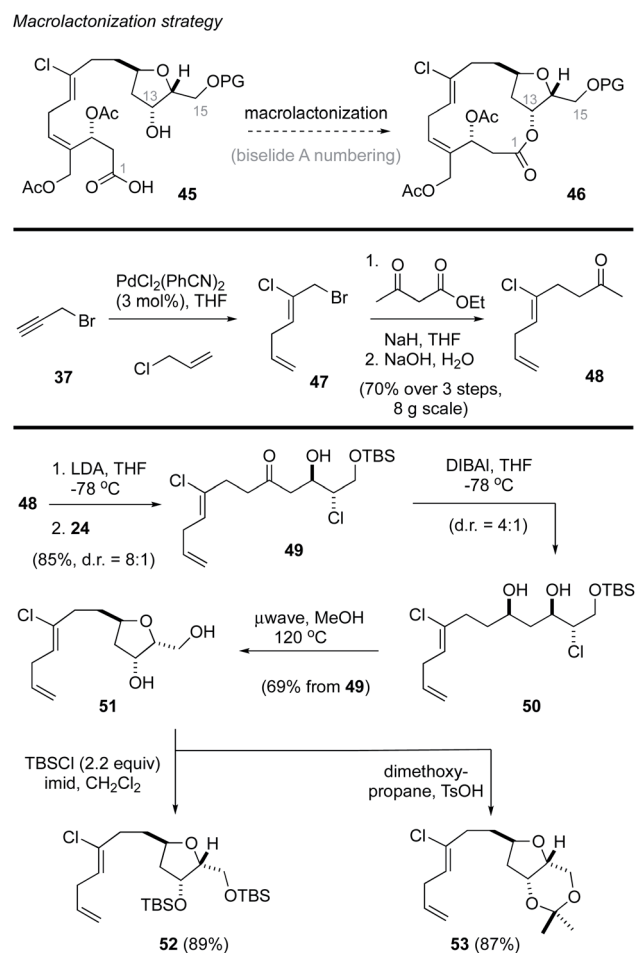
Scheme 2 A relay ring closing metathesis strategy for biselide A.

Considering Hovey had reported that functional groups adjacent to the site of RRCM (*e.g.*, alkene, alcohol, ketone) play a significant role in determining the ultimate configuration of the alkene,<sup>17</sup> we also prepared the diastereomeric ester **43** to assess the impact of C3 configuration on the RRCM reaction. Thus, ester *ent*-**33** was synthesized *via* a Sharpless asymmetric epoxidation using (–)-DIPT and progressed to the C3 epimer **43** in the same manner outlined for **41**. Unfortunately, RRCM reaction of **43** again gave the *E,Z*-isomer **44** as the only macrocyclization product.

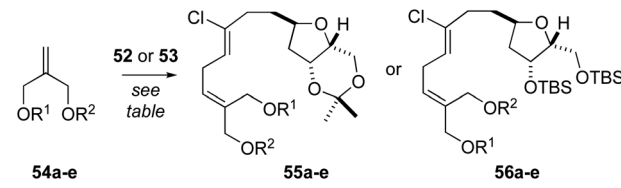
## Macrolactonization approach to biselide A

Based on these results, we revised our approach and instead pursued a macrolactonization strategy to form the 14-membered ring (Scheme 3, **45** → **46**).<sup>49</sup> Toward this goal, the methyl ketone **48** was prepared using Kaneda's procedure<sup>44</sup> in three steps from propargyl bromide (**37**). A subsequent lithium aldol reaction<sup>22,38</sup> with the enantiomerically enriched  $\alpha$ -chloroaldehyde **24** provided the keto-chlorohydrin **49** in excellent yield as a mixture of diastereomers. DIBAL reduction of the carbonyl function in **49** then gave the chlorodiol **50** as a 4 : 1 mixture of diastereomers that were not separated. Heating this mixture in methanol (120 °C)<sup>23</sup> in a sealed vessel in a microwave effected the removal of the TBS protecting group followed by cyclization to afford the THF **51**, which was isolated as a single diastereomer in excellent yield. Attempts to avoid the removal of the TBS protecting group by addition of buffers (*e.g.*, pH 7 phosphate) or base (*e.g.*, 2,6-di-*tert*-butyl-4-methylpyridine) were not successful. Notwithstanding, the diol could be readily protected as the corresponding bis-TBS ether **52** or acetonide **53** in good yield.

At this point, we examined diastereoselective cross metathesis reactions to incorporate both the C3–C4 fragment of biselide A along with the C20 hydroxymethyl group using various

Scheme 3 Synthesis of the tetrahydrofurans **52** and **53**.



**Table 2** Cross metathesis reactions of 2-methylene-1,3-propanediol and derivatives


Entry <sup>a</sup>	Alkene	R <sup>1</sup>	R <sup>2</sup>	THF	Product	Yield (%)
1	54a	TES	H	53	55a <sup>b</sup>	30
2	54b	TBS	H	53	55b <sup>c</sup>	33
3	54c	H	H	53	55c	17
4	54d	TBS	TBS	53	55d	16
5 <sup>d</sup>	54d	TBS	TBS	53	55d	<10 <sup>e</sup>
6	54e	C(CH <sub>3</sub> ) <sub>2</sub>		53	55e	60
7 <sup>f</sup>	54e	C(CH <sub>3</sub> ) <sub>2</sub>		53	55e	77
8	54e	C(CH <sub>3</sub> ) <sub>2</sub>		52	56e	46

<sup>a</sup> Conditions: **52** or **53** (1 equiv.) was dissolved in toluene (0.05 M), **54a-e** was added (5 equiv.), reaction mixture was sparged with N<sub>2</sub>, heated to 60 °C and Hoveyda-Grubbs catalyst (2<sup>nd</sup> generation, 5 mol%) was added. The resulting mixture was heated at reflux for 2 h. <sup>b</sup> *E* : *Z* ratio = 1 : 1. <sup>c</sup> *E* : *Z* ratio = 1 : 1.5. <sup>d</sup> Stewart-Grubbs catalyst was used (5 mol%). <sup>e</sup> The major product was the dimer of **53** (~50% yield). <sup>f</sup> 10 equiv. of **54e** was added.

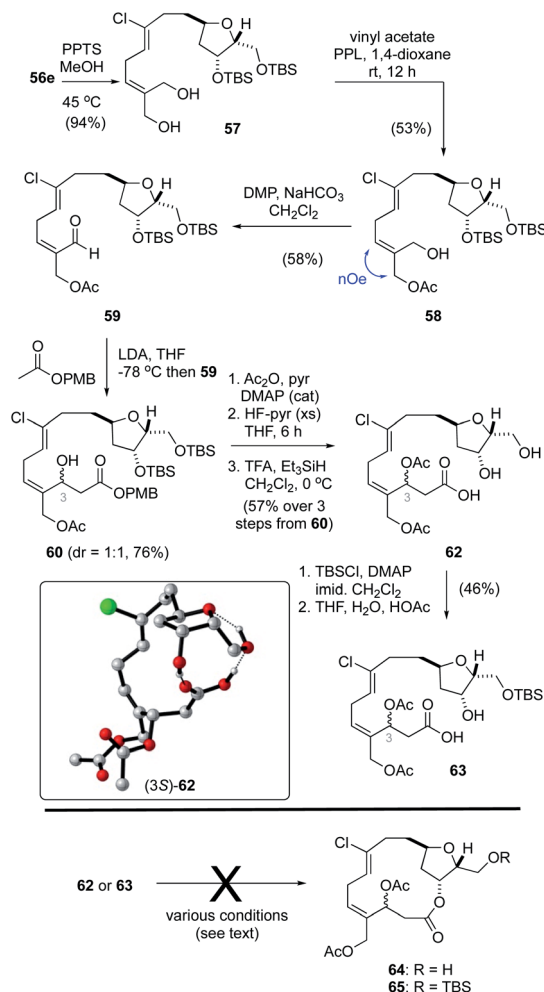
derivatives of 2-methylene-1,3-propanediol. As summarized in Table 2, cross metathesis of **52** with mono-TES or mono-TBS protected diols **54a** and **54b** gave little *E/Z* selectivity. In fact, the metathesis reaction with **54b** favoured the undesired *Z*-isomer (entries 1 and 2). We also investigated cross metathesis reactions involving diol **54c** (entry 3) or the protected diols **54d** and **54e** (entries 4 to 6). From these studies the acetonide **54e** proved to be the best metathesis partner and gave the diene **56e** in 77% yield (entry 7). A similar cross metathesis reaction using the bis-TBS ether **52** gave the acetonide **56e** (entry 8).

While regioselective deprotection of the bis-acetonide **55e** (Table 2, entry 8) was unsuccessful, the acetonide protecting group in bis-silyl ether **56e** was readily removed to afford the diol **57** (Scheme 4). From here, following a procedure reported by Imai,<sup>50</sup> treatment of **57** with vinyl acetate and porcine pancreatic lipase (PPL) in 1,4-dioxane gave the mono-acetate **58** in 56% yield. The *E*-configuration of the alkene function in **58** was confirmed by NOE analysis. Oxidation of the allylic alcohol using Dess–Martin periodinane (DMP)<sup>40</sup> afforded the unstable aldehyde **59** that was prone to isomerization. As such, this material was reacted directly with the lithium enolate derived from ethyl acetate to afford a near equal mixture of hydroxy esters (not shown). Unfortunately, we were unable to hydrolyze the ethyl ester function without significant degradation and, as such, a small collection of related esters were prepared including *O*<sup>t</sup>Bu, OPMB, OPh and the *S*<sup>t</sup>Bu thioester. Of these, only the *p*-methoxybenzyl ester<sup>17</sup> **60** was hydrolyzed without effecting significant degradation. Thus, acylation of the C3–OH function, removal of the TBS protecting groups and ester hydrolysis gave the seco-acid **62**. Following re-protection of both alcohol functions in **62** as TBS ethers, the secondary alcohol

could be selectively unmasked in a mixture of THF–H<sub>2</sub>O–HOAc (2 : 1 : 0.1), affording seco-acid **63**.

With the seco-acids **62** and **63** in hand, macrolactonization attempts using basic (*e.g.*, Yamaguchi<sup>51</sup> and Boden–Keck<sup>52</sup>), acidic (*e.g.*, Trost<sup>53</sup> and Yamamoto<sup>54</sup>) and near neutral (*e.g.*, modified Mukaiyama<sup>55</sup> and Corey–Nicolaou<sup>56</sup>) conditions were explored. Unfortunately, none of these reactions gave the desired macrocycles **64** or **65**, and instead resulted primarily in decomposition. Hayakawa and Kigoshi have reported that<sup>21</sup> macrolactonization of a less labile seco-acid, in which the alcohol functions at C3/C20 are protected as silyl ethers and not acetates, was successful using Yamamoto conditions. Considering as well that the choice of protecting group at C15 also plays a key role in related macrolactonizations,<sup>13</sup> advancing seco-acids **62** or **63** to the desired macrocycles **64** or **65** would require several tedious protecting group manipulations including replacement of the acetate functions at C3 and C20 that are required for biside A with orthogonal protecting groups to that at C15, and thus add significantly to the overall length of the process.

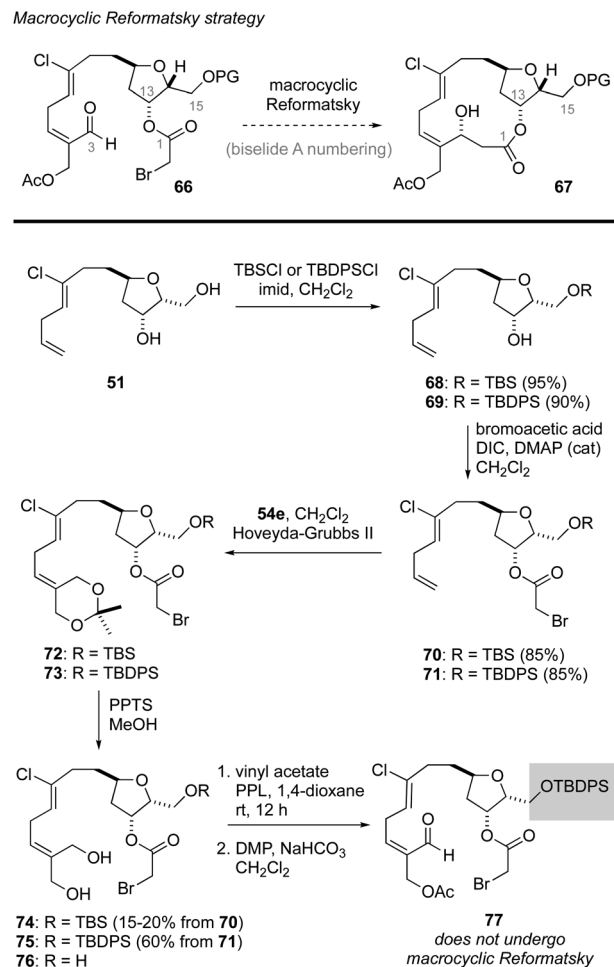
Curiously, during these studies we noticed that protons assigned to the C1–C13 region of the molecule were distinct for

**Scheme 4** Synthesis of the seco acids **62** and **63**.

the C3 epimers in both **62** and **63** when spectra were recorded in  $\text{CDCl}_3$  but not in  $\text{CD}_3\text{OD}$ . Considering that 8 bonds separate the C3 stereocenter from the THF core, the distinct resonances for each diastereomer suggested that these compounds adopt conformations dominated by hydrogen bonding between the carboxylic acid and the THF function. In this way, the relative configurations of C3 impacts the overall conformation and chemical shift values for protons throughout the molecule in  $\text{CDCl}_3$ . In  $\text{CD}_3\text{OD}$ , where hydrogen bonding would be disrupted, C3 epimers had nearly identical  $^1\text{H}$  NMR spectra. Further, when the seco-acid **62** was converted into the corresponding methyl ester (TMSCHN<sub>2</sub>) the C3 epimers had identical  $^1\text{H}$  NMR spectra in  $\text{CDCl}_3$ . To probe the role of hydrogen bonding in these seco-acids a molecular dynamics conformational search of both C3 epimers of **62** was carried out using the Tinker molecular modelling package.<sup>57</sup> Conformers within 4 kcal mol<sup>-1</sup> of their respective minima were then further refined by geometry optimization in Gaussian 09<sup>58</sup> at the PCM<sup>59</sup> (solvent:  $\text{CHCl}_3$ )–PM6<sup>60</sup> level of theory (see ESI† for details). Using the PM6 Gibbs free energies we then visually examined conformations within 2 kcal mol<sup>-1</sup> of their respective minima to interrogate the different modes of intramolecular hydrogen bonding within the seco-acid. For both C3 epimers, the lowest energy conformations included ones where hydrogen bonding between the carboxylic acid and the C2-, C3-hydroxy functions and/or tetrahydrofuran oxygen constrain the molecule in a pseudo-macrocyclic conformation (e.g., Scheme 4, inset). Unfortunately, attempts to exploit the biased pseudo-macrocyclic conformation of the seco-acid through hydrogen bond-templated macrolactonization promoted by various acid catalysts in aprotic solvents (e.g.,  $\text{CHCl}_3$ ) also failed to provide the desired macrocycle.

### Macrocyclic Reformatsky approach to biselide A

Having failed to assemble the macrocyclic core of biselide A via RRCM or macrolactonization, we finally focused on a macrocyclic Reformatsky reaction that had proven successful in Kigoshi's first synthesis of *ent*-haterumalide methyl ester (Fig. 2).<sup>13</sup> As summarized in Scheme 5, we expected that the bromoacetate **77** could be accessed directly from intermediates generated in our earlier studies. Towards this goal, monoprotection of the chlorodiol **51** gave the TBS ether **68**, which was converted into the bromoacetate **70** in excellent overall yield. Next, cross metathesis with acetonide-protected 2-methylene-1,3-propanediol **54e** (Table 2) gave the orthogonally protected tetrol **72**. Unfortunately, attempts to remove the acetonide protecting group from this intermediate resulted in low and variable yields of the desired diol **74** (15–20%), which was accompanied by triol **76** as the major product. Thus, we returned to diol **51** and protected the primary alcohol function at C13 as a TBDPS ether. We were pleased to find that with this choice of protecting group, the diol **75** was accessible in excellent overall yield following an identical sequence of reactions. Enzymatic acylation<sup>50</sup> of the *E*-allyl alcohol function followed by oxidation gave the Reformatsky macrocyclization substrate **77**, which was prone to isomerization and degradation as noted



Scheme 5 Synthesis of the bromoacetate **77**.

above for the related aldehyde **59** (Scheme 4). With freshly prepared aldehyde **77** in hand, the key ring closure reaction was investigated using rhodium catalysis as reported by Honda.<sup>61</sup> Disappointingly, using these standard conditions as well as variants where concentration, temperature and catalyst loading were examined gave only mixtures of degradation products. Considering the noted influence of the C15 protecting group on productivity of macrocyclizations within this family of molecules,<sup>13,62</sup> and the failure of a related C15 TBDPS-ether to undergo ring closure *via* macrolactonization,<sup>13</sup> we reluctantly returned to the TBS ether **68** and exhaustively examined methods to advance this material to the corresponding bromoacetate **74** in improved yield.

Following the cross-metathesis reaction between the acetonide-protected 2-methylene-1,3-propanediol **54e** and TBS ether **68**, we suspected that incomplete removal of ruthenium catalyst contributed to the partial degradation and low yields of diol **74**. Grubbs<sup>63</sup> and others<sup>64–67</sup> have noted that highly coloured residual ruthenium catalysts can promote isomerization and decomposition of metathesis products. Several strategies for removing the ruthenium catalysts have been reported and were evaluated separately by us, including stirring the crude metathesis reaction mixture with (i) DMSO or  $\text{Ph}_3\text{PO}$  prior to



purification,<sup>64</sup> (ii) activated carbon followed by filtration through silica gel or neutral alumina,<sup>65</sup> (iii)  $\text{Pb}(\text{OAc})_4$  prior to filtration through silica gel or neutral alumina,<sup>66</sup> or (iv) an isocyanide ( $\text{CNCH}_2\text{CO}_2\text{K}$ ) prior to filtration through silica gel or neutral alumina. Unfortunately, none of these procedures completely removed the brown colour from the product, which proved to be a reasonable predictor for stability. As a last resort, we explored removal of the ruthenium by-products by size exclusion chromatography (Sephadex LH-20 resin, MeOH). Here, we found that the first few column fractions contained all of the coloured ruthenium by products and none of the acetone 72, and that the isolated acetone was a clear colourless oil.

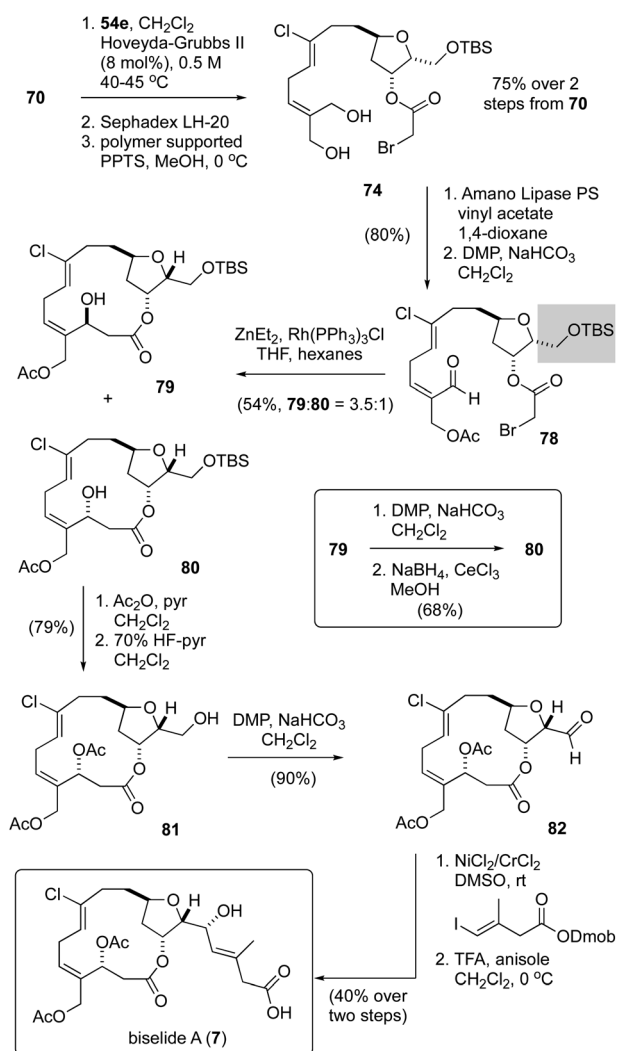
With ruthenium-free metathesis product 72, removal of the acetone protecting group proceeded cleanly to provide the diol 74 in now reproducibly excellent yield (75%) over two steps (Scheme 6). From here, a straightforward sequence of acetylation and oxidation gave the aldehyde 78. At this point we examined the macrocyclic Reformatsky reaction using the conditions described by Honda<sup>61</sup> and Kigoshi.<sup>13</sup> Now, with the

TBS protected C13 alcohol, we were pleased to find that the macrocycle was formed in 54% yield as a 3.5 : 1 mixture of separable C3-epimers 79 and 80, further highlighting the critical importance of the C15 protecting group to macrocyclizations in this family of natural products. While a Mitsunobu reaction<sup>68</sup> of the (3*S*)-epimer 79 gave a mixture of elimination, stereochemical inversion and stereochemical retention products, a sequence of oxidation and reduction (see inset) converted 79 cleanly into the desired (3*R*)-epimer 80. Macrocyclic stereocontrol in a related reduction was also noted by Hoyer.<sup>17</sup> In an attempt to improve the yield and diastereoselectivity of the Reformatsky reaction, we examined several solvents ( $\text{CH}_2\text{Cl}_2$ , THF, MTBE), reaction temperatures ( $-20^\circ\text{C}$ ,  $0^\circ\text{C}$ , rt), addition rates of aldehyde 78, and reaction concentrations but failed to improve significantly on this outcome. Finally, acetylation and removal of the TBS protecting group gave the alcohol 81, a compound previously reported by Hayakawa and Kigoshi in their synthesis of biselide A.<sup>21</sup> Following the same sequence of reactions used by these researchers (*i.e.*, NHK reaction<sup>14–16</sup> and deprotection), the total synthesis of biselide A (7) was completed. At this point, we were pleased to find that the spectroscopic data recorded on our synthetic material ( $^1\text{H}$ -,  $^{13}\text{C}$ - NMR, HRMS) was identical in all regards to that reported by Hayakawa and Kigoshi<sup>21</sup> for their synthetic material as well as to that of the natural product.<sup>9</sup> Thus, we were able to access biselide A in ~2% overall yield *via* a synthetic route that required only 18 steps in the longest linear sequence starting from propane diol (Table 1), or 20 steps from L-serine (Scheme 1).

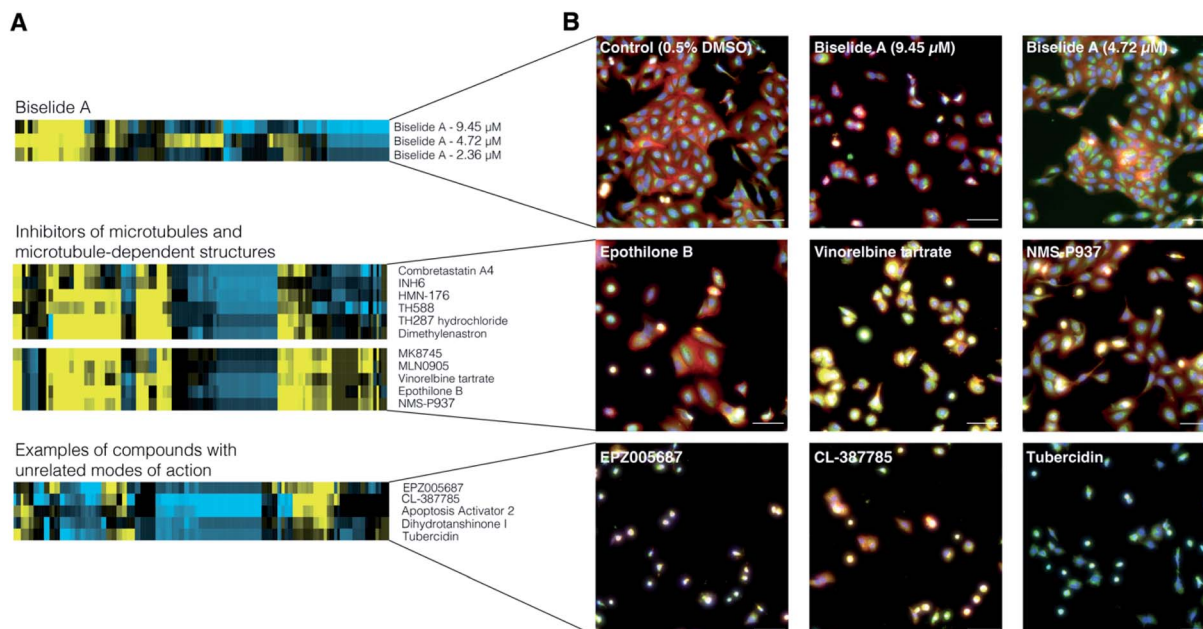
### Biological testing of synthetic biselide A

To examine the effect of biselide A on mammalian cell development, the synthetic natural product was subjected to image-based phenotypic screening using the Cell Painting protocol.<sup>69</sup> Here, hundreds of features, including fluorescence intensity, cell number, and texture, are extracted from images and used to generate a biological activity profile (or fingerprint) of each treatment condition. This fingerprint can then be compared to those of reference compounds to identify similar phenotypes associated with disruption of cell development.<sup>70</sup> A dilution series of biselide A ( $9.5\ \mu\text{M}$  to  $0.3\ \text{nM}$ ,  $16 \times$  two-fold dilutions) was applied to cultures of U2OS human osteosarcoma cells, incubated overnight, treated with a set of five fluorescent stains that stain structural features and sub-cellular organelles, and imaged using an automated high-content fluorescence microscope (Molecular Devices ImageXpress). Hierarchical clustering of fingerprints from biselide A-treated cells with fingerprints from the TargetMol reference library (Fig. 3) identified two concentrations that clustered in a region enriched in reference compounds that impact microtubule organization and dynamics.

Microtubules (MTs) are hollow cylindrical filaments comprised of tubulin dimers. MTs form a variety of highly ordered structures (arrays) that perform key functions within cells, including intracellular transport, cell migration, regulation of cell morphology, and cell division. Cell division is



Scheme 6 Completion of the synthesis of biselide A (7).



**Fig. 3** Cell Painting results for biselide A (7). (A) Cell Painting fingerprints for dilution series of biselide A. Yellow = positive deviation from control values, blue = negative deviation from control values. Comparison of Cell Painting fingerprints for active biselide A concentrations and representative related reference compounds. (B) Original Cell Painting images including control and biselide A (top row), representative TargetMol compounds with related MOAs (middle row) and TargetMol compounds with other, unrelated MOAs (bottom row). Images are composite images from all fluorescence channels.

dependent on the mitotic spindle, a structure consisting of both MTs and associated proteins, such as polo-like kinase 1<sup>71,72</sup> and Aurora kinase,<sup>73,74</sup> which are involved in spindle MT assembly and organization.

Example Cell Painting profiles and images are presented in Fig. 3A and B, respectively. Biselide A clustered with known compounds that can be categorized into two classes based on their impact on MT arrays. Class I compounds (which includes combretastatin A,<sup>75</sup> TH588, TH287 hydrochloride,<sup>76</sup> vinorelbine tartrate,<sup>77</sup> and epothilone B<sup>78</sup>) directly bind to tubulin dimers in all MT arrays, including the spindle, within the cell. Class II compounds (which include INH6,<sup>79</sup> HMN-176,<sup>80</sup> dimethylenastron,<sup>81</sup> MK8745,<sup>82</sup> MLN905,<sup>83</sup> and NMS-P937<sup>84</sup>) target non MT-components of the mitotic spindle. Though these compounds have different targets, they result in similar cellular phenotypes due to their impact on MT arrays. Compounds in class I will impact MT dynamics in all MT arrays and cause MT spindle damage in a dose dependent manner,<sup>75</sup> whereas class II compounds largely affect MT array organization and MT dynamics within the spindle.

Clustering of biselide A in a region enriched in compounds known to target MTs and MT dependent structures suggest that biselide A may perturb MT associated processes, such as cell division, through mechanisms related to regulation of MT cytoskeleton organization.

## Conclusion

We report several generations of synthetic strategies aimed towards the total synthesis of the marine macrolide biselide A.

Ultimately, an enantioselective  $\alpha$ -chloroaldehyde-based approach was used to assemble the tetrahydrofuran core and the 14-membered macrocycle was constructed using a macrocyclic Reformatsky reaction. Notably, all 5 stereocenters in the molecule are installed using substrate-based stereocontrol that originates from a single  $\alpha$ -chloroaldehyde. This concise approach compares well to contemporary syntheses of members of the haterumalide and biselide family of natural products. We also provide the first biological testing data on any synthetic member of this family and show that biselide A disrupts cell division through a mechanism related to regulation of microtubule cytoskeleton organization. This work should support medicinal chemistry efforts directed at anti-cancer macrolides of this general structure class.

## Author contributions

V. R. C. and D. K. completed the total synthesis, M. T., H. F. and B. K. developed methods for THF synthesis, metathesis and explored macrocyclization reactions, D. W. carried out molecular modeling studies, J. H. and S. K. were responsible for biological testing, R. G. L. planned and supervised the biological testing and assisted with manuscript preparation, R. B. planned and supervised the synthetic work and managed manuscript preparation.

## Conflicts of interest

There are no conflicts to declare.





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