Total Synthesis of Amphidinolide X and Its 12*Z*-Isomer by Formation of the C12–C13 Trisubstituted Double Bond via Ring-Closing Metathesis

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Abstract: Amphidinolide X, a 16-membered cytotoxic macrodiolide, and its 12*Z*-isomer have been synthesized via ring-closing metathesis (RCM) for assembling the C12–C13 trisubstituted double bond. A 29:71 *E*/*Z* mixture was obtained from the *seco* substrate appended with a bulky C8-ODPS group in 50–65% combined yields by using 20 mol% of the second-generation Grubbs initiator and the corresponding indenylidene ruthenium complex. Amphidinolide X and 12*Z*-isomer exhibit similar cytotoxicity (IC_{50}: 7.6–13.9 μg/mL) against A549, KB, and HL60 cell lines.

Key words: *anti*-selective aldehyde crotylation, macrodiolide, ring-closing metathesis, microwave, trisubstituted olefin

Amphidinolide X[1] (**1**, Scheme 1) belongs to a family of biologically significant secondary metabolites featured with versatile macrocyclic lactones of 12- to 29-membered rings.[2] Compound **1** is a unique 16-membered macrodiolide and was isolated from cultures of marine dinoflagellates *Amphidinium* sp. (strain Y-42), exhibiting cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cell lines with IC_{50} values of 0.6 and 7.5 μg/mL, respectively.[1] It was proposed that **1** may be biogenetically related to the 17-membered amphidinolide Y[3] (**2**, Scheme 1), which exists as an equilibrium mixture of 6-keto and 6(9)-hemiacetal forms (**2a/2b** = 5:1 to 9:1) in $CDCl_3$. Exposure of **2** to $Pb(OAc)_4$ formed **1** as the oxidative cleavage product.[1] These two molecules possess a common *trans*-fused tetrasubstituted tetrahydrofuran ring, and trisubstituted and conjugated *E*-double bonds. Fürstner and co-workers have completed the first total syntheses of amphidinolide X and Y[4] by using the classic macrolactonization as the ring-forming operation.[5] We have recently accomplished the second total synthesis of amphidinolide Y by ring-closing metathesis (RCM)[6] of the densely functionalized alkenes for assembling the trisubstituted *E*-double bond at C11–C12 (Scheme 1).[7,8] It was found that the remote endocyclic appendages at C6 and C9 exerted a profound influence on the reactivity toward RCM. We took a similar strategy for construction of the 16-membered macrodiolide **1** as outlined in Scheme 1. Disconnection of the two ester bonds and the C12–C13 double bond according to RCM gave three fragments **3**–**5**, of which the tetrahydrofuran fragment **5** has been synthesized in our previous study.[7,9] The fragment **3** would be prepared by *anti*-selective aldehyde crotylation using chiral boron[10a,b] and silicon[11] reagents. The hydroxyl group in the chiral ester (*R*)-**6** was selected as a masked C8-ketone. The bisacid fragment **4** could be obtained from the chiral alcohol (*S*)-**7** by homologation at C5 and the Wittig olefination at C3. We report here on total synthesis of amphidinolide X (**1**) and its 12*Z*-isomer via RCM and preliminary cytotoxicity of both macrodiolides.

Scheme 2 describes the synthesis of the C7–C12 fragment **3**. The reaction of (*S*,*S*)-diisopropyl tartrate (*E*)-crotylboronate (*S*,*S*)-**10**[10c] with the known aldehyde (*R*)-**8**[12] derived from (*R*)-3-hydroxybutyric acid methyl ester (*R*)-**6** gave a 83:17 mixture of the two *anti*-homoallyl alcohols **3** and **3′** in favor of the desired stereomer **3**.[10d] We used the chiral silicon reagent (*S*,*S*)-**9** developed by Leighton et al.[11,13] for the same crotylation of the chiral aldehyde (*R*)-**8**. The desired homoallyl alcohol **3** was obtained in 59% yield and in a diastereomeric ratio of >99:1. The results suggest a nearly complete stereocontrol by the chiral silicon reagent without influence arising from the stereogenic β-carbon of the aldehyde.

The acid **11** was prepared from the known alcohol (*S*)-**7**[14] in 73% overall yield in three steps (Scheme 3). Condensation of **11** with the chiral homoallyl alcohol **3** was performed under the Yamaguchi conditions[15] to afford the ester **12** in 90% yield. Removal of the PMB group in **12** by DDQ (82%) and oxidation of the resultant alcohol **13** by DMP (80%) afforded the aldehyde **14**. The latter was then subjected to the Wittig olefination with the ylide, $Ph_3P{=}CHCO_2Me$, to form the methyl ester **15** in 90% yield. Selective cleavage of a methyl ester in the presence of other esters could be effected by using LiI in pyridine at heating temperatures for more than 24 hours.[16] We used a modified procedure by treating **15** with LiI (10 equiv) in pyridine at 180 °C for one hour under microwave heating[16b] to afford the acid **16** in 85% isolated yield. Condensation of the acid **16** with the known fragment **5**[7] afforded the *seco* intermediate **17** (90%) which was treated with HF·pyridine to remove the *tert*-butyldiphenylsilyl (DPS) group, affording the C8-free alcohol **18** in 65%

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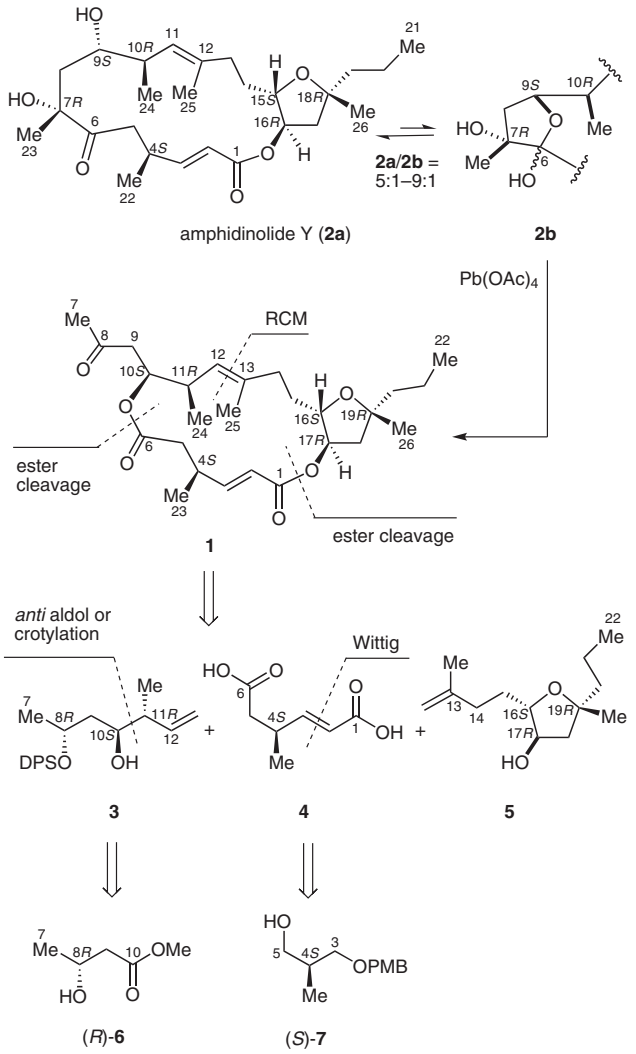
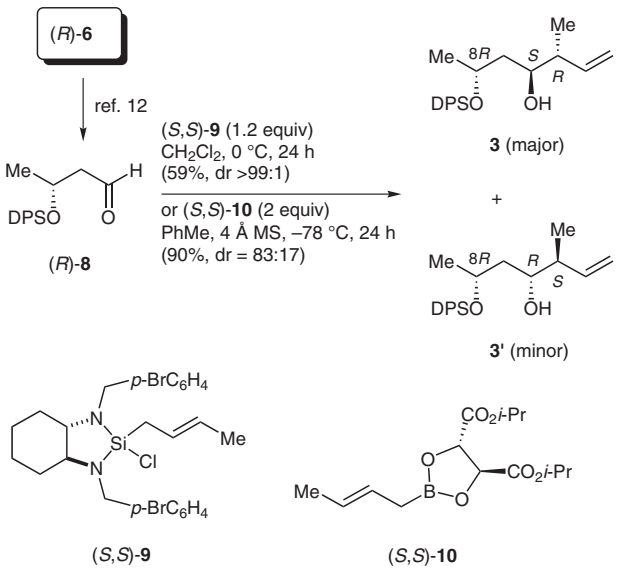


Scheme 1 Structures of amphidinolide X (**1**) and Y (**2**) and retrosynthetic bond disconnections of amphidinolide X



Scheme 2 Synthesis of the C7–C12 fragment **3**

yield. Finally, oxidation of **18** using DMP[19] furnished the ketone **19** in 93% yield.

In our total synthesis of amphidinolide Y (**2**),[7] we found that the second-generation Grubbs initiator **23** promoted formation of the trisubstituted *E*-C11–C12 double bond while Schrock's molybdenum catalyst was inactive with only recovered starting materials. We examined the RCM reactions of **17**–**19** using three initiators **22**–**24** (Scheme 4) and the results are summarized in Table 1. In the presence of 20 mol% of the first-generation Grubbs initiator **22**, the substrate **17** did not form any RCM product with or without added Ti(O*i*-Pr)$_4$[17] (Table 1, entries 1 and 2). When the second-generation Grubbs initiator **23** was used, the substrate **17** furnished the RCM products **20** and **21** in 50% combined yield as a 71:29 (*Z*/*E*) mixture (Table 1, entry 3). It is interesting to find that the indenylidene ruthenium complex **24**[18] could promote RCM of **17** to afford a 65% yield of **20** and **21** and in the same *Z*/*E* ratio (Table 1, entry 4). In contrast to the C8-protected **17**, the C8-free alcohol **18** decomposed upon exposure to the initiator **23** while the C8-ketone **19** afforded exclusively (12*Z*)-amphidinolide X [(12*Z*)-**1**] in 85% yield (Table 1, entries 5 and 6).[19] Finally, the mixture of **20**/**21** was subjected to desilylation (HF·pyridine, 75%) and oxidation (DMP, 85%),[20] giving pure (12*Z*)-amphidinolide X {[α]$_D^{17}$ –16.0 (*c* 1.00, $CHCl_3$)} and amphidinolide X (**1**) {[α]$_D^{17}$ –26.8 (*c* 1.00, $CHCl_3$)}. The latter agrees with the reported [α]$_D^{17}$ values of –12 (*c* 1.00, $CHCl_3$)[1] and –25.6 (*c* 1.00, $CHCl_3$).[4a]

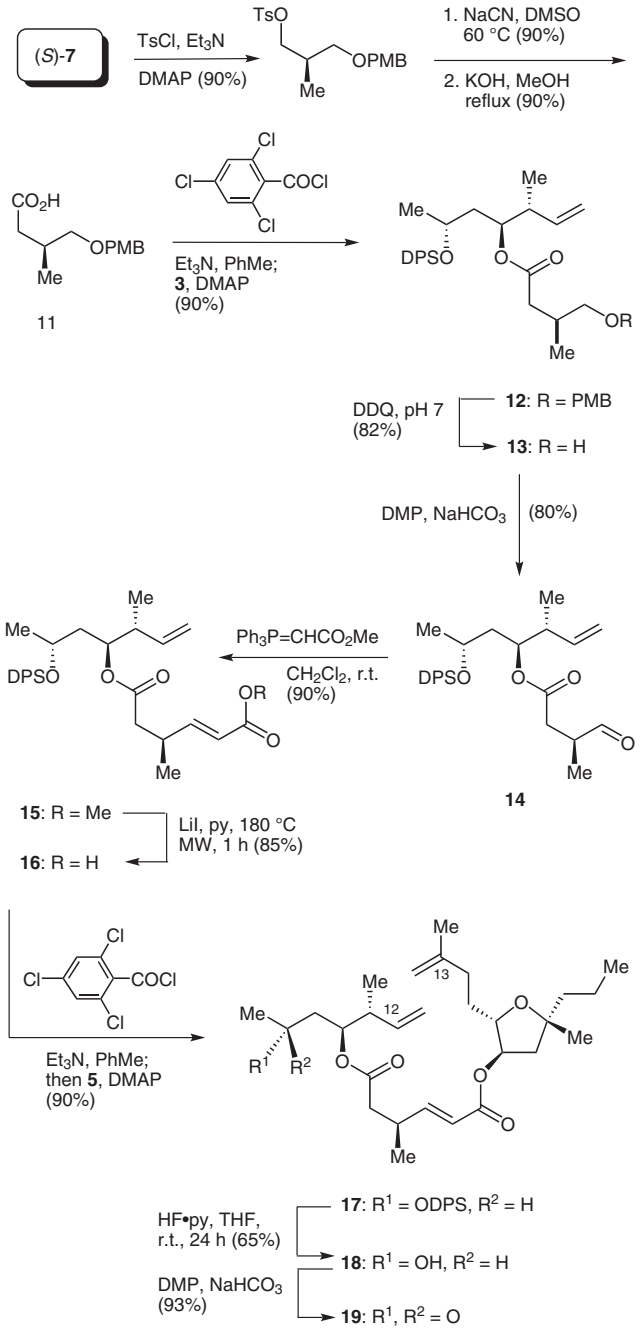
Table 2 shows our preliminary assay results of synthetic amphidinolide X (**1**) and the 12*Z*-isomer using A549, KB, and HL60 cancer cell lines. The data reveal that **1** and (12*Z*)-**1** exhibit similar cytotoxicity (IC_{50}: 7.6–13.9 μg/mL), indicating that the geometry of the trisubstituted alkene is not essential for cytotoxic activity.

In summary, we have accomplished total synthesis of amphidinolide X (**1**) and its 12*Z*-isomer via formation of the trisubstituted double bond by RCM. A bulky DPSO group at the remote exocyclic C8 position is found helpful in for-

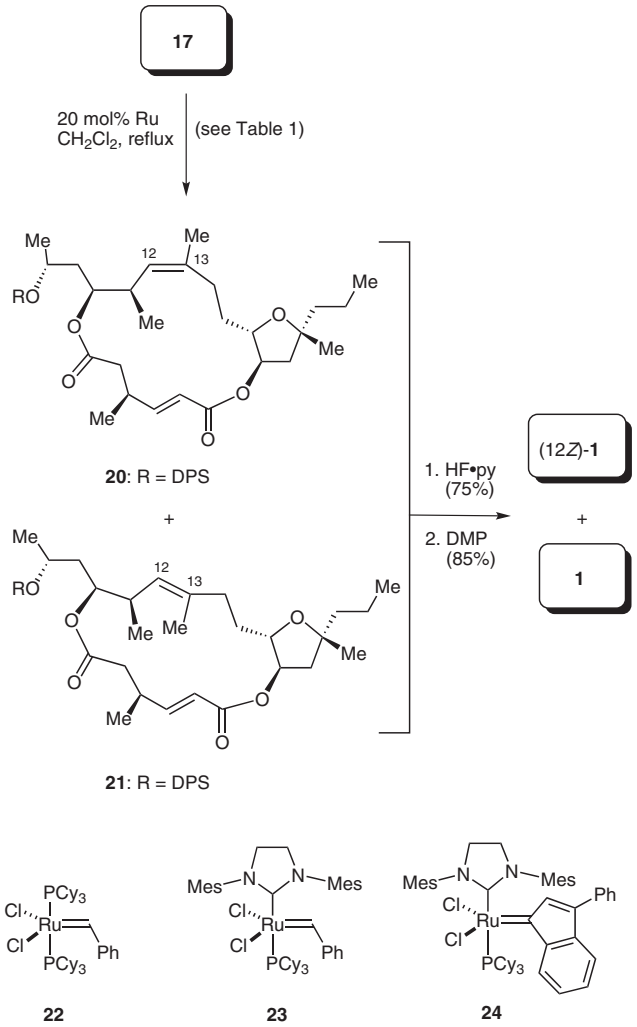
Table 1 Results of RCM Reactions of **17**–**19** in Refluxing CH_2Cl_2

Entry	Substrate	Cat.[a]	Time (d)	Yield (%)(%)[b]	Ratio (*Z*/*E*)[c]
1	**17**	**22**	3	NR[d]	–
2	**17**	**22**	4[e]	NR[d]	–
3	**17**	**23**	6	**20** + **21**: 50	71:29
4	**17**	**24**	6	**20** + **21**: 65	71:29
5	**18**	**23**	3	–[f]	–
6	**19**	**23**	4	(12*Z*)-**1**: 85	100:0

[a] The catalyst was added in portions.
[b] Isolated yield.
[c] Estimated values based on ^{1}H NMR spectra of the reaction mixtures.
[d] No reaction with recovery of **17**.
[e] Ti(O*i*-Pr)$_4$ (30 mol%) was added.
[f] Decomposed to give unidentified byproduct(s).



Scheme 3 Synthesis of the *seco* intermediates **17**–**19**



Scheme 4 The RCM reaction of **17** and synthesis of (12*Z*)-**1** and **1**

Table 2 The IC_{50} (μg/mL) Data of Amphidinolide X (**1**) and (12*Z*)-**1**

Compound	IC_{50} (A549)[a]	IC_{50} (KB)[b]	IC_{50} (HL60)[c]
1	10.93 ± 1.55	8.53 ± 0.92[d]	11.31 ± 0.84
(12*Z*)-**1**	13.85 ± 1.06	12.86 ± 1.32	7.61 ± 1.01
taxol	0.013 ± 0.002	0.003 ± 0.0004	<0.003

[a] Non-small-cell lung cancer cell line.
[b] Human epidermoid carcinoma cell line.
[c] Human leukemia cell line.
[d] A value of 7.5 μg/mL was reported for natural **1** (see ref. 1).

mation of the 12*E*-alkene as compared to the exclusive formation of the 12*Z*-isomer from the C8-ketone substrate.[21] The preference for trisubstituted *Z*-alkene in assembling the skeleton of amphidinolide X via RCM seems the synergetic effect of a smaller ring system and the macrodiolide moiety.

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(13) We used noncrystallized form of the reagent. An 85:15 mixture of (*S*,*S*)-**9** and its *Z*-isomer was prepared from an 85:15 mixture of *E*- and *Z*-crotyl chloride obtained from Aldrich according to the literature procedure (ref. 11). The *Z*-isomer of (*S*,*S*)-**9** reacted with (*R*)-**8** to produce a *syn*-homoallyl alcohol which is different from **3′**. The diastereomeric ratio of >99:1 was estimated according to the ^{1}H NMR spectrum of the product mixture.
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(19) **Procedure for Synthesis of (12*Z*)-Amphidinolide X via Ring-Closing Metathesis of the *seco* Ketone 19 Using Second-Generation Grubbs Initiator 23**
To a degassed, refluxing solution of the *seco* ketone **19** (30.0 mg, $6.3\cdot10^{-2}$ mmol) in anhyd CH_2Cl_2 (100 mL) under a nitrogen atmosphere was added the second-generation Grubbs initiator **23** (2.7 mg, $0.3\cdot10^{-2}$ mmol). The resulting clear, pale pink solution changed to a clear yellow color after refluxing for 24 h. Three additional portions of **23** (2.7 mg, $0.3\cdot10^{-2}$ mmol; a total of $1.2\cdot10^{-2}$ mmol) were added after 24, 48, and 72 h, respectively. After refluxing for a total of 4 d, the reaction mixture was concentrated to <1 mL on a rotary evaporator, and the remaining mixture was purified directly by flash column chromatography over SiO_2 [eluting with 3% EtOAc in PE (bp 60–90 °C)] to give (12*Z*)-amphidinolide X [(12*Z*)-**1**, 24.0 mg, 85%]. (12*Z*)-Amphidinolide X [(12*Z*)-**1**]: colorless oil; $[\alpha]_D^{17}$ –16.0 (*c* 1.00, $CHCl_3$). IR (film): 2964, 1731, 1655, 1454, 1377, 1315, 1269, 1215, 1167, 1049 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.92 (dd, *J* = 16.0, 6.4 Hz, 1 H), 5.81 (dd, *J* = 16.0, 1.6 Hz, 1 H), 5.18 (dt, *J* = 8.8, 4.0 Hz, 1 H), 4.88 (d, *J* = 10.4 Hz, 1 H), 4.82 (dt, *J* = 8.8, 7.2 Hz, 1 H), 3.74 (ddd, *J* = 10.0, 7.6, 2.8 Hz, 1 H), 2.96–2.89 (m, 1 H), 2.85–2.80 (m, 1 H), 2.66–2.58 (m, 2 H), 2.53 (dd, *J* = 14.8, 4.8 Hz, 1 H), 2.42–2.33 (m, 2 H), 2.26–2.19 (m, 1 H), 2.16 (s, 3 H), 1.89–1.82 (m, 2 H), 1.70 (s, 3 H), 1.64 (dd,

J = 13.2, 7.2 Hz, 1 H), 1.49–1.47 (m, 2 H), 1.36–1.30 (m, 2 H), 1.26 (s, 3 H), 1.15 (d, *J* = 7.6 Hz, 3 H), 1.05 (d, *J* = 6.8 Hz, 3 H), 0.91 (t, *J* = 7.6 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.4, 171.3, 166.0, 151.7, 137.3, 126.3, 120.0, 81.9, 79.5, 78.0, 72.8, 44.8, 43.8, 43.1, 40.0, 35.9, 33.9, 31.4, 30.1, 27.3, 26.7, 23.5, 19.6, 17.8, 14.7, 14.6. MS (+ESI): *m/z* (rel. int.) = 471 (100) [M + Na^+]. HRMS (+ESI): *m/z* calcd for $C_{26}H_{40}O_6Na^+$ [M + Na^+], 471.2717; found: 471.2719.

(20) **Procedure for the Synthesis of Amphidinolide X via Ring-Closing Metathesis of 17 Using Second-Generation Grubbs Initiator 23 as the Key Step**

To a degassed, refluxing solution of **17** (150.0 mg, 0.21 mmol) in anhyd CH_2Cl_2 (150 mL) under a nitrogen atmosphere was added the second-generation Grubbs initiator **23** (8.9 mg, $1.1\cdot10^{-2}$ mmol). The resulting clear, pale pink solution changed to a clear yellow color after refluxing for 24 h. Three additional portions of **23** (8.9 mg, $1.1\cdot10^{-2}$ mmol; a total of $4.4\cdot10^{-2}$ mmol) were added after 24, 48, and 72 h, respectively. After refluxing for a total of 6 d, the reaction mixture was concentrated to <1 mL on a rotary evaporator and the remaining mixture was purified directly by flash column chromatography over SiO_2 [eluting with 3% EtOAc in PE (bp 60–90 °C)] to give a 71:29 mixture of the *Z* and *E* RCM products **20** and **21** (70.0 mg, 50%) as a colorless oil. The *Z*/*E* ratio was determined by the integration of the corresponding signals in the ^{1}H NMR spectrum. An pure sample of isomer **20** was obtained by flash column chromatography [eluting with 3% EtOAc in PE (bp 60–90 °C)] of the product mixture.

Compound **20**: colorless oil; $[\alpha]_D^{17}$ –35.9 (*c* 2.0, $CHCl_3$). IR (film): 2929, 1733, 1653, 1456, 1428, 1379, 1268, 1167, 1112, 1086 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68–7.65 (m, 4 H), 7.42–7.32 (m, 6 H), 6.93 (dd, *J* = 16.0, 6.0 Hz, 1 H), 5.75 (dd, *J* = 16.0, 1.6 Hz, 1 H), 5.02–5.00 (m, 1 H), 4.86 (d, *J* = 10.8 Hz, 1 H), 4.78 (dt, *J* = 8.4, 7.2 Hz, 1 H), 3.82–3.78 (m, 1 H), 3.74–3.70 (m, 1 H), 2.97–2.89 (m, 1 H), 2.74–2.67 (m, 1 H), 2.43–2.36 (m, 2 H), 2.30–2.22 (m, 2 H), 1.91–1.71 (m, 4 H), 1.67 (s, 3 H), 1.64–1.61 (m, 2 H), 1.48–1.44 (m, 2 H), 1.36–1.30 (m, 2 H), 1.25 (s, 3 H), 1.14 (d, *J* = 6.8 Hz, 3 H), 1.02 (d, *J* = 6.0 Hz, 3 H), 1.00 (s, 9 H), 0.96 (d, *J* = 7.2 Hz, 3 H), 0.91 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.3, 166.2, 151.8, 136.3 (2×), 136.1 (2×), 135.7, 135.3, 134.2, 129.7, 129.6, 127.7 (2×), 127.6 (2×), 127.4, 119.9, 81.7, 79.8, 77.8, 74.3, 67.6, 44.8, 44.0, 40.1, 38.5, 36.1, 33.4, 31.3, 27.2 (3×), 27.1, 26.8, 24.2, 23.3, 19.5, 19.3, 17.9, 14.8, 14.4. MS (+ESI): *m/z* (rel. int.) = 711 (100) [M + Na^+]. HRMS (+ESI): *m/z* calcd for $C_{42}H_{60}O_6SiNa^+$ [M + Na^+], 711.4051; found: 711.4054.

A plastic tube was charged with the 71:29 mixture of **20** and **21** (50.0 mg) in THF (2 mL) followed by adding 70% hydrogen fluoride pyridine (2 mL) at r.t. The resultant mixture was stirred for 24 h at r.t. and the reaction was quenched carefully with sat. aq Na_2CO_3. The reaction mixture was extracted with Et_2O and the combined organic layer was dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography over SiO_2 [eluting with 20% EtOAc in PE (bp 60–90 °C)] to give a mixture of the alcohol (34.0 mg, 75%) as a colorless oil. The structure of the alcohol was confirmed by mass spectrometry data. MS (+ESI): *m/z* (rel. int.) = 473 (100) [M + Na^+]. HRMS (+ESI): *m/z* calcd for $C_{26}H_{42}O_6Na^+$ [M + Na^+]: 473.2874; found: 473.2880.

To a solution of the above mixture (34.0 mg, $7.5\cdot10^{-2}$ mmol) in CH_2Cl_2 (1 mL) at r.t. was added $NaHCO_3$ (19 mg, 0.225 mmol) followed by carefully adding a solution of Dess–Martin periodinane in CH_2Cl_2 (0.3 M, 0.5 mL, 0.15 mmol). The resultant mixture was stirred at r.t. for 4 h followed by treating with sat. aq $Na_2S_2O_3$. The reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhyd Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography over SiO_2 [eluting with 15% EtOAc in PE (bp 60–90 °C)] to give amphidinolide X (**1**, 8.0 mg, 23%) and (12*Z*)-amphidinolide X [(12*Z*)-**1**, 21.0 mg, 62%].

Amphidinolide X (**1**): colorless oil; $[\alpha]_D^{17}$ –26.8 (*c* 1.00, $CHCl_3$). IR (film): 2963, 1717, 1654, 1453, 1377, 1265, 1186, 1039 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.12 (dd, *J* = 15.8, 7.2 Hz, 1 H), 5.79 (dd, *J* = 15.8, 1.6 Hz, 1 H), 5.20 (m, 2 H), 4.95 (d, *J* = 10.3 Hz, 1 H), 3.96 (dt, *J* = 11.2, 3.6 Hz, 1 H), 2.78 (m, 1 H), 2.69 (dd, *J* = 16.6, 6.1 Hz, 1 H), 2.69 (m, 1 H), 2.58 (dd, *J* = 13.6, 3.9 Hz, 1 H), 2.58 (dd, *J* = 16.5, 7.5 Hz, 1 H), 2.41 (dd, *J* = 13.3, 6.3 Hz, 1 H), 2.18 (m, 1 H), 2.17 (m, 1 H), 2.14 (s, 3 H), 2.12 (m, 1 H), 1.94 (tt, *J* = 13.5, 3.3 Hz, 1 H), 1.75 (dd, *J* = 13.9, 2.4 Hz, 1 H), 1.55 (s, 3 H), 1.54 (m, 1 H), 1.50 (m, 2 H), 1.35 (m, 2 H), 1.30 (s, 3 H), 1.14 (d, *J* = 6.9 Hz, 3 H), 0.92 (t, *J* = 7.5 Hz, 3 H), 0.91 (d, *J* = 7.1 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 205.5, 170.7, 165.8, 153.2, 135.6, 126.1, 120.3, 83.0, 80.6, 78.5, 74.3, 47.2, 44.3, 43.6, 41.5, 35.6, 35.4, 33.1, 30.5, 30.5, 24.7, 18.2, 17.9, 17.6, 15.5, 14.7. MS (+ESI): *m/z* (rel. int.) = 471 (100) [M + Na^+]. HRMS (+ESI): *m/z* calcd for $C_{26}H_{40}O_6Na^+$ [M + Na^+]: 471.2717; found: 471.2699.

(21) Remote control of alkene geometry by endocyclic groups in ring-closing metathesis has been reported. For examples of formation of disubstituted macrocyclic alkenes, see: (a) Meng, D.; Su, D.-S.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733. (b) Fürstner, A.; Thiel, O. R.; Blanda, G. *Org. Lett.* **2000**, *2*, 3731. (c) Fürstner, A.; Dierkes, T.; Thiel, O. R.; Blanda, G. *Chem. Eur. J.* **2001**, *7*, 5286. (d) Aïssa, C.; Riveiros, R.; Ragot, J.; Fürstner, A. *J. Am. Chem. Soc.* **2003**, *125*, 15512. (e) Couladouros, E. A.; Mihou, A. P.; Bouzas, E. A. *Org. Lett.* **2004**, *6*, 977. (f) Castoldi, D.; Caggiano, L.; Panigada, L.; Sharon, O.; Costa, A. M.; Gennari, C. *Angew. Chem. Int. Ed.* **2005**, *44*, 588. (g) Matsumura, T.; Akiba, M.; Arai, S.; Nakagawa, M.; Nishida, A. *Tetrahedron Lett.* **2007**, *48*, 1265. (h) Mohapatra, D. K.; Ramesh, D. K.; Giardello, M. A.; Chorghade, M. S.; Gurjara, M. K.; Grubbs, R. H. *Tetrahedron Lett.* **2007**, *48*, 2621. (i) For examples of formation of trisubstituted macrocyclic alkenes, see: Meng, D.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073. (j) Nicolaou, K. C.; Vassilikogiannakis, G.; Montagnon, T. *Angew. Chem. Int. Ed.* **2002**, *41*, 3276. (k) Nicolaou, K. C.; Montagnon, T.; Vassilikogiannakis, G.; Mathison, C. J. N. *J. Am. Chem. Soc.* **2005**, *127*, 8872. (l) Vassilikogiannakis, G.; Margaros, I.; Tofi, M. *Org. Lett.* **2004**, *6*, 205. (m) Alhamadsheh, M. M.; Gupta, S.; Hudson, R. A.; Perera, L.; Tillekeratne, L. M. V. *Chem. Eur. J.* **2008**, *14*, 570. (n) Trost, B. M.; Dong, G.; Vance, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 4540. (o) Ramírez-Fernández, J.; Collado, I. G.; Hernández-Galán, R. *Synlett* **2008**, 339; see also ref. 7.