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Thrombomodulin induction in cultured human endothelial cells by 9-*cis*-locked retinoic acid analogues

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Abstract—9-*cis*-Retinoic acid (RA) analogues devised to lock the 9-*cis* double bond by ring formation were synthesized using two stereoselective carbon–carbon bond formation reactions as key steps. The palladium-mediated Suzuki reaction was adopted to construct a 7*E*-double bond (RA numbering) and the Horner–Emmons olefination was employed for stereoselective 11*E*-double bond (RA numbering) formation. The synthesized 9-*cis*-RA analogues that are locked by five-membered ring systems (cyclopentene, dihydrofuran, and dihydrothiophene) were shown to have comparable thrombomodulin induction activities to that of 9-*cis* RA. Conformational analysis of these compounds showed their similarity to 9-*cis* RA in the spatial orientation of the side chain and the terminal carboxy group.

1. Introduction

Retinoic acid (RA) is well known to play a critical role in many aspects of cell proliferation and differentiation, and has proved useful for the treatment of dermatologic diseases and certain cancers.[1] We have demonstrated that all-*trans*-RA (ATRA) causes an increase in thrombomodulin (TM) antigen in human umbilical vein endothelial cells (HUVECs) in vitro.[2,3] TM is an endothelial membrane surface glycoprotein and represents one of the most important antithrombotic factors in the anticoagulant system. TM forms a 1:1 stoichiometric complex with thrombin and then thrombin loses its procoagulant functions such as fibrin formation. Additionally, the TM–thrombin complex markedly promotes activation of protein C, which proteolytically degrades coagulation factors.[4,5] Since TM functions as an antithrombotic agent, RA has the ability to change the coagulant balance by controlling TM expression.

Recently, much attention has been paid to 9-*cis*-RA, because it has been identified as a second endogenous RA that binds both retinoic acid receptors (RARs) and retinoid X receptors (RXRs) with nanomolar affinity.[6–8] This finding prompted us to investigate the structure–activity relationships of RA analogues to TM induction. Figure 1 shows our preliminary observations of TM induction by geometric isomers of RAs (ATRA, 9-*cis*-RA, 11-*cis*-RA, and 13-*cis*-RA). The TM expression depended on the amount of all of Ras,[9] but a significant increase was observed at 0.1 μM 9-*cis*-RA, which was most potent among examined. Thus, our attention was centered around the structural modification which fixes the *cis*-double bond of 9-*cis*-RA.

2. Results and discussion

2.1. Molecular design

Our design of 9-*cis*-RA analogues presented herein was based on locking its 9-*cis*-double bond by introducing an appropriate ring system.[10]

As shown in Figure 2, two modes of ring formation can be applied to lock the 9-*cis*-double bond. Mode A forms

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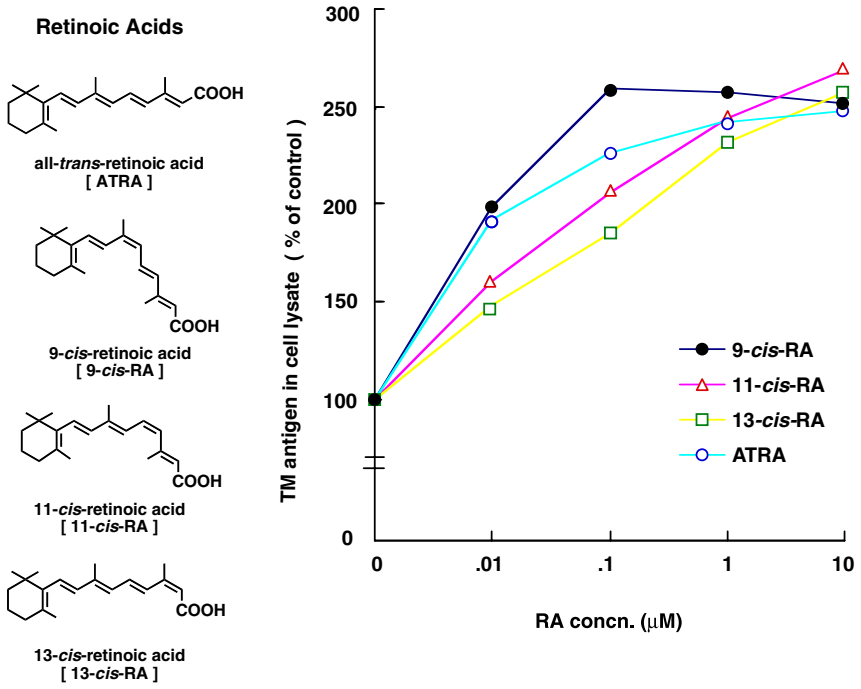


Figure 1. Thrombomodulin induction in human endothelial cells by geometric isomers of RAs.

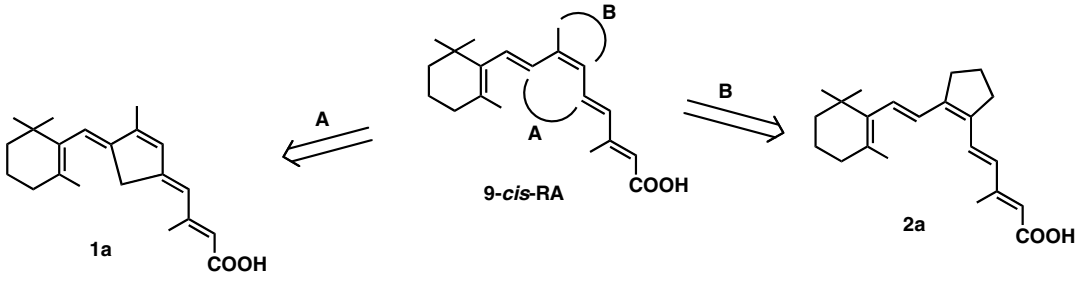


Figure 2. Two modes of ring formation of 9-*cis*-locked RA.

a ring system inside the molecule, and mode B forms one outside. Some groups used mode A ring formation to lock the 11-*cis*-double bond in their studies on the mechanism of vision in which *cis–trans* isomerization of retinal tissue plays an important role.[11–14] Successful results were obtained in those studies, although mode A ring formation was considered to alter the three-dimensional skeleton of the parent *cis*-RA. Molecular mechanics calculations have been widely employed to investigate stable conformations of biologically interesting molecules, and Dawson's group has adopted this technique to discuss the functionally active conformation of 9-*cis*-RA based on comparison with RAR- and RXR-selective retinoids.[15,16] Mohamadi et al. conducted a similar computational analysis[17] of 9-*cis*-locked RAs **1a** and **2a** having five-membered ring systems as representative molecules for our design. Monte Carlo conformational searches[18] of **1a** and **2a** showed that they have different conformational properties. The most stable conformer of 9-*cis*-RA, which was selected as the functionally active conformer based on Jong et al.'s assumption,[15] is superimposed on the corresponding similar conformers of **1a** or **2a** to visualize their differences (polytube model in Fig. 3, colored: 9-*cis*-RA and black: 9-*cis*-locked analogue).

Apparently, ring formation in **1a** causes distinct locations of the side chain and the terminal carboxyl group.

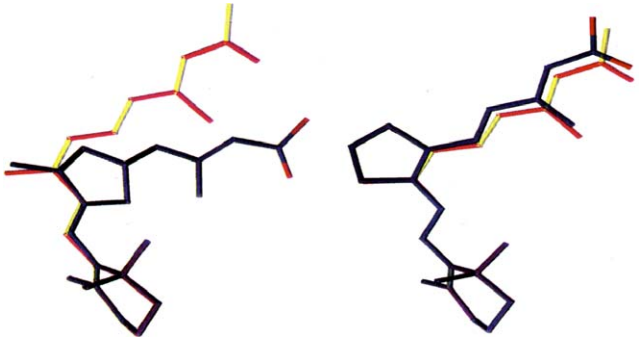


Figure 3. Molecular design of 9-*cis*-locked RA by inducing a ring system. Left, **1a** superimposed on the most stable conformer of 9-*cis*-RA; right, **2a** superimposed on the most stable conformer of 9-*cis*-RA.

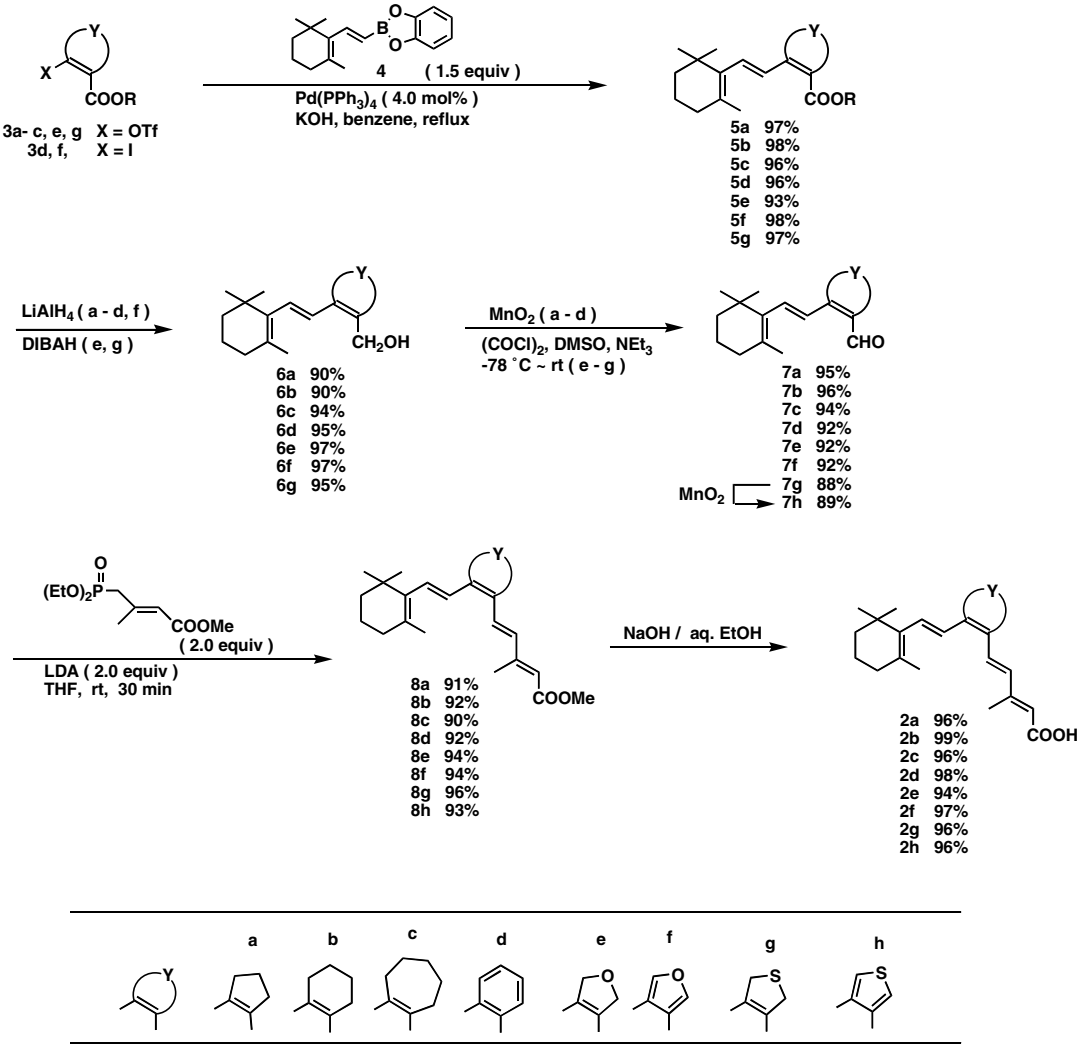
On the contrary, mode B ring formation (**2a**) retains these locations nearly unchanged. Employment of a six-membered or seven-membered ring system resulted in analogous conformational properties. Katsuta et al. have reported the synthesis of 9-*cis*-RA analogues employing mode A ring formation (six-membered ring) and showed that it was less effective than 9-*cis*-RA.[19] Their result is consistent with our computational consideration because the carboxyl group of their derivative was also calculated to be differently positioned from that of 9-*cis*-RA. Since **2a** was found to have a similar conformational property based on the inspection of this computational molecular modeling, we focused our attention on the 'outer locked' 9-*cis*-RAs (mode B ring formation). Recently, the cyclohexene derivative **2b** (Ro 25-6603), which is one of our target molecules, has been reported to be an RXR-selective ligand.[20,21] Similarly, analogues of 9-*cis*-RA incorporating an alicyclic ring, which included our target molecules **2a**–**c**, have been synthesized and their binding affinities for the RXR receptor were determined.[22] Conformationally defined 6-*s*-*trans*-RA analogues employing a similar ring formation have also been reported.[23]

In the first stage of this study, we prepared cycloalkane and benzene derivatives **2a**–**d**. After performing biological evaluations showing that the cyclopentene derivative **2a** was most effective (vide infra), we next examined the incorporation of heteroatoms into five-membered ring systems (**2e**–**h**).

2.2. Chemistry

9-*cis*-Locked RA analogues **2a**–**h** were synthesized using two stereoselective carbon–carbon bond formation reactions as key steps[22] (Scheme 1).

In the first step, the Suzuki reaction of alkenyl triflates (**3a**–**c**, **e**, and **g**) or aryl iodides (**3d** and **f**) with alkenylborane **4** was employed to construct the 7*E*-double bond (RA numbering). This palladium-catalyzed cross-coupling has been known to proceed with complete retention of stereochemistry of the double bond,[24,25] and completely stereochemically defined coupling products **5a**–**g** were obtained in high yield (90%-quantitative). The esters **5a**–**g** thus obtained were converted to alcohols **6a**–**g** using an appropriate reducing reagent.



Scheme 1. Reagents: (a) $LiAlH_4$, THF (for **6a**–**d**, f); DIBAL, CH_2Cl_2, (for **6e**, g); (b) MnO_2, pet. ether (for **7a**–**d**); $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, (for **7e**–**g**); $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, then MnO_2, pet. ether (for **7h** from **6g**); (c) NaOH, aq EtOH.

In the case of dihydrofuran and dihydrothiophene derivatives (**6e** and **g**), DIBAL was used in place of $LiAlH_4$ because ring cleavage of these heterocycles was observed. After conversion of **6a**–**g** to aldehydes **7a**–**g** in MnO_2 or Swern oxidations, Horner–Emmons olefination resulted in a stereoselective coupling reaction to form an 11*E*-double bond (RA numbering) in 90–98% yield.[26] Thiophene aldehyde **7h** was prepared by oxidative aromatization of **7g** and was similarly converted into **8h**. Although a small amount (ca. <5% estimated by ^1H NMR) of stereoisomers was formed in the olefination, they were easily removed by recrystallization at the final stage. Hydrolysis of esters **8a**–**h** by NaOH in aqueous EtOH afforded the desired acids **2a**–**h**. In this straightforward synthesis, RA analogues were prepared stereoselectively in high overall yield (65–81%).

2.3. Biological activity

HUVECs were treated with various concentrations of ATRA, 9-*cis*-RA, and synthesized RA analogues **2a**–**d** for 24 h, and TM antigen levels in cell lysates were measured in an enzyme immunoassay.[27]

As shown in Figure 4, the effects of 9-*cis*-RA and cyclopentene derivative **2a** were dose-dependent and the TM antigen levels increased to 150% of the baseline level at the dose of RAs of 0.001 μM and to 180% at 0.01 μM. Cyclohexene derivative **2b** showed a similar activity to 9-*cis*-RA at 0.001 μM, but lower activity was observed at higher concentrations (1–10 μM). The other derivatives **2c** and **d** were less potent in this assay.

The effects of heterocyclic analogues **2e**–**h** were also measured at the concentrations of 0.001 μM, 0.1 μM, and 10 μM (Fig. 5). Dihydrofuran and dihydrothiophene derivatives **2e** and **g** increased TM antigen levels to 200% of the baseline level at 0.1 μM, which was comparable to the increase induced by **2a**. Aromatization of heterocycles (**2f** and **h**) significantly lowered TM induction potency.

2.4. Discussion

Based on the result that 9-*cis*-RA induced TM most effectively, we designed RA analogues that retain the conformational property of 9-*cis*-RA. The computational conformational searches of RA analogues showed that **2a**–**c** could have a low-energy conformation similar to the 'active conformation' of 9-*cis*-RA. Incorporation of a five-membered ring system especially modifies the spatial orientation of the side chain relative to 9-*cis*-RA, as shown in Figure 2. On the other hand, a similar conformation of the aromatic analogue **2d** was calculated to locate ca. +1 kcal/mol above the global minimum, and the distinct conformation was found to be stable. This conformational analysis suggested a relation between the conformational properties and the activity of TM induction. The five-membered ring system (in **2a**, **e**, and **g**), which mimicked the most stable conformation of 9-*cis*-RA, could be used as an effective lock with retention of biological activity. The low potency of TM induction by the aromatic analogues **2d**, **f**, and **h** was also consistent with the conformational properties mentioned above. Although it was difficult to determine the relation between ring size and activity, it appears plausible that the lower activity of **2b** and **c** was the result of changes in steric bulk caused by introduction of the six- or seven-membered ring system.

Since the analogues described herein are designed to mimic 9-*cis*-RA, which activates both RARs and RXRs, it is conceivable that they are pan-agonists (activating both RARs and RXRs). However, recent reports on RAR- and RXR-selective ligands,[22,23] in which the cyclohexene derivative **2b** was shown to be highly

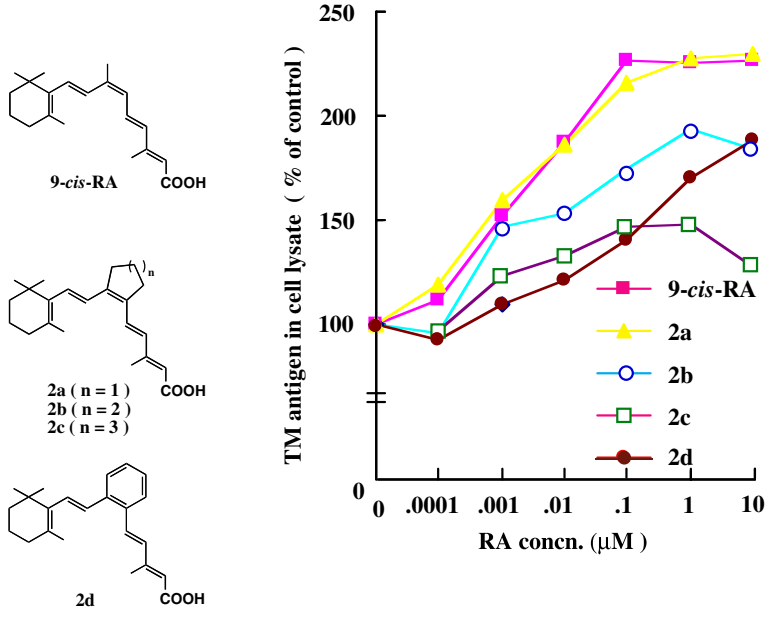


Figure 4. TM induction by 9-*cis*-locked RA analogues (**2a**–**d**).

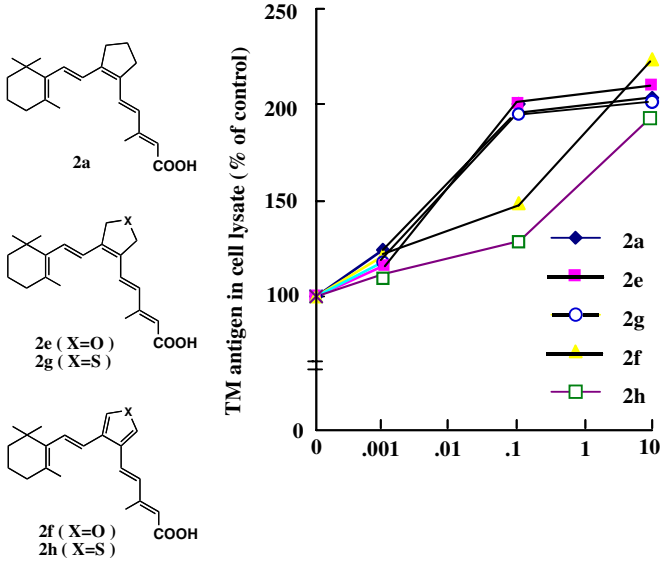


Figure 5. TM induction by 9-*cis*-locked RA analogues (**2e**–**h**).

RXR selective, have suggested that the 9-*cis*-locked analogues are also RXR selective. Although there are many reports on the RXR selectivity of 9-*cis*-RA,[28,29] we have not clarified which receptor(s), RAR and/or RXR, is involved in the expression of TM induction; a study concerning the precise role of RAs in the TM induction is now underway.

3. Conclusion

Molecular design aided by computational chemistry efficiently creates 9-*cis*-locked RA analogues that retain the 'active conformation' of the parent 9-*cis*-RA using appropriate ring formation. The analogues thus designed were synthesized stereoselectively and, as expected, induced TM with potency comparable to that of 9-*cis*-RA. It is also noteworthy that the synthesis of the analogues is very efficient (65–88% overall yield) and thus provides a large quantity of the compounds for various types of biological evaluation.

3.1. Experimental

3.1.1. General. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-8000 spectrometer. ^{1}H NMR spectra were recorded on a JEOL GSX-400 spectrometer (400 MHz). High and low mass spectra were conducted on an JEOL SX-102A spectrometer. Elemental analysis was performed by Perkin-Elmer Series II CHNS/O analyzer 2400. Tetrahydrofuran and benzene were distilled from sodium metal prior to use. Methylene chloride was distilled from CaH_2. Column chromatography was performed by using BW-820 (Fuji Silysia) or Wakogel C-200, and thin-layer chromatography was carried out on 0.25-mm E. Merck precoated silica-gel glass plate (Art. 5715).

3.1.2. Computer modeling. Monte Carlo conformational searches[18] were performed using MacroModel 4.0[17] with the MM2* derivative of the MM2 forcefield.

3.1.3. Ethyl 2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclopent-1-enecarboxylate (5a).[22] To a solution of $Pd(PPh_3)_4$ 28 mg (0.024 mmol) in benzene (2 ml) was added enol triflate **3a** 172 mg (0.60 mmol) in 3 ml of benzene. After stirring for 15 min under Ar, 0.9 M benzene solution of alkenylborane **4**[28] 1.0 ml (0.90 mmol) and 4.0 M KOH aqueous solution 0.23 ml (0.90 mmol) were added at room temperature. The mixture was heated to reflux for 1 h, and the residual organoborane was oxidized by 3 M NaOH (0.1 ml) and 30% H_2O_2 (0.1 ml) for 1 h at room temperature. The reaction mixture was filtered through Celite, and the filtrate was extracted with ether. The organic layer was washed with water and brine, and dried over $MgSO_4$. The solvent was evaporated and the crude residue was purified by silica gel column chromatography (hexane/ethyl acetate 40:1 as an eluent) to afford **5a** 167 mg (97% yield) as a colorless oil. IR (neat) 2930, 1701, 1227, 1107 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (6H, s, $C(CH_3)_2$), 1.30 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 1.47 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.77 (3H, s, CH_3), 1.88 (2H, m, CH_2), 2.04 (2H, t, J = 6.3 Hz, CH_2), 2.73 (4H, t, J = 7.6 Hz, $2(CH_2)$), 4.21 (2H, q, J = 7.0 Hz, $COOCH_2CH_3$), 6.21 (1H, d, J = 16.5 Hz, C=CH), 7.34 (1H, d, J = 16.5 Hz, C=CH). MS (EI) m/z 288 (M^+).

Similar reactions of **3b**, **3c**, **3e**, and **3g** gave the following compounds.

3.1.4. Ethyl 2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohex-1-enecarboxylate (5b).[22] Yield: 98%. IR (neat) 2930, 1709, 1208 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (6H, s, $C(CH_3)_2$), 1.30 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 1.46 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.67 (4H, m, $2(CH_2)$), 1.72 (3H, d, J = 0.6 Hz, CH_3), 2.01 (2H, t, J = 6.2 Hz, CH_2), 2.39

(4H, m, 2(CH_2)), 4.21 (2H, q, J = 7.0 Hz, COOCH_2CH$_3$), 6.32 (1H, d, J = 16.3 Hz, C=CH), 6.83 (1H, d, J = 16.3 Hz, C=CH). MS (EI) m/z 302 (M^+).

3.1.5. Ethyl 2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohept-1-enecarboxylate (5c).[22] Yield: 95%. IR (neat) 2924, 1703, 1200 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (6H, s, C(CH_3)$_2$), 1.29 (3H, t, J = 7.1 Hz, COOCH$_2$CH_3), 1.46 (2H, m, CH_2), 1.51–1.64 (6H, m, 3(CH_2)), 1.71 (3H, d, J = 0.6 Hz, CH_3), 1.76 (2H, m, CH_2), 2.01 (2H, t, J = 6.3 Hz, CH_2), 2.53 (4H, m, 2(CH_2)), 4.20 (2H, q, J = 7.1 Hz, COOCH_2CH$_3$), 6.33 (1H, d, J = 16.2 Hz, C=CH), 6.74 (1H, d, J = 16.2 Hz, C=CH). MS (EI) m/z 316 (M^+).

3.1.6. Methyl 4-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrofuran-3-carboxylate (5e). Yield: 93%. IR (neat) 2930, 1715, 1237 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (6H, s, C(CH_3)$_2$), 1.47 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.77 (3H, s, CH_3), 2.05 (2H, t, J = 6.2 Hz, CH_2), 3.77 (3H, s, $COOCH_3$), 4.89 (2H, t, J = 4.2 Hz, OCH_2), 4.99 (2H, t, J = 4.2 Hz, OCH_2), 6.26 (1H, d, J = 16.6 Hz, C=CH), 7.19 (1H, d, J = 16.6 Hz, C=CH). MS (EI) m/z 276 (M^+).

3.1.7. Methyl 4-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrothiophen-3-carboxylate (5g). Yield: 97%. IR (neat) 2928, 1715, 1599, 1435, 1219, 1103 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (6H, s, C(CH_3)$_2$), 1.47 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.76 (3H, d, J = 0.6 Hz, CH_3), 2.05 (2H, t, J = 6.1 Hz, CH_2), 3.77 (3H, s, $COOCH_3$), 4.06 (2H, m, SCH_2), 4.13 (2H, m, SCH_2), 6.41 (1H, dd, J = 16.5, 0.6 Hz, C=CH), 7.43 (1H, d, J = 16.5 Hz, C=CH). MS (EI) m/z 292 (M^+).

3.1.8. Methyl 2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]benzoate (5d). To a solution of $Pd(PPh_3)_4$ 17 mg (0.015 mmol) in benzene (1 ml) was added methyl *o*-iodobenzoate **3d** 78 mg (0.30 mmol) in 1 ml of benzene. After stirring for 15 min under Ar, 0.9 M benzene solution of alkenylborane **4**[24] 0.50 ml (0.45 mmol) and 4.0 M KOH aqueous solution 0.34 ml (1.4 mmol) were added at room temperature. The mixture was heated to reflux for 2 h, and the residual organoborane was oxidized by 3 M NaOH (0.1 ml) and 30% H_2O_2 (0.1 ml) for 1 h at room temperature. The reaction mixture was filtered through Celite, and the filtrate was extracted with ether. The organic layer was washed with water and brine, and dried over $MgSO_4$. The solvent was evaporated and the crude residue was purified by silica gel column chromatography (hexane/ethyl acetate 30:1 as an eluent) to afford **5d** 82 mg (96% yield) as a colorless oil. IR (neat) 2924, 1723, 1244, 1076 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (6H, s, C(CH_3)$_2$), 1.50 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.80 (3H, d, J = 0.9 Hz, CH_3), 2.04 (2H, t, J = 6.3 Hz, CH_2), 3.89 (3H, s, $COOCH_3$), 6.57 (1H, dd, J = 16.2, 0.9 Hz, C=CH), 7.06 (1H, d, J = 16.2 Hz, C=CH), 7.27 (1H, dt, J = 1.1, 7.6 Hz, Ar), 7.47 (1H, dt, J = 1.1, 7.6 Hz, Ar), 7.61 (1H, dd, J = 1.1, 7.6 Hz, Ar), 7.86 (1H, dd, J = 1.1, 7.6 Hz, Ar). MS (EI) m/z 284 (M^+).

Similar reactions of **3f** gave the following compound.

3.1.9. Methyl 4-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]furan-3-carboxylate (5f). Yield: 98%. IR (neat) 2928, 1728, 1148, 1094 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (6H, s, C(CH_3)$_2$), 1.48 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.77 (3H, s, CH_3), 2.03 (2H, t, J = 6.3 Hz, CH_2), 3.83 (3H, s, $COOCH_3$), 6.49 (1H, d, J = 16.6, C=CH), 6,55 (1H, d, J = 16.6 Hz, C=CH), 7.55 (1H, d, J = 1.7 Hz, Ar), 7.97 (1H, d, J = 1.7 Hz, Ar). MS (EI) m/z 274 (M^+).

3.1.10. 2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclopent-1-enemethanol (6a).[22] To a suspension of $LiAlH_4$ 65 mg (1.7 mmol) in THF (5 ml) was added dropwise ester **5a** 119 mg (0.41 mmol) in THF (3 ml) at 0 °C and the reaction mixture was stirred for 30 min. Saturated aqueous Na_2SO_4 solution (1 ml) was slowly added to this mixture, and precipitate was removed by filtration through Celite. The filtrate was dried over $MgSO_4$ and concentrated in vacuo. Alcohol **6a** 92 mg (90% yield) was obtained as a colorless oil after purification by silica gel column chromatography (hexane/ethyl acetate 8:1 as an eluent) IR (neat) 3312, 2926, 1456, 1030 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (6H, s, C(CH_3)$_2$), 1.46 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.71 (3H, d, J = 0.9 Hz, CH_3), 1.90 (2H, m, CH_2), 2.01 (2H, t, J = 6.1 Hz, CH_2), 2.58 (4H, t, J = 7.5 Hz, 2(CH_2)), 4.33 (2H, s, CH_2OH), 6.05 (1H, d, J = 15.8 Hz, C=CH), 6.35 (1H, d, J = 158 Hz, C=CH). MS (EI) m/z 246 (M^+).

Similar reactions of **5b**–**5d** and **5f** gave the following compounds.

3.1.11. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]cyclohex-1-enemethanol (6b).[22] Yield: 90%. IR (neat) 3227, 2924, 1458 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (6H, s, C(CH_3)$_2$), 1.46 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.66 (4H, m, 2(CH_2)), 1.70 (3H, d, J = 0.6 Hz, CH_3), 2.00 (2H, t, J = 6.1 Hz, CH_2), 2.26 (4H, m, 2(CH_2)), 4.23 (2H, s, CH_2OH), 6.11 (1H, dd, J = 16.2, 0.6 Hz, C=CH), 6.48 (1H, d, J = 16.2 Hz, C=CH). MS (EI) m/z 260 (M^+).

3.1.12. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]cyclohept-1-enemethanol (6c).[22] Yield: 94%. IR (neat) 3252, 2923, 1451 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (6H, s, C(CH_3)$_2$), 1.45–1.55 (6H, m, 3(CH_2)), 1.62 (2H, m, CH_2), 1.70 (3H, d, J = 0.6 Hz, CH_3), 1.78 (2H, m, CH_2), 2.02 (2H, t, J = 6.1 Hz, CH_2), 2.42 (4H, m, 2(CH_2)), 4.26 (2H, s, CH_2OH), 6.11 (1H, d, J = 15.9 Hz, C=CH), 6.38 (1H, d, J = 15.9 Hz, C=CH). MS (EI) m/z 256 (M^+-H_2O).

3.1.13. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]benzyl alcohol (6d). Yield: 95%. IR (neat) 3330, 2926, 1455, 1099 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (6H, s, C(CH_3)$_2$), 1.50 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.78 (3H, d, J = 0.9 Hz, CH_3), 2.04 (2H, t, J = 6.3 Hz, CH_2), 4.76 (2H, s, CH_2OH), 6.58 (1H, dd, J = 16.2, 0.9 Hz, C=CH), 6.65 (1H, d, J = 16.2 Hz, C=CH), 7.24 (1H, dt, J = 1.4, 7.4 Hz, Ar), 7.30 (1H,

dt, J = 1.4, 7.4 Hz, Ar), 7.37 (1H, dd, J = 1.4, 7.4 Hz, Ar), 7.54 (1H, dd, J = 1.4, 7.4 Hz, Ar). MS (EI) m/z 256 (M^+).

3.1.14. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]furan-3-methanol (6f). Yield: 92%. IR (neat) 3330, 2928, 1458, 1055 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.75 (3H, d, J = 0.9 Hz, CH_3), 2.02 (2H, t, J = 6.1 Hz, CH_2), 4.64 (2H, s, CH_2OH), 6.17 (1H, d, J = 16.4, C=CH), 6.48 (1H, d, J = 16.4 Hz, C=CH), 7.39 (1H, d, J = 1.5 Hz, Ar), 7.45 (1H, d, J = 1.5 Hz, Ar), MS (EI) m/z 246 (M^+).

3.1.15. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrofuran-3-methanol (6e). To a solution of ester **5e** 104 mg (0.015 mmol) in 3 ml CH_2Cl_2 was added 1.76 M DIBAL in hexane 0.64 ml (1.13 mmol) at −78 °C and the reaction mixture was stirred for 30 min at the same temperature. Methanol 0.3 ml and saturated aqueous Rochelle salt 4 ml were sequentially added to this mixture. After being stirred for 90 min at room temperature, the two-phase mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated and the crude residue was purified by silica gel column chromatography (hexane/ethyl acetate 2:1 as an eluent) to afford **5e** 90 mg (97% yield) as a colorless oil. IR (neat) 3407, 2928, 1456, 1362, 1071 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (6H, s, $C(CH_3)_2$), 1.46 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.71 (3H, s, CH_3), 2.01 (2H, t, J = 6.3 Hz, CH_2), 4.40 (2H, s, CH_2OH), 4.84 (2H, d, J = 3.4 Hz, OCH_2), 4.86 (2H, d, J = 3.4 Hz, OCH_2), 5.91 (1H, d, J = 16.3 Hz, C=CH), 6.24 (1H, d, J = 16.3 Hz, C=CH). MS (EI) m/z 248 (M^+).

Similar reactions of **5g** gave the following compound.

3.1.16. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrothiophen-3-methanol (6g). Yield: 97%. IR (neat) 3395, 2930, 1456, 1109 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.00 (6H, s, $C(CH_3)_2$), 1.46 (2H, m, CH_2), 1.62 (2H, m, CH_2), 1.70 (3H, s, CH_3), 2.01 (2H, t, J = 6.1 Hz, CH_2), 3.99 (4H, m, CH_2SCH_2), 4.37 (2H, s, CH_2OH), 6.07 (1H, d, J = 16.2 Hz, C=CH), 6.33 (1H, d, J = 16.2 Hz, C=CH). MS (EI) m/z 264 (M^+).

3.1.17. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-cyclopent-1-enecarbaldehyde (7a). To a solution of alcohol **6a** 126 mg (0.51 mmol) in 20 ml of petroleum ether (boiling range 30–60 °C) was added 898 mg of MnO_2. After being stirred for 3.5 h at room temperature, the reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo to afford aldehyde **7a** 119 mg (95%) as a yellow oil. IR (neat) 2930, 1659, 1611, 1208 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.75 (3H, d, J = 0.6 Hz, CH_3), 1.92 (2H, m, CH_2), 2.06 (2H, t, J = 6.1 Hz, CH_2), 2.68 (2H, t, J = 7.5 Hz, CH_2), 2.82 (2H, m, CH_2), 6.52 (1H, dd, J = 15.9, 0.9 Hz, C=CH), 6.98 (1H, d, J = 15.9 Hz, C=CH), 10.19 (1H, s, CHO). MS (EI) m/z 244 (M^+).

Similar reactions of **6b–d** gave the following compounds.

3.1.18. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-cyclohex-1-enecarbaldehyde (7b). Yield: 96%. IR (neat) 2932, 1661, 1154 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.66–1.72 (6H, m, $3(CH_2)$), 1.73 (3H, d, J = 0.9 Hz, CH_3), 2.04 (2H, t, J = 6.3 Hz, CH_2), 2.32 (2H, t, J = 6.1 Hz, CH_2), 2.49 (2H, m, CH_2), 6.46 (1H, dd, J = 15.9, 0.9 Hz, C=CH), 6.96 (1H, d, J = 15.9 Hz, C=CH), 10.31 (1H, s, CHO). MS (EI) m/z 258 (M^+).

3.1.19. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-cyclohept-1-enecarbaldehyde (7c). Yield: 86%. IR (neat) 2924, 1659, 1165 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.43–1.50 (4H, m, $2(CH_2)$), 1.57–1.66 (4H, m, CH_2 and CH_2), 1.74 (3H, d, J = 0.9 Hz, CH_3), 1.80 (2H, m, CH_2), 2.04 (2H, t, J = 6.4 Hz, CH_2), 2.58 (4H, m, $2(CH_2)$), 6.40 (1H, dd, J = 15.9, 0.9 Hz, C=CH), 6.77 (1H, d, J = 15.9 Hz, C=CH), 10.13 (1H, s, CHO). MS (EI) m/z 272 (M^+).

3.1.20. 2-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-benzaldehyde (7d). Yield: 92%. IR (neat) 2928, 1698, 1595, 1188 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (6H, s, $C(CH_3)_2$), 1.51 (2H, m, CH_2), 1.66 (2H, m, CH_2), 1.81 (3H, d, J = 0.9 Hz, CH_3), 2.06 (2H, t, J = 6.1 Hz, CH_2), 6.60 (1H, dd, J = 16.1, 0.9 Hz, C=CH), 7.17 (1H, d, J = 16.1 Hz, C=CH), 7.38 (1H, dt, J = 7.6, 1.3 Hz, Ar), 7.55 (1H, dt, J = 7.6, 1.3 Hz, Ar), 7.59 (1H, dd, J = 7.6, 1.3 Hz, Ar), 7.84 (1H, dd, J = 7.6, 1.3 Hz, Ar), 10.33 (1H, s, CHO). MS (EI) m/z 254 (M^+).

3.1.21. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrofuran-3-carbaldehyde (7e). To a solution of oxalyl chloride 0.11 ml (1.3 mmol) in CH_2Cl_2 (4 ml) was added DMSO 0.18 ml (2.5 mmol) at −78 °C under Ar and the mixture was stirred for 3 min. To this solution alcohol **6e** 240 mg (0.97 mmol) in CH_2Cl_2 (3 ml) was added and stirring was continued for an additional 15 min. Triethylamine 0.68 ml (4.9 mmol) was added, and the reaction mixture was stirred for 5 min and allowed to warm to room temperature. Water 10 ml was added and the aqueous layer was reextracted with CH_2Cl_2. The combined organic layer was washed with brine and dried ($MgSO_4$). Purification on silica gel column chromatography (hexane/ethyl acetate 10:1 as an eluent) afforded **7e** 218 mg (92%) as a colorless oil. IR (neat) 2932, 1659, 1613, 1225 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.77 (3H, s, CH_3), 2.07 (2H, t, J = 6.3 Hz, CH_2), 4.93 (2H, t, J = 3.9 Hz, OCH_2), 5.05 (2H, t, J = 3.9 Hz, OCH_2), 6.38 (1H, d, J = 16.2 Hz, C=CH), 6.85 (1H, d, J = 16.2 Hz, C=CH), 10.11 (1H, s, CHO). MS (EI) m/z 246 (M^+).

Similar reactions of **6a** and **6g** gave the following compounds.

3.1.22. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]furan-3-carbaldehyde (7f). Yield: 92%. IR (neat) 2928, 1694, 1530, 1146 cm^{-1}. ^1H NMR (400 MHz,

$CDCl_3$) δ 1.06 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.77 (3H, s, CH_3), 2.03 (2H, t, J = 6.1 Hz, CH_2), 6.48 (1H, d, J = 16.6 Hz, C=CH), 6.70 (1H, d, J = 16.6 Hz, C=CH), 7.57 (1H, s, Ar), 8.01 (1H, d, J = 1.6 Hz, Ar), 10.01 (1H, s, CHO). MS (EI) m/z 244 (M^+).

3.1.23. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrothiophen-3-carbaldehyde (7g). Yield: 88%. A yellow crystalline solid, mp 56–58 °C IR (nujol) 2932, 1665, 1456, 1173 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.76 (3H, s, CH_3), 2.07 (2H, t, J = 6.4 Hz, CH_2), 4.02 (2H, t, J = 3.7 Hz, SCH_2), 4.22 (2H, t, J = 3.7 Hz, SCH_2), 6.52 (1H, d, J = 16.0 Hz, C=CH), 6.99 (1H, d, J = 16.0 Hz, C=CH), 10.17 (1H, s, CHO). MS (EI) m/z 262 (M^+).

3.1.24. 4-[(*E*)-2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]thiophen-3-carbaldehyde (7h). To a stirred solution of aldehyde **7g** 102 mg (0.39 mmol) in 15 ml of petroleum ether (boiling range 30–60 °C) was added 998 mg MnO_2. After being stirred for 6 h at room temperature, the reaction mixture was filtered though Celite and the filtrate was concentrated in vacuo. Purification on silica gel column chromatography (hexane/ethyl acetate 10:1 as an eluent) afforded **7h** 89 mg (89%) as a yellow oil. IR (neat) 2928, 1686, 1435, 911 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.08 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.79 (3H, s, CH_3), 2.04 (2H, t, J = 6.3 Hz, CH_2), 6.59 (1H, d, J = 16.4 Hz, C=CH), 6.93 (1H, d, J = 16.4 Hz, C=CH), 7.33 (1H, dd, J = 32, 0.8 Hz, Ar), 8.09 (1H, d, J = 3.2 Hz, Ar), 10.03 (1H, s, CHO). MS (EI) m/z 260 (M^+).

3.1.25. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclopent-1-enyl]penta-2,4-dienoate (8a). To LDA in THF (3 ml) prepared from 0.11 ml (0.75 mmol) of diisopropylamine and 0.51 ml of 1.5 M BuLi solution in hexane (0.75 mmol) was added the phosphate[26] 188 mg (0.75 mmol) at −78 °C under Ar and the reaction mixture was stirred for 15 min. A solution of aldehyde **7a** 119 mg (0.38 mmol) in THF (2 ml) was then added at the same temperature, and the reaction mixture was allowed to warm to room temperature and stirred for further 15 min in the dark. Saturated aqueous NH_4Cl solution was added and the mixture was extracted with ether, washed with brine, and dried ($MgSO_4$). The solvent was removed, and chromatography of the residue on silica gel (hexane/ethyl acetate 30:1 as an eluent) afforded methyl ester **8a** 150 mg (91%) as a yellow crystalline solid, mp 77–79 °C. IR (nujol) 2924, 1719, 1154 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.75 (3H, d, J = 0.9 Hz, CH_3), 1.93 (2H, m, CH_2), 2.05 (2H, t, J = 6.3 Hz, CH_2), 2.37 (3H, d, J = 0.9 Hz, CH_3), 2.66 (4H, m, 2(CH_2)), 3.71 (3H, s, $COOCH_3$), 5.81 (1H, s, C=CH), 6.19 (1H, d, J = 15.8 Hz, C=CH), 6.20 (1H, d, J = 15.8 Hz, C=CH), 6.58 (1H, d, J = 15.8 Hz, C=CH), 7.12 (1H, d, J = 15.8 Hz, C=CH). MS (EI) m/z 340 (M^+). HRMS Calcd for $C_{23}H_{32}O_2$: 340.2403. Found: 340.2409.

Similar reactions of **7b**–**7h** gave the following compounds.

3.1.26. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohex-1-enyl]penta-2,4-dienoate (8b). Yield: 91%. A yellow oil. IR (nujol) 2926, 1715, 1605, 1154 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.69 (4H, m, 2(CH_2)), 1.75 (3H, d, J = 0.6 Hz, CH_3), 2.04 (2H, t, J = 6.3 Hz, CH_2), 2.32 (2H, m, CH_2), 2.36 (3H, d, J = 1.2 Hz, CH_3), 2.40 (2H, m, CH_2), 3.71 (3H, s, $COOCH_3$), 5.81 (1H, s, C=CH), 6.23 (1H, d, J = 15.8 Hz, C=CH), 6.28 (1H, d, J = 15.8 Hz, C=CH), 6.40 (1H, d, J = 15.8 Hz, C=CH), 7.34 (1H, d, J = 15.8 Hz, C=CH). MS (EI) m/z 354 (M^+).

3.1.27. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohept-1-enyl]penta-2,4-dienoate (8c). Yield: 92%. A yellow oil. IR (nujol) 2924, 1715, 1605, 1154 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.47–1.54 (6H, m, 3(CH_2)), 1.64 (2H, m, CH_2), 1.76 (3H, s, CH_3), 1.77 (2H, m, CH_2), 2.05 (2H, t, J = 6.1 Hz, CH_2), 2.36 (3H, d, J = 0.9 Hz, CH_3), 2.53 (4H, m, 2(CH_2)), 3.71 (3H, s, $COOCH_3$), 5.81 (1H, s, C=CH), 6.23 (1H, d, J = 15.9 Hz, C=CH), 6.30 (1H, d, J = 15.9 Hz, C=CH), 6.62 (1H, d, J = 15.9 Hz, C=CH), 7.27 (1H, d, J = 15.9 Hz, C=CH). MS (EI) m/z 360 (M^+).

3.1.28. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]phenyl]penta-2,4-dienoate (8d). Yield: 92%. A yellow-green oil. IR (nujol) 2926, 1719, 1456, 1156 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.08 (6H, s, $C(CH_3)_2$), 1.51 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.80 (3H, d, J = 0.9 Hz, CH_3), 2.05 (2H, t, J = 6.3 Hz, CH_2), 2.40 (3H, d, J = 0.9 Hz, CH_3), 3.73 (3H, s, $COOCH_3$), 5.90 (1H, s, C=CH), 6.49 (1H, dd, J = 16.2, 0.9 Hz, C=CH), 6.62 (1H, d, J = 16.2 Hz, C=CH), 6.70 (1H, d, J = 15.9 Hz, C=CH), 7.22–7.30 (3H, m, C=CH, Ar), 7.46 (1H, dd, J = 1.5, 7.6 Hz, Ar), 7.50 (1H, d, J = 7.6 Hz, Ar). MS (EI) m/z 350 (M^+).

3.1.29. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]2,5-dihydrofuran-3-yl]penta-2,4-dienoate (8e). Yield: 94%. A yellow crystalline solid, mp 78–81 °C. IR (nujol) 2926, 1717, 1595, 1154 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.76 (3H, s, CH_3), 2.05 (2H, t, J = 6.1 Hz, CH_2), 2.36 (3H, d, J = 0.9 Hz, CH_3), 3.72 (3H, s, $COOCH_3$), 4.89 (2H, d, J = 3.5 Hz, OCH_2), 4.93 (2H, d, J = 3.5 Hz, OCH_2), 5.82 (1H, s, C=CH), 6.02 (1H, d, J = 16.2 Hz, C=CH), 6.03 (1H, d, J = 16.2 Hz, C=CH), 6.44 (1H, d, J = 16.2 Hz, C=CH), 6.94 (1H, d, J = 16.2 Hz, C=CH). MS (EI) m/z 342 (M^+). HRMS Calcd for $C_{22}H_{30}O_3$: 342.2195. Found: 342.2196.

3.1.30. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]furan-3-yl]penta-2,4-dienoate (8f). Yield: 96%. A yellow oil. IR (neat) 2928, 1717, 1613, 1237, 1157 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.77 (3H, s, CH_3), 2.04 (2H, t, J = 6.1 Hz, CH_2), 2.37 (3H, s,

CH_3), 3.72 (3H, s, $COOCH_3$), 5.80 (1H, s, C=CH), 6.17 (1H, d, J = 16.5 Hz, C=CH), 6.38 (1H, d, J = 16.5 Hz, C=CH), 6.61 (1H, d, J = 16.2 Hz, C=CH), 6.84 (1H, d, J = 16.2 Hz, C=CH), 7.45 (1H, s, Ar), 7.55 (1H, s, Ar). MS (EI) m/z 340 (M^+).

3.1.31. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrothiophen-3-yl]penta-2,4-dienoate (8g). Yield: 94%. A yellow crystalline solid, mp 145–146 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.75 (3H, s, CH_2), 2.05 (2H, t, J = 6.0 Hz, CH_2), 2.37 (3H, d, J = 0.9 Hz, CH_2), 3.72 (3H, s, $COOCH_3$), 4.01 (2H, m, SCH_2), 4.06 (2H, m, SCH_2), 5.84 (1H, s, C=CH), 6.20 (2H, d, J = 16.0 Hz, C=CH), 6.56 (1H, d, J = 16.0 Hz, C=CH), 7.08 (1H, d, J = 16.0 Hz, C=CH). MS (EI) m/z 358 (M^+). HRMS Calcd for $C_{22}H_{30}O_2S$: 358.1997. Found: 358.1996.

3.1.32. Methyl (*E*,*E*)-3-methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]-thiophen-3-yl]penta-2,4-dienoate (8h). Yield: 93%. A yellow oil. IR (neat) 2928, 1717, 1613, 1237, 1156 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (6H, s, $C(CH_3)_2$), 1.50 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.78 (3H, s, CH_3), 2.05 (2H, t, J = 6.1 Hz, CH_2), 2.39 (3H, d, J = 0.9 Hz, CH_3), 3.72 (3H, s, $COOCH_3$), 5.86 (1H, s, H-14), 6.36 (1H, d, J = 16.2 Hz, C=CH), 6.48 (1H, d, J = 16.2 Hz, C=CH), 6.68 (1H, d, J = 16.0 Hz, C=CH), 6.98 (1H, d, J = 16.0 Hz, C=CH), 7.24 (1H, d, J = 3.2 Hz, Ar), 7.39 (1H, d, J = 3.2 Hz, Ar). MS (EI) m/z 356 (M^+).

3.1.33. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclopent-1-enyl]penta-2,4-dienoic acid (2a)[22]. To a stirred solution of ester **8a** 162 mg (0.48 mmol) in 3 ml of ethanol was added 3.0 ml of 6.0 M KOH in the dark under Ar, and the reaction mixture was heated to 60 °C for 1 h. After hydrolysis was complete (by TLC), ethanol was evaporated in vacuo, and the mixture was cooled to 0 °C and acidified with 10% aqueous HCl. The organics were extracted with ether, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified by column chromatography on silica gel (CH_2Cl_2/ether 5:1) to give 149 mg of desired acid **2a** (89%) as a yellow solid. Recrystallization from CH_2Cl_2/hexane afforded yellow needles, mp 193–194 °C. IR (nujol) 3445, 2924, 1684, 1156 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.64 (2H, m, CH_3), 1.76 (3H, s, CH_3), 1.94 (2H, m, CH_2), 2.05 (2H, t, J = 6.0 Hz, CH_2), 2.38 (3H, s, CH_2), 2.67 (4H, m, 2(CH_2)), 5.84 (1H, s, C=CH), 6.20 (1H, d, J = 15.8 Hz, C=CH) 6.23 (1H, d, J = 15.8 Hz, C=CH), 6.59 (1H, d, J = 15.8 Hz, C=CH), 7.16 (1H, d, J = 15.8 Hz, C=CH). Anal. Calcd for $C_{22}H_{30}O_2$/0.75H_2O: C, 77.72; H, 9.34. Found: C, 77.75; H, 9.34.

Similar reactions of **8b**–**h** gave the following compounds.

3.1.34. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohex-1-enyl]penta-2,4-dienoic acid (2b).[22] Yield: 99%. Yellow needles, mp 165–167 °C (CH_2Cl_2/hexane). IR (nujol) 3445, 2924, 1682, 1651 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.70 (4H, m, 2(CH_2)), 1.75 (3H, s, CH_3), 2.05 (2H, t, J = 6.1 Hz, CH_2), 2.33 (2H, m, CH_2), 2.37 (3H, d, J = 0.9 Hz, CH_3), 2.40 (2H, m, CH_2), 5.83 (1H, s, C=CH), 6.24 (1H, d, J = 15.8 Hz, C=CH), 6.31 (1H, d, J = 15.8 Hz, C=CH), 6.71 (1H, d, J = 15.8 Hz, C=CH), 7.38 (1H, d, J = 15.8 Hz, C=CH). MS (EI) m/z 340 (M^+). Anal. Calcd for $C_{23}H_{32}O_2$/0.75H_2O: C, 78.03; H, 9.54. Found: C, 77.65; H, 9.21.

3.1.35. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohept-1-enyl]penta-2,4-dienoic acid (2c)[22]. Yield: 96%. Yellow needles, mp 143–145 °C (CH_2Cl_2/hexane). IR (nujol) 3445, 2924, 1684 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.47–1.55 (6H, m, 3$C(CH_3)_2$), 1.64 (2H, m, CH_2), 1.76 (3H, s, CH_3), 1.78 (2H, m, CH_2), 2.05 (2H, t, J = 6.1 Hz, CH_2), 2.37 (3H, s, CH_3), 2.53 (4H, m, 2(CH_2)), 5.83 (1H, s, C=CH) 6.24 (1H, d, J = 15.8 Hz, C=CH), 6.33 (1H, d, J = 15.8 Hz, C=CH), 6.62 (1H, d, J = 15.8 Hz, C=CH), 7.30 (1H, d, J = 15.8 Hz, C=CH). MS (EI) m/z 354 (M^+). Anal. Calcd for $C_{24}H_{34}O_2$/0.5H_2O: C, 79.29; H, 9.70. Found: C, 78.90; H, 9.65.

3.1.36. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]phenyl]penta-2,4-dienoic acid (2d). Yield: 98%. Yellow plates, mp 176–178 °C (CH_2Cl_2/hexane). IR (neat) 3445, 2924, 1682, 1458 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (6H, s, $C(CH_3)_2$), 1.51 (2H, m, CH_2), 1.66 (2H, m, CH_2), 1.81 (3H, s, CH_3), 2.06 (2H, t, J = 6.3 Hz, CH_2), 2.42 (3H, s, CH_3), 5.93 (1H, s, C=CH), 6.50 (1H, d, J = 16.2 Hz, C=CH), 6.62 (1H, d, J = 16.2 Hz, C=CH) 6.74 (1H, d, J = 15.9 Hz, C=CH), 7.23–7.32 (2H, m, Ar), 7.32 (1H, d, J = 15.9 Hz, C=CH), 7.47 (1H, dd, J = 7.6, 1.0 Hz, Ar), 7.52 (1H, dd, J = 7.6, 1.0 Hz, Ar). MS (EI) m/z 336 (M^+). Anal. Calcd for $C_{23}H_{28}O_2$: C, 82.10; H, 8.39. Found: C, 81.84; H, 8.41.

3.1.37. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrofuran-3-yl]penta-2,4-dienoic acid (2e). Yield: 94%. Yellow needles, mp 224–226 °C (MeOH). IR (neat) 2926, 1694, 1591, 1458, 1181 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (6H, s, $C(CH_3)_2$), 1.48 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.76 (3H, s, CH_3), 2.05 (2H, t, J = 6.0 Hz, CH_2), 2.37 (3H, s, CH_3), 4.91 (2H, d, J = 3.5 Hz, OCH_2), 4.94 (2H, d, J = 3.5 Hz, OCH_2), 5.85 (1H, s, C=CH) 6.02 (2H, d, J = 16.0 Hz, 2(C=CH)), 6.44 (1H, d, J = 16.0 Hz, C=CH), 6.98 (1H, d, J = 16.0 Hz, C=CH). MS (EI) m/z 328 (M^+). Anal. Calcd for $C_{21}H_{28}O_3$: C, 82.10; H, 8.39. Found: C, 76.47; H, 8.73.

3.1.38. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]furan-3-yl]penta-2,4-dienoic acid (2f). Yield: 97%. Colorless needles, mp 175–177 °C (MeOH). IR (nujol) 2928, 1692, 1599, 1574, 1456, 1061 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.78 (3H, s, CH_3), 2.04 (2H, t, J = 6.0 Hz, CH_2), 2.38 (3H, d, J = 0.6 Hz, CH_3), 5.83 (1H, s, C=CH), 6.17 (1H, d, J = 16.2 Hz, C=CH), 6.39 (1H, d, J = 16.2 Hz, C=CH) 6.40 (1H,

d, $J = 16.0$ Hz, C=CH), 6.88 (1H, d, $J = 16.0$ Hz, C=CH), 7.46 (1H, s, Ar), 7.57 (1H, d, $J = 1.2$ Hz, Ar). MS (EI) *m/z* 326 (M^+). Anal. Calcd for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found: C, 77.11; H, 7.96.

3.1.39. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]-2,5-dihydrothiophen-3-yl]penta-2,4-dienoic acid (2g). Yield: 96%. Yellow needles, mp 231–234 °C (AcOEt). IR (nujol) 3422, 2924, 1688, 1590, 1458, 1197 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (6H, s, $C(CH_3)_2$), 1.49 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.75 (3H, s, CH_3), 2.07 (2H, t, $J = 6.3$ Hz, CH_2), 2.38 (3H, d, $J = 0.6$ Hz, CH_3), 4.01 (2H, d, $J = 3.4$ Hz, SCH_2), 4.07 (2H, d, $J = 3.4$ Hz, SCH_2), 5.87 (1H, s, C=CH) 6.21 (1H, d, $J = 16.0$ Hz, C=CH), 6.23 (1H, d, $J = 16.0$ Hz, C=CH), 6.56 (1H, d, $J = 16.0$ Hz, C=CH), 7.12 (1H, d, $J = 16.0$ Hz, C=CH). MS (EI) *m/z* 344 (M^+). Anal. Calcd for $C_{21}H_{28}O_2S$: C, 73.21; H, 8.19; S, 9.31. Found: C, 72.96; H, 8.38; S, 9.32.

3.1.40. (*E*,*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]thiophen-3-yl]penta-2,4-dienoic acid (2h). Yield: 97%. Colorless needles, mp 172–174 °C (AcOEt/hexane). IR (neat) 2926, 1684, 1595, 1256, 1176 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.08 (6H, s, $C(CH_3)_2$), 1.50 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.79 (3H, s, CH_3), 2.05 (2H, t, $J = 6.2$ Hz, CH_2), 2.40 (3H, d, $J = 0.9$ Hz, CH_3), 5.89 (1H, s, C=CH), 6.36 (1H, d, $J = 16.2$ Hz, C=CH), 6.48 (1H, d, $J = 16.2$ Hz, C=CH) 6.71 (1H, d, $J = 16.2$ Hz, C=CH), 7.02 (1H, d, $J = 16.2$ Hz, C=CH), 7.25 (1H, d, $J = 3.4$ Hz, Ar), 7.39 (1H, d, $J = 3.4$ Hz, Ar). MS (EI) *m/z* 356 (M^+). Anal. Calcd for $C_{21}H_{26}O_2S$: C, 73.64; H, 7.65; S, 9.36. Found: C, 73.57; H, 7.82; S, 9.16.

3.2. Measurement of thrombomodulin antigen

9-*cis*-RA analogues were dissolved in dimethyl sulfoxide to a stock concentration of 10 mM and stored at −80 °C. All experiments were performed in subdued light, and tubes containing RA analogues were covered with aluminum foil. Controls were run using the same concentration of dimethyl sulfoxide as present and this concentration of diluent had no effect.

Endothelial cells were isolated from human umbilical cord veins, and were cultured in Dulbecco's modified Eagle's medium (Flow Laboratories) supplemented with 20% fetal calf serum (FCS; Filtron), 20 μg/ml endothelial cell growth supplement (Collaborative Research), 100 μg/ml heparin (Sigma), 50 μg/ml penicillin, and 50 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C as described previously.[3] Cells were used for the experiments within three passages. The confluent cells on 96 well gelatin-coated plate (Falcon; Becton Dickinson) were incubated in above culture medium containing retinoids for 24 h. After the incubation, conditioned medium was discarded and the monolayer was used for the assay of thrombomodulin antigen.

Cells were washed three times with Hanks' balanced salt solution (pH 7.4), and then solubilized for 1 h at 4 °C with 50 mM Tris–HCl (pH 7.4) containing 0.15 M NaCl, 0.5% Triton X-100, and 1 mM benzamidine hydrochloride. Triton X-100-insoluble material was removed by centrifugation (18,000*g* for 15 min at 4 °C), and TM antigen levels in the cell lysates were measured by an enzyme immunoassay using mouse monoclonal anti-human TM antibodies as previously described. Purified human placental TM was used as a standard.

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