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Synthesis and biological activity of isopentenyl diphosphate analogues

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Abstract—A series of analogues of isopentenyl diphosphate (IPP) having a dicarboxylate moiety in place of the diphosphate were synthesized and investigated as inhibitors of undecaprenyl diphosphate (UPP) synthase and protein farnesyltransferase (PFTase). PFTase is involved in control of cell proliferation and is known to be inhibited by certain maleic acid derivatives bearing long alkyl substituents ($\geq$12 carbons, e.g., chaetomellic acid). UPP synthase is a potential target for antimicrobial agents and utilizes isopentenyl diphosphate (IPP) as a substrate. A number of dicarboxylate-containing IPP analogues were prepared in 2–5 steps from commercially available starting materials with good overall yield (20–78%). These syntheses involved the conjugate addition of an organocuprate to dimethyl acetylenedicarboxylate (DMAD) followed by basic ester hydrolysis. The *E*-pentenylbutanedioic acid **32** showed inhibition of UPP synthase with an IC_{50} of 135 μM. Compound **30** displays competitive inhibition of PFTase with a K_i of 287 μM.

The prevalence of bacterial resistance to current antibiotics has encouraged the search for new antimicrobial compounds active against pathogenic microorganisms. Enzymes involved in bacterial cell wall biosynthesis are attractive targets for the development of new antibacterial agents. Undecaprenyl diphosphate synthase catalyzes the condensation of eight molecules of isopentenyl diphosphate (IPP) with farnesyl diphosphate (FPP) to produce undecaprenyl diphosphate (UPP), whose function is to transport lipid II across the membrane for bacterial cell wall biosynthesis.[1] Inhibitors of UPP synthase may give some insight on its specificity as well as its molecular mechanism for the formation of UPP. Recently, a crystal structure of UPP synthase and a proposed substrate-enzyme binding model were reported.[2,3] Further understanding of this enzyme will provide insight for the development of new antibacterial agents. In the present study we targeted inhibition of UPP synthase by dicarboxylate analogues of IPP because certain alkyl-substituted dicarboxylates are potent inhibitors of protein farnesyltransferase (PFTase).[4–6]

PFTase catalyzes the transfer of the C_{15} farnesyl unit from farnesyl diphosphate (FPP) to the cysteine residue located in a tetrapeptide CAAX (A = aliphatic, X = methionine or serine) sequence at the carboxyl terminus of proteins.[7] Prenylation of proteins is an important post-translational modification of ribosomally produced proteins and is important for cell proliferation.[8] Examples of proteins that undergo prenylation would include the family of small G proteins, in particular Ras, oncogenic proteins found in a variety of tumor types including breast, ovarian and pancreatic.[9] As prenylation of Ras is required for its activity, there has been a considerable effort in discovering new PFTase inhibitors as a potential treatment of cancer.

To date, most of the inhibitors developed are focused on peptidomimetics that contain the CAAX recognition motif. Much less attention has been paid to inhibitors that resemble farnesyl diphosphate. For example, chaetomellic acid A (**1**) is a inhibitor of PFTase and competes for the FPP binding site with an IC_{50} of 17 μM (Fig. 1).[10] Recently, two natural products, CJ-13,981 (**2**) and CJ-13,982 (**3**), containing polyanionic functionality were isolated and were shown to inhibit squalene synthase, an enzyme that catalyzes the dimerization of two FPP molecules to form squalene, an intermediate in cholesterol biosynthesis.[11] Inhibitors designed as prenyl

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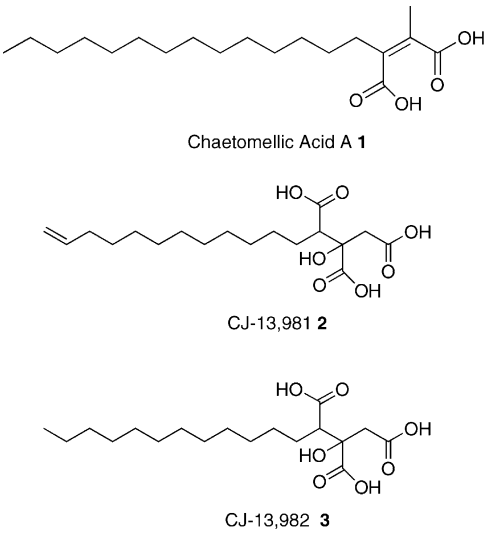


Figure 1. Polyanionic natural compounds.

diphosphate analogues have been employed in substrate specificity studies and may lead to new drugs.[12] We now report the synthesis of a series of new inhibitors that contain a dicarboxylate moiety as inhibitors of enzymes that utilize diphosphates. Analogues of isopentenyl diphosphate (IPP) containing a dicarboxylate moiety were attractive because of IPP's importance in the biosynthesis of a variety of isoprenoids, including FPP.

Initial targets containing maleic acid units and structurally rigid succinic acid units were designed as analogues of IPP. Such units can achieve a spacing of negatively charged oxygen atoms that is within 0.1 Å of that in the corresponding diphosphate moiety (Fig. 2).[13]

1. Results and discussion

Previously, we reported a new synthetic route to chaetomellic acid A and its analogues.[10] The key step in this synthetic sequence involved the conjugate addition of an organocuprate to dimethyl acetylenedicarboxylate (DMAD). It was observed that use of copper (I) bromide dimethyl sulfide complex ($CuBr{\cdot}Me_2S$) gave the preferential formation of product with the two esters having a *cis* relationship.

1.1. Synthesis of diphosphate analogues

Compounds **7** and **12** were selected as initial synthetic targets because of their similarity to IPP. Their synthesis is shown in Scheme 1. Treatment of the commercially available 3-methyl-3-buten-1-ol (**4**) with triphenylphosphine and *N*-bromosuccinimide gave bromide **5**.[14] Formation of the Grignard reagent followed by its addition to a suspension of $CuBr{\cdot}Me_2S$ in THF produced the corresponding organocuprate, which was subsequently added to DMAD to provide the desired diester **6**. Ester hydrolysis using lithium hydroxide afforded the desired dicarboxylate **7**. Compound **12** was made in a similar manner, starting with ethyl-4-methyl-4-pentenoate (**8**). Reduction of the ester with lithium aluminum hydride gave the alcohol **9**,[15] which was then converted to bromide **10** in two steps via the corresponding tosylate.[16] The protected diester **11** was made as previously described by the conjugate addition to DMAD. Subsequent base hydrolysis gave **12** in quantitative yield.

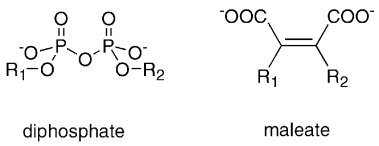
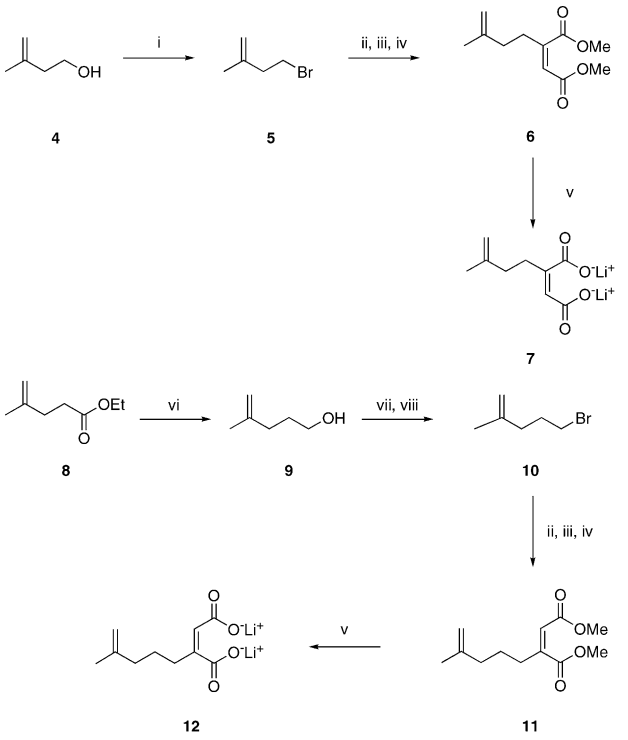


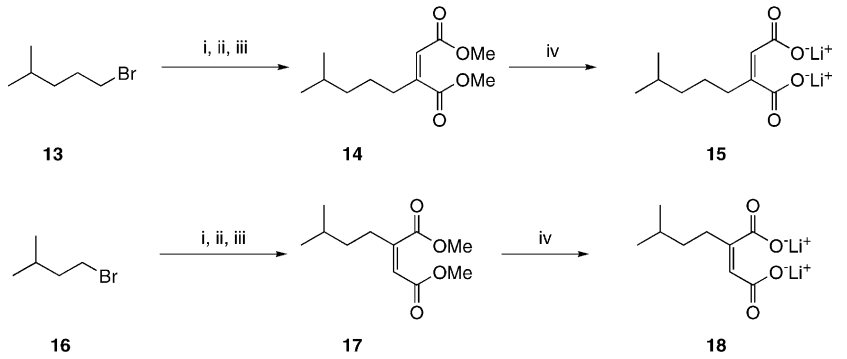
Figure 2. Comparison of diphosphate and maleates.



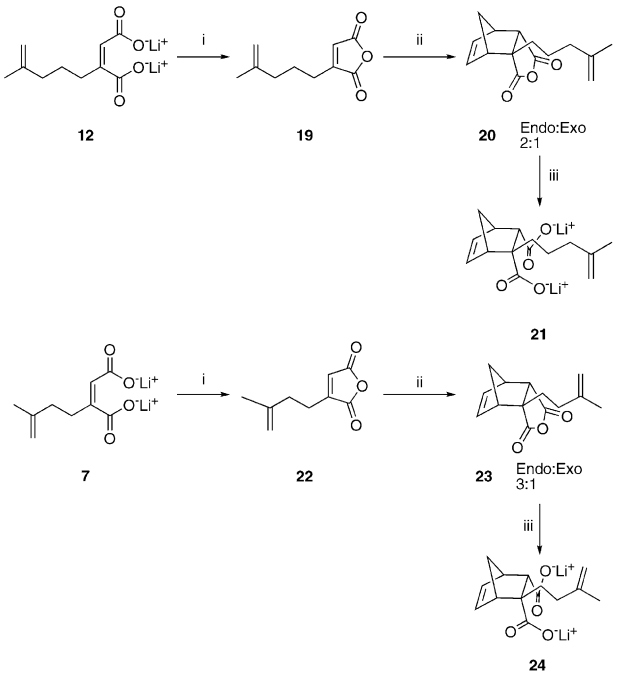
Scheme 1. Synthesis of IPP analogues. Reagents and conditions: (i) NBS, PPh_3, CH_2Cl_2, 16 h 40%; (ii) Mg, THF, Δ, 2 h; (iii) $CuBr{\cdot}Me_2S$, THF, −40 °C, 2 h; (iv) DMAD, THF, −78 °C, 40% for **6** and 62% for **11** (over three steps); (v) LiOH, H_2O/THF, quant for **7** and **12**; (vi) $LiAlH_4$, THF, 88%; (vii) TsCl, NEt_3, DMAP, CH_2Cl_2; (viii) LiBr, acetone, 18 h, 60% (over two steps).

To probe the importance of the external methylene group for IPP binding to the enzyme active site, saturated analogues of IPP were also synthesized as shown in Scheme 2. Starting from 1-bromo-3-methyl-1-butane (**13**), the corresponding organocuprate was formed and added to DMAD. Conversion of the dimethyl ester to the lithium dicarboxylate **15** was achieved using 1.0 M lithium hydroxide. In a similar process, **18** was prepared from 1-bromo-4-methyl-4-pentene (**16**).

Inhibitors based on succinic acid were also made to determine if selective inhibition of *cis*- or *trans*-prenyltransferases would be achieved. Compounds **21** and **24** were synthesized in seven steps using a Diels–Alder reaction as the key transformation (Scheme 3). Conversion of the dilithium salt **12** to its anhydride **19** with dilute hydrochloric acid and subsequent reaction with



Scheme 2. Synthesis of saturated analogues. Reagents and conditions: (i) Mg, THF, Δ, 2 h; (ii) CuBr·Me_2S, THF, −40 °C, 2 h; (iii) DMAD, THF, −78 °C, 60% for **14** and 82% for **17** (over three steps); (iv) LiOH, H_2O/THF, quant for **15** and **18**.



Scheme 3. Synthesis of rigid IPP analogues. Reagents and conditions: (i) HCl, Et_2O, 80% for **19** and 76% for **22**; (ii) cyclopentadiene, toluene, Δ, 69% for **20** and 41% for **23**; (iii) LiOH, H_2O/THF, quant for **21** and **24**.

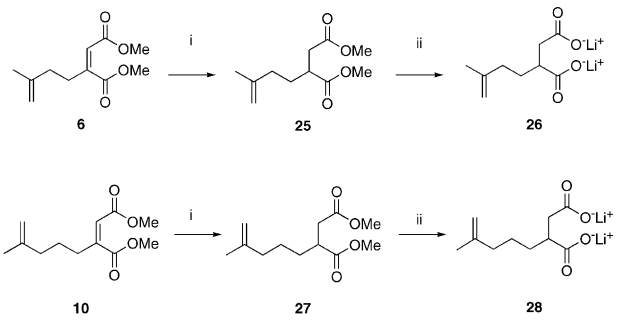
cyclopentadiene gave the cycloadduct **20**. NMR analysis of the reaction mixture indicated preferential formation of the endo adduct in a ratio of 2:1 to its exo product. The stereochemical assignments of the cycloadduct products were determined by using 2D TROESY NMR experiments. In particular, interaction was observed for the endo isomer between the bridged methylene and the hydrogen α- to the carbonyl of the anhydride.

Anhydride **20** was then hydrolyzed using lithium hydroxide to afford **21**. In a similar manner, treatment of the lithium dicarboxylate **7** with dilute HCl gave anhydride **22** in good yield. Conversion into the bicyclic **23** derivative was achieved by reaction with cyclopentadiene in toluene. Basic hydrolysis of anhydride **23** gave the desired compound **24**.

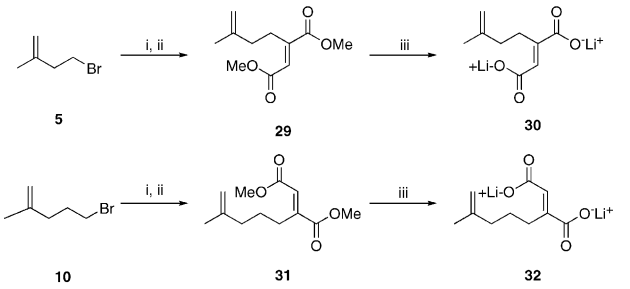
The installment of a double bond between the two dicarboxylates introduces restriction to the movement of the oxygen atoms in the dicarboxylate and this lack of rotational freedom may not allow the molecule to achieve the correct geometrical or spatial arrangement for efficient binding to the active site of the enzyme. To investigate the effect of increasing the rotational freedom within the two dicarboxylates, compounds **26** and **28** were prepared.

As shown in Scheme 4, syntheses of the desired compounds **26** and **28** can be achieved via the conjugate reduction of the corresponding substituted dimethyl maleate. Initial attempts at this reduction utilizing either dissolving metal reduction conditions (Li and $NH_{3(l)}$) or ruthenium-catalyzed reductions using triethylsilane did not yield the desired product. Analysis of the reaction mixtures with both sets of reagents indicated formation of many side products. Recently, copper hydrides such as hexa-μ-hydrohexakis(triphenylphosphine) hexacopper have been used as gentle reducing agents of α,β-unsaturated esters, ketones and aldehydes.[17] Therefore, treatment of dimethyl (*Z*)-2-(2-methyl)butenylbutenedioate (**6**) with hexa-μ-hydrohexakis(triphenylphosphine) hexacopper gave the desired compound **25** in 61% yield. Hydrolysis of the dimethyl esters with lithium hydroxide generated the desired lithium salt **26**. In a similar manner compound **10** was reduced and subsequent base mediated removal of the protective groups afforded **28** in quantiative yield.

In addition to synthesizing analogues that contain dicarboxylates with a *cis* relationship, two analogues with *trans* dicarboxylates were also prepared (Scheme



Scheme 4. Synthesis of flexible compounds. Reagents and conditions: (i) $[(C_6H_5)_3PCu]_6$, toluene, 61% for **25** and **27**; (b) LiOH, H_2O/THF, quant for **26** and **28**.



Scheme 5. Synthesis of *trans* IPP analogues. Reagents and conditions: (i) Mg, THF, Δ, 2 h; (ii) DMAD, THF, −78 °C, 8% for **29** and 3% for **31**; (d) LiOH, H_2O/THF, quantitative for **30** and **32**.

5). As was previously described, the addition of an organocopper reagent, prepared from CuBr·Me_2S, to DMAD gave primarily *cis* addition. The *trans* compounds were accessed by the addition of the corresponding organomagnesium reagent to DMAD, followed by basic hydrolysis to give the desired compounds **30** and **32**. The yields for these compounds are unoptimized.

1.2. Biological evaluation of the IPP analogues

Ten analogues of IPP (**7**, **12**, **15**, **18**, **21**, **24**, **26**, **28**, **30**, **32**) were tested against PFTase and UPP synthase. Recombinant yeast protein farnesyltransferase was expressed in *Escherichia coli* and purified on a Ni(II) column to homogeneity.[18] The enzyme activity was conveniently monitored using a continuous flourometric assay using dansyl-Gly-Val-Ile-Ala at an enzyme concentration of 1.0 nM.[19] The majority of compounds tested, proved to be only poor inhibitors of PFTase with IC_{50} values of greater than 1 mM. As shown in Table 1, the bicyclic-based inhibitors **21** and **24** showed improved inhibition with IC_{50}'s of 812 and 804 μM, respectively. Analogue **30**, with the dicarboxylates having a *trans* relationship displayed the best inhibition of PFTase with an IC_{50} of 384 μM. Compound **30** was also shown to be a competitive inhibitor of PFTase (Fig. 3) against FPP with a $K_i = 287 \pm 30$ μM.

Table 1. IC_{50} values for IPP analogues

Compd	PFTase IC_{50} (μM)[a]	UPP synthase IC_{50} (μM)[b]
7	>1000	>1000
12	>1000	>1000
15	>1000	>1000
18	>1000	>1000
21	812±28	936±43
24	804±46	>1000
26	>1000	>1000
28	>1000	>1000
30	384±33	492±20
32	>1000	135±18

[a] IC_{50} conditions: 1.0 nM PFTase, 50 mM Tris–HCl, 12 mM $MgCl_2$, 12 μM $ZnCl_2$, 5.8 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0 dansyl-Gly-Cys-Val-Ile-Ala was the peptide substrate.
[b] IC_{50} conditions: 0.3 μM UPPase, 100 mM Tris–HCl pH 8.5, 2 mM $MgCl_2$, 5 μM FPP and 45 μM [1-^{14}C] IPP (55 Ci/mol).

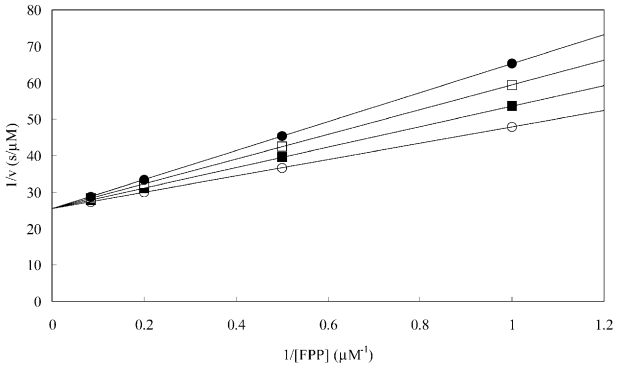


Figure 3. Inhibition of PFTase by compound **30**. Double-reciprocal plot with FPP as the varied substrate at fixed concentrations of **30**. Concentrations of analogue **30** were 100 (●), 200 (□), 300 (■), and 400 (○) μM. Dansyl-Gly-Val-Ile-Ala was held constant at 2.4 μM. PFTase (1.0 nM) was used to initiate reactions. Double-reciprocal plot was generated by fitting the data to the appropriate form of the Michaelis–Menten equation.

Inhibition studies with *E. coli* UPP synthase involve a radioactive assay. After incubation of [1-^{14}C] IPP, FPP and inhibitor with UPP synthase, the reaction products are chromatographed on silica gel TLC. The plate with radiolabeled products was exposed to a film and the radioactivity distribution was determined by phosphor autoradiography. The results show that the majority of the dicarboxylates are poor inhibitors of UPP synthase. Fortunately, compounds **30** and **32** with the *trans* dicarboxylates show modest inhibition with an IC_{50} of 492 and 135 μM, respectively.

The lack of inhibition by these diphosphate mimics may be due to poor binding of the substrate to the active site of the enzyme. Recently, a co-crystal structure of protein farnesyltransferase indicated that the non-bridging oxygen atoms of the two phosphates in diphosphate moiety of the bound FPP molecule do not reflect in a plane through the bridging oxygen.[20,21] For binding to the enzyme active site, specific geometric or spatial arrangement of the diphosphate may be required, which these dicarboxylates can not achieve in solution.

2. Conclusion

In summary, we have described the synthesis of a series of ten analogues of diphosphates. Although these compounds generally show only weak inhibition of PFTase and UPP synthase, it was shown that **32** is a modest inhibitor of UPP synthase. Synthesis of other mimics of diphosphates and their interactions with enzymes that utilize diphosphates is currently underway.

3. Experimental

3.1. General

All reagents were purchased from Sigma or Aldrich and were used without further purification unless otherwise stated. All solvents were dried and distilled prior to use according to standard procedures.[22] Copper(I) bromide

dimethyl sulfide complex was purified as previously reported by House et al.[23] ^{1}H NMR data is reported to the nearest 0.01 and ^{13}C NMR data to the nearest 0.1 ppm.

3.2. Inhibition studies with protein farnesyl tranferase

Recombinant yeast PFTase was produced in *E. coli* and purified by chromatography on a Ni(II) column as previously described.[18] Catalytic rate constants were measured using a fluorescence assay that continuously monitored farnesylation of dansylated pentapeptide using a Spex Fluoromax model spectroflourimeter with $\lambda_{ex} = 340$ (slit width = 5.1 nm) and $\lambda_{em} = 486$ nm (slit width = 5.1 nm) and 3 mm square cuvettes. For PFTase, assays (250 μL) were conducted at 30 °C in 50 mM Tris–HCl, 12 mM $MgCl_2$, 12 μM $ZnCl_2$, 5.8 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0. Dansyl-Gly-Cys-Val-Ile-Ala was the peptide substrate. Reaction mixture were preincubated at 30 °C for 5 min before the reaction was initiated by PFTase previously diluted with assay buffer containing 1 mg/mL bovine serum albumin. Initial rates were measured from the linear region of each run, and all measurements were made in duplicate. Rates were measured in counts/second per second and converted to units of s^{-1} using a conversion factor calculated from the slope of a line generated in a plot of concentration of synthetic dansyl-Gly-((*S*)-farnesyl)Cys-Val-Ile-Ala versus fluorescence intensity.

3.3. Inhibition studies with undecaprenyl diphosphate synthase

Recombinant his_6-tagged UPP synthase from *E. coli* was overproduced and purified by Ni (II) affinity chromatography essentially as described for PFTase.[18] UPP synthase activity was measured by incorporation of radioactivity into UPP from [1-^{14}C] IPP. Reaction mixtures (250 μL) contained 100 mM Tris–HCl (pH 8.5), 2 mM $MgCl_2$, 5 μM FPP, 45 μM [1-^{14}C] IPP (55 Ci/mol), and inhibitor. UPP synthase (0.3 μM) and the samples were incubated at 37 °C for 10 min before being quenched. The reaction mixtures were chromatographed on silica gel TLC plates and the enzymatic product was visualized by phosphor autoradiography.

3.4. General procedure for preparation of *cis*-maleyl substituted dicarboxylates

In a flame dried, argon flushed round-bottom flask was added freshly ground magnesium turnings (1.2 equiv) and THF (3 mL). To this was then added a solution of bromide (1.0 equiv) in THF (5 mL) dropwise, and a crystal of iodine. The reaction mixture was refluxed for 2 h and cooled to room temperature. The prepared Grignard was then added dropwise to a suspension of cuprous bromide-dimethyl sulfide complex (1.2 equiv) in THF (10 mL) at −40 °C. The resulting solution was stirred at −40 °C for 2 h and then cooled to −78 °C, and freshly distilled DMAD (1 equiv) in THF (5 mL) was added dropwise to give a dark red-brown mixture. After 1 h, the reaction mixture was quenched with a saturated solution of ammonium chloride (5 mL, adjusted to pH 8 with 10% ammonia) and allowed to warm to room temperature. After 30 min, the mixture is partitioned between water and ether. The aqueous layer was extracted with ether (3×15 mL) and the combined organic extracts were washed with an additional saturated aqueous NH_4Cl solution (25 mL), and brine (25 mL). Drying over $MgSO_4$ and concentration in vacuo gave an amber oil. Purification by flash column chromatography gave diester as a colorless oil.

3.4.1. Dimethyl (*Z*)-2-(2-methyl)butenylbutenedioate (6). The general procedure was followed. Thus 4-bromo-2-methyl-1-butene (1.0 g, 6.71 mmol), magnesium turnings (300 mg, 12.08 mmol), $CuBr{\cdot}Me_2S$ (1.38 g, 6.71 mmol), DMAD (800 mg, 5.6 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, petroleum ether/ether, 9:1) gave **6** as an oil (490 mg, 41%). IR (film) 3077, 2953, 1732, 1651, 1436 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.82 (m, 1H), 4.76 (m, 1H), 4.70 (m, 1H), 3.82 (s, 3H), 3.71 (s, 3H), 2.49 (m, 2H), 2.19 (m, 2H), 1.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 165.4, 150.0, 143.5, 119.6 111.2, 52.4, 51.9, 34.9, 32.5, 22.4; HRMS (ES +ve) calcd $[M+Na]^+$ for $C_{11}H_{16}O_4Na$ 235.0946, found 235.0944.

3.4.2. Dimethyl (*Z*)-2-(2-methyl)pentenylbutenedioate (11). The general procedure was followed. Thus 1-bromo-4-methyl-4-pentene (1.0 g, 6.13 mmol), magnesium turnings (298 mg, 12.26 mmol), $CuBr{\cdot}Me_2S$ (1.63 g, 6.13 mmol), DMAD (0.63 mL, 4.85 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, Petroleum ether/ether, 9:1) gave **11** (680 mg, 62%) as an oil. IR (film) 3074, 2951, 1728, 1649, 1436 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.8 (t, 1H, $J = 1.5$ Hz), 4.71 (m, 1H), 4.65 (m, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 2.32 (m, 2H), 2.03 (m, 2H), 1.67 (s, 3H), 1.58 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.4, 165.4, 119.4, 110.8, 52.3, 51.8, 36.8, 33.5, 24.8, 22.2; HRMS (ES +ve) calcd for $[M+Na]^+$ $C_{12}H_{18}O_4Na$ 249.1103, found 249.1108.

3.4.3. Dimethyl (*Z*)-2-(2-methyl)pentylbutenedioate (14). The general procedure was followed. Thus 1-bromo-4-methyl-4-pentene (1.0 g, 6.06 mmol), magnesium turnings (300 mg, 12.12 mmol), $CuBr{\cdot}Me_2S$ (1.25 g, 6.06 mmol), DMAD (0.63 mL, 4.85 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, Petroleum ether/ether, 9:1) gave **14** (830 mg, 60%) as an oil. IR (film) 2954, 2870, 1728, 1651, 1436 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.79 (t, 1H, $J = 1.5$ Hz), 3.81 (s, 3H), 3.70 (s, 3H), 2.32 (m, 2H), 1.50 (m, 3H), 1.19 (m, 2H), 0.85 (d, 6H, $J = 6.6$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 165.5, 151.1, 119.0, 52.3, 51.8, 38.1, 34.6, 27.8, 24.8, 22.5; HRMS (ES +ve) calcd for $[M+Na]^+$ $C_{12}H_{20}O_4Na$ 251.1259, found 251.1261.

3.4.4. Dimethyl (*Z*)-2-(2-methyl)butylbutenedioate (17). The general procedure was followed. Thus 1-bromo-3-methyl-1-butane (1.0 g, 6.62 mmol), magnesium turnings (322 mg, 13.24 mmol), $CuBr{\cdot}Me_2S$ (1.76 g, 6.62 mmol), DMAD (784 mg, 5.52 mmol) were reacted as before. Purification of the crude product by flash col-

umn chromatography (SiO_2, petroleum ether/ether, 9:1) gave **17** (970 mg, 82%) as an oil. IR (film) 2955, 2871, 1731, 1635, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.79 (t, 1H, $J=1.4$ Hz), 3.81 (s, 3H), 3.69 (s, 3H), 2.33 (m, 2H), 1.56 (m, 3H), 1.35 (m, 2H) 0.87 (d, 6H, $J=8.7$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 165.5, 151.3, 118.9, 52.3, 51.8, 35.9, 32.4, 27.5 22.3; HRMS (ES +ve) calcd for $[M+Na]^+$ $C_{11}H_{18}O_4Na$ 237.1103, found 237.1096.

3.5. General procedure for preparation of *trans*-maleyl substituted dicarboxylates

In a flame dried, argon flushed round-bottom flask was added freshly ground magnesium turnings (1.2 equiv) and THF (3 mL). To this was then added a solution of bromide (1.0 equiv) in THF (5 mL) dropwise, and a crystal of iodine. The reaction mixture was refluxed for 2 h and cooled to room temperature. The prepared Grignard was then added dropwise to a stirring solution of freshly distilled DMAD (1 equiv) at −78 °C to give dark brown-red mxture. After 1 h, the reaction mixture was quenched with a saturated solution of ammonium chloride (5 mL, adjusted to pH 8 with 10% ammonia) and allowed to warm to room temperature. After 30 min, the mixture is partitioned between water and ether. The aqueous layer was extracted with ether (3×15 mL) and the combined organic extracts were washed with an additional saturated aqueous NH_4Cl solution (25 mL), and brine (25 mL). Drying over $MgSO_4$ and concentration in vacuo gave an amber oil. Purification by flash column chromatography gave diester as a colorless oil.

3.5.1. Dimethyl (*E*)-2-(2-methyl)butenylbutenedioate (29). The general procedure was followed. Thus 4-bromo-2-methyl-1-butene (750 mg, 5.03 mmol), magnesium turnings (245 mg, 10.07 mmol), and DMAD (0.56 mL, 4.57 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, hexanes/ethyl acetate, 10:1) gave **29** as an oil (78 mg, 8%). IR (film) 3075, 2953, 1725, 1645, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.73 (s, 1H), 4.70 (m, 1H), 4.67 (m, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 2.91 (m, 2H), 2.13 (m, 2H), 1.74 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2, 165.9, 147.8, 144.8, 126.5, 110.7, 52.6, 51.8, 37.2, 26.7, 22.3; HRMS (ES +ve) calcd for $[M+H]^+$ $C_{11}H_{17}O_4$ 213.1121, found 213.1122.

3.5.2. Dimethyl (*E*)-2-(2-methyl)pentenylbutenedioate (31). The general procedure was followed. Thus 1-bromo-4-methyl-4-pentene (750 mg 4.60 mmol), magnesium turnings (223 mg, 9.20 mmol) and DMAD (0.38 mL, 2.80 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, hexanes/ethyl acetate, 10:1) gave **31** as an oil (30 mg, 3%). IR (film) 3073, 2929, 1725, 1649, 1436, 1373 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.74 (s, 1H), 4.71 (m, 1H), 4.68 (m, 1H), 3.79 (s, 3H), 3.76 (s, 3H), 2.74 (m, 2H), 2.07 (t, 2H, $J=7.6$ Hz), 1.70 (s, 3H), 1.58 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.3, 166.0, 148.1, 145.3, 126.3, 110.1, 52.4, 51.6, 37.7, 27.6, 26.9, 22.2; HRMS (EI) calcd for $[M^{+\cdot}]$ $C_{12}H_{18}O_4$ 226.1205, found 226.1200.

3.6. General procedure for the formation of anhydrides

Acidification of a stirring solution of dilithium salts in water (5 mL) with 1.0 N HCl at 0 °C and extraction with ether gave the anhydride. Purification by flash column chromatography gave the corresponding anhydride as a white solid.

3.6.1. (*Z*)-2-(2-Methyl)pentenylbutenedioic acid, di-carbonic anhydride (19). The acidification of di-lithium salt **12** (100mg 0.471 mmol) gave salt **19** (90 mg, 99%) as a white powder: IR 2939, 1843, 1772, 1700, 1652, 1436, 1376 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.88 (s, 1H), 4.74 (s, 1H), 4.68 (s, 1H), 2.40 (t, 2H, $J=7.0$ Hz), 2.06 (t, 2H, $J=7.5$ Hz), 1.70 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.3, 170.5, 150.9, 144.3, 119.9, 110.9, 36.8, 33.8, 24.8, 22.2; HRMS (EI) calcd for $[M^{+\cdot}]$ $C_{10}H_{13}O_3$ 180.0786, found 180.0781.

3.6.2. (*Z*)-2-(2-Methyl)butenylbutenedioic acid, di-carbonic anhydride (22). The acidification of di-lithium salt **7** (100 mg 0.471 mmol) gave anhydride **22** (90 mg, 99%) as a colorless oil: IR 2916, 1697, 1648, 1424, 1380 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.90 (s, 1H), 4.78 (s, 1H), 4.71 (s, 1H), 2.54 (m, 2H), 2.40 (t, 2H, $J=6.9$ Hz), 1.73 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.9, 170.5, 150.4, 143.2, 120.4, 111.5, 34.9, 32.5, 22.4; HRMS (EI) calcd for $[M^{+\cdot}]$ $C_9H_{10}O_3$ 166.0630, found 166.0628.

3.7. General procedure for the basic hydrolysis of dimethyl esters to lithium salts

To the di-ester (50 mg) in THF–H_2O (2 mL, 1:1) was added 1.0 N LiOH (3 equiv) and the mixture was stirred at 50 °C and monitored for the consumption of starting material by TLC. The solvent was removed in vacuo and the remaining residue was dissolved in H_2O (4 mL). Freeze-drying of the aqueous layer gave the respective lithium salt.

3.7.1. (*Z*)-2-(2-Methyl)butenylbutenedioic acid, di-lithium salt (7). The hydrolysis of ester **6** (100 mg, 0.471 mmol) gave salt **7** (90 mg, 99%) as a white powder: IR 3379, 3079, 2968, 2937, 1648, 1555, 1419 cm^{-1}; ^1H NMR (CD3OD, 300 MHz) δ 5.51 (m, 1H), 4.70 (m, 2H), 2.39 (m, 2H), 2.23 (m, 2H), 1.73 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 179.0, 174.8, 151.3, 147.2, 120.3, 109.9, 35.5, 33.1, 21.9; HRMS (ES–ve) calcd for $[M-H]^-$ $C_9H_{11}O_4$ 183.0657, found 183.0663.

3.7.2. (*Z*)-2-(2-Methyl)pentenylbutenedioic acid, di-lithium salt (12). The hydrolysis of ester **11** (100 mg 0.471 mmol) gave salt **12** (90 mg, 99%) as a white powder: IR 3379, 3079, 2968, 2937, 1648, 1555, 1419 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.48 (m, 1H), 4.70 (m, 2H), 2.21 (t, 2H, $J=8.0$ Hz), 2.08 (t, 2H, $J=7.4$ Hz), 1.72 (s, 3H), 1.59 (dpent, 2H, $J=1.4$ Hz, 7.4 Hz); ^{13}C NMR (D_2O, 75 MHz) δ 179.9, 175.4, 152.6, 148.6, 120.7, 110.4, 37.4, 34.9, 25.8, 22.3; HRMS (ES–ve) calcd for $[M-H]^-$ $C_9H_{11}O_4$ 197.0814, found 197.0819.

3.7.3. (*Z*)-2-(2-Methyl)pentylbutenedioic acid, di-lithium salt (15). The hydrolysis of ester **14** (100 mg 0.438

mmol) gave salt **15** (93 mg, 99%) as a white powder: IR 3433, 2954, 2870, 2845, 1640, 1586, 1428 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.48 (m, 1H), 2.21 (m, 2H), 1.45 (m, 3H), 1.23 (m, 2H), 0.88 (d, 6H, $J=6.6$ Hz); ^{13}C NMR (D_2O, 125 MHz) δ 179.4, 174.8, 152.6, 119.8, 38.4, 35.3, 37.6, 25.3, 22.5, 22.4; HRMS (ES–ve) calcd for $[M-H]^-$ $C_{10}H_{15}O_4$ 199.0970, found 199.0976.

3.7.4. (*Z*)-2-(2-Methyl)butylbutenedioic acid, di-lithium salt (18). The hydrolysis of ester **17** (99 mg 0.462 mmol) gave salt **18** (102 mg, 94%) as a white powder: IR 3409, 2954, 2871, 1638, 1574, 1428 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.48 (m, 1H), 2.23 (m, 2H), 1.58 (m, 1H), 1.32 (m, 2H), 0.87 (d, 6H, $J=6.6$ Hz); ^{13}C NMR (D_2O, 75 MHz) δ 180.0, 175.5, 153.4, 120.2, 37.1, 33.4, 27.9, 22.5; HRMS (ES–ve) calcd for $[M-H]^-$ $C_9H_{13}O_4$ 185.0814, found 185.0819.

3.7.5. 2-(4-Methyl-pent-4-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dioic acid, dilithium salt (21). The hydrolysis of the anhydride **20** (16 mg, 0.069 mmol) gave salt **21** (25 mg, 99%) as a white powder. IR 3373, 3074, 2967, 1704, 1649, 1551, 1416 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 6.29 (dd, 1H, $J=2.99$ Hz, 5.55 Hz), 6.18 (dd, 1H, $J=2.97$ Hz, 5.45 Hz), 4.59 (m, 2H), 2.83 (b, 1H) 2.79 (b, 1H), 2.64 (d, 1H. $J=3.05$ Hz), 2.07 (m, 3H), 1.73 (4H, m), 1.57 (d, 1H, $J=9.35$ Hz), 1.28 (td, 1H, $J=1.76$ Hz, 8.52 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 184.3, 183.3, 149.0, 138.0, 136.6, 110.1, 64.5, 51.9, 49.5, 47.7, 47.2, 42.8, 38.5, 24.5, 22.5; HRMS (ES-ve) calcd for $[M-H]^-$ $C_{15}H_{19}O_4$ 263.1278, found 263.1276.

3.7.6. 2-(3-Methyl-but-3-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dicarbonic anhydride (24). The hydrolysis of the anhydride **23** (16 mg, 0.065 mmol) gave salt **24** (24 mg, 99%) as a white powder.: IR 3417, 3375, 3078, 2988, 2967, 2884, 1646, 1576, 1469 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 6.31 (d,d, 1H, $J=5.50$ Hz, 2.93 Hz), 6.18 (d,d, 1H, $J=5.50$ Hz, 2.93 Hz), 4.76 (m, 2H), 2.81 (m, 2H), 2.63 (d, 2H, $J=3.05$ Hz), 2.04 (dt, 2H, $J=3.30$ Hz, 7.33 Hz), 1.95 (dt, 1H, $J=4.76$ Hz, 12.46 Hz), 1.72 (s, 3H), 1.51 (m, 4H), 1.27 (dt, 1H, $J=1.70$ Hz, 8.71 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 184.3, 183.1, 149.5, 137.8, 136.8, 109.7, 64.4, 61.7, 49.7, 17.6, 47.1, 41.6, 34.9, 22.7; HRMS (ES +ve) calcd for $[M+Na]^+$ $C_{14}H_{18}O_4Na$ 273.1097 found 273.1094.

3.7.7. (*Z*)-2-(2-Methyl)butenylbutane dioic acid, dilithium salt (26). The general procedure was followed. The hydrolysis of ester **25** (21 mg, 0.098 mmol) gave salt **26** (24 mg, quantitative) as a white powder. IR 3372, 2937, 1580, 1433 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 4.75 (m, 2H), 2.56 (m, 1H), 2.45 (dd, 1H, $J=5.2$ Hz, 14.4 Hz), 2.18 (dd, 1H, $J=4$ Hz, 14 Hz), 2.03 (m, 2H), 1.72 (s, 3H), 1.59 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 185.5, 182.6, 148.6, 110.4, 46.8, 42.1, 36.2, 31.1, 22.5; HRMS (ES−ve) calcd for $[M-H]^-$ $C_9H_{13}O_4$ 185.0808, found 185.0809.

3.7.8. (*Z*)-2-(2-Methyl)pentenylbutane dioic acid, dilithium salt (28). The general procedure was followed. The hydrolysis of ester **27** (21 mg, 0.098 mmol) gave salt **28** (24 mg, quantitative) as a white powder. IR 3356, 2936, 1573, 1427 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 4.72 (m, 2H), 2.54 (m, 1H), 2,41 (dd, 1H, $J=5.4$ Hz, 14.3 Hz), 2.14 (dd, 1H, $J=9.5$ Hz, 14.3 Hz), 2.04 (m, 2H), 1.70 (s, 3H), 1.42 (m, 4H) ^{13}C NMR (D_2O, 100 MHz) δ 185.6, 182.6, 148.9, 110.1, 46.9, 42.0, 37.9, 32.5, 25.8, 24.1, 22.3; HRMS (ES–ve) calcd for $[M-H]^-$ $C_{10}H_{15}O_4$ 199.0963, found 199.0964.

3.7.9. (*E*)-2-(2-Methyl)butenylbutenedioic acid, di-lithium salt (30). The hydrolysis of ester **29** (14 mg 0.066 mmol) gave salt **30** (14 mg, 99%) as a white powder: IR 3362, 2938, 1648, 1567, 1390 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 6.34 (s, 1H), 4.71 (s, 1H), 4.69 (s, 1H), 2.59 (t, 2H, $J=8.1$ Hz), 2.09 (t, 2H, $J=7.5$ Hz), 1.69 (s, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 178.1, 177.2, 147.8, 144.4, 129.4, 110.7, 37.3, 28.0, 22.3; HRMS (ES–ve) calcd for $[M-H]^-$ $C_9H_{11}O_4$ 183.0652, found 183.0653.

3.7.10. (*E*)-2-(2-Methyl)pentenylbutenedioic acid, di-lithium salt (32). The hydrolysis of ester **31** (15 mg 0.066 mmol) gave salt **32** (15 mg, 99%) as a white powder: IR 3328, 2935, 1566, 1502, 1428 cm^{-1}; ^1H NMR (D2O, 400 MHz) δ 6.29 (s, 1H), 4.69 (m, 2H), 2.40 (t, 2H, $J=7.82$ Hz), 1.98 (t, 2H, $J=7.5$ Hz), 1.66 (s, 3H), 1.47 (p, 2H, $J=7.7$ Hz); ^{13}C NMR (CD3OD, 75 MHz) δ 178.4, 177.3, 148.7, 144.8, 129.0, 110.3, 37.7, 29.1, 27.1, 22.3; HRMS (ES–ve) calcd for $[M-H]^-$ $C_{10}H_{13}O_4$ 197.0808, found 197.0806.

3.8. General procedure for Diels–Alder reaction

To a stirring solution of butenedioic acid anhydride (1 equiv) in toluene (2 mL) was added freshly prepared cyclopentadiene (5 equiv). The resulting reaction mixture was stirred at reflux for 5 h. Analysis of the reaction mixture by TLC showed disappearance of starting material. Solution was cooled to room temperature and solvent was removed in vacuo. Purification of the crude product by flash column chromatography gave the desired cycloadduct.

3.8.1. 2-(4-Methyl-pent-4-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dicarbonic anhydride (20). The general procedure was used. Thus, (*Z*)-2-(3-methyl-pent-3-enyl)-butenedioic acid, anhydride **19** (50 mg, 0.277 mmol) and cyclopentadiene (0.11 mL, 1.385 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, hexanes/ethyl acetate, 10:1) gave **20** as an oil (40 mg, 59%). IR 3073, 2943, 1771, 1650, 1459; ^1H NMR (CD_2Cl_2, 500 MHz) δ 6.34 (d,d, 1H, $J=5.65$ Hz, $J=2.90$ Hz), 6.26 (d,d, 1H, $J=5.65$, 2.90 Hz), 4.72 (m, 1H), 4.66 (m, 1H), 3.42 (m, 1H), 3.19 (d, 1H, $J=4.58$ Hz), 3.03 (m, 1H), 2.07 (m, 3H), 1.78 (m, 2H), 1.69 (s, 3H), 1.64 (t, d $J=13.13$ Hz, $J=4.12$ Hz), 1.56 (m, 2H), 1.45 (m, 2H); ^3C NMR (CD_2Cl_2, 75 MHz) δ 174.8, 171.4, 145.1, 137.8, 135.9, 110.9, 59.7, 51.9, 51.3 (2C), 47.2, 38.0, 35.5, 24.2, 22.2; HRMS (EI) calcd for $[M^{+\cdot}]$ $C_{15}H_{18}O_3$ 246.1256 found 246.1254.

3.8.2. 2-(3-Methyl-but-3-enyl)-bicyclo[2.2.1]hept-5-ene-2,3-dicarbonic anhydride (23). The general procedure was used. Thus (*Z*)-2-(3-methyl-but-3-enyl)-butenedioic

acid, anhydride **22** (80 mg, 0.484 mmol) and cyclopentadiene (0.175 mL, 2.42 mmol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, hexanes/ethyl acetate, 15:1) gave **23** (60 mg, 54%) as an amber oil. IR 3073, 2952, 1779, 1649, 1457 cm^{-1}; ^1H NMR (CD_2Cl_2, 500 MHz) δ 6.36 (d,d, 1H, $J = 5.71$ Hz, 2.99 Hz), 6.29 (d,d, 1H, $J = 5.85$ Hz, 2.86 Hz), 4.77 (m, 1H), 4.72 (m, 1H), 3.44 (m, 1H), 3.24 (d, 1H, $J = 4.75$ Hz), 3.06 (m, 1H), 2.30 (m, 1H), 2.16 (m, 1H), 2.07 (m, 1H), 1.81 (m, 3H), 1.74 (s, 3H). ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 174.6, 171.3, 144.4, 137.5, 135.9, 111.3, 59.5, 52.3, 51.4, 51.1, 47.4, 34.6, 34.0, 22.4; HRMS (EI) calcd for [$M^{+\cdot}$] $C_{14}H_{16}O_3$ 232.1099, found 232.1096.

3.9. General procedure for the conjugate reduction of maleyl substituted diester

In a flame-dried, argon-flushed round-bottom flask was added maleyl substituted diester. In an inert atmosphere, $[(Ph_3P)CuH]_6$ was weighed and added to round bottom flask under positive N_2 pressure. Deoxygenated toluene (2 mL) containing water (50 μL) was added, and the resultant red solution was stirred at room temperature until starting material had been consumed by TLC analysis. The cloudy red-brown reaction mixture was opened to air, and stirring was continued for 1 h. Filtration of mixture through Celite and resulting filtrate was concentrated in vacuo gave crude product which was purified by flash chromatography.

3.9.1. Dimethyl (*Z*)-2-(2-methyl)butenylbutanedioate (25). The general procedure was followed. Thus dimethyl (*Z*)-2-(2-methyl)butenylbutanedioate **6** (25 mg, 0.118 mmol) and $[(Ph_3P)CuH]_6$ (115 mg, 0.06 mol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, hexanes/ethyl acetate, 10:1) gave **25** (17 mg, 67%) as an oil. IR 3075, 2952, 1738, 1650, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.71 (m, 1H), 4.65 (m, 1H), 3.68 (s, 3H), 3.65 (3s, 3H), 2.82 (m, 1H), 2.70 (dd, 1H, $J = 9.1$ Hz, 16.3 Hz), 2.44 (dd, 1H, $J = 5.1$ Hz, 16.2 Hz), 2.0 (t, 2H, $J = 7.8$ Hz), 1.79 (m, 1H), 1.68 (s, 3H), 1.63 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.3, 172.3, 144.5, 110.7, 51.9, 51.7, 40.7, 35.8, 35.0, 19.8, 22.3; HRMS (EI) calcd for [$M^{+\cdot}$] $C_{11}H_{18}O_4$ 214.1205, found 214.1204.

3.9.2. Dimethyl (*Z*)-2-(2-methyl)pentenylbutanedioate (27). The general procedure was followed. Thus, dimethyl (*Z*)-2-(2-methyl)pentenylbutenedioate **27** (100 mg, 0.442 mmol) and $[(Ph_3P)CuH]_6$ (350 mg, 0.18 mol) were reacted as before. Purification of the crude product by flash column chromatography (SiO_2, hexanes/ethyl acetate, 10:1) gave **25** (62 mg, 61%) as an oil. IR 3074, 2951, 1738, 1650, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.68 (m, 1H), 4.63 (m, 1H), 3.67 (s, 3H), 3.64 (s, 3H), 2.84 (m, 1H), 2.70 (ddd, 1H, $J = 2.3$ Hz, 9.2 Hz, 16.5 Hz), 2.41 (ddd, 1H, $J = 2.1$ Hz, 5.2 Hz, 16.4 Hz), 1.98 (t, 2H, $J = 7.4$ Hz), 1.66 (s, 3H), 1.60 (m, 1H), 1.42 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.3, 172.3, 145.0, 110.2, 51.7 (2C), 41.0, 37.3, 35.8, 31.4, 24.7, 22.2; HRMS (ES+ve) calcd for $[M+H]^+$ $C_{12}H_{20}O_4Na$ 251.1254, found 251.1256.

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