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Authors: Manish Bhattacharjee, Rajesh K. Jena, Mohitosh Mandal, and Y Rajesh

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[(*para*-cymene)Ru(dppp)Cl][PF_6] Catalyzed Stereospecific Synthesis of O- Dienyl Esters and Evaluation of the Anti-cancer Activity of a Long Chain Fatty Acid O-Dienyl Ester

Rajesh K. Jena,[a] Yetirajam Rajesh,[b] Mahitosh Mandal[b] and Manish Bhattacharjee*[a]

Abstract: O-dienyl esters are obtained via regio- and stereospecific addition of both aromatic and aliphatic carboxylic acids to terminal propargylic alcohols, with the sequential formation of a carbon-oxygen bond and dehydration, in the presence of [(p-cymene)Ru(dppp)Cl][PF_6] (1). Also, cytotoxicity and anticancer activity of the newly synthesized O-dienyl ester, (Z)-3-methylbuta-1,3-dienyl oleate has been investigated.

Introduction

Fatty acids and their derivatives comprise an important class of biologically active compounds. Some of the major fatty acids such as linoleic acid (LA), arachidonic acid (ARA), palmitoleic acid (PA), and oleic acid (OA) and their methyl and ethyl esters were investigated for antimicrobial activity against oral pathogens.[1] It has been reported that conjugated linoleic acid and its methyl esters derivatives show various biological activities, including antioxidant activity, anti-bacterial activity, and free radical scavenging effect.[2] Biosynthetic gene cluster, known as fatty acid enol esters (fee) was isolated from cultured bacteria, and this has been shown to produce fatty acid enol esters.[3] Fatty acids and their derivatives have been fund to have anti-cancer activity.[4] Also, fatty acids and their derivatives are precursors of many biomolecules which have roles in regulating blood pressure, preventing heart disease and some chronic diseases.[5]

Dienyl esters have been shown to be useful precursors in organic synthesis,[6] especially for the regio- and stereo-selective generation of enolates.[7] On the other hand, in spite of their usefulness as 1,3-diene monomer,[8] very few examples of the selective formation of enol esters are known. Ruthenium complexes have been used for the catalytic addition of carboxylic acids to terminal propargylic alcohols. The reaction involves the Markovnikov addition of the carboxylic acid to the C≡C bond of the propargylic alcohols, and the product β-oxo esters are obtained in high yield.[9] Reports on the anti-Markovnikov addition of carboxylic acids to terminal propargylic alcohols are not very common. It has been shown that [(dppe)Ru(η^3-CH_2-C(Me)=CH_2)$_2$] catalyzes the anti-Markovnikov addition of benzoic acid to 2-propyn-1-ols and 3-hydroxy-1-propen-1-yl benzoates are obtained.[10] From literature survey, we found that there was no repot on the synthesis of dienyl ester from the direct addition of carboxylic acid with terminal propargylic alcohols. Dixneuf et al. reported the synthesis of dienyl esters or diene from the addition of carboxylic acids with 3-methylbut-3-en-1-yne in the presence of [(dppb)Ru(η^3-CH_2-C(Me)=CH_2)$_2$] as a precursor.[11]

We have been working on ruthenium catalyzed selective addition reaction to terminal alkynes.[12-14] Recently we have reported ruthenium catalyzed selective enol ester synthesis from the addition of aromatic and aliphatic carboxylic acid to terminal alkynes using cationic ruthenium phosphine complexes. Also we have reported synthesis, structure and catalytic properties of the cationic complex, [Ru(dppp)$_2$(CH_3CN)Cl][BPh_4] {dppp = diphenylphosphino-propane}. This complex can catalyze oxidative coupling of terminal alkynes in the presence of catalytic amount of $Ag(NO_3)$.[15] The complex also catalyzes activation of terminal alkyne for the synthesis of long-chain fatty acids enol ester[16] and one pot synthesis of dienyl ester from the addition of carboxylic acid to propargylic alcohol and its atom transfer radical polymerization.[17] It may be noted that in the [Ru(dppp)$_2$(CH_3CN)Cl][BPh_4] catalyzed reaction of propargylic alcohols with carboxylic acids, only aromatic carboxylic acids were found to be effective. Also, Z-dienyl esters were obtained as the major product along with some E- isomer.

We wanted to modify the catalyst structure, so that, we could carry out the reaction of aliphatic carboxylic acids, including fatty acids with propargylic alcohols. One of the objectives of the present work was to synthesize O-dienyl esters of fatty acids and explore their biological properties. Herein we report the synthesis and structure of [(p-cymene)Ru(dppp)Cl][PF_6] (**1**) and its catalytic properties towards the anti-Markovnikov addition of carboxylic acids to propargylic alcohols. Also, we report here the results of our initial studies on the anti-cancer activities and cytotoxicity of the newly synthesized dienyl ester, (Z)-3-methylbuta-1,3-dienyl oleate.

Results and Discussion

The compound has been synthesized from the reaction of [{Ru(η^6-p-cymene)Cl_2}$_2$] with diphenylphosphinopropane (dppp) in 1:3.5 ratio in acetonitrile in the presence of NH_4PF_6. The compound has been characterized by elemental analyses, IR, and ^{1}H and ^{31}P NMR spectroscopy. It may be noted that the complex, [(p-cymene)Ru(dppp)Cl]Cl and its catalytic activity towards hydrogenation of styrene has been reported.[18] Synthesis by the procedure reported in the literature leads to the

[a] Mr. R. K. Jena, Prof. Dr. M. Bhattacharjee
Department of Chemistry
Indian Institute of Technology
Kharagpur 721302, India
E-mail: mxb@iitkgp.ernet.in
http://www.iitkgp.ac.in/department/CY/faculty/cy-mxb
[b] Mr. Y. Rajesh, Dr. M. Mandal
School of Medical Science & Technology
Indian Institute of Technology
Kharagpur 721302, India

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formation of by-products including phosphine-bridged bimetallic complexes and bis-diphosphine species resulting from arene displacement.[19, 20] To avoid the formation of the dimeric species, a large excess of diphosphine was used, and lower temperatures were applied to prevent loss of the arene.

The elemental analyses are in good accordance with the formulation of the compound. The ^{1}H NMR data of this complex is in agreement with the literature data.[18] The ^{31}P NMR spectrum ($CDCl_3$) of **1** shows two peaks at 25.3, and 25.1 ppm due to ruthenium coordinated dppp ligand and one septet appears at -143.88 due to PF_6^- counter anion.

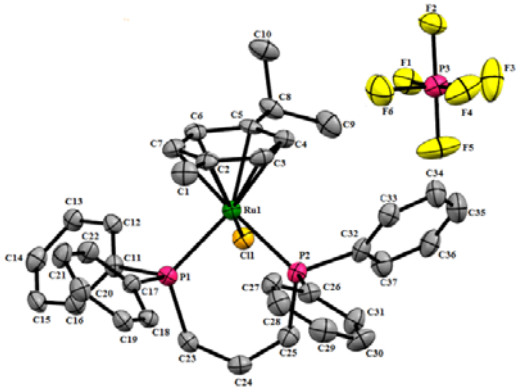
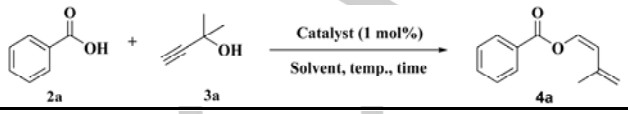


Figure 1 ORTEP view of [(*para*-Cymene)Ru(dppp)Cl]PF6 with thermal ellipsoids drawn in 30% probability. Hydrogen atoms and solvent toluene have been omitted for clarity

The complex, **1** crystallizes in orthorhombic space group $P2_12_12_1$ (Table S1; ESI). The asymmetric unit contains one cationic ruthenium complex (Fig. 1), one hexafluorophosphate anion and one free toluene. The ruthenium center is in a distorted tetrahedral coordination environment. The basal positions of one ruthenium complex occupied by two phosphorus from dppp ligand, P1 and P2 {P1–Ru1 = 2.3383(13) Å; P2–Ru1 = 2.3429(12) Å; P(1)-Ru(1)-P(2) = 90.47(4)^o}, one chlorine Cl1{Cl1-Ru1 = 2.3974(11) Å} and p-cymene ligand. The PF_6^- anion is found outside the coordination sphere and does not show any bonding interaction with ruthenium(II) center.

We were interested in studying the catalytic properties of **1** towards the addition of carboxylic acids to terminal propargylic alcohol. We have shown that cationic ruthenium complexes are capable of activating alkynes towards addition reactions and we wanted to explore the efficiency of **1** as the catalyst for the addition of terminal propargyl alcohol to both aromatic and aliphatic carboxylic acids. Thus the scope of the **1** catalyzed dienyl ester forming addition reaction of various carboxylic acids with propargylic alcohols was examined. We have reported that the cationic ruthenium(II) complex, $[Ru(dppp)_2(CH_3CN)Cl][BPh_4]$ can catalyze the anti-Markovnikov addition of carboxylic acids to secondary and tertiary terminal propargylic alcohols having β-protons with respect to –OH group and produce *Z*-dienyl esters as the major product (*vide supra*). We choose the reaction between benzoic acid (**2a**) and 2-methyl-3-butyn-2-ol (**3a**) in toluene in the presence of 1 mol% of **1** as a model reaction. The addition reaction of 1 mmol of **2a** with 1 mmol of **3a** in the presence of 0.01 mmol of **1** led to the complete conversion of the starting propargylic alcohol to (*Z*)-buta-1,3-dienyl benzoate (**4a**) (Table 1).

Table 1 Optimization of the Reaction Conditions[a]

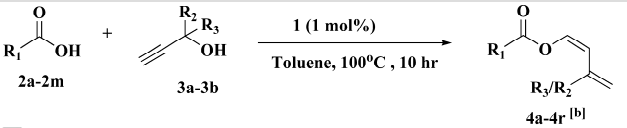


2a 3a 4a

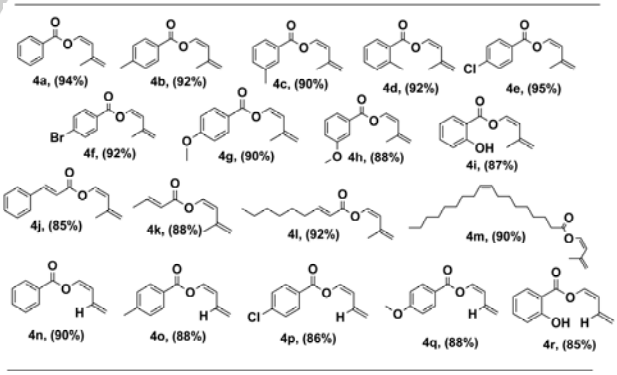
Entry	Solvent	Temp (°C)	Time (h)	Yield [b]	Z/E[c]
1	Acetonitrile	80	10	Tarce	-
2	Dioxane	90	10	30	-
3	Toluene	50	12	Trace	-
4	Toluene	80	12	40	4:1
5	Toluene	90	12	75	95:5
6	Toluene	100	10	94	*Z* only

[a]Reaction conditions: **2a** (1.0 mmol), **3a** (1.0 mmol), **1** (0.01 mmol) in solvent (4.0 mL). [b]Isolated. [c]From NMR spectroscopy

Table 2 Reaction of addition of carboxylic acids to terminal propargylic alcohols [a]



2a-2m 3a-3b 4a-4r [b]



[a]Reagents and conditions: **2** (1.0 mmol), **3** (1.0 mmol), **1** (0.01mmol %), toluene (4 mL), and 100 °C, 10 h. [b]Isolated

We then examined the substrate scope. Thus, we tried the reaction of different aromatic acid derivatives with various propargylic alcohols in the presence of 1 mol% of **1** in toluene at 100 °C (Table 2). For example, the reaction of 2-methyl-3-butyn-2-ol (**3a**) with various carboxylic acids {benzoic acid (**2a**),

p-toluic acid (**2b**), m-toluic acid (**2c**), o-toluic acid (**2d**), p-chlorobenzoic acid (**2e**), p-bromobenzoic acid (**2f**), p-methoxy benzoic acid (**2g**), m- methoxy benzoic acid (**2h**), salicylic acid (**2i**), cinnamic acid (**2j**)} afforded the corresponding Z-dienyl esters {**4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **4g**, **4h**, **4i**, **4j**}, in very good to excellent yield (Table 2). Similarly the reaction between but-3-yn-2-ol (**3b**) with different carboxylic acids {**2a**, **2b**, **2e**, **2g**, **2j**} afforded the corresponding Z-dienyl esters {**4n**, **4o**, **4p**, **4q**, **4r**} in very good yield (Table 2).

As mentioned earlier, one of our objectives was to explore the efficacy of **1** as a catalyst for the addition of aliphatic carboxylic acids, including fatty acids to propargylic alcohols. Thus next we tried the reaction of some aliphatic carboxylic acids such as medium and long chain fatty acids, and in all cases, we isolated Z-dienyl esters in good yield. For example, the reaction of 2-methyl-3-butyn-2-ol (**3a**) with crotonic acid (**2k**), 2-nonenoic acid (**2l**) and oleic acid (**2m**) afforded Z-dienyl esters **4k**, **4l**, and **4m** in 88, 92 and 90% yield, respectively (Table 2).

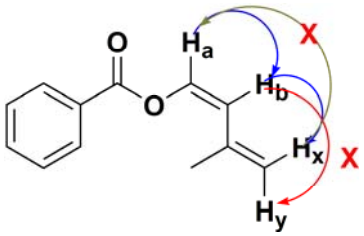


Figure 2 NOE interactions observed in the NOESY spectrum of **4a**

All the compounds have been characterized by ^{1}H, ^{13}C NMR spectroscopy, and the newly synthesized compounds, **4k**, **4l**, and **4m** have been further characterized by high-resolution mass spectrometry (HRMS). The geometry of the double bond was determined based on NOESY measurements. The Z-configuration of **4a** was established based on the NOESY studies. NOEs between vinyl-H_b [δ = 5.48 ppm] and vinyl-H_a [δ = 7.27 ppm] and that between vinyl-H_b [δ = 5.48 ppm] and geminal proton H_x [δ = 5.12 ppm] of **4a** is seen in the NOESY spectrum. However, no NOE is seen between vinyl- H_b [δ = 5.48 ppm] and geminal proton H_y [δ = 5.00 ppm] (Figure 2).

Based on the *in situ* NMR studies we proposed a reaction mechanism for the dienyl ester formation of the reaction between aromatic carboxylic acids and propargylic alcohols.[17] We propose that, the mechanism of the reaction is similar that reported in our earlier communication.[17]

The reaction between **2a** and **3a** was monitored by gas chromatography (GC). Benzoic acid (**2a**) has a retention time 13.8 min and 2-methyl-3-butyn-2-ol (**3a**) has a retention time 2.5 min and the product, (Z)-buta-1,3-dienyl benzoate (**4a**) has a retention time 19.0 min. The reaction was monitored by GC every 30 min for five hours and finally after the completion of the reaction (10 hrs.). After 90 minutes a new product appeared having retention time, 10.30 min. and the concentration increased gradually with time up to 5 hours. After five hours the concentration started decreasing. The GC peak for **4a** appeared after 2.5 hours and the concentration increased with time. After 10 hours all the peaks disappeared except the peak for **4a**. Thus we can conclude that the product with retention time 10.3 min is hydroxyl enol ester resulting from the initial anti-Markovnikov addition, which undergoes dehydration to give the product **4a**.

It has been shown that natural products containing 1,3- diene or fatty acid esters show significant biological activities such as antioxidant activity, anti-bacterial activity and free radical scavenging effect.[21] These reports have prompted us to study the biological activities of the newly synthesized O-dienyl ester, (Z)-3-methylbuta-1,3-dienyl oleate, **4m**.

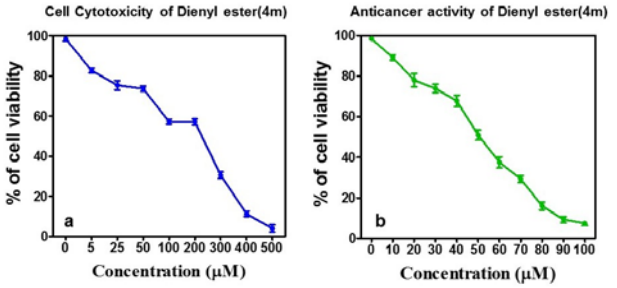


Figure 3 (a) Cell cytotoxicity assay on HaCaT Cells (Normal Human Cells) (b) anticancer activity on U87MG Cells (Human Cancer Cells) of (Z)-3-methylbuta-1,3-dienyl oleate, **4m**. Values are presented as mean ± SD.

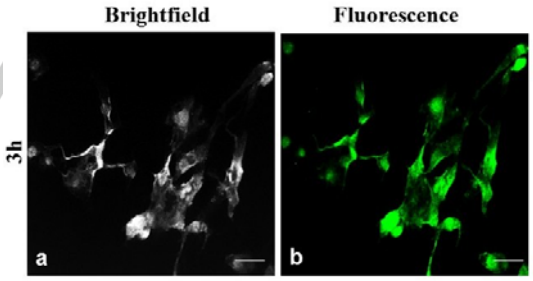


Figure 4 Confocal fluorescence and brightfield images of U87MG cells after 3 h of incubation with **4m** exhibited green fluorescence indicating successful internalization (a and b) (scale bar = 20 μm)

The cytotoxicity assay of **4m** was performed *in vitro* using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, a yellow tetrazole) assay in the HaCaT cells (Normal Human Cells). The cytotoxic effect of each treatment was expressed as the percentage of cell viability relative to the untreated control cells. The percentage of cell viability was plotted versus the concentration. It was observed that cell viability remains 50% at 212 μM of **4m** (Figure 3a). Thus it is concluded that compound is biocompatible in nature.[22]

Then to assess the anticancer activity of the compounds, MTT assay was performed in the U87MG cells (Human Cancer Cells). The anticancer activity is expressed in terms of IC_{50} i.e., the half maximal inhibitory concentration measuring quantitatively the effectiveness of a compound inhibiting the biological process of the cell by half. The IC_{50} of **4m** was found to be 32.7 μM (Figure 3b).

The cellular uptake property of **4m** was investigated by cell imaging studies. The cancer cells (U87MG) were treated with 32.7 μM of **4m**. Confocal microscopy study confirms the cellular internalization of **4m** into U87MG cells within 3 h of treatment (green coloration, Figure 4b).

The U87MG cells were treated with **4m** to determine the time dependent pattern of cell death. During 48 h of incubation, changes in cell morphology were determined by phase contrast microscopy (Figure 5). The cellular shrinkage, increase in fragmentation into membrane-bound bodies and remarkable reduction in number of viable cells was observed during 48h of incubation.

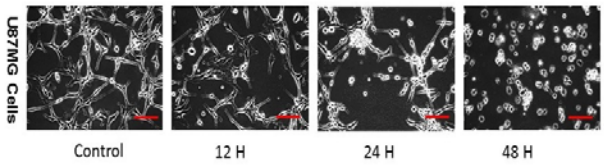


Figure 5 Time dependent effect of (Z)-3-methylbuta-1,3-dienyl oleate, **4m** on U87MG cell morphology.

Conclusion

In summary, we have synthesized a simple chiral cationic ruthenium complex, $[(\eta^6\text{-p-cymene})Ru(\eta^2\text{-dppp})Cl][PF_6]$, using commercially available cheap chemicals. The complex is stable in air and moisture. This compound can catalyze the anti-Markovnikov addition of aromatic and aliphatic carboxylic acids to terminal propargylic alcohols to form stereo specifically Z-dienyl esters. The initial biological activity evaluation exhibits that octadec-9-enoic acid 3-methyl-buta-1,3-dienyl ester is biocompatible in nature with potent anticancer activity. This study opens up a new window in biologics and pharmaceutical industry.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on a 400 and 100 MHz, respectively. NOESY spectra were recorded with a 600 MHz spectrometer. Infrared spectra were recorded on KBr plates as thin films. Solvents and reagents used were reagent grade products. HRMS data were recorded on TOF MS in ESI+ mode in a methanol− water mixture.

Synthesis of $[(\eta^6$-*p*-Cymene)$Ru(\eta^2$-dppp)Cl][PF_6] (1)

A sample of $\{Ru(\eta^6\text{-arene})Cl_2\}_2$] (1 mmol) and 1,3-bis(diphenylphosphino) propane (3.5 mmol) were dissolved in acetonitrile (15 mL) and the reaction solution was heated at 65°C for about 3 hr. Then after 3 hr NH_4PF_6 (3 mmol) was added and heated for another 0.5 hr. The color of the reaction solution turned brown to yellow- orange. After that the reaction solution was filtered and concentrated to get yellow- orange crystalline solid. The solid was washed with hexane (3–4 times), filtered and dried in vacuum. The compound was finally re-crystallized from toluene-acetonitrile mixture. Yield: 0.800 g (87%). ^1H NMR (400 MHz, $CDCl_3$, ppm): 0.84 (d, *J* = 6.8 Hz, 6H), 1.39 (s, 3H), 2.22 – 2.30 (m, 4H), 2.37 (m, 1H), 3.02 (m, 2H) 5.67 (d, *J* = 6.0 Hz, 2H), 5.78 (d, *J* = 6.0 Hz, 2H), 7.18–7.66 (m, 20H); ^{31}P NMR (161.98 MHz, CDCl3, ppm): 25.3 and 25.1, -143.8 (septet) ppm; IR (KBr) (cm^{-1}): 495, 557, 648, 745, 999, 1097, 1192, 1310, 1435, 1810, 1960, 2252, 2965, 3065, 3337 cm^{-1}; HRMS (ESI^+): *m/z* calculated for $C_{37}H_{40}Cl_1P_2Ru$ (without solvent C_7H_8 and PF_6^- anion): 683.1337; found: 683.1328.

Single crystal X-ray diffraction

Crystal data for **1**: $C_{44}H_{48}ClF_6P_3Ru$, Mw = 920.25, 293(2) K, Orthorhombic, *P212121*, *a* = 11.9981(16) Å, *b* = 18.036(2) Å, *c* = 19.087(3) Å, *V* = 4130.4(9) $Å^3$. *Z* = 4, λ= 0.71073A, reflection collected/unique = 48131/7388 R_{int} = 0.0983, R1 = 0.0390, wR2 = 0.0786 for 6122 observed reflections [I>2σ(I)], The structures were solved by direct methods and refined by least square methods on F^2 employing WinGx (L. J. Farrugia, *J. Appl. Crystallogr.* **1999**, *32*, 837.) package and the relevant programs {SHELX-97 (G. M Sheldrick,. *Acta Crystallogr.* **2008**, *A64*, 112.) and ORTEP-3 (L. J. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.)} implemented therein. Non-hydrogen atoms were refined anisotropically and hydrogen atoms on C-atoms were fixed at calculated positions and refined using a riding model. CCDC 1513732 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam. ac.uk/data_request/cif

General procedure for the synthesis of O-dienyl esters

The alkyne (1 mmol), carboxylic acid (1 mmol) and **1** (0.01 mmol) were taken in 5 cm^3 toluene in a 10 mL round bottomed flask with a magnetic bar fitted with a reflux condenser under air. The reaction mixture was stirred for 10h at 100°C. The reactions were monitored by TLC. After the completion of the reaction, the reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The analytically pure products were isolated by a column chromatography on silica gel (hexanes/EtOAc).

Z)-3-methylbuta-1,3-dienyl benzoate ***(4a)***[17]*:* Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4a** as a light yellow oil (0.175 g, 94% yield).^1H NMR (400 MHz, $CDCl_3$, ppm) δ 2.20 (s, 3H), 5.01 (d, *J* = 1.2 Hz 1H), 5.12 (d, *J* = 1.4 Hz 1H), 5.48 (d, *J* = 7.2 Hz, 1H), 7.27 (d, *J* = 7.2 Hz, 1H), 7.42-7.51 (m, 2H), 7.60-7.64 (m, 1H), 8.11(d, *J* = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 23.1, 115.1, 117.9, 128.8, 129.1, 130.1, 133.3, 133.8, 139.3, 163.6.

(Z)-3-methylbuta-1,3-dienyl-4-methylbenzoate ***(4b)***[17]: Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4b** as a colourless oil (0.185 g, 92% yield).^1H NMR (400 MHz, $CDCl_3$, ppm) δ 2.20 (s, 3H), 2.43 (s,3H), 4.99 (d, *J* = 1.2 Hz, 1H), 5.15 (d, *J* =1.0 Hz, 1H), 5.46 (d, *J* = 7.2 Hz, 1H), 7.26-7.30 (m, 3H), 7.98 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 21.8, 23.0, 114.8, 117.6, 126.3, 129.3, 130.1, 133.4, 139.3, 144.6, 163.6.

*(Z)-3-methylbuta-1,3-dienyl-3-methylbenzoate (**4c**)*[17]:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4c** as a colorless oil (0.181 g, 90% yield) .^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.19 (s, 3H), 2.42 (s, 3H), 4.99 (d, *J* = 1.2 Hz, 1H), 5.11 (d, *J* =1.0 Hz, 1H), 5.48 (d, *J* = 7.2 Hz, 1H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.91 (d, *J* = 7.2 Hz, 1H), 7.37 (t, *J* = 7.6, 1H), 7.42 (d, *J* = 7.2 Hz, 1H), 7.62 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 21.4, 23.0, 114.9, 117.7, 127.2, 128.6, 129.0, 130.7, 133.4, 134.5, 138.6, 139.2, 163.7.

*(Z)-3-methylbuta-1,3-dienyl-2-methylbenzoate (**4d**)*[17]:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4d** as a colorless oil (0.185 g, 92% yield).^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.14 (s, 3H), 2.65 (s, 3H), 4.97 (d, *J* = 1.2 Hz, 1H), 5.10 (d, *J* =1.0 Hz, 1H), 5.46 (d, *J* = 7.2 Hz, 1H), 7.25 (d, *J* =8.4, 1H), 7.99 (d, *J* = 7.2, 1H), 7.45-7.47 (m, 2H), 7.30 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 21.9, 23.0, 114.7, 117.6, 126.0, 128.1, 130.8, 132.1, 132.8, 133.3, 139.3, 141.7, 163.8.

*(Z)-3-methylbuta-1,3-dienyl-4-chlorobenzoate (**4e**)*[17]: Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4e** as a white solid (0.210 g, 95% yield).^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.16 (s, 3H), 5.00 (d, *J* = 1.4 Hz, 1H), 5.10 (d, *J* =0.8 Hz, 1H), 5.48 (d, *J* = 7.2 Hz, 1H), 7.23 (d, *J* = 7.2 Hz, 1H), 7.44-7.47 (m, 2H), 8.01- 8.03 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 23.0, 115.3, 118.1, 127.6, 129.2, 131.4, 133.2, 139.0, 140.4, 162.7.

*(Z)-3-methylbuta-1,3-dienyl-4-bromobenzoate (**4f**)*[17]:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4f** as a white solid (0.244 g, 92% yield).^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.17 (s, 3H), 5.01 (d, *J* = 1.4 Hz, 1H), 5.11 (d, *J* = 0.9 Hz, 1H), 5.50 (d, *J* = 7.2 Hz, 1H), 7.25 (d, *J* = 7.2 Hz, 1H), 7.62-7.64 (m, 2H), 7.94-7.96 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 23.0, 115.3, 118.1, 127.6, 129.2, 131.4, 133.2, 139.0, 140.4, 162.7.

*(Z)-3-methylbuta-1,3-dienyl-4-methoxybenzoate (**4g**)*[17]:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4g** as a colorless oil (0.196 g, 90% yield). ^{1}H NMR (200 MHz, $CDCl_3$, ppm) δ 2.19 (s, 3H), 3.88 (s,3H), 4.98 (d, *J* = 1.2 Hz, 1H), 5.10 (d, *J* =1.0 Hz, 1H), 5.44 (d, *J* = 7.4 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 1H), 7.24-7.28 (m, 2H), 8.03- 8.08 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$, ppm) δ 23.1, 55.6, 114.1, 114.6, 117.5, 121.4, 132.2, 133.5, 139.3, 163.3, 164.1.

*(Z)-3-methylbuta-1,3-dienyl-3-methoxybenzoate (**4h**)*[17]:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4h** as a colorless oil (0.191 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.20 (s, 3H), 3.86 (s, 3H), 4.99 (d, *J* = 1.2 Hz, 1H), 5.11 (d, *J* =1.0 Hz, 1H), 5.49 (d, *J* = 7.2 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.7 (d, *J* = 7.6, 1H), 7.39 (t, *J* = 8.0, 1H), 7.26 (d, *J* = 6.4, 1H), 7.61 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ 23.0, 55.5, 114.4, 115.0, 117.9, 120.4, 122.5, 129.8, 130.3, 133.3, 139.2, 159.8, 163.4.

*(Z)-3-methylbuta-1,3-dienyl 2-hydroxybenzoate (**4i**)*[17]:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4i** as a white solid (0.177 g, 87% yield).^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.18 (s, 3H), 5.03 (d, *J*=1.2 Hz, 1H), 5.13 (d, *J* = 1.0 Hz, 1H), 5.54 (d, *J* = 7.2 Hz, 1H), 7.02 (d, *J* = 8.0, 1H), 7.88 (d, *J* = 7.6, 1H), 7.22 (m, 1H), 7.51 (m, 1H), 6.93 (m, 1H), 10.52 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ 22.9, 111.5, 116.0, 118.0, 118.6, 119.7, 130.0, 132.3, 136.5, 138.8, 162.4, 167.2.

*(Z)-3-methylbuta-1,3-dienyl cinnamate (**4j**)*[17]*:* Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4j** as a colorless oil (0.124 g, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.15 (s, 3H), 4.98 (d, *J* = 1.2 Hz, 1H), 5.10 (d, *J* =1.0 Hz, 1H), 5.43 (d, *J* = 7.2 Hz, 1H), 6.46 (d, *J* =16 Hz, 1H), 7.18 (d, J=7.2 Hz, 1H), 7.41-7.55 (m, 2H), 7.57-7.59 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 22.9, 114.7, 116.9, 117.6, 128.5, 129.1, 130.9, 133.4, 139.4, 146.9, 163.7, 171.3.

*(E)-(Z)-3-methylbuta-1,3-dienyl-but-2-enoate (**4k**)*:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4k** as a colourless oil (0.133 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 1.93 (d, J = 6.8 Hz, 3H), 2.10 (s, 3H), 4.93 (d, *J* = 1.2 Hz, 1H), 5.04 (d, *J* =1.0 Hz, 1H), 5.35 (d, *J* = 7.2 Hz, 1H), 5.87 (d, J = 15.6 Hz, 1H), 7.05-7.14 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 18.4, 22.8, 114.4, 117.4, 121.8, 133.2, 139.4, 147.2, 163.1. HRMS (ESI): *m*/*z* calculated for $C_9H_{12}O_2$ $[M + H]^+$ 153.0871; found 153.0910.

*(E)-(Z)-3-methylbuta-1,3-dienyl-non-2-enoate (**4l**)*:

Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4l** as a colourless oil (0.204 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) ^{1}H NMR (400Hz,CDCl3,ppm) δ 0.83 (t, 3H), 1.26 (m, 6H), 1.44 (m, 2H), 2.07 (s, 3H), 2.22 (m, 2H), 4.93 (d, *J* = 1.2 Hz, 1H), 5.04 (d, *J* =1.0 Hz, 1H), 5.36 (d, *J* = 7.2 Hz, 1H), 5.84 (d, J = 15.6 Hz, 1H), 7.05-7.13 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 14.2, 22.6, 28.1, 28.9, 29.8, 31.7, 114.4, 117.3, 120.2, 133.3, 139.4, 152.3, 163.2. HRMS (ESI): *m*/*z* calculated for $C_9H_{12}O_2$ $[M + H]^+$ 223.1653; found 223.1707.

*(Z)-3-methylbuta-1,3-dienyl oleate (**4m**):* Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4m** as a colourless oil (0.313 g, 90% yield). ^{1}H NMR (400Hz, CDCl3, ppm) δ 0.86 (t, 3H), 1.26 (m, 20H), 1.67 (m, 2H), 2.01 (m, 7H), 2.75 (t, 2H),), 4.92 (d, *J* = 1.2 Hz, 1H), 5.03 (d, *J* =1.0 Hz, 1H), 5.32 (d, *J* = 7.2 Hz, 1H), 5.40 (m, 2H), 7.02 (d, *J* = 7.2 Hz 1H, 2H).^{13}CNMR(100MHz,$CDCl_3$, ppm)δ 14.2, 20.3, 22.7, 22.8, 24.7, 24.9, 25.7, 27.3, 27.4, 29.0, 29.3, 29.4, 29.7, 29.9, 32.0, 34.3, 117.3, 128.0, 129.8, 130.2, 130.3, 133.2, 139.3, 170.4. HRMS (ESI): *m*/*z* calculated for $C_9H_{12}O_2$ $[M + H]^+$ 349.3062; found 349.3118.

*(Z)-buta-1,3-dienyl benzoate (**4n**)*[17]*:* Reaction was performed according to the general procedure, and the mixture was purified using *(*hexane/AcOEt = 98/2 as eluent to give **4n** as a colorless oil (0.156 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.17 (d, *J* = 10.4 Hz, 1H), 5.30 (d, *J* = 17.2 Hz, 1H), 5.63 (dd, *J* = 4.4, 6.4 Hz, 1H), 6.84-6.94 (m, 1H), 7.31 (d, *J* = 6.0 Hz, 1H), 7.44-7.51 (m, 2H), 7.59-7.63 (m, 1H),8.09-8.15 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 114.2, 118.1, 128.8, 129.1, 129.2, 130.2, 133.9, 134.5, 163.2.

*(Z)-buta-1,3-dienyl 4-methylbenzoate (**4o**)*[17]: Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4o** as a colorless oil (0.165 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 2.44 (s, 3H), 5.16 (d, J = 10.2 Hz, 1H), 5.29 (d, J = 17.6 Hz, 1H), 5.61 (dd, *J* = 4.4, 6.4 Hz, 1H), 6.84-6.94 (m, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 7.32-7.66 (m, 2H), 8.01 (d, J = 8.0 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 21.9, 113.9, 117.9, 126.4, 129.2, 129.5, 130.2, 134.6, 144.7, 163.2.

*(Z)-buta-1,3-dienyl 4-chlorobenzoate (**4p**)*[17]: Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4p** as a white solid (0.178 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.18 (d, *J* = 10.4 Hz, 1H), 5.31 (d, *J* = 17.2 Hz, 1H), 5.64 (dd, *J* = 4.4, 6.4 Hz, 1H), 6.83-6.87 (m, 1H), 7.26-7.30 (m, 1H), 7.46 (d, *J* = 8.0 Hz, 2H),8.05 (d, *J*= 8.0 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 114.4, 118.4, 128.9, 129.1, 129.2, 131.4, 134.3, 140.4, 163.2.

*(Z)-buta-1,3-dienyl 4-methoxybenzoate (**4q**)*[17]: Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4q** as a colorless oil (0.178 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 3.87 (s, 3H), 5.14 (d, *J* = 10.4 Hz, 1H), 5.27 (d, *J* = 17.2 Hz, 1H), 5.59- 5.63 (m, 1H), 6.83-6.92 (m, 1H), 7.15 (d, *J* = 7.2 Hz, 1H), 7.27-7.41 (m, 2H), 7.63- 7.74 (m, 2H).^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 55.6, 114.1, 114.6, 118.1, 120.2, 122.5, 129.7, 130.4, 134.5, 159.8, 163.1.

*(Z)-buta-1,3-dienyl 2-hydroxybenzoate (**4r**)*[17]: Reaction was performed according to the general procedure, and the mixture was purified using hexane/AcOEt = 98/2 as eluent to give **4r** as a white solid (0.161g, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 5.21 (d, *J* = 10.4 Hz, 1H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.68 (dd, *J* = 4.4, 6.4 Hz, 1H), 6.84-6.94 (m, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 7.50-7.54 (m, 2H), 7.95-7.97 (d, *J* = 8.0 Hz, 1H), 10.48 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 114.2, 118.1, 128.8, 129.1, 129.2, 130.2, 133.9, 134.5, 163.2.

General procedure for the study of anticancer activity:

Cell cytotoxicity assay of (Z)-3-methylbuta-1,3-dienyl oleate on HaCaT cells (Normal Human Cells)

The cytotoxicity assay of (Z)-3-methylbuta-1,3-dienyl oleate (**4m**) *in vitro* was measured using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay on the normal human cells (HaCaT). The cells growing in the log phase were seeded into two 96-well cell-culture plate at 1 × 10^4 cells / mL. Different concentrations of Dienyl ester (4m) were added into the wells with an equal volume of incomplete DMEM medium in the control wells. The cells were then incubated for 48 h at 37 °C in 5% CO_2. Thereafter, fresh incomplete DMEM medium containing 0.40 mg / ml MTT was added to the 95 well plates and incubated for 4 h at 37 °C in 5% CO_2. Formazan crystals thus formed were dissolved in DMSO after decanting the earlier media, and the absorbance was recorded at 595 nm.

Anticancer activity of (Z)-3-methylbuta-1,3-dienyl oleate (4m) on U87MG cells (Human Cancer Cells)

The anticancer activity *in vitro* was measured using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) assay on the cancer cells U87MG. The cells growing in the log phase were seeded into a 96-well cell-culture plate at 1 × 10^4 cells mL^{-1}. Different concentrations of Dienyl ester (4m) were added into the wells with an equal volume of incomplete MEM medium in the control wells. The cells were then incubated for 48 h at 37 °C in 5% CO_2. After that, fresh MEM medium containing 0.40 mg / ml MTT was added to the 95 well plates and incubated for 4 h at 37 °C in 5% CO_2. Formazan crystals thus formed were dissolved in DMSO after decanting the earlier media and the absorbance was recorded at 595 nm.

Cellular uptake study in U87MG cells

The cells (1 × 10^3 cells / mL) were seeded on cover slips in MEM medium. After 24 h cells were treated with Dienyl ester (**4m**) and then incubated for 3 h. Cells were fixed in 3.7% paraformaldehyde. The slides were washed again in PBST twice for 10 min and mounted with D.P.X mountant. Fluorescence images were acquired using confocal fluorescence microscope after 3 h of incubation.

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Supporting Information

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author

Keywords: Ruthenium • Dienyl ester • Propargylic alcohol • carboxylic acid • Fatty acid

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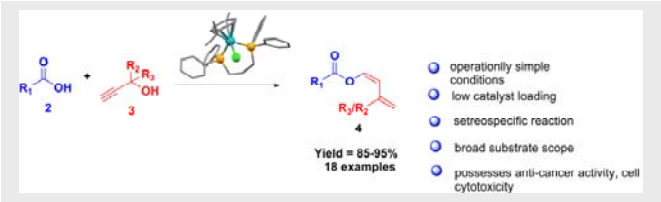

(*Z*) O-Dienyl esters have been obtained from [(η^6-*p*-Cymene)Ru(η^2-dppp)Cl]$^+$ catalyzed reaction of propargylic alcohols with carboxylic acids, including fatty acids. Anti cancer activity of one of the fatty acid dienyl esters has been investigated.

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[(*para*-cymene)Ru(dppp)Cl][PF_6] Catalyzed Stereospecific Synthesis of O- Dienyl Esters and Evaluation of the Anti-cancer Activity of a Long Chain Fatty Acid O-Dienyl Ester