Visualization of vitamin A metabolism

Koenders, S.T.A.

Chapter 3

Design and Synthesis of an Activity-based Retinoid Probe

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Introduction

Retinoic acid was synthesized for the first time in 1946 by the Dutch chemists Arens and Van Dorp while working for Organon International.[1] In the year thereafter the first commercially viable synthesis was developed by Otto Isler while in employment of Hoffman-La Roche.[2] Progress in the field of organic synthesis opened up new synthesis routes towards retinoids with the development of reactions such as the Wittig reaction and the Julia olefination.[3] The synthesis of retinoids on an industrial scale is still performed using these transformations, while research into new routes using cross-couplings or cross-metathesis reactions is still ongoing.[4]

In cells retinoic acid is formed via the oxidation of retinal. The enzymes catalyzing this reaction are called retinaldehyde dehydrogenases. The human genome expresses three of these: ALDH1A1, ALDH1A2 and ALDH1A3.[5,6] Nucleophilic attack of their catalytic cysteine on the aldehyde of retinal results in a hemithioacetal. Hydride abstraction by nicotinamide adenine dinucleotide (NAD^+) with concomitant deprotonation results in net dehydrogenation and formation of a thioester adduct.[5] Hydrolysis of this thioester leads to the formation of retinoic acid.

ALDH1A1 and ALDH1A3 have been reported as cancer stem cell biomarkers[7] and ALDH1A1 activity confers resistance against chemo- and radiation therapy.[8–10] The ability to discern the contribution of specific retinaldehyde dehydrogenases to the global aldehyde dehydrogenase (ALDH) activity would be helpful to understand the underlying biology and to determine which ALDHs are potential therapeutic targets in cancer. Retinaldehyde dehydrogenases have a variable and inducible cellular expression pattern. Their activity is regulated by post-translational modifications, such as the phosphorylation of threonine residues by Aurora kinase A[11] and acetylation of a lysine residue.[12]

Activity-based protein profiling (ABPP) has become one of the key methodologies to map enzyme activities on a global scale in living systems, such as cells and animals.[13,14] ABPP is a technology that relies on activity-based chemical probes that covalently and irreversibly react with the catalytic nucleophile in the active site of an enzyme in their native biological context.[15] Since this process requires a catalytically active protein, these chemical probes report on the abundance of active enzymes. ABPP enables the determination of target engagement and selectivity profiling of drug candidates in a physiologically relevant environment, which enhances the therapeutic relevance of the observed interaction profile.[19-21] An activity-based probe (ABP) generally consists of an electrophilic warhead, a scaffold that is recognised by the protein (family) of interest and a fluorophore or biotin as reporter group. To date, no ABP for retinaldehyde dehydrogenases has been developed, despite the fact that the catalytic mechanism employed by these enzymes during substrate processing involves a covalent intermediate.

The development of an activity-based probe to profile cellular retinaldehyde dehydrogenase activity would enable the study of retinoic acid production in cells and the selectivity profiling of ALDH inhibitors. This chapter describes the design and synthesis of **LEI-945**, a first-in-class activity-based probe based on the natural substrate retinal to profile retinaldehyde dehydrogenases.

Results and discussion

Design of the retinal-based probe LEI-945

Taking advantage of the fact that covalent enzyme-substrate intermediates emerge during catalysis an ABP based on the structure of retinal was designed. It was hypothesized that the aldehyde in retinal could be exchanged with a vinyl ketone to trap the nucleophilic catalytic cysteine through conjugate addition (**Fig. 3.1**).

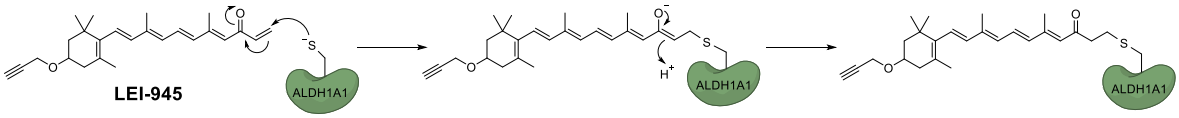


Fig. 3.1 | Covalent interaction of LEI-945 with ALDH1A1. The vinyl ketone warhead of **LEI-945** undergoes nucleophilic attack by the catalytic cysteine of ALDH1A1. The resulting thioether cannot by hydrolysed and results in an irreversible covalent interaction between **LEI-945** and ALDH1A1.

Docking of the retinal-derived vinyl ketone into the crystal structure of ALDH1A1 (PDB: 4WP7)[19] suggested that the nucleophile (the catalytic cysteine thiol) and the electrophile (the unsaturated ketone) would be in close proximity. It pointed as well to a suitable position, which is a solvent exposed position where probe contact with the enzyme active site is negligible, for installation of a bioorthogonal ligation handle (**Fig. 3.2**). An alkyne ligation handle was selected as it is expected to minimally affect the lipophilicity and physico-chemical properties of the probe.[20,21] This led to the design of **LEI-945** as a potential two-step retinaldehyde dehydrogenase ABP (**Scheme 3.1**).

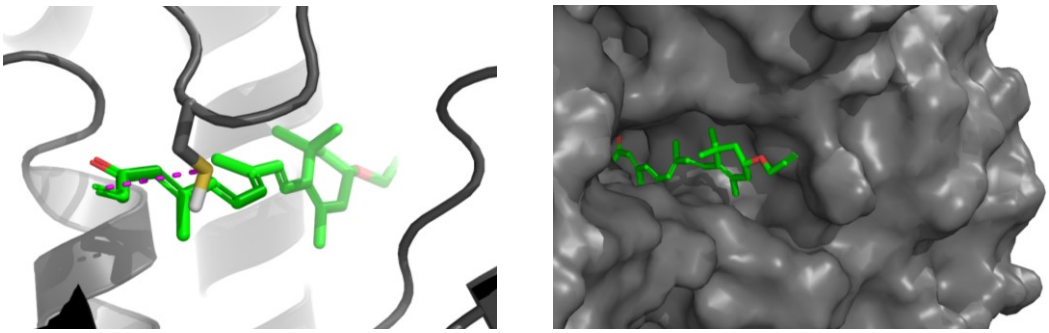
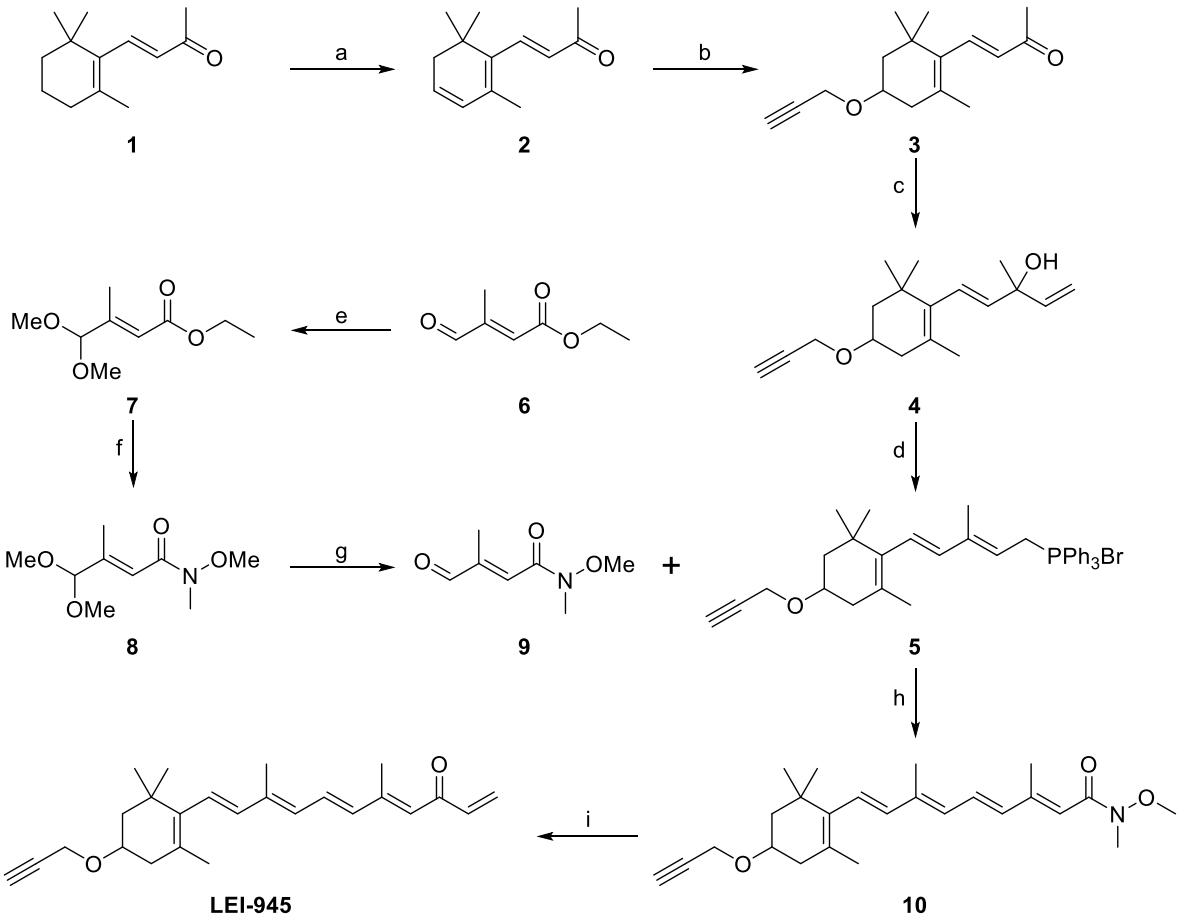


Fig. 3.2 | Docking pose of LEI-945 in the crystal structure of ALDH1A1. The left panel shows the vinyl ketone warhead in close proximity to the catalytic cysteine (yellow; C303) of ALDH1A1. The right panel shows the binding pocket of ALDH1A1 with the alkyne ligation handle solvent exposed.

Synthesis of the retinal-based probe LEI-945

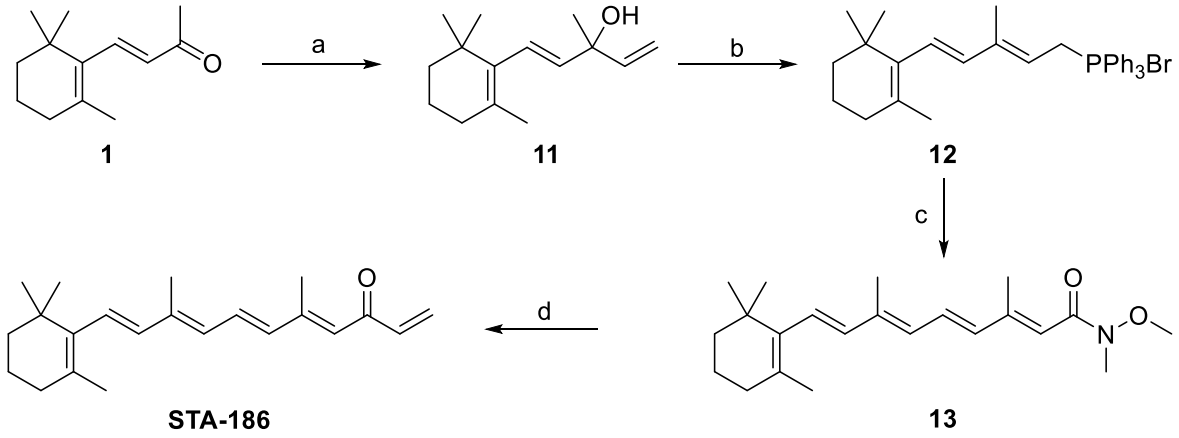
LEI-945 was synthesized using a convergent synthesis starting from commercially available β-ionone **1** and ethyl 3-methyl-4-oxocrotonate **6** (**Scheme 3.1**). Bromination of **1** using recrystallized *N*-bromosuccinimide (NBS), followed by dehydrobromination of 3-bromo-β-ionone with Na_2CO_3 afforded 3-dehydro-β-ionone **2** in 81% yield. As previously described by Henbest *et al.* for this substrate the amount of NBS in this reaction is critical.[22] A slight surplus of NBS is necessary to fully consume the starting material as some NBS will be lost in side reactions such as the bromination of the methyl ketone. Too much NBS, however, will lead to more side reactions consuming the product and substrate. Purification of NBS through recrystallization is, therefore, required to permit accurate determination of the amounts of NBS added.

Conjugate addition of propargyl alcohol to 3-dehydro-β-ionone **2** following a modification of the literature procedure[23] provided alkyne **3** in 23% yield. It should be noted that rapid degradation of 3-dehydro-β-ionone **2** was observed even when stored at -20 °C and the intermediate should, therefore, be used straightaway. Grignard reaction of **3** and vinylmagnesium bromide quantitatively afforded tertiary alcohol **4**. Addition of triphenylphosphine hydrobromide in methanol provided phosphonium salt **5** in 78% yield.[24] Intermediate **9** required for the key Wittig reaction towards the retinal scaffold was synthesised from commercially available ethyl 3-methyl-4-oxocrotonate **6** as follows. Treatment of **6** with trimethyl orthoformate and catalytic acid, yielded acetal **7** in quantitative yield. The ester in **7** was converted into Weinreb amide **8** using *N,O*-dimethylhydroxylamine hydrochloride also in quantitative yield.



Scheme 3.1 | Synthesis of retinal-based probe LEI-945. Reagents and conditions: a) NBS, AIBN, CCl_4, 80 °C, 2 h, then Na_2CO_3, DMF, 81% over two steps; b) H_2SO_4, propargyl alcohol, 4 °C, 18 h, 23%; c) vinylmagnesium bromide, THF, 18 h, quant.; d) PPh_3HBr, 18 h, 78%; e) trimethyl orthoformate, *p*TsOH, MeOH, 2 h, quant.; f) *N,O*-dimethylhydroxylamine hydrochloride, *i*-PrMgCl, THF, 3 h, quant.; g) TFA, DCM/H_2O, 2 h, quant.; h) *n*-BuLi, THF, -78 °C to rt, 2 h, 46% (1:1 E/Z); i) vinyl magnesium bromide, THF, 2 h, 43% (7:3 E/Z).

The acetal in **8** was quantitatively deprotected using aqueous TFA, yielding aldehyde **9**. Phosphonium salt **5** was deprotonated at -78 °C using *n*-BuLi after which addition of aldehyde **9** and stirring at room temperature afforded compound **10** as a 1:1 E/Z mixture. Finally, treatment of Weinreb amide **10** with vinylmagnesium bromide furnished **LEI-945** in 20% yield over the last two steps. The E/Z mixture was inseparable and the product prone to isomerization under the influence of light. As retinoids are rapidly converted *in vivo* into their biological equilibrium of stereoisomers, **LEI-945** was used as the reported E/Z mixture.[25,26]



Scheme 3.2 | Synthesis of mechanism-based inhibitor STA-186. Reagents and conditions: a) vinylmagnesium bromide, THF, 18 h, 95%; b) PPh_3HBr, 18 h, 90%; c) *n*-BuLi, **9**, THF, -78 °C to rt, 2 h, 43% (1:1 E/Z); d) vinyl magnesium bromide, THF, 2 h, 29% (2:1 E/Z).

The retinal-based probe **LEI-945** was stored in aliquots as DMSO stocks at -80 °C under nitrogen. When stored under these conditions stocks can be kept for at least up to two years. DMSO was chosen instead of ethanol, because ethanol is converted into acetaldehyde *in situ*. As acetaldehyde is also a substrate of ALDH1A1 this might interfere with efficient probe labelling.

As a control compound the inhibitor analogue of probe **LEI-945** was made, starting from β-ionone **1** in a similar way (**Scheme 3.2**). Wittig salt **12** was made via a Grignard using β-ionone **1** and vinylmagnesium bromide followed by treatment with triphenylphosphine hydrobromide in 86% over two steps. Wittig salt **26** was then coupled with aldehyde **9** and the warhead introduced sequentially in 43% and 29% yield, respectively. Inhibitor **STA-186** should be stored and handled under the same conditions as probe **LEI-945**.

Conclusion

In conclusion, **LEI-945** and **STA-186**, were synthesized using a convergent synthesis starting from β-ionone **1** and ethyl 3-methyl-4-oxocrotonate **6**. A key step in the chemical route towards **LEI-945** is the introduction of the ligation handle. This step is performed early on in the synthesis, forming the key intermediate Wittig salt **5**. Using this key intermediate, other clickable retinoids can be synthesized, which are described in **Chapter 7**. The validation and applications of **LEI -945** as an activity-based probe for retinaldehyde dehydrogenases are described in **Chapters 4-6**.

Acknowledgements

Lindsey Burggraaff is kindly acknowledged for performing the docking studies.

Experimental procedures

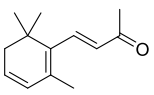
Docking

All calculations were performed using the Schrödinger Suite.[27] The X-ray structure of ALDH1A1 was extracted from the PDB (PDB ID: 4WP7).[19,28] The centroid of the co-crystalized inhibitor CM026 was used to define the binding pocket coordinates. The protein was prepared for docking using the protein preparation tool: metals and ions were removed, and hydrogens and missing side chains were added. Retinoic acid and **LEI-945** were docked into the CM026 binding pocket of ALDH1A1. A constraint was used in docking of retinoic acid for hydrogen bond formation with either the backbone of Cys303 or the side chain of Asn170.

Synthetic methods

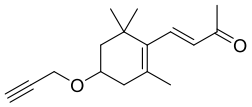
General remarks. All reactions were performed using oven or flame-dried glassware and dry solvents. Reagents were purchased from Sigma Aldrich, Acros, Biosolve, VWR, Fluka, Fischer Scientific and Merck and used as received unless stated otherwise. Tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were stored over 4 Å molecular sieves before use. All moisture sensitive reactions were performed under a nitrogen atmosphere. TLC analysis was performed using Merck aluminium sheets (TLC silica gel 60/Kieselguhr F_{254}). Compounds were visualized using a solution of $KMnO_4$ (7.5 g), K_2CO_3 (50 g), 10% NaOH (6 mL) in H_2O (1 L). Column chromatography was performed using Screening Device B.V. silica gel (particle size 40 – 63 µm, pore diameter of 60 Å) with the indicated eluents. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AV-400 (400 MHz and 101 MHz, respectively) or Bruker AV-500 MHz (500 MHz and 150 MHz, respectively) using $CDCl_3$ as solvent. Chemical shifts are reported in ppm (δ) relative to the residual solvent peak or tetramethylsilane. Coupling constants are given in Hz. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 – 2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass", or with a Synapt G2-Si (Waters), equipped with an electrospray ion source in positive mode (ESI-TOF), injection via NanoEquity system (Waters), with LeuEnk (m/z = 556.2771) as "lock mass". Eluents used: MeCN:H_2O (1:1 v/v) supplemented with 0.1% formic acid. The high-resolution mass spectrometers were calibrated prior to measurements with a calibration mixture (Thermo Finnigan). The retinoids were handled under dark conditions using amber coloured flasks or aluminium foil when containing more than 3 conjugated double bonds. Retinoid intermediates were stored in the dark under nitrogen at -30 °C and final compounds as powder or as DMSO stock at -80 °C under nitrogen atmosphere. For further information on retinoid handling and storage we refer to the review from Barua and Furr.[29]

(*E*)-4-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)but-3-en-2-one (2):



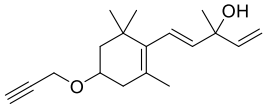
A solution of purified β-ionone (1.0 g, 5.2 mmol), recrystallized NBS (1.2 g, 6.8 mmol) and AIBN (43 mg, 0.26 mmol) in tetrachloromethane (50 mL) was refluxed at 80 °C for 2 hours and was then allowed to cool down.[22] The solution was filtered and then DMF (12.5 mL) and Na_2CO_3 (1.3 g) were added. The tetrachloromethane was distilled off and the mixture cooled to room temperature. Et_2O (100 mL) was added and the solution was filtered. The organic layer was washed with 1 M HCl and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (Et_2O/pentane) afforded the title compound **2** (0.80 g, 4.2 mmol, 81%) as an orange oil. R_f (10% Et_2O in pentane) = 0.45. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, *J* = 16.4 Hz, 1H), 6.20 (d, *J* = 16.4 Hz, 1H), 5.88 (s, 2H), 2.31 (s, 3H), 2.12 (d, *J* = 2.2 Hz, 2H), 1.91 (s, 3H), 1.08 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.3, 135.9, 133.2, 132.7, 130.3, 129.6, 128.2, 50.4, 39.9, 32.8, 26.5, 20.3.

(*E*)-4-(2,6,6-Trimethyl-4-(prop-2-yn-1-yloxy)cyclohex-1-en-1-yl)but-3-en-2-one (3):



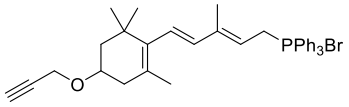
Compound **2** (11.6 g, 61 mmol) was dissolved in propargyl alcohol (180 mL, 3.2 mol) and stirred at 4 °C under a nitrogen atmosphere.[23] Then concentrated sulfuric acid (2.0 mL, 38 mmol) was added and the reaction was stirred overnight at 4 °C. The reaction mixture was then poured onto ice water and then 50% NaOH aq. was added. After stirring for several minutes the mixture was extracted with Et_2O and the organic layer washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the residue by column chromatography (EtOAc/pentane) afforded the title compound **3** (3.5 g, 14 mmol, 23%; 32% yield based on 29% starting material recovered) as an orange oil and recovered compound **2** (3.3 g, 17 mmol, 29%). R_f (10% EtOAc in pentane) = 0.45. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 16.4 Hz, 1H), 6.11 (d, *J* = 16.4 Hz, 1H), 4.22 (d, *J* = 2.4 Hz, 2H), 3.92 – 3.82 (m, 1H), 2.47 – 2.41 (m, 2H), 2.30 (s, 3H), 2.13 – 2.10 (m, 1H), 1.90 – 1.84 (m, 1H), 1.78 (s, 3H), 1.50 – 1.41 (m, 1H), 1.13 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.5, 142.2, 135.7, 132.4, 132.1, 80.2, 74.1, 71.0, 55.3, 44.5, 39.6, 36.6, 30.0, 28.5, 27.3, 21.6. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{16}H_{22}O_2$: 247.16926, found 247.16918.

(*E*)-3-Methyl-1-(2,6,6-trimethyl-4-(prop-2-yn-1-yloxy)cyclohex-1-en-1-yl)Penta-1,4-dien-3-ol (4):



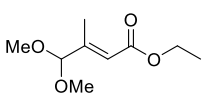
To a stirred solution of **3** (3.0 g, 12 mmol) in dry THF (120 mL) at 0 °C under nitrogen atmosphere was added vinylmagnesium bromide (1 M solution in THF, 18 mL, 18 mmol). The mixture was stirred overnight at room temperature and was then cooled to 0 °C. Sat. NH_4Cl aq. was added and the mixture was stirred for 30 minutes. The mixture was extracted with Et_2O. The organic layer washed with brine and concentrated under reduced pressure affording the title compound **4** (3.5 g, 12 mmol, quant.) as an orange oil. R_f (10% EtOAc in pentane) = 0.4. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 – 5.95 (m, 2H), 5.55 (dd, *J* = 16.2, 1.2 Hz, 1H), 5.26 (dd, *J* = 17.3, 1.2 Hz, 1H), 5.08 (dd, *J* = 10.6, 1.2 Hz, 1H), 4.22 (d, *J* = 2.4 Hz, 2H), 3.90 – 3.80 (m, 1H), 2.43 – 2.41 (m, 1H), 2.41 – 2.34 (m, 1H), 2.07 – 1.98 (m, 1H), 1.87 – 1.80 (m, 1H), 1.68 (s, 3H), 1.42 (s, 4H), 1.05 (s, 3H), 1.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.0, 139.7, 136.8, 125.3, 124.8, 112.2, 80.3, 73.9, 73.5, 71.6, 55.1, 44.2, 38.8, 36.6, 30.0, 28.3, 28.0, 21.2. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{18}H_{26}O_2$-H_2O: 257.18999, found: 257.18994.

Bromo((2*E*,4*E*)-3-methyl-5-(2,6,6-trimethyl-4-(prop-2-yn-1-yloxy)cyclohex-1-en-1-yl)Penta-2,4-dien-1-yl)triphenyl-λ^5-phosphane (5):



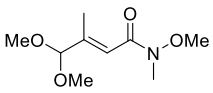
To a stirred mixture of **4** (3.0 g, 11 mmol) in MeOH (60 mL) was added triphenylphosphine hydrobromide (4.1 g, 12 mmol).[24] The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. H_2O was added and the product extracted with DCM. The organic layer was washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by column chromatography (EtOAc followed by MeOH) affording title compound **5** (5.1 g, 8.5 mmol, 78%) as a yellow/orange foam. R_f (10% MeOH in DCM) = 0.6. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.86 (m, 6H), 7.80 – 7.77 (m, 3H), 7.70 – 7.67 (m, 6H), 5.93 (s, 2H), 5.41 – 5.32 (m, 1H), 5.07 – 4.86 (m, 2H), 4.20 (d, *J* = 2.4 Hz, 2H), 3.82 (dddd, *J* = 11.9, 9.2, 5.5, 3.4 Hz, 1H), 2.41 (t, *J* = 2.4 Hz, 1H), 2.40 – 2.33 (m, 1H), 2.05 – 1.97 (m, 1H), 1.84 – 1.78 (m, 1H), 1.64 (s, 3H), 1.44 – 1.39 (m, 1H), 1.37 (d, *J* = 3.5 Hz, 3H), 1.01 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.5 (d, *J* = 13.8 Hz), 137.0, 136.3, 134.8, 133.9 (d, *J* = 9.7 Hz), 130.2 (d, *J* = 12.4 Hz), 127.5, 126.3, 118.3 (d, *J* = 85.2 Hz), 113.4 (d, *J* = 11.4 Hz), 73.9, 71.4, 55.1, 44.1, 39.0, 36.6, 30.0, 28.5, 24.9 (d, *J* = 48.5 Hz), 21.5, 12.8. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{36}H_{40}OP^+$: 519.28113, found: 519.28065.

Ethyl (*E*)-4,4-dimethoxy-3-methylbut-2-enoate (7):



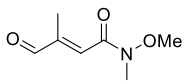
To a stirred solution of trimethyl orthoformate (8.6 mL, 77 mmol) and ethyl 3-methyl-4-oxocrotonate **6** (10 g, 70 mmol) in MeOH (40 mL) was added *p*TsOH (268 mg, 1.4 mmol). The mixture was stirred for 2 hours and then H_2O (100 mL) was added. The product was extracted with Et_2O (3x200 mL). The combined organic layers were washed with $NaHCO_3$ aq., brine and dried over Na_2SO_4, filtered and carefully concentrated affording title compound **7** (13.1 g, 69 mmol, quant.) as a colourless oil. R_f (10% Et_2O in pentane) = 0.9. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.03 (s, 1H), 4.61 (s, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.31 (s, 6H), 2.11 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 152.2, 118.3, 104.8, 59.7, 53.1, 14.1, 13.8.

(*E*)-*N*,4,4-Trimethoxy-*N*,3-dimethylbut-2-enamide (8):



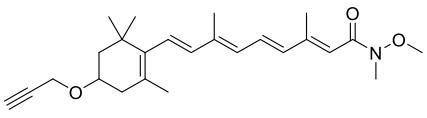
To a stirred solution of **7** (2.0 g, 11 mmol) in dry THF (30 mL) under N_2 at 0 °C was added *N,O*-dimethylhydroxylamine hydrochloride (1.6 g, 16 mmol) followed by *i*-PrMgCl (16 mL, 32 mmol, 2 M in THF). The reaction was stirred for 3 hours at room temperature and was then quenched with sat. NH_4Cl aq. The mixture was extracted with Et_2O and the organic layer washed with brine, dried over Na_2SO_4, filtered and concentrated affording title compound **8** (2.2 g, 11 mmol, quant.) as a yellowish oil. R_f (10% Et_2O in pentane) = 0.05; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (s, 1H), 4.64 (s, 1H), 3.69 (s, 3H), 3.32 (s, 6H), 3.23 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 117.1, 105.2, 67.9, 61.5, 53.2, 14.1. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_9H_{17}NO_4$: 204.12303, found: 204.12299.

(*E*)-*N*-methoxy-*N*,3-dimethyl-4-oxobut-2-enamide (9):



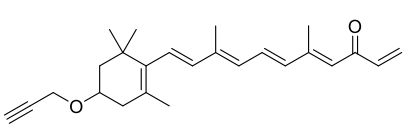
To a stirred solution of **8** (0.50 g, 2.5 mmol) in DCM (20 mL) at room temperature was added a solution of TFA (2.5 mL) in H_2O (2.5 mL). The reaction mixture was stirred for 2 hours and then K_2CO_3 was added until the pH was 8. The water layer was then extracted with DCM. The combined organic layers washed with brine, dried over $MgSO_4$, filtered and concentrated affording title compound **9** (378 mg, 2.5 mmol, quant.) as a yellow oil. R_f (50% EtOAc in pentane) = 0.5. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1H), 7.01 (s, 1H), 3.74 (s, 3H), 3.30 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.6, 165.8, 147.9, 135.3, 61.9, 31.9, 10.7. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_7H_{11}NO_3$: 158.08117, found 158.08116.

(2*E*,4*E/Z*,6*E*,8*E*)-*N*-Methoxy-*N*,3,7-trimethyl-9-(2,6,6-trimethyl-4-(prop-2-yn-1-yloxy)cyclohex-1-en-1-yl)nona-2,4,6,8-tetraenamide (10):



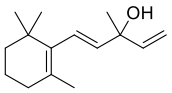
To a stirred solution of **5** (0.50 g, 0.83 mmol) in dry THF (4 mL) at -78 °C under N_2 was added *n*-BuLi (0.5 mL, 1.6 M in hexane, 0.8 mmol). The mixture was stirred for 30 minutes at -78 °C and then **9** (0.12 g, 0.76 mmol) dissolved in dry THF (1 mL) was added. The reaction was stirred for 1 hour at -78 °C and was then allowed to reach room temperature in 2 hours. Then the reaction was quenched with sat. NH_4Cl aq. and extracted with Et_2O. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated. Purification of the residue by column chromatography (EtOAc/pentane) afforded title compound **10** (139 mg, 0.35 mmol, 46%; 1:1 4E/Z) as a yellow oil. R_f (50% Et_2O in pentane) = 0.8. NMR spectra are obtained from the mixture of stereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 – 5.92 (m, 6H), 4.22 (s, 2H), 3.92 – 3.81 (m, 1H), 3.68 (d, *J* = 8.3 Hz, 3H), 3.23 (s, 3H), 2.47 – 2.38 (m, 2H), 2.35 – 2.28 (m, 3H), 2.13 – 2.06 (m, 1H), 2.01 – 1.95 (m, 3H), 1.88 – 1.81 (m, 1H), 1.74 – 1.70 (m, 3H), 1.50 – 1.40 (m, 1H), 1.07 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 138.7, 138.4, 138.3, 138.1, 137.6, 132.7, 130.1, 129.7, 127.9, 127.1, 127.0, 126.6, 126.4, 216.3, 118.0, 117.5, 80.3, 73.9, 71.5, 61.5, 55.1, 44.4, 39.2, 36.8, 30.2, 28.6, 21.6, 19.1, 13.9, 12.8, 12.4. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{25}H_{35}NO_3$: 398.26897, found 398.26875.

(4*E*,6*E/Z*,8*E*,10*E*)-5,9-Dimethyl-11-(2,6,6-trimethyl-4-(prop-2-yn-1-yloxy)cyclohex-1-en-1-yl)undeca-1,4,6,8,10-Pentaen-3-one (LEI-945):



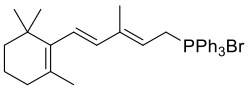
To a solution of **10** (0.10 g, 0.25 mmol) in THF (1 mL) at 0 °C under N_2 was added vinyl magnesium bromide (0.27 mmol, 0.30 mL) dropwise. The solution was then stirred for 2 hours at room temperature and then quenched with sat. NH_4Cl aq. The mixture was then extracted with Et_2O and the organic layer washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification of the residue by column chromatography (EtOAc/pentane) afforded title compound **LEI-945** (39 mg, 0.11 mmol, 43%; 7:3 6E/Z) as a yellow oil. The E/Z mixture was inseparable and prone to further isomerization under the influence of light. As retinoids are rapidly converted *in vivo* into their biological equilibrium of stereoisomers, **LEI-945** was used as the reported E/Z mixture.[25,26] R_f (10% EtOAc in pentane) = 0.9. NMR spectra are obtained from the mixture of stereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 – 6.96 (m, 1H), 6.61 – 5.96 (m, 7H), 5.80 – 5.70 (m, 1H), 4.22 (s, 2H), 3.92 – 3.82 (m, 1H), 2.47 – 2.41 (m, 2H), 2.40 – 2.35 (m, 3H), 2.14 – 2.05 (m, 1H), 2.02 – 1.97 (m, 3H), 1.89 – 1.82 (m, 1H), 1.74 (s, 3H), 1.49 – 1.41 (m, 1H), 1.08 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.4, 153.1, 139.8, 138.6, 138.5, 138.3, 138.0, 137.6, 136.1, 132.4, 132.3, 130.1, 129.8, 127.9, 127.8, 127.1, 126.8, 126.5, 125.0, 124.7, 86.8, 73.9, 71.5, 55.2, 44.4, 39.3, 36.8, 30.2, 28.6, 21.7, 14.5, 12.9. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{25}H_{32}O_2$: 365.24751, found 365.24749.

(*E*)-3-Methyl-1-(2,6,6-trimethylcyclohex-1-en-1-yl)Penta-1,4-dien-3-ol (11):



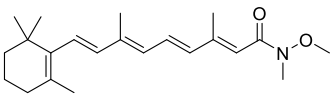
The title compound **11** was synthesized from β-ionone **1** (0.80 g, 4.2 mmol) according to the procedure described for compound **4**. This yielded **11** (0.88 g, 4.0 mmol, quant.) as a yellow oil. R_f (10% Et_2O in pentane) = 0.3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.12 – 5.94 (m, 2H), 5.57 – 5.49 (m, 1H), 5.32 – 5.21 (m, 2H), 5.12 – 5.03 (m, 1H), 1.97 (t, *J* = 5.2 Hz, 2H), 1.66 (s, 3H), 1.62 – 1.57 (m, 2H), 1.47 – 1.43 (m, 2H), 1.42 (s, 3H), 0.98 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.2, 138.9, 136.8, 128.4, 125.7, 112.0, 73.6, 39.3, 34.0, 32.6, 28.7, 28.0, 21.3, 19.2.

Bromo((2*E*,4*E*)-3-methyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)Penta-2,4-dien-1-yl)triphenyl-λ^5-phosphane (12):



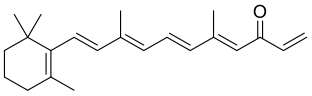
The title compound **12** was synthesized from **11** (0.88 g, 4.0 mmol) according to the procedure described for compound **5**. This yielded **12** (1.95 g, 3.6 mmol, 90%) as a yellow foam. R_f (10% MeOH in DCM) = 0.5. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.70 (m, 15H), 6.07 – 5.89 (m, 2H), 5.43 – 5.35 (m, 1H), 4.89 – 4.75 (m, 2H), 1.97 (s, 2H), 1.63 (s, 3H), 1.58 (s, 2H), 1.46 – 1.40 (m, 2H), 1.37 (d, *J* = 3.2 Hz, 3H), 0.96 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.1 (d, *J* = 13.7 Hz), 136.4, 134.4, 133.2 (d, *J* = 9.7 Hz), 129.7 (d, *J* = 12.4 Hz), 128.9, 127.9, 117.4 (d, *J* = 85.2 Hz), 112.1 (d, *J* = 11.6 Hz), 38.6, 33.4, 32.1, 28.2, 24.1 (d, *J* = 48.9 Hz), 21.0, 18.5, 12.1.

(2*E*,4*E/Z*,6*E*,8*E*)-*N*-Methoxy-*N*,3,7-trimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenamide (13):



The title compound **13** was synthesized from **9** (0.13 g, 0.83 mmol) and **12** (0.50 g, 0.92 mmol) as described for compound **10**. This yielded **13** (0.12 g, 0.36 mmol, 43%; 1:1 4E/Z mixture) as a yellow oil. R_f (50% EtOAc in pentane) = 0.8. NMR spectra are obtained from the mixture of stereoisomers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 – 5.89 (m, 6H), 3.67 (s, 3H), 3.23 (s, 3H), 2.35 – 2.29 (m, 3H), 2.04 – 2.01 (m, 2H), 2.00 – 1.96 (m, 3H), 1.72 – 1.69 (m, 3H), 1.65 – 1.58 (m, 2H), 1.50 – 1.45 (m, 2H), 1.04 – 1.01 (m, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 168.2, 139.0, 138.8, 137.7, 137.6, 137.4, 136.1, 132.3, 129.9, 129.8, 129.6, 128.2, 128.0, 126.1, 117.9, 117.3, 61.6, 60.4, 39.5, 34.2, 33.0, 28.9, 21.7, 19.2, 19.2, 13.9, 12.8, 12.4. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{22}H_{33}NO_2$: 344.25841, found 344.25830.

(4*E*,6*E/Z*,8*E*,10*E*)-5,9-Dimethyl-11-(2,6,6-trimethylcyclohex-1-en-1-yl)undeca-1,4,6,8,10-Pentaen-3-one (STA-186):



The title compound **STA-186** was synthesized from **13** (100 mg, 0.29 mmol) as described for compound **LEI-945**. This yielded **STA-186** (25.7 mg, 83 µmol, 29%; 2:1 6E/Z mixture). R_f (20% EtOAc in pentane) = 0.9. NMR spectra are obtained from the mixture of stereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 – 7.04 (m, 1H), 6.53 – 6.12 (m, 7H), 5.75 – 5.70 (m, 1H), 2.39 – 2.10 (m, 3H), 2.06 – 1.99 (m, 5H), 1.77 – 1.71 (m, 3H), 1.66 – 1.59 (m, 2H), 1.51 – 1.44 (m, 2H), 1.03 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.1, 152.9, 139.9, 138.2, 137.4, 136.9, 135.4, 132.2, 129.3, 128.8, 126.4, 124.2, 122.4, 39.3, 33.9, 32.8, 28.6, 21.4, 18.9, 14.2, 12.6. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{22}H_{30}O$: 311.23694, found 311.23667.

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