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PAPER

Synthesis and evaluation of new polyenic compounds as potential PPARs modulators†‡

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In order to identify new leads for the treatment of type 2 diabetes, polyenic molecules **A** and **B** derived from nipecotic acid and dienol derivatives **C** have been prepared and their effect on PPARs transcriptional activity evaluated and compared to that of rosiglitazone, WY14,643 and GW501516. Among the synthesized compounds, dienol **39** is the most active, increasing WY14,643 PPARα response and demonstrating partial agonist properties on rosiglitazone PPARγ.

The increasing incidence of obesity associated with type 2 diabetes, and its consequences in terms of cardiovascular morbidity and mortality is a real public health problem.[1] Based on this issue, it is obvious that losing weight as well as restoration of serum glucose and serum lipid parameters to normal levels are of prime importance. Thus, the regulation of energy balance, glucose and lipid homeostasis is important for the treatment of this metabolic syndrome. The identification of receptors critically involved in this balance is crucial to enable the synthesis of new therapeutic agents for the treatment of the metabolic syndrome. Among these receptors, attention has been paid to the members of the peroxisome proliferation-activated receptor (PPAR) family.

PPARs are activated by endogenous saturated and unsaturated fatty acids and their metabolites, as well as synthetic ligands.[2] Three subtypes of PPARs have been identified to date: PPARα, PPARγ and PPARδ. Their effects are exerted through ligand-dependent transcription of an important set of genes encoding proteins that regulate nutrient metabolism, energy homeostasis and cell differentiation. PPARα is mostly expressed in the tissues involved in lipid oxidation[3] and PPARγ is mainly found in skeletal muscle[4] and adipose tissues and plays a pivotal role in adipogenesis, glucose and lipid homeostasis, insulin sensitivity, and inhibition of inflammatory responses.[5]

In the last years, efforts have been directed toward identifying second-generation insulin sensitizers by using several approaches including dual PPARα/γ agonists like glitazar compounds and Selective Modulator PPARγ agonists: SPPARM compounds. The therapeutic objective was to avoid thiazolidine-dione side effects like those observed in clinical studies with rosiglitazone (AvandiaR), edema formation, fat mass increase and heart failure.

In order to find new leads for the treatment of type 2 diabetes, polyenic molecules such as **A** and **B** derived from nipecotic acid and dienol **C** have been prepared (Fig. 1) in order to compare their biological activities on PPARs. Herein, we would like to report the synthesis of molecules of type **A**, **B** and **C** as well as the biological tests on PPARs.

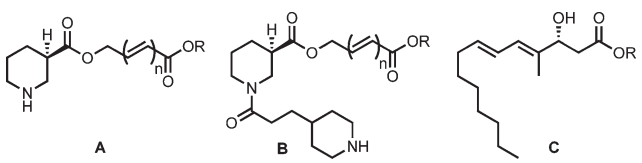

Fig. 1

Synthesis of polyenes A and B

The synthesis of compounds of type **A** and **B** was envisaged by esterification of nipecotic acid derivatives **D** by polyunsaturated esters of type **E**. The esters **E** would be prepared *via* stereoselective elimination of 1,3-polyacetates **F**. α,β-Unsaturated esters **F** would result from a cross-metathesis reaction between an alkyl acrylate and terminal olefin **G**, which could be obtained through an iterative sequence of reactions (oxidative cleavage–allylation–acetylation sequence) applied to homoallylic alcohol **H** resulting from the opening of protected glycidol **I**.

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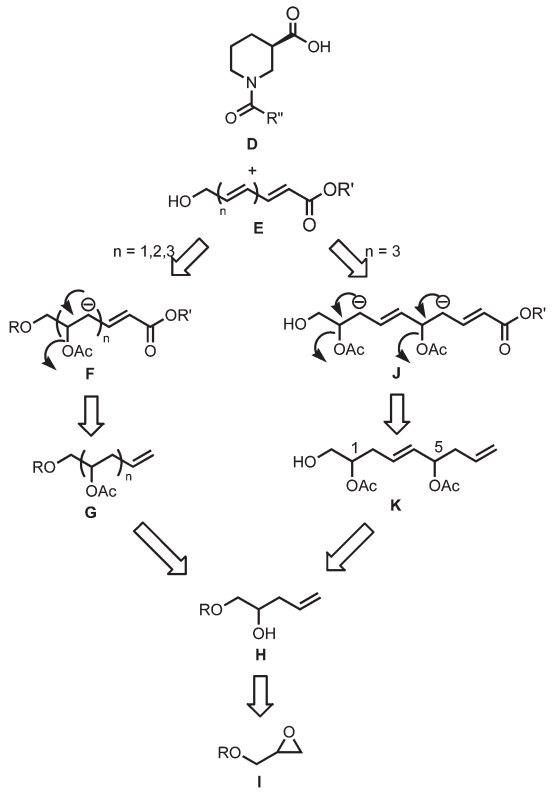
Furthermore, tetraenic esters (n = 3) could alternatively be derived from an elimination applied to activated 1,5-diacetates **J** which would result from a stereo- and regioselective cross-metathesis between an alkyl acrylate and the terminal olefin present in **K**. Diacetate **K** would derive from homoallylic alcohol **H** (Scheme 1).

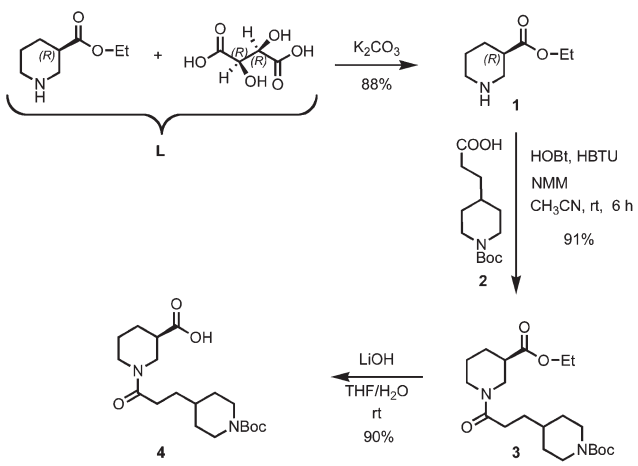


Scheme 1

Synthesis of the piperidinic core

The synthesis of compounds of type **B** required the obtention of optically active (*R*)-nipecotic acid ethyl ester **1**. The latter was formed by treatment of the commercially available (*R*)-(−)-nipecotate ester–L-tartrate salt **L** with potassium carbonate (88% yield). A peptide coupling between **1** and protected piperidinic acid **2**[6] was then achieved (HOBt, HBTU, NMM, CH_3CN) furnishing amide **3** in 91% yield. Saponification of ethyl ester **3** with lithium hydroxide ultimately provided the desired carboxylic acid **4** in 90% yield (Scheme 2).

In order to have access to piperidinic acid **D**, the *N*-Boc-(*R*)-(−)-nipecotic acid **7** was synthesized from the commercially available racemic nipecotic acid **5**. The resolution of the latter was achieved by crystallization with optically active (*R*)-(−)-10-camphorsulphonic acid.[7] Successive recrystallizations in acetone–H_2O (6 : 1) allowed the formation of ammonium salt **6** in low yield (8% yield). Further treatment of this salt with di-*tert*-butyldicarboxylate in the presence of Et_3N in refluxing methanol ultimately gave rise to the desired *N*-Boc-(*R*)-nipecotic acid **7** (92% yield) (Scheme 3).

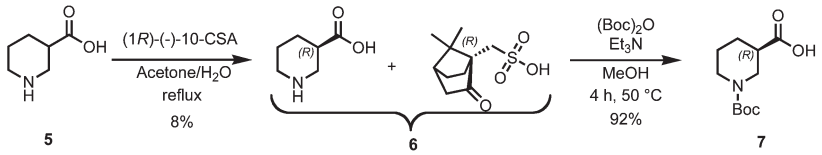


Scheme 2

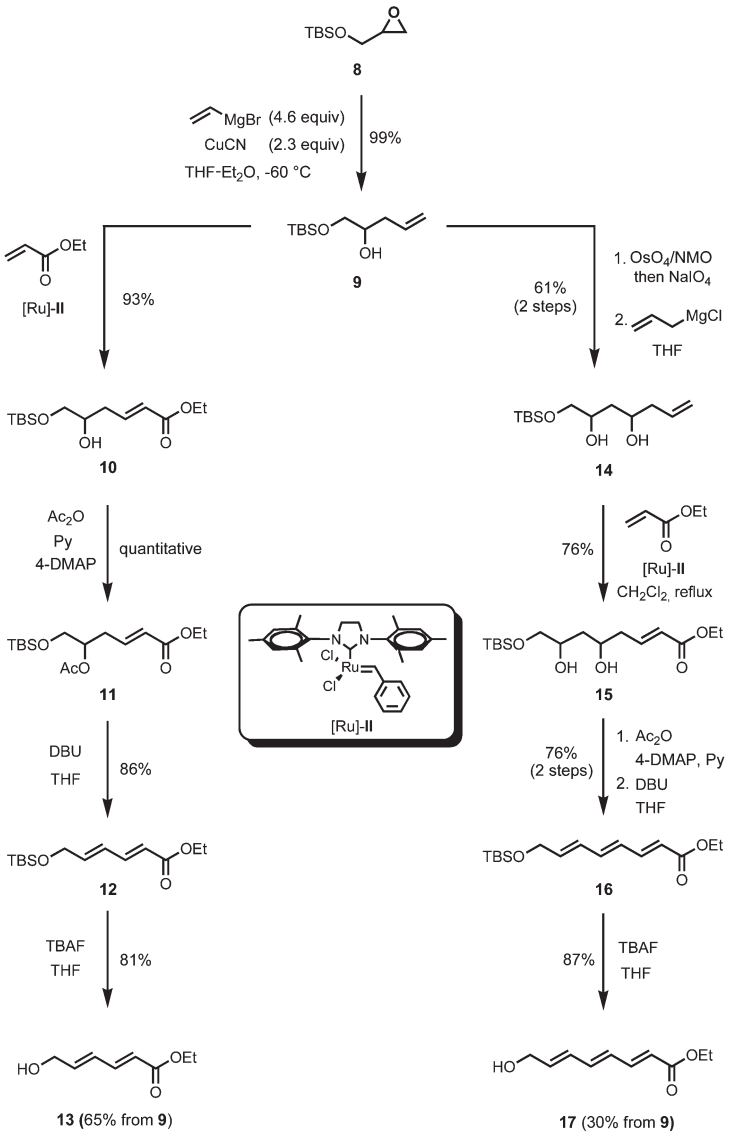
Synthesis of polyenic esters of type E

Following our retrosynthetic scheme (Scheme 1), the dienic ester **13** (n = 1) was synthesized from homoallylic alcohol **9**, resulting from regioselective opening of protected glycidol **8** using a vinylcuprate generated *in situ* (vinylmagnesium bromide, CuCN, −60 °C, THF–Et_2O, 99%).[8] A stereoselective cross-metathesis between alkene **9** and ethyl acrylate (3 equiv), in the presence of the second generation Grubbs catalyst [Ru]-**II**[9] (3 mol%) in refluxing CH_2Cl_2, led to α,β-unsaturated ester **10** (93% yield) which was transformed in three steps into ω-hydroxy dienic ester **13**. Conversion of alcohol **10** to the corresponding acetate **11** (4-DMAP, Py, Ac_2O, 86%) followed by a β-elimination produced protected dienic ester **12** (quant. yield), which was deprotected upon treatment with TBAF in THF to ultimately give the desired dienic ester **13** (81% yield) (Scheme 4).

A similar strategy was implemented to access trienic ester **17** (n = 2). Thus, trienic ester **17** was obtained in four steps from 1,3-diol **14**, prepared in two steps from olefin **9** (oxidative cleavage–allylation sequence). After performing, as previously, a cross-metathesis (ethyl acrylate, [Ru]-**II**, CH_2Cl_2, reflux), followed by acetylation (Ac_2O, 4-DMAP, Py), elimination (DBU, THF) and deprotection (TBAF, THF) the desired unsaturated trienic ester **17** was isolated with an overall yield of 50% (Scheme 4).



Scheme 3



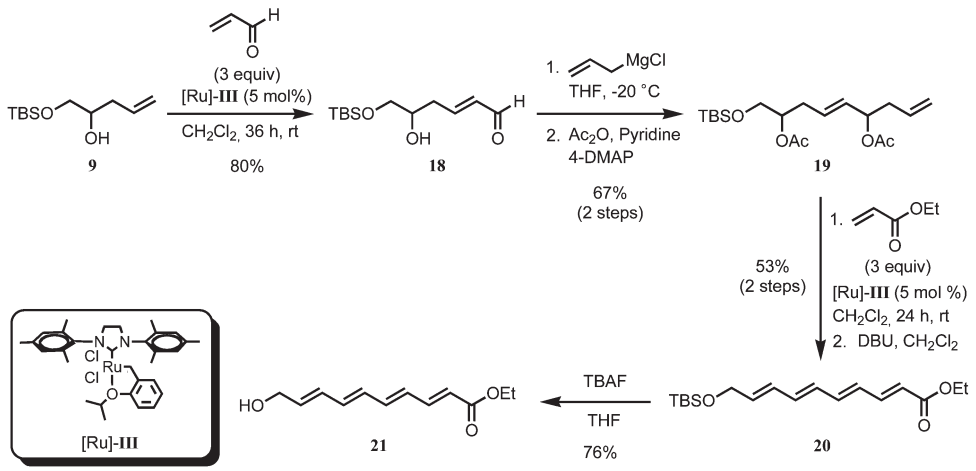
Scheme 4

When the same synthetic strategy was applied to diol **14** to produce higher homolog tetraenic ester **21** (oxidative cleavage–allylation–acetylation–CM–elimination–deprotection), the overall yield turned out to be low (10%). To circumvent this issue, an alternative strategy was devised. It involved the synthesis of 1,5-diacetate **20** which was prepared from allylic alcohol **9**. Thus, the latter was transformed into α,β-unsaturated aldehyde **18** by performing a cross-metathesis involving acrolein (3 equiv) and induced by the Grubbs–Hoveyda catalyst [Ru]-**III**[10] (5 mol%, CH_2Cl_2, rt, 36 h, 80%). After allylation (allylMgBr, THF, −20 °C) and acetylation (Ac_2O, Py, 4-DMAP) the desired 1,5-diacetate **19** was isolated (67% for the two steps). Diacetate **19** was subsequently subjected to a three step sequence (cross-metathesis–elimination–deprotection) as previously to furnish the desired tetraenic ester **21**, *via* silyl ether **20**, which was therefore obtained from **9** with an overall yield of 21.7% (Scheme 5).

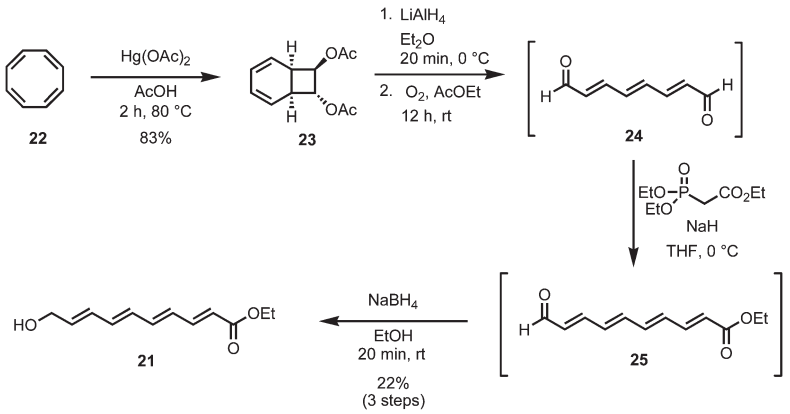
The synthesis of tetraenic ester **21** could be further improved starting from commercially available cyclooctatetraene **22**. An electrocyclic ring formation followed by a $Hg(OAc)_2$-catalyzed *trans*-acetylation (AcOH, 2 h, 80 °C) allowed the formation of crystalline compound **23** in 83% yield.[11] Subsequent acetate reduction by $LiAlH_4$, (Et_2O, rt), followed by stirring the resulting *trans*-1,2-diol in EtOAc under ambient air (O_2) gave rise to a ring fragmentation followed by isomerization of the double bonds to give the very unstable and light-sensitive dialdehyde **24**. The latter was directly treated with one equivalent of sodium triethylphosphonoacetate, in THF at 0 °C to give monoester-monoaldehyde **25** which upon reduction with sodium borohydride furnished the desired tetraenic alcohol **21** (22% yield from **23**).[12] This last synthetic route is undoubtedly the shortest, the most efficient and the most suitable for scale-up (Scheme 6).

Coupling reactions

With the nipecotic acid derivatives **D** and the hydroxypolyenic esters **E** in hands, coupling reactions were performed in order to access compounds **A** and **B**.



Scheme 5



Scheme 6

We first turned our attention towards the synthesis of polyenes of type **A**. A coupling reaction between **7** and **13** as well as **7** and **17** was achieved under Mitsunobu conditions[13] (PPh_3, DIAD, THF, rt) and led, after TFA-mediated deprotection, to the corresponding esters **26** and **27** in good yields (Scheme 7). It is worth pointing out that treatment of **28** with trifluoroacetic acid (TFA) to cleave the *N*-Boc protecting group present on tetraene **28**, formed upon coupling of carboxylic acid **7** and tetraenic alcohol **21**, resulted in a complete degradation which prevented isolation of the desired compound **29**.

Similarly, compounds **B** were synthesized using Mitsunobu conditions followed by an acid-mediated *N*-Boc-cleavage step. Thus, compounds **30** and **31** were respectively formed by coupling reactions between **4** and **13**, and **4** and **17** (Scheme 8).

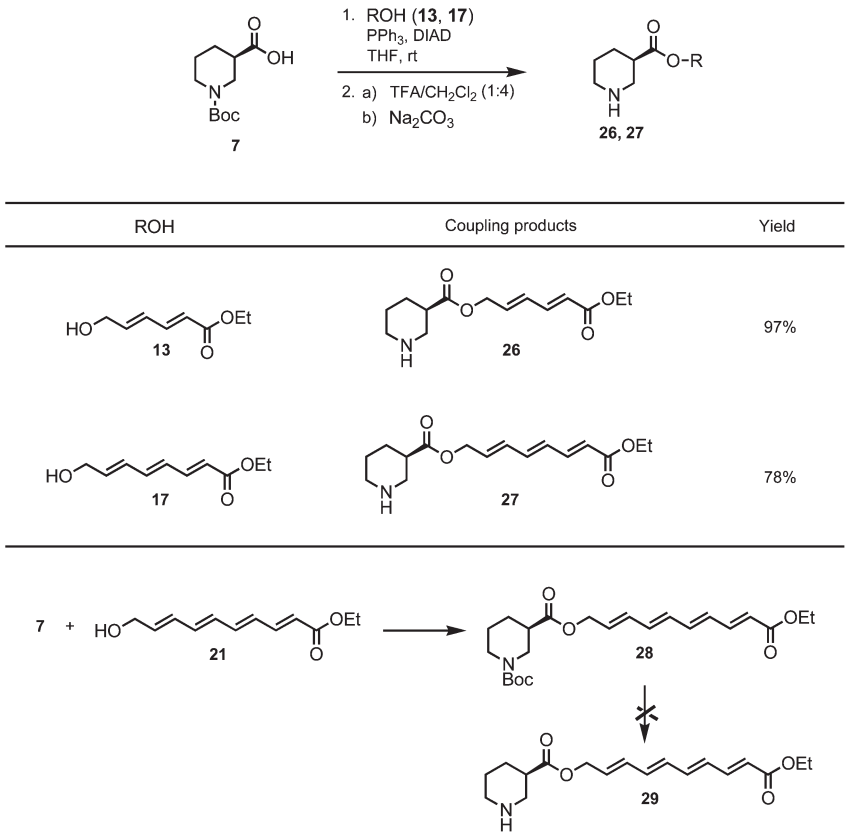
Synthesis of dienol C

The synthesis of (*E*,*E*)-dienol **C** should result from a palladium-catalyzed Stille cross-coupling between alkenyl metal **M** and vinyl halide **N**. Besides, the required optically active β-hydroxyester **N** could be obtained by addition of a chiral titanium enolate onto an aldehyde of type **O** (Scheme 9).

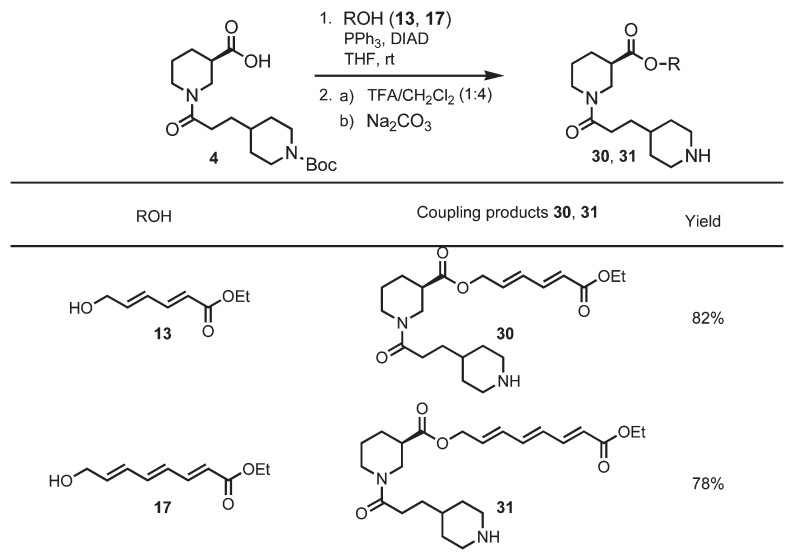
Vinyl stannane **34** (of type **M**) was prepared *via* a stereo- and regioselective hydrozirconation of 1-nonyne **32** by utilizing the Schwartz reagent Cp_2ZrHCl, generated *in situ* ($[Cp_2ZrCl_2]$, DIBAL-H, THF),[14] followed by iodolysis (I_2, THF) to give (*E*)-alkenyl iodide **33** as a single stereoisomer in 89% yield. Subsequent halogen–metal exchange with *tert*-BuLi, followed by trapping the resulting vinyl lithium species with tri-*n*-butyltin chloride gave rise to the desired vinylstannane **34** in 95% yield (Scheme 10).

The requisite aldehyde **37** (of type **O**) was obtained by MnO_2 oxidation of the known allylic alcohol **36**,[15] prepared upon stereo- and regioselective zirconium-assisted carboalumination of propargylic alcohol **35** followed by iodolysis.[16] The resulting aldehyde **37** was directly treated with the optically active titanium enolate **IV**,[17] generated *in situ* from *tert*-butylacetate lithium enolate and $CpTi(ODAG)_2Cl$ titanium complex derived from D-glucose, which furnished β-hydroxyester **38** of *R*-configuration in 71% yield and with high optical purity (ee >95%)[18] (Scheme 11).

Coupling of vinyl stannane **34** and alkenyl iodide **38** was achieved by performing a $[PdCl_2(CH_3CN)_2]$-catalyzed Stille



Scheme 7



Scheme 8

cross-coupling in DMF, which successfully led to the desired (*E*,*E*)-diene. It is noteworthy that protection of the free hydroxyl group in **38** was not required to perform the coupling. Saponification of the *tert*-butyl ester (NaOH, MeOH, 70 °C) ultimately provided the desired dienic β-hydroxyacid **39**[19] in 52% yield (two steps) (Scheme 12).

Results and discussion

(1) Human PPARs transactivation activity: evaluation of potential direct agonist activity

The effect of compounds **27**, **30**, **31** as well as **39** on PPAR transcriptional activity using chimaeric protein constructs were

performed on a chimera human PPAR/Gal4 gene reporter luciferase system to appreciate the maximal transactivation response for each compound. Therefore the compounds were compared to the PPAR classical references, WY14,643 for subtype α, rosiglitazone for subtype γ and GW501516 for subtype β/δ. The effects of the four compounds on the direct activation of the three different PPARs subtypes were tested using concentrations from 0.001 μM to 3 μM. No agonist activity was detected on the different PPAR subtypes with compounds **27**, **30** and **31**.

Only compound **39** was a weak dual PPARα and PPARγ activator, with a maximal agonist response equal to 30% of the reference compound at a concentration of 3 μM (Fig. 2). The reference compounds used were WY14,643 on PPARα (100% activation at 10 μM, Fig. 2A) and rosiglitazone on PPARγ (100% activation at 0.1 μM, Fig. 2B). EC_{50} values of compound **39** were respectively equal to 1 ± 0.02 μM on (h)PPARα and 0.08 ± 0.006 μM on (h)PPARγ. It is worth pointing out that compound **39** has no agonist activity on (h)PPAR β/δ.

The results summarizing the evaluation of the potential agonist activity of the compounds on the human PPAR subtypes are reported in Table 1.

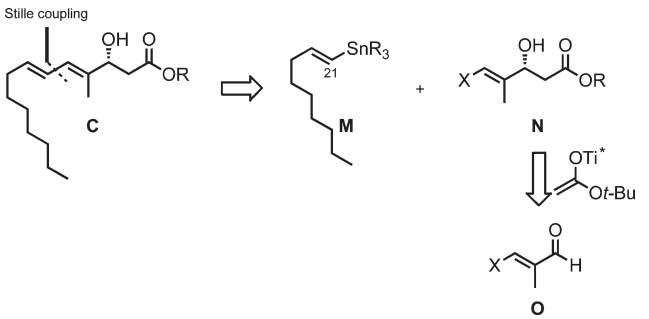
(2) Human PPARs transactivation activity: evaluation of potential indirect antagonist activity

In a second protocol, the different compounds were tested at concentrations from 0.001 μM to 3 μM for functional potency on the different PPAR reference responses to detect a potential pharmacological effect in term of antagonist or modulator properties.

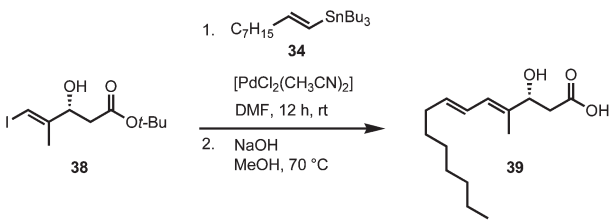
The different (h)PPAR subtypes were respectively activated with their reference compounds, WY14,643 (10 μM) for PPARα, rosiglitazone (0.1 μM) for PPARγ and GW501516 (1 μM) for PPAR β/δ. The results demonstrated that all the compounds showed a significant increase of the WY14,643 PPARα response, from 130% with compound **31** to 262% with compound **39**, which was the most potent indirect (h)PPARα activator (Fig. 3A). Interestingly, compound **39** showed antagonist properties on rosiglitazone PPARγ activation. About 50% of inhibition of the rosiglitazone response at a concentration of 1 μM was obtained with compound **39** (Fig. 3B). These results seemed to confirm the weak PPARγ partial agonism observed in the first protocol. However, only a partial inhibition was observed at a concentration of 3 μM. The analysis of the results showing a synergistic effect of compound **39** on the WY14,643 PPARα response suggests a mechanism of action different from that observed for rosiglitazone's effect on PPARγ. The results summarizing the evaluation of the potential antagonist activity of the compounds on the human PPAR subtypes are reported in Table 2.

As opposed to **39**, compounds **27**, **30** and **31** showed an increase of the rosiglitazone PPARγ response, with a maximal effect obtained with compound **30** (+180%).

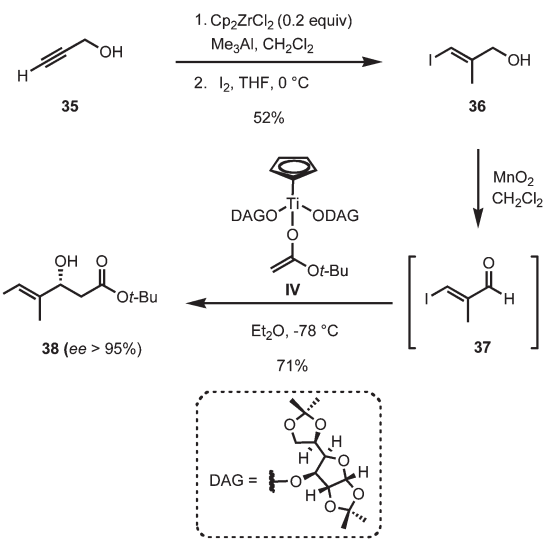
With all the compounds no effect on the GW501516 PPARδ/β response was observed, contrary to what was seen with PPARα and PPARγ.



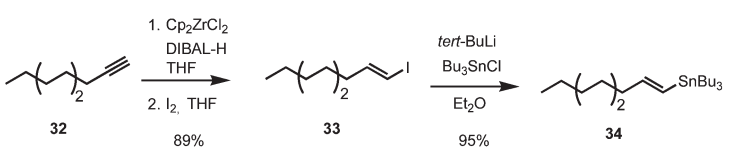
Scheme 9



Scheme 12



Scheme 11



Scheme 10

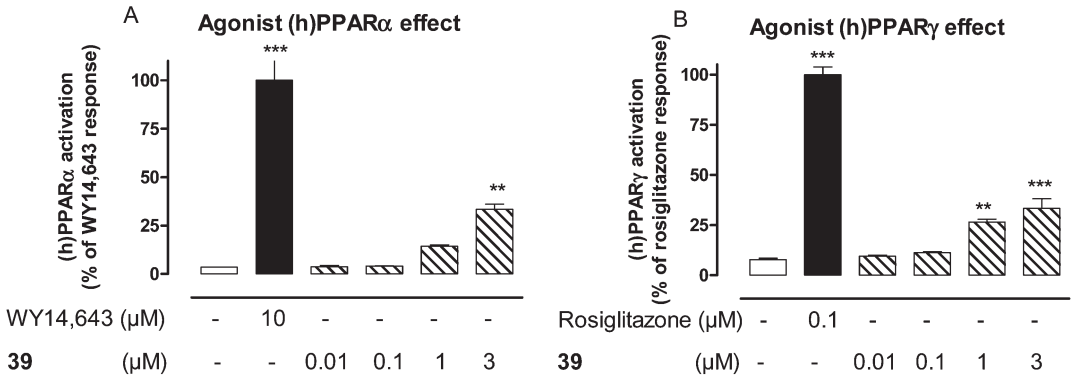


Fig. 2 *In vitro* evaluation of agonist activity for compound **39** in cell-based transactivation assay against (A) human PPARα/Gal_4 and (B) human PPARγ/Gal_4 receptors. The results are expressed as a percentage (mean ± S.E.M.) of maximal effect of the reference compound for each subtype. Results are expressed as the mean of three values ± S.E.M. Asterisks indicate significant differences at $^{**}p$ <0.01, $^{***}P$ <0.001 between compound effect and basal control value without activation.

Table 1 *In vitro* evaluation of agonist activity for compounds in cell-based transactivation assay against human PPARα/Gal_4, PPARγ/Gal_4 and PPARδβ/Gal_4 receptors

Compounds	hPPARα/Gal_4 activation E_{max} % of reference compound	hPPARγ/Gal_4 activation E_{max} % of reference compound	hPPARδ/β/Gal_4 activation E_{max} % of reference compound
WY14,643 (10 μM)	**100** ± **2*****	NT	NT
Rosiglitazone (0.1 μM)	NT	**100** ± **3*****	NT
GW501516 (1 μM)	NT	NT	**100** ± **1*****
27	NA	NA	NA
30	NA	NA	NA
31	NA	NA	NA
39	**29** ± **4****	**28** ± **2*****	NA

(NT = Not Tested, NA = Not Active). The results are expressed as a percentage (mean ± S.E.M.) of maximal effect of the reference compound for each subtype. Values are expressed as the mean of three values. Asterisks indicate significant differences at $^{*}p$ <0.05, $^{**}P$ <0.01 between compound effect and basal control value without activation.

Conclusion

The synthesis of trienic and tetraenic compounds **27**, **30** and **31**, derived from nipecotic acid, was achieved by using a cross-metathesis–elimination sequence as the key step. β-Hydroxy acid **39** was prepared by using two key steps: a palladium-catalyzed Stille cross-coupling between a vinyl iodide and a vinyl stannane to elaborate the (*E*,*E*)-diene and the addition of an optically active titanium enolate to an aldehyde to control the stereogenic center. The effect of these compounds on PPARs transcriptional activity was evaluated and compared to that of rosiglitazone, WY14,643, GW501516. We have shown that, among the synthesized compounds, **39** was the only compound to be a weak PPARα and PPARγ activator, giving partial agonist response (30% at 3 μM). We have also shown that all the synthesized compounds increased the WY14,643 PPARα response and that the most active compound is **39**. In addition, we have demonstrated that **39** possesses partial agonist properties on rosiglitazone PPARγ response and that **27**, **31** and **32** increased the rosiglitazone PPARγ response. Further studies should focus on the precise mechanism of action for compound **39** which offers a promising *in vitro* profile.

In vivo tests will be performed to evaluate the pharmacological effect of this compound on glucose (PPARγ partial agonist) and on lipid metabolism (PPARα effect). The pharmacological activity of **39** will be focused in advanced studies to validate the SPPARMγ/α hypothesis based on the activation of only a subset of the PPARγ and PPARα function induced by endogenous ligand in order to retain the desired metabolic benefits, while reducing the undesirable side effects.

Experimental part

1 Synthesis and structural determination of the compounds

General methods. TLC was performed on Merck $60F_{254}$ silica gel plates and visualized either with a UV lamp (254 nm), or by using a solution of *p*-anisaldehyde–sulfuric acid–acetic acid in EtOH followed by heating. Flash chromatography was performed with Merck Geduran Si60 silica gel (40–63 UM). Infrared (IR) spectra were recorded on a Perkin-Elmer 298 or on a Bruker TENSOR™ 27 (IRFT), wave-numbers are indicated in cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker AC 300 at 300 MHz or on a Bruker AVANCE 400 at 400 MHz and data are reported as follows: chemical shift in ppm from tetramethylsilane as an internal standard, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, m = multiplet or overlap of non equivalent resonances, br = broad, app = apparent), integration. ^{13}C NMR spectra were recorded on a Bruker AC 300 at 75 MHz or on a Bruker AVANCE 400 at 100 MHz and data are reported as follows: chemical shift in ppm from tetramethylsilane with the solvent as an internal indicator ($CDCl_3$ δ 77.0 ppm), multiplicity with respect to proton (deduced from DEPT experiments, s = quaternary C, d = CH, t = CH_2, q = CH_3). Mass spectra with electronic impact (MS) were recorded from a Hewlett-Packard tandem 5890A GC (12 m capillary column) – 5971 MS (70 eV). High resolution mass spectra (HRMS) were performed by the Groupe de

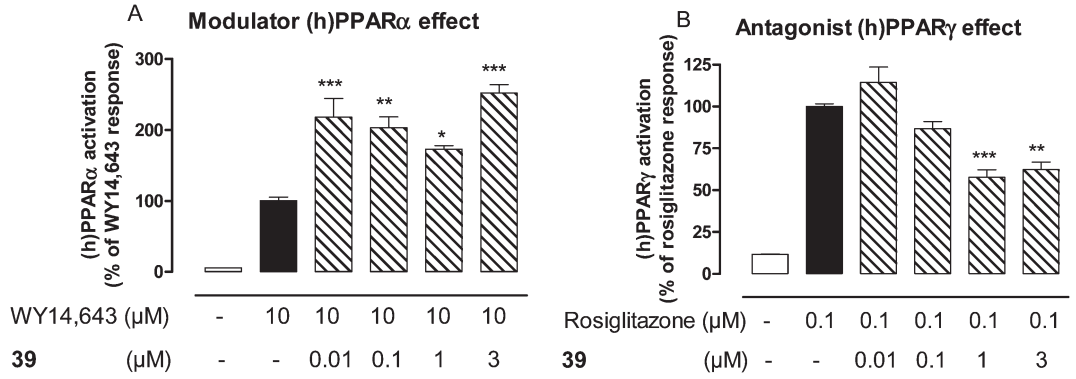


Fig. 3 *In vitro* evaluation of antagonist activity for compound **39** in cell-based transactivation assay against (A) human PPARα/Gal_4 and (B) human PPARγ/Gal_4 receptors. The results are expressed as a percentage (mean ± S.E.M.) of maximal effect of the reference compound for each subtype. Results are expressed as the mean of three values ± S.E.M. Asterisks indicate significant differences at $^{**}p$ <0.01, $^{***}P$ <0.001 between compound effect and basal control value without activation.

Table 2 *In vitro* evaluation of modulator activity for compounds in cell-based transactivation assay against human PPARα/Gal4, PPARγ/Gal4 and PPARδβ/Gal4 receptors

Compounds	hPPARα/Gal_4 activation % of reference compound	hPPARγ/Gal_4 activation % of reference compound	hPPARδ/β/Gal_4 activation % of reference compound
WY14,643 (10 μM)	100 ± 5	NT	NT
Rosiglitazone (0.1 μM)	NT	100 ± 4	NT
GW501516 (1 μM)	NT	NT	100 ± 3
27	222 ± 5*	168 ± 4*	115 ± 6 ns
30	276 ± 12*	180 ± 7*	119 ± 5 ns
31	130 ± 5*	125 ± 2*	108 ± 7 ns
39	262 ± 9***	54 ± 3**	115 ± 4 ns

(NT = Not tested). The effect of compounds was evaluated on the modulation of the response of reference compound for the three PPAR subtypes. The results are expressed as a percentage of the effect of the reference compound for each subtype. Values are expressed as the mean of triplicate values. Asterisks indicate significant differences at $^{*}p$ <0.05, $^{**}P$ <0.01 and $^{***}p$ <0.001. ns indicates a non significant effect *vs.* reference compound response.

Spectrométrie de Masse de l'Université Pierre et Marie Curie (Paris). Optical rotations were measured with a Perkin Elmer model 343 polarimeter with a 1 dm path length. All the reactions were performed under an argon atmosphere.

Ethyl (3*R*)-piperidine-3-carboxylate (1). To a solution of commercially available salt ethyl (*R*)-(−)-nipecotate–L-tartrate **L** (6.0 g, 19.5 mmol) in water (150 mL) was added a saturated aqueous solution of K_2CO_3 (150 mL). After stirring vigorously for 10 min at rt, the reaction mixture was extracted with Et_2O (3 × 150 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give free amine **1** (2.71 g, 88%) as a yellow oil. The physical and spectral properties for compound **1** were in accordance with those described in the literature;[21] $[\alpha]_D^{20}$ −1.22 (*c* 0.94, $CHCl_3$) {Lit[21] $[\alpha]_D^{20}$ −0.95 (*c* 1.2, $CHCl_3$)}; IR (neat): 3327, 2936, 2855, 2812, 1724, 1445, 1372, 1310, 1253, 1178, 1119, 1027, 855, 749 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 4.07 (q, *J* = 7.1 Hz, 2H), 3.10 (br dd, *J* = 12.4 Hz and *J* = 3.6 Hz, 1H), 2.87 (dt, *J* = 12.3 Hz and *J* = 3.9 Hz, 1H), 2.75 (dd, *J* = 12.4 Hz and *J* = 9.3 Hz, 1H), 2.58 (ddd, *J* = 12.4 Hz, *J* = 10.3 Hz and *J* = 3.0 Hz, 1H), 2.37 (m, 1H), 1.93 (m, 1H), 1.67–1.53 (m, 3H), 1.40 (m, 1H), 1.20 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.3 (s), 60.2 (t), 48.6 (t), 46.4 (t), 42.5 (d), 27.4 (t), 25.5 (t), 14.2 (q); MS (EI, 70 eV) *m/z* (%): 157 ($M^{+\cdot}$, 32), 128 (M − Et^+, 89), 112 (36), 110 (14), 101 (15), 100 (13), 84 (100), 83 (31), 82 (36), 73 (21), 68 (16), 57 (54), 56 (64), 55 (37), 54 (10).

***tert*-Butyl 4-(2-carboxyethyl)piperidine-1-carboxylate (2).** To a solution of 3-(4-piperidinyl)propanoic acid (100 mg, 0.636 mmol, 1.0 equiv) and K_2CO_3 (176 mg, 1.27 mmol, 2.0 equiv) in water (1.25 mL) at 0 °C was added a solution of di-*tert*-butyl-dicarbonate (139 mg, 0.636 mmol, 1.0 equiv) in THF (1.25 mL). After 4 h at rt, THF was removed *in vacuo* and the resulting aqueous layer was washed with Et_2O (5 mL). The layers were separated and the aqueous phase was acidified by adding a saturated aqueous solution of 1 M HCl until pH ≈2 was obtained. The aqueous phase was extracted with EtOAc (3 × 10 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give *N*-Boc piperidine **2** (130 mg, 80%) as pale yellow crystals; IR (neat): 3182, 2913, 1733, 1664, 1477, 1439, 1368, 1249, 1153, 858, 765, 639 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.0 (br s, 1H), 4.08 (m, 2H), 2.65 (br t_{app}, *J* = 12.3 Hz, 2H), 2.36 (t, *J* = 7.8 Hz, 2H), 1.67–1.35 (m, 5H), 1.43 (s, 9H), 1.08 (br ddd, *J* = 16.5 Hz, *J* = 12.5 Hz and *J* = 4.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.2 (s), 154.9 (s), 79.5 (s), 43.9 (br t, 2C), 35.4 (d), 31.8, 31.3, 31.2, 31.1 (t, 4C), 28.5 (q, 3C).

***tert*-Butyl 4-{3-[(3*R*)-3-(ethoxycarbonyl)-1-piperidinyl]-3-oxopropyl}-1-piperidinecarboxylate (3).** To a solution of ethyl ester of (*R*)-(−)-nipecotic acid **1** (102 mg, 0.649 mmol, 1.0 equiv) in CH_3CN (10 mL) at 0 °C were successively added carboxylic acid **2** (167 mg, 0.649 mmol, 1.0 equiv), HBTU (246 mg, 0.649 mmol, 1.0 equiv), HOBt (88 mg, 0.649 mmol, 1.0 equiv) and *N*-methylmorpholine (214 μL, 1.947 mmol, 3.0 equiv). After 6 h at rt, the reaction mixture was hydrolyzed by adding water (5 mL) and CH_3CN was removed *in vacuo*. EtOAc

(15 mL) was then added, the layers were separated and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were successively washed with a 5 M HCl aqueous solution (15 mL), a saturated aqueous solution of $NaHCO_3$ (15 mL), brine (15 mL) and then dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (Petroleum ether–EtOAc: 50 : 50) furnished compound **3** (233 mg, 91%) as a foam. The physical and spectral data for this compound were in accordance with those described in the literature;[22] $[\alpha]_D^{20}$ −23.4 (*c* 0.65, DMSO); IR (neat): 2921, 2858, 1726, 1682, 1625, 1469, 1427, 1366, 1276, 1246, 1161, 839, 770, 736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, rotamers): δ 4.50 (br dd, J = 13.1 Hz and J = 3.5 Hz, 0.5H), 4.16–3.90 (m, 4H + 0.5H), 3.67 (m, 1H), 3.34 (dd, J = 13.5 Hz and J = 9.0 Hz, 0.5H), 3.04–2.92 (m, 2 × 0.5H), 2.80 (dd, J = 13.0 Hz and J = 10.5 Hz, 0.5H), 2.59 (br t_{app}, J = 12.2 Hz, 2H), 2.42–2.20 (m, 3H), 1.97 (m, 1H), 1.78–1.25 (m, 8H), 1.37 (s, 9H), 1.18 (q_{app}, J = 7.2 Hz, 3H), 1.12–0.95 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz, rotamers): δ 173.1 and 172.8 (s, 1C), 171.4 and 171.3 (s, 1C), 154.8 (s), 79.1 (s), 60.8 and 60.5 (t, 1C), 47.2 and 45.9 (t, 2C), 43.7 (br d), 43.5 and 41.9 (t, 1C), 41.7 and 41.1 (d, 1C), 35.6 (d), 31.9, 31.7 and 31.6 (d, 3C), 30.3 and 30.2 (t), 28.4 (q, 3C), 27.2 (t), 25.0 and 23.8 (t), 14.1 (q). HRMS (ESI): calcd for $C_{21}H_{36}O_5N_2Na$ $[M + Na]^+$: 419.2516; found: 419.2509.

(3*R*)-1-{3-[1-(*tert*-Butoxycarbonyl)-4-piperidinyl]propanoyl}-3-piperidinecarboxylic acid (4). To a solution of ester **3** (1.08 g, 2.72 mmol, 1.0 equiv) in THF (20 mL) at 0 °C was added an aqueous solution of LiOH (172 mg, 4.08 mmol, 1.5 equiv) in water (20 mL). After stirring for 3 h at rt, EtOAc (50 mL) was added to the mixture and the pH of the solution was adjusted to pH ≈4 upon addition of an aqueous 4 M HCl solution. The layers were separated and the aqueous phase was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give carboxylic acid **4** (895 mg, 90%) as a white solid. The physical and spectral data for this compound are in accordance with those described in the literature;[22] m.p. = 165–167 °C; IR (neat): 2917, 2849, 1721, 1682, 1622, 1427, 1366, 1276, 1245, 1160, 839 cm^{-1}; ^1H NMR (300 MHz, CD_3CN, rotamers): δ 4.38 (br d, J = 12.9 Hz, 0.5H), 4.02 (br d, 2H), 3.90–3.67 (m, 1H + 0.5H), 3.41 (dd, J = 13.6 Hz and J = 8.8 Hz, 0.5H), 3.16–3.0 (m, 2 × 0.5H), 2.88 (m, 0.5H), 2.68 (br m, 2H), 2.51 (m, 0.5H), 2.45–2.31 (m, 3H), 1.97 (br m, 1H + 0.5H), 1.80–1.34 (m, 8H), 1.42 (s, 9H), 1.12–0.85 (m, 2H); ^{13}C NMR (100 MHz, CD_3CN, rotamers): δ 174.1 and 173.8 (s, 1C), 171.8 and 171.7 (s, 1C), 154.3 (s), 78.5 (s), 46.7, 45.5 (t, 2C), 43.5 (br d), 43.0 and 41.3 (t, 1C), 40.6 and 40.4 (d, 1C), 35.1 (d), 31.5, 31.4, 31.3 (d, 3C), 29.8 and 29.5 (t, 1C), 27.3 (q, 3C), 26.6 (t), 24.4 and 23.3 (t, 1C); HRMS (ESI): calcd for $C_{19}H_{32}N_2O_5Na$ $[M + Na]^+$: 391.2214; found: 391.2203.

1-*tert*-Butyl ester of (3*R*)-piperidine-1,3-dicarboxylic acid (7). To a solution of racemic nipecotic acid **5** (32.3 g, 250 mmol, 1.0 equiv) in acetone (500 mL) was added (1*R*)-(−)-10-camphorsulfonic acid (58.1 g, 250 mmol, 1.0 equiv). The mixture was heated to reflux and H_2O (≈75–85 mL) was added dropwise until a complete dissolution of the solid was observed. Stirring was maintained at rt for 15 h and the resulting suspension was filtered to give a white solid. The latter was recrystallized three times in an acetone–H_2O (6 : 1) mixture thus allowing the isolation of the (1*R*)-(−)-10-camphorsulfonic acid salt of (*R*)-(−)-nipecotic acid **6** (7.51 g, 8%). The specific optical rotation obtained for this salt {$[\alpha]_D^{20}$ −26.0 (*c* 1.1, MeOH)} was in accordance with the one reported in the literature {Lit.[7] $[\alpha]_D^{20}$ −25.9 (*c* 1.1, MeOH)}.

To a solution of the (1*R*)-(−)-10-camphorsulfonic acid salt of (*R*)-(−)-nipecotic acid **6** (975 mg, 2.69 mmol, 1.0 equiv) in MeOH (11 mL) were successively added di-*tert*-butyl-dicarbonate (706 mg, 3.24 mmol, 1.2 equiv) and Et_3N (1.9 mL, 13.49 mmol, 5.0 equiv) at 0 °C. The reaction mixture was heated at 50 °C and, after stirring for 4 h, the reaction mixture was concentrated *in vacuo* to remove the bulk of MeOH. The residue was dissolved in water (5 mL) and the resulting solution was acidified upon addition of a saturated aqueous solution of $KHSO_4$ until pH ≈2–3 was obtained. The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic layers were successively washed with an aqueous solution of 1 M HCl, brine (20 mL), then dried over $MgSO_4$, filtered and concentrated under reduced pressure to give optically enriched *N*-Boc-(*R*)-(−)-nipecotic acid **7** (563 mg, 92%) as a white solid. The physical and spectral properties for this compound were in accordance with those described in the literature;[23] m.p. = 168–170 °C; $[\alpha]_D^{20}$ −52.4 (*c* 0.98, MeOH) {Lit.[23] $[\alpha]_D^{20}$ −52.2 (*c* 1.15, MeOH)}; IR (neat): 3150, 2975, 2902, 1730, 1653, 1433, 1392, 1364, 1270, 1239, 1214, 1144, 847, 766, 758, 635 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 10.81 (br s, 1H), 4.09 (br m, 1H), 3.87 (br dt, J = 13.4 Hz and J = 4.0 Hz, 1H), 3.02 (br m, 1H), 2.83 (br t_{app}, J = 12.2 Hz, 1H), 2.46 (m, 1H), 2.05 (m, 1H), 1.74–1.56 (m, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.0 (s), 154.8 (s), 80.0 (s), 45.5 (br t), 44.0 (br t), 41.1 (d), 28.4 (q, 3C), 27.2 (t), 24.1 (t).

1-(*tert*-Butyldimethylsilyloxy)-4-penten-2-ol (9). To a solution of CuCN (2.19 g, 24.4 mol, 2.3 equiv) in Et_2O (35 mL) at −78 °C was added vinyl magnesium bromide (1.0 M in THF, 48.9 mL, 48.9 mmol, 4.6 equiv) dropwise over 15 min. The heterogeneous mixture was cooled to −20 °C until all CuCN was dissolved (around 20 min), then the mixture was cooled down to −78 °C. A solution of commercially available silylated glycidol **8** (2.0 g, 2.22 mL, 10.6 mmol, 1.0 equiv) in Et_2O (35 mL) was then added dropwise to the mixture *via* cannula. The internal temperature was then slowly raised to −60 °C and the mixture was stirred at this temperature for 6 h. The reaction mixture was hydrolyzed by adding a saturated aqueous solution of NH_4Cl containing 10% aqueous ammonia (50 mL) and the resulting mixture was vigorously stirred for 1 h. The layers were separated and the aqueous phase was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered then concentrated under reduced pressure to give allylic alcohol **9** (2.29 g, 99%) as a colorless oil with a satisfying degree of purity. This compound was used in the following step with no further purification: IR (neat): 3400, 2920, 2840, 1640, 1460, 1360, 1255, 1100, 910, 835, 775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.84 (ddt, J = 17.1 Hz, J = 10.1 Hz and J = 7.1 Hz, 1H), 5.16–5.05 (m, 2H), 3.70 (m, 1H), 3.63 (dd, J = 9.9 Hz and J = 3.8 Hz, 1H), 3.46

(dd, J = 9.9 Hz and J = 7.0 Hz, 1H), 2.24 (tt_{app}, J = 6.7 Hz and J = 1.2 Hz, 2H), 2.12 (br s, 1H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.5 (d), 117.4 (t), 71.1 (d), 66.5 (t), 37.6 (t), 25.9 (q, 3C), 18.3 (s), −5.4 (q, 2C); MS (EI, 70 eV) m/z (%): 175 (4), 159 (M − tBu$^+$, 8), 141 (8), 117 (16), 105 (27), 89 (8), 76 (9), 75 (100), 73 (24), 67 (29), 59 (8).

Ethyl (*E*)-6-(*tert*-butyldimethylsilyloxy)-5-hydroxy-2-hexenoate (10). To a solution of alkene **9** (2.41 g, 11.16 mmol, 1.0 equiv) in CH_2Cl_2 (60 mL) were successively added ethyl acrylate (3.6 mL, 33.47 mmol, 3.0 equiv) and the second generation Grubbs-catalyst [Ru]-**II** (285 mg, 0.33 mmol, 0.03 equiv). The mixture was refluxed and after 4 h the reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (gradient Petroleum ether–EtOAc: 90 : 10 then 70 : 30) furnished α,β-unsaturated ester **10** (2.99 g, 93%) as a brown oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.98 (dt, J = 15.7 Hz and J = 7.3 Hz, 1H), 5.91 (dt, J = 15.6 Hz and J = 1.4 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 3.79 (m, 1H), 3.64 (dd, J = 9.9 Hz and J = 3.6 Hz, 1H), 3.46 (dd, J = 9.9 Hz and J = 3.6 Hz, 1H), 2.37 (br t_{app}, J = 7.4 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 6H); MS (EI, 70 eV) m/z (%): 243 (2), 231 (M − tBu$^+$, 4), 186 (10), 185 (70), 157 (15), 117 (77), 114 (15), 111 (31), 105 (15), 103 (14), 89 (13), 83 (29), 75 (100), 73 (40), 55 (12).

Ethyl (*E*)-5-acetoxy-6-(*tert*-butyldimethylsilyloxy)-2-hexenoate (11). To a solution of alcohol **10** (218 mg, 0.756 mmol, 1.0 equiv) in pyridine (61 µL, 0.756 mmol, 1.0 equiv) at 0 °C were successively added acetic anhydride (286 µL, 3.02 mmol, 4.0 equiv) dropwise then 4-dimethylaminopyridine (19 mg, 0.15 mmol, 0.2 equiv). After stirring for 15 h at rt, the reaction mixture was hydrolyzed upon addition of a saturated aqueous solution of $NaHCO_3$ (15 mL). Diethyl ether was added (30 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered then concentrated under reduced pressure to give acetate **11** (261 mg, 100%) as a pale yellow oil. This compound was used in the following step without further purification; IR (neat): 2940, 2925, 2850, 1740, 1720, 1660, 1460, 1470, 1370, 1320, 1235, 1175, 1040, 980, 840, 780 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.88 (dt, J = 15.6 Hz and J = 7.4 Hz, 1H), 5.87 (dt, J = 15.6 Hz and J = 1.4 Hz, 1H), 4.94 (br $quint_{app}$, J = 6.0 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.68–3.58 (m, 2H), 2.62–2.42 (m, 2H), 2.04 (s, 3H), 1.27 (t, J = 7.1 Hz, 3H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (s), 166.2 (s), 143.5 (d), 124.1 (d), 72.7 (d), 63.5 (t), 60.3 (t), 33.3 (t), 25.8 (q, 3C), 21.1 (q), 18.2 (s), 14.2 (q), −5.5 (q, 2C); MS (EI, 70 eV) m/z (%): 285 (M − OEt$^+$, 4), 273 (M − tBu$^+$, 5), 231 (8), 185 (12), 118 (9), 117 (100), 111 (10), 75 (27), 73 (12).

Ethyl (2*E*,4*E*)-6-(*tert*-butyldimethylsilyloxy)-2,4-hexadienoate (12). To a solution of acetate **11** (90 mg, 0.272 mmol, 1.0 equiv) in THF (1 mL) at 0 °C was added DBU (90 µL, 0.60 mmol, 2.2 equiv) dropwise. After stirring for 36 h at rt, the reaction mixture was hydrolyzed by addition of a saturated aqueous solution of NH_4Cl (5 mL). The aqueous layer was extracted with Et_2O (3 × 10 mL) and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Petroleum ether–Et_2O: 90 : 10) furnished dienic ester **12** (63 mg, 86%) as a viscous dark yellow oil; IR (neat): 2930, 2857, 1707, 1471, 1369, 1303, 1253, 1179, 1096, 1030, 979, 834, 777 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (dd, J = 15.4 Hz and J = 11.3 Hz, 1H), 6.39 (m, 1H), 6.16 (dt, J = 15.2 Hz and J = 4.2 Hz, 1H), 5.86 (d, J = 15.3 Hz, 1H), 4.29 (br dd, J = 4.2 Hz and J = 1.7 Hz, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H), 0.91 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7 (s), 140.3 (d), 139.5 (d), 135.4 (d), 132.8 (d), 61.8 (t), 61.6 (t), 25.6 (q, 3C), 18.0 (s), 14.1 (q), −4.7 (q, 2C); MS (EI, 70 eV) m/z (%): 270 (M$^{+\cdot}$, 20), 213 (33), 185 (28), 167 (22), 157 (18), 141 (26), 139 (10), 131 (25), 115 (7), 103 (78), 75 (100), 73 (49), 66 (17), 65 (10), 59 (10), 57 (8).

Ethyl (2*E*,4*E*)-6-hydroxy-2,4-hexadienoate (13). To a solution of silyl ether **12** (552 mg, 2.04 mmol, 1.0 equiv) in THF (55 mL) at 0 °C was added n-Bu_4NF (1 M in THF, 2.25 mL, 2.25 mmol, 1.1 equiv) and the mixture was stirred for 1 h at rt. The reaction mixture was poured in water (50 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Petroleum ether–EtOAc: 60 : 40) furnished alcohol **13** (258 mg, 81%) as a viscous colorless oil which crystallized upon standing in the freezer at −20 °C; IR (neat): 3400, 2980, 2860, 1710, 1650, 1620, 1370, 1180, 1135, 1090, 1000, 875, 795 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (dd, J = 15.4 Hz and J = 10.9 Hz, 1H), 6.40 (br dd, J = 15.3 Hz and J = 10.9 Hz, 1H), 6.21 (dt, J = 15.4 Hz and J = 4.9 Hz, 1H), 5.87 (d, J = 15.5 Hz, 1H), 4.28 (br d, J = 4.2 Hz, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.93 (br s, 1H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.1 (s), 143.7 (d), 141.1 (d), 127.8 (d), 121.4 (d), 62.6 (t), 60.4 (t), 14.2 (q); MS (EI, 70 eV) m/z (%): 156 (M$^{+\cdot}$, 23), 128 (12), 127 (73), 111 (43), 110 (46), 109 (19), 99 (98), 97 (28), 88 (8), 84 (22), 83 (83), 82 (67), 81 (100), 79 (17), 71 (11), 69 (10), 67 (10), 66 (24), 65 (16), 56 (11), 55 (99), 54 (19), 53 (54), 52 (11), 51 (16).

Ethyl (2*E*,4*E*,6*E*)-8-(*tert*-butyldimethylsilyloxy)-2,4,6-octatrienoate (16). To a solution of alkene **9** (3.0 g, 13.86 mmol, 1.0 equiv) in an acetone–H_2O mixture (9 : 1, 70 mL) at 0 °C were successively added *N*-methylmorpholine *N*-oxide (1.62 g, 13.86 mmol, 1.0 equiv) followed by OsO_4 (4 wt% in H_2O, 852 µL, 0.01 equiv) dropwise. After 15 h at rt, water (30 mL) and $NaIO_4$ (12 g, 55.4 mmol, 4.0 equiv) were successively added to the reaction mixture. After 30 min at rt, solid $Na_2S_2O_3$ (3 g) and Florisil® (10 g) were added and the resulting suspension was stirred for 30 min, then filtered over a plug of cotton to remove osmium salts. The filtrate was concentrated *in vacuo*, Et_2O was added (75 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 75 mL) and the combined organic layers were successively washed with a saturated aqueous solution of $Na_2S_2O_3$ (50 mL), brine (75 mL) then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The obtained crude aldehyde thus obtained was not purified but directly used in the following step.

To a solution of the afore-mentioned aldehyde (13.86 mmol, 1.0 equiv) in THF (45 mL) at −20 °C was added allylmagnesium chloride (2 M in THF, 20.8 mL, 41.6 mmol, 3.0 equiv)

dropwise. After 3 h at this temperature, the reaction mixture was hydrolyzed upon addition of a saturated aqueous solution of NH_4Cl (30 mL). Ethyl acetate (50 mL) was added followed by an aqueous solution of 10% HCl (15 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Petroleum ether–EtOAc: 70 : 30) allowed us to obtain homoallylic alcohol **14** (2.22 g, 61% over 2 steps) as a mixture of two diastereomers which was directly used in the following step.

To a solution of alkene **14** (2.22 g, 6.52 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL), were successively added ethyl acrylate (2.8 mL, 25.6 mmol, 3.0 equiv) and the second generation Grubbs-catalyst [Ru]-**II** (218 mg, 0.256 mmol, 0.03 equiv). After 20 h at rt, the reaction mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (gradient Petroleum ether–EtOAc: 80 : 20 then 60 : 40) furnished α,β-unsaturated ester **15** (1.15 g, 76%) as a mixture of two diastereomers which was directly used in the following step.

To a solution of 1,3-diol **15** (1.03 mg, 3.1 mmol, 1.0 equiv) in pyridine (500 µL, 6.2 mmol, 2.0 equiv) at 0 °C were successively added acetic anhydride (2.35 mL, 24.8 mmol, 8.0 equiv) dropwise then 4-DMAP (152 mg, 1.23 mmol, 0.4 equiv). After 15 h at rt, the reaction mixture was hydrolyzed upon addition of a saturated aqueous solution of $NaHCO_3$ (20 mL). Et_2O was added (50 mL), the layers were separated and the aqueous phase was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the corresponding acetate (1.28 g, 99%) as a pale yellow oil which was not purified but directly used in the following step.

To a solution of the previously obtained 1,3-diacetate (1.10 g, 2.64 mmol, 1.0 equiv) in THF (10 mL) at 0 °C was added DBU (1.7 mL, 11.6 mmol, 4.4 equiv) dropwise. After 24 h at rt, the reaction mixture was hydrolyzed upon addition of a saturated aqueous solution of NH_4Cl (10 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (short column, Petroleum ether–EtOAc: 80 : 20) furnished trienic ester **16** (606 mg, 77%) as a viscous yellow oil which crystallized upon standing in the freezer at −20 °C; IR (neat): 2929, 2856, 1710, 1619, 1463, 1253, 1216, 1178, 1117, 1003, 834, 775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.29 (dd, J = 15.3 Hz and J = 11.3 Hz, 1H), 6.56 (dd, J = 14.7 Hz and J = 10.8 Hz, 1H), 6.34 (ddt, J = 15.0 Hz, J = 10.9 Hz and J = 1.7 Hz, 1H), 6.28 (dd, J = 15.0 Hz and J = 11.3 Hz, 1H), 5.97 (dt, J = 15.0 Hz and J = 4.7 Hz, 1H), 5.85 (d, J = 15.3 Hz, 1H), 4.26 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H), 0.91 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.1 (s), 144.4 (d), 140.1 (t), 137.7 (d), 129.3 (d), 128.6 (d), 120.7 (d), 63.1 (t), 60.2 (t), 25.9 (q, 3C), 18.4 (s), 14.3 (q), −5.2 (q, 2C); MS (EI, 70 eV) m/z (%): 296 ($M^{+\cdot}$, 11), 251 (8), 240 (14), 239 (M − tBu$^+$, 73), 211 (33), 193 (35), 181 (9), 167 (10), 165 (26), 149 (13), 145 (10), 131 (10), 129 (10), 120 (10), 119 (44), 107 (19), 103 (43), 93 (15), 92 (19), 91 (98), 89 (12), 79 (16), 77 (22), 75 (100), 73 (86), 65 (13), 59 (15), 57 (12).

Ethyl (2*E*,4*E*,6*E*)-8-hydroxyocta-2,4,6-trienoate (17). To a solution of **16** (306 mg, 1.03 mmol, 1.0 equiv) in THF (30 mL) at 0 °C was added *n*-Bu_4NF (1 M in THF, 1.24 mL, 1.24 mmol, 1.2 equiv). After 1 h at rt, the reaction mixture was poured into H_2O (20 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered then concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Petroleum ether–EtOAc: 60 : 40) furnished alcohol **17** (164 mg, 87%) as yellow crystals; IR (neat): 3483, 2924, 1694, 1617, 1369, 1299, 1258, 1234, 1177, 1134, 1001 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.29 (dd, J = 15.3 Hz and J = 11.3 Hz, 1H), 6.56 (dd, J = 15.0 Hz and J = 10.6 Hz, 1H), 6.34 (m, 1H), 6.29 (dd, J = 15.3 Hz and J = 11.1 Hz, 1H), 6.03 (dt, J = 15.1 Hz and J = 5.4 Hz, 1H), 5.87 (d, J = 15.3 Hz, 1H), 4.24 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.84 (br s, 1H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.1 (s), 144.2 (d), 139.7 (d), 136.9 (d), 130.0 (d), 129.9 (d), 121.2 (d), 62.9 (t), 60.3 (t), 14.3 (q).

Ethyl (2*E*,4*E*,6*E*,8*E*)-10-(*tert*-butyldimethylsilyloxy)-deca-2,4,6,8-tetraenoate (20). To a solution of alkene **9** (480 mg, 2.22 mmol, 1.0 equiv) in CH_2Cl_2 (15 mL) were successively added acrolein (445 µL, 6.65 mmol, 3.0 equiv) and the Grubbs–Hoveyda catalyst [Ru]-**III** (67 mg, 0.111 mmol, 0.05 equiv). After 36 h at rt, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (Petroleum ether–EtOAc: 80 : 20) to give α,β-unsaturated aldehyde **18** (434 mg, 80%) as a brown oil.

To a solution of aldehyde **18** (310 mg, 1.27 mmol, 1.0 equiv) in THF (5 mL) at −20 °C was added allylmagnesium chloride (2 M in THF, 1.9 mL, 3.8 mmol, 3.0 equiv) dropwise. After 3 h at this temperature, the reaction mixture was hydrolyzed by addition of a saturated aqueous solution of NH_4Cl (20 mL). EtOAc (30 mL) was added followed by a 10% aqueous solution of HCl (15 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel furnished the corresponding homoallylic alcohol (298 mg, 82%) as a mixture of two diastereomers (pale yellow oil) which was directly used in the following step.

To a solution of previously obtained 1,5-diol (278 mg, 0.970 mmol, 1.0 equiv) in pyridine (156 µL, 1.94 mmol, 2.0 equiv) at 0 °C were successively added acetic anhydride (734 µL, 7.76 mmol, 8 equiv) dropwise then 4-dimethylaminopyridine (47 mg, 0.39 mmol, 0.4 equiv). After 15 h at rt, the reaction mixture was hydrolyzed upon addition of a saturated aqueous solution $NaHCO_3$ (15 mL). Et_2O (30 mL) was added and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 30 mL) and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Petroleum ether–EtOAc: 95 : 5) furnished 1,5-diacetate **19** (303 mg, 82%) as a mixture of two diastereomers which was directly used in the following step.

To a solution of alkene **19** (285 mg, 0.697 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) were successively added ethyl acrylate (228 µL, 2.10 mmol, 3.0 equiv) and Grubbs–Hoveyda catalyst

[Ru]-**III** (21 mg, 0.04 mmol, 0.05 equiv). After 24 h at rt, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel (Petroleum ether–EtOAc: 90 : 10) to give α,β-unsaturated ester (230 mg, 75%) as a brown oil which was directly used in the following elimination step.

To a solution of previously obtained 1,5-diacetate (211 mg, 0.480 mmol, 1.0 equiv) in THF (2 mL) at 0 °C was added DBU (314 µL, 2.10 mmol, 4.4 equiv) dropwise. After 36 h at rt, the reaction mixture was hydrolyzed by addition of a saturated aqueous solution of NH_4Cl (5 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered then concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (petroleum ether–EtOAc: 95 : 5) furnished **20** (110 mg, 71%) as a dark yellow oil; IR (neat): 2955, 2928, 2855, 1709, 1620, 1597, 1463, 1255, 1124, 1007, 835, 776 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.31 (dd, J = 15.4 Hz and J = 11.6 Hz, 1H), 6.58 (dd, J = 14.8 Hz and J = 10.5 Hz, 1H), 6.50–6.20 (m, 4H), 5.89 (dt, J = 14.5 Hz and J = 4.7 Hz, 1H), 5.86 (d, J = 15.3 Hz, 1H), 4.26 (m, 2H), 4.20 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H), 0.91 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.1 (s), 144.4 (d), 140.6 (d), 136.6 (d), 135.9 (d), 131.2 (d), 129.8 (d), 129.2 (d), 120.6 (d), 63.3 (t), 60.2 (t), 25.9 (q), 18.4 (s), 14.3 (q), −5.3 (q, 2C); MS (EI, 70 eV) m/z (%): 322 ($M^{+\cdot}$, 23), 293 (M − Et^+, 4), 265 (M − tBu$^+$, 36), 219 (17), 191 (12), 159 (12), 145 (28), 133 (10), 118 (18), 117 (100), 115 (26), 105 (15), 103 (29), 91 (28), 77 (10), 75 (60), 73 (67), 59 (9); HRMS (ESI): calcd for $C_{18}H_{30}O_3SiNa$ $[M + Na]^+$: 345.1856; found: 345.1702.

(1*R*,6*S*,7*R*,8*R*)-8-Acetoxy-bicyclo[4.2.0]octa-2,4-dien-7-yl acetate (23). To a vigorously stirred suspension of $Hg(OAc)_2$ (15.3 g, 48.0 mmol, 1.0 equiv) in acetic acid (40 mL) was added cyclooctatetraene **22** (5 g, 48.0 mmol, 1.0 equiv). The mixture was heated at 80 °C for 2 h and was then allowed to cool down to rt. The mercury formed during the reaction was then filtered over cotton, and H_2O (400 mL) was added to the filtrate. The resulting mixture was stirred for 10 min until a solid appeared, which was filtered and dried *in vacuo* to give diacetate **23** (8.85 g, 83%) as an analytically pure orange solid. The spectral characteristics for this compound were in accordance with those reported in the literature;[11] m.p. = 64 °C {Lit.[11] m.p. = 34 °C}; IR (neat): 2911, 1731, 1367, 1215, 1049, 942, 901, 844, 744, 685, 620 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.92 (m, 1H), 5.86–5.73 (m, 2H), 5.45 (br dd, J = 9.8 Hz and J = 3.6 Hz, 1H), 5.33–5.24 (m, 2H), 3.50 (m, 1H), 2.65 (m, 1H), 2.08 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4 (s), 169.8 (s), 126.3 (d), 125.0 (d), 123.0 (d), 122.5 (d), 78.6 (d), 78.5 (d), 34.2 (d), 33.8 (d), 20.8 (q), 20.6 (q); HRMS (ESI): calcd for $C_{12}H_{14}O_4Na$ $[M + Na]^+$: 245.0784; found: 245.0785.

Ethyl (2*E*,4*E*,6*E*,8*E*)-10-hydroxydeca-2,4,6,8-tetraenoate (21)

*From silyl ether **20**.* To a solution of the previously obtained silyl ether **20** (55 mg, 0.17 mmol, 1.0 equiv) in THF (5 mL) at 0 °C was added tetra-*n*-butylammonium fluoride (1 M in THF, 187 µL, 0.187 mmol, 1.1 equiv) dropwise. After 1 h of stirring at rt and in the dark, the reaction mixture was poured into water (5 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 10 mL) and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel allowed isolation of alcohol **21** (27 mg, 76%) as a bright yellow solid, which slowly decomposes in $CDCl_3$.

*Synthesis from 1,2-diacetate **23** in 4 steps.* To a solution of diacetate **23** (6.0 g, 27.0 mmol, 1.0 equiv) in Et_2O (240 mL) at 0 °C was added $LiAlH_4$ (1.23 g, 32.4 mmol, 1.2 equiv) dropwise. After 20 min at rt, EtOAc (500 mL) and a 1% aqueous solution of H_3PO_4 (350 mL) were successively added. The layers were separated and the aqueous phase was extracted with EtOAc (2 × 350 mL). The combined organic layers were successively washed with an aqueous solution of 2% NaOH (2 × 250 mL), brine (300 mL) then dried over $MgSO_4$ and filtered. The resulting filtrate was poured into an Erlenmeyer wrapped around with aluminium foil and the solution was stirred for 12 h in the dark. The filtrate was then concentrated *in vacuo* which furnished the crude dialdehyde **24** (2.06 g, 15.1 mmol) as yellow/orange crystals, extremely sensitive to air and light, which were not purified but directly used in the following step.

To a suspension of NaH (660 mg, 16.5 mmol, 1.1 equiv) [washed with hexane (twice)] in THF (100 mL) at 0 °C was added triethylphosphonoacetate (3.0 mL, 15.1 mmol, 1.0 equiv) dropwise and in the dark. After 20 min at rt, the resulting solution of triethylphosphonoacetate ylide was added dropwise *via* a syringe pump over 45 min to a solution of dialdehyde **24** (2.06 g, 15.1 mmol, 1.0 equiv) in THF (65 mL), cooled down to 0 °C. Upon completion of the addition, stirring was pursued for 1 h at 0 °C and the reaction mixture was then hydrolyzed by adding a saturated aqueous solution of $NaHCO_3$ (50 mL). EtOAc (100 mL) was added and the layers were separated. The aqueous phase was extracted with EtOAc (2 × 100 mL) and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting tetraenic aldehyde **25** (yellow solid) was not purified but directly used in the following step.

To a solution of aldehyde **25** (15.1 mmol, 1.0 equiv) in ethanol at 95% (200 mL) was added sodium borohydride (2.28 g, 60.4 mmol, 4.0 equiv) in one portion at rt. After 20 min, the reaction mixture was hydrolyzed by adding water (50 mL) and the bulk of ethanol was then removed *in vacuo*. EtOAc (200 mL) and water (200 mL) were successively added and the layers were separated. The aqueous phase was extracted with EtOAc (2 × 200 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (gradient Petroleum ether–EtOAc: 95 : 5 then 80 : 20) furnished ω-hydroxylated tetraenic ester **21** (1.25 g, 22% over 3 steps) as a yellow solid which was stored in the dark in the freezer at −20 °C; IR (neat): 3527, 2927, 1695, 1618, 1595, 1371, 1304, 1243, 1224, 1174, 1149, 1125, 1003, 868, 714 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.30 (dd, J = 15.0 Hz and J = 11.3 Hz, 1H), 6.57 (dd, J = 14.9 Hz and J = 10.3 Hz, 1H), 6.45–6.23 (m, 4H), 5.96 (dt, J = 14.3 Hz and J = 5.7 Hz, 1H), 5.86 (d, J = 15.2 Hz, 1H), 4.23 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.69 (br s, 1H), 1.29 (t, J = 11.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.1 (s), 144.2 (d), 140.3 (d), 136.0 (d), 134.9 (d), 132.0 (d),

130.7 (d), 130.3 (d), 120.9 (d), 63.2 (t), 60.4 (t), 14.3 (q); MS (EI, 70 eV) *m/z* (%): 208 ($M^{+\cdot}$, 95), 179 (M − Et^+, 7), 163 (21), 161 (11), 145 (14), 135 (10), 134 (18), 133 (67), 131 (19), 125 (36), 119 (27), 118 (25), 117 (83), 116 (21), 115 (43), 112 (12), 107 (41), 106 (17), 105 (99), 104 (14), 103 (38), 97 (29), 93 (10), 91 (100), 84 (13), 81 (17), 80 (11), 79 (72), 78 (32), 77 (59), 67 (19), 66 (12), 65 (25), 63 (10), 55 (26), 53 (15), 51 (16); HRMS (ESI): calcd for $C_{12}H_{16}O_3Na$ $[M + Na]^+$: 231.0992; found: 231.0990.

General procedure for esterification under Mitsunobu conditions

To a solution of carboxylic acid **4** or **7** (1.0 equiv) in THF (0.05–0.1 M) and alcohol (1.0 equiv) were successively added triphenylphosphine (1.25 equiv) in one portion and diethylazodicarboxylate (1.25 equiv) dropwise. After 12 h at rt, the solution was concentrated *in vacuo*, EtOAc was then added and the resulting solution was hydrolyzed upon addition of a saturated aqueous solution of $NaHCO_3$. The layers were separated and the aqueous phase was extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel furnished the desired ester.

[(2*E*,4*E*)-5-Ethoxycarbonylpenta-2,4-dienyl] (3*R*)-piperidine-3-carboxylate (26). Following the general esterification procedure using *N*-Boc-(*R*)-(−)-nipecotic acid **7** (75 mg, 0.326 mmol) and ω-hydroxylated dienic ester **13** (51 mg, 0.326 mmol) in THF (5 mL), in the presence of triphenylphosphine (107 mg, 0.408 mmol) and diethylazodicarboxylate (81 µL, 0.408 mmol), and after purification by flash chromatography on silica gel (Petroleum ether–EtOAc: 70 : 30), 118 mg of 1-*tert*-butyl and 3-[(2*E*,4*E*)-5-ethoxycarbonylpenta-2,4-dienyl] (3*R*)-piperidine-1,3-dicarboxylate (98%) are obtained as a colorless oil.

To a solution of 1-*tert*-butyl and 3-[(2*E*,4*E*)-5-ethoxycarbonylpenta-2,4-dienyl]-(3*R*)-piperidine-1,3-dicarboxylate (153 mg, 0.417 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added trifluoroacetic acid (1.25 mL) dropwise. After 15 min at rt, the reaction mixture was concentrated under reduced pressure and the resulting ammonium trifluoroacetate was diluted in $CHCl_3$ (20 mL). A saturated aqueous solution of Na_2CO_3 (20 mL) was then added to the previous solution and the resulting biphasic mixture was transferred to a separatory funnel and shaken vigorously. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give piperidine **26** (110 mg, 99%) as a viscous yellow oil; $[\alpha]_D^{20}$ −6.0 (*c* 0.47, $CHCl_3$); IR (neat): 2922, 2852, 2808, 1713, 1649, 1620, 1447, 1368, 1299, 1265, 1233, 1178, 1137, 1096, 1030, 1000, 975, 868, 733 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.23 (dd, *J* = 15.4 Hz and *J* = 11.0 Hz, 1H), 6.35 (dd, *J* = 15.4 Hz and *J* = 11.0 Hz, 1H), 6.09 (dt, *J* = 15.4 Hz and *J* = 5.7 Hz, 1H), 5.89 (d, *J* = 15.4 Hz, 1H), 4.66 (br d, *J* = 5.5 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.77 (br s, 1H), 3.23 (m, 1H), 3.02 (br m, 1H), 2.84 (m, 1H), 2.72–2.50 (m, 2H), 2.02 (m, 1H), 1.75–1.44 (m, 3H), 1.27 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3 (s), 166.6 (s), 143.0 (d), 135.0 (d), 130.6 (d), 122.7 (d), 65.4 (t), 60.4 (t), 47.8 (t), 45.9 (t), 37.9 (d), 27.0 (t), 24.7 (t), 14.2 (q); MS (EI, 70 eV) *m/z* (%): 267 ($M^{+\cdot}$, 1), 222 (M − CO_2Et^+, 14), 140 (8), 128 (100), 110 (8), 84 (21), 83 (10), 82 (13), 67 (11), 57 (9), 56 (15), 55 (15); HRMS (ESI): calcd for $C_{14}H_{22}O_4N$ $[M + H]^+$: 268.1543; found: 268.1539.

[(2*E*,4*E*,6*E*)-7-Ethoxycarbonylhepta-2,4,6-trienyl] (3*R*)-piperidine-3-carboxylate (27). Following the general esterification procedure using *N*-Boc-(*R*)-(−)-nipecotic acid **7** (43 mg, 0.186 mmol) and ω-hydroxylated trienic ester **17** (34 mg, 0.186 mmol) in THF (3 mL), in the presence of triphenylphosphine (60 mg, 0.233 mmol) and diethylazodicarboxylate (46 µL, 0.233 mmol), and after purification by flash chromatography on silica gel (Petroleum ether–EtOAc: 80 : 20), 58 mg of 1-*tert*-butyl and 3-[(2*E*,4*E*,6*E*)-7-ethoxycarbonylhepta-2,4,6-trienyl] (3*R*)-piperidine-1,3-dicarboxylate (79%) were obtained as a colorless oil.

To a solution of 1-*tert*-butyl and 3-[(2*E*,4*E*,6*E*)-7-ethoxycarbonylhepta-2,4,6-trienyl] (3*R*)-piperidine-1,3-dicarboxylate (56 mg, 0.142 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added trifluoroacetic acid (500 µL) dropwise. After 15 min at rt, the reaction mixture was concentrated under reduced pressure and the resulting ammonium trifluoroacetate was diluted in $CHCl_3$ (10 mL). A saturated aqueous solution of Na_2CO_3 (10 mL) was then added, the layers were separated and the aqueous phase was extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were washed with brine, (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give trienic piperidine **27** (42 mg, 99%) as a viscous yellow oil; $[\alpha]_D^{20}$ −12.8 (*c* 0.125, $CHCl_3$); IR (neat): 3323, 2936, 2857, 1705, 1620, 1445, 1367, 1339, 1299, 1259, 1220, 1777, 1132, 1004, 852, 718 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.27 (dd, *J* = 15.3 Hz and *J* = 11.0 Hz, 1H), 6.52 (dd, *J* = 14.8 Hz and *J* = 10.7 Hz, 1H), 6.38–6.26 (m, 2H), 5.91 (dt, *J* = 15.2 Hz and *J* = 6.1 Hz, 1H), 5.88 (d, *J* = 15.4 Hz, 1H), 4.64 (br d, *J* = 6.1 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.16 (br dd, *J* = 12.4 Hz and *J* = 3.4 Hz, 1H), 2.92 (br dt, *J* = 12.2 Hz and *J* = 3.6 Hz, 1H), 2.81 (dd, *J* = 12.3 Hz and *J* = 9.3 Hz, 1H), 2.63 (m, 1H), 2.48 (m, 1H), 2.19 (br s, 1H), 1.99 (m, 1H), 1.73–1.59 (m, 2H), 1.46 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.9 (s), 166.9 (s), 143.8 (d), 138.9 (d), 132.6 (d), 131.1 (d), 131.0 (d), 121.9 (d), 64.1 (t), 60.4 (t), 48.4 (t), 46.3 (t), 42.3 (d), 27.3 (t), 25.3 (t), 14.3 (q); MS (EI, 70 eV) *m/z* (%): 293 ($M^{+\cdot}$, 4), 248 (M − OEt^+, 3), 220 (M − CO_2Et^+, 3), 166 (15), 128 (100), 119 (15), 110 (8), 92 (8), 91 (37), 84 (19), 82 (9), 77 (9), 56 (14), 55 (16); HRMS (ESI): calcd for $C_{16}H_{24}O_4N$ $[M + H]^+$: 294.1700; found: 294.1698.

1-*tert*-Butyl and 3-[(2*E*,4*E*,6*E*,8*E*)-9-ethoxycarbonylnona-2,4,6,8-tetraenyl] (3*R*)-piperidine-1,3-dicarboxylate (28). Following the general esterification procedure using *N*-Boc-(*R*)-(−)-nipecotic acid **7** (55 mg, 0.240 mmol) and ω-hydroxyled tetraenic ester **21** (50 mg, 0.240 mmol) in THF (4 mL), in the presence of triphenylphosphine (79 mg, 0.300 mmol) and diethylazodicarboxylate (60 µL, 0.300 mmol), and after purification by flash chromatography on silica gel (Petroleum ether–EtOAc: 85 : 15), 55 mg of ester **28** (55%) were obtained as a viscous yellow oil; $[\alpha]_D^{20}$ −23.9 (*c* 2.35, $CHCl_3$); IR (neat): 2932, 2924, 2860, 1729, 1687, 1422, 1366, 1264, 1240, 1148, 1007,

734, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 (dd, J = 15.2 Hz and J = 11.5 Hz, 1H), 6.56 (dd, J = 14.7 Hz and J = 10.1 Hz, 1H), 6.42–6.26 (m, 4H), 5.92–5.79 (m, 1H), 5.88 (d, J = 15.2 Hz, 1H), 4.63 (br d, J = 6.4 Hz, 2H), 4.19 (q, J = 7.1 Hz, 2H), 4.15–4.03 (m, 1H), 3.90 (br d, J = 12.9 Hz, 1H), 2.97 (br m, 1H), 2.81 (ddd, J = 13.3 Hz, J = 11.2 Hz and J = 3.1 Hz, 1H), 2.46 (m, 1H), 2.04 (m, 1H), 1.74–1.65 (m, 2H), 1.61 (m, 1H), 1.44 (s, 9H), 1.29 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1 (s), 167.0 (s), 154.6 (s), 144.1 (d), 140.0 (d), 135.4 (d), 133.6 (d), 133.0 (d), 130.8 (d), 129.0 (d), 121.3 (d), 79.7 (s), 64.5 (t), 60.3 (t), 43–46 (br t, 2C), 41.4 (d), 28.4 (q, 3C), 27.4 (t), 24.2 (t), 14.3 (q); HRMS (ESI): calcd for $C_{23}H_{33}O_6NNa$ $[M + Na]^+$: 442.2200; found: 442.2194.

(2*E*,4*E*)-5-Ethoxycarbonylpenta-2,4-dienyl (3*R*)-1-[3-(4-piperidinyl)propanoyl]-3-piperidinecarboxylate (30). Following the general protocol using carboxylic acid **4** (387 mg, 1.06 mmol) and ω-hydroxylated dienic ester **13** (165 mg, 1.06 mmol) in THF (25 mL), in the presence of triphenylphosphine (347 mg, 1.32 mmol) and diethylazodicarboxylate (262 µL, 1.32 mmol), and after purification by flash chromatography on silica gel (gradient CH_2Cl_2–Et_2O: 90 : 10 then 65 : 35), 438 mg of *tert*-butyl 4-(3-{(3*R*)-3-[(2*E*,4*E*)-5-ethoxycarbonylpenta-2,4-dienyloxycarbonyl]-1-piperidinyl}-3-oxo-propyl)-1-piperidinecarboxylate (82%) were obtained as a colorless oil.

To a solution of *tert*-butyl 4-(3-{(3*R*)-3-[(2*E*,4*E*)-5-ethoxycarbonylpenta-2,4-dienyloxycarbonyl]-1-piperidinyl}-3-oxo-propyl)-1-piperidinecarboxylate (115 mg, 0.227 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added trifluoroacetic acid (750 µL) dropwise. After 15 min at rt, the reaction mixture was concentrated under reduced pressure and the resulting ammonium trifluoroacetate was diluted in $CHCl_3$ (15 mL). A saturated aqueous solution of Na_2CO_3 (15 mL) was then added, the layers were separated and the aqueous phase was extracted with $CHCl_3$ (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered then concentrated *in vacuo* to give piperidine **30** (93 mg, 100%) as a viscous yellow oil; $[\alpha]_D^{20}$ −32.6 (*c* 0.43, $CHCl_3$); IR (neat): 3437, 2918, 2854, 1712, 1635, 1439, 1233, 1169, 1137, 1010, 974, 857, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, rotamers): δ 7.17 (m, 1H), 6.30 (dd, J = 14.2 Hz and J = 11.2 Hz, 1H), 6.03 (m, 1H), 5.85 (d, J = 15.3 Hz, 1H), 4.61 (t_{app}, J = 6.2 Hz, 2H), 4.49 (br d, J = 13.1 Hz, 0.5H), 4.12 (q, J = 7.0 Hz, 2H), 4.10–3.80 (m, 0.5H + 2H), 3.68 (m, 1H), 3.35 (dd, J = 13.6 Hz and J = 8.9 Hz, 0.5H), 3.10–2.92 (m, 2 × 0.5H), 2.85 (m, 0.5H), 2.54 (br t_{app}, J = 11.9 Hz, 2H), 2.43 (m, 1H), 2.27 (m, 2H), 2.00 (m, 1H), 1.80–1.29 (m, 8H), 1.21 (t, J = 7.1 Hz, 3H), 1.24–1.00 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz, rotamers): δ 172.6 and 172.3 (s, 1C), 171.4 (s), 166.6 and 166.5 (s, 1C), 142.9 and 142.7 (d, 1C), 134.8 and 134.5 (d, 1C), 131.0 and 130.6 (d, 1C), 122.9 and 122.6 (d, 1C), 64.1 and 63.8 (t, 1C), 60.4 and 60.3 (t, 1C), 47.1, 46.0 (t, 2C), 45.9 (t), 43.4 and 41.8 (t, 1C), 41.6 and 41.0 (d, 1C), 35.4 (d), 32.4, 32.1 (t, 3C), 30.2 and 30.1 (t, 1C), 27.2 (t), 24.9 and 23.8 (t, 1C), 14.2 (q); HRMS (ESI): calcd for $C_{22}H_{35}O_5N_2$ $[M + H]^+$: 407.2541; found: 407.2535.

[(2*E*,4*E*,6*E*)-7-Ethoxycarbonylhepta-2,4,6-trienyl] (3*R*)-1-[3-(4-piperidinyl)propanoyl]-3-piperidinecarboxylate (31). Following the general protocol using carboxylic acid **4** (123 mg, 0.335 mmol) and ω-hydroxylated trienic ester **17** (61 mg, 0.335 mmol) in THF (8 mL), in the presence of triphenylphosphine (110 mg, 0.419 mmol) and diethylazodicarboxylate (83 µL, 0.419 mmol), and after purification by flash chromatography on silica gel (gradient Petroleum ether–EtOAc: 70 : 30 then 50 : 50), 139 mg of *tert*-butyl 4-(3-{(3*R*)-3-[(2*E*,4*E*,6*E*)-7-ethoxycarbonylhepta-2,4,6-trienyloxycarbonyl]-1-piperidinyl}-3-oxopropyl)-1-piperidinecarboxylate (78%) were obtained as a viscous yellow oil.

To a solution of t*ert*-butyl 4-(3-{(3*R*)-3-[(2*E*,4*E*,6*E*)-7-ethoxycarbonylhepta-2,4,6-trienyloxycarbonyl]-1-piperidinyl}-3-oxopropyl)-1-piperidinecarboxylate (92 mg, 0.173 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added trifluoroacetic acid (750 µL) dropwise. After 15 min at rt, the reaction mixture was concentrated under reduced pressure and the resulting ammonium trifluoroacetate was diluted in $CHCl_3$ (15 mL). A saturated aqueous solution of Na_2CO_3 (15 mL) was then added and the biphasic mixture was transferred to a separatory funnel and shaken vigorously. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered then concentrated *in vacuo* to give piperidine **31** (75 mg, 100%) as a viscous yellow oil; $[\alpha]_D^{20}$ −27.9 (*c* 1.12, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.28 (dd, J = 15.2 Hz and J = 11.0 Hz, 1H), 6.53 (m, 1H), 6.39–6.25 (m, 2H), 5.97–6.83 (m, 2H), 4.66 (t_{app}, J = 6.5 Hz, 2H), 4.59 (m, 0.5H), 4.19 (q, J = 7.1 Hz, 2H), 4.19–3.95 (m, 0.5H + 2H), 3.75 (m, 1H), 3.43 (m, 0.5H), 3.15–2.99 (m, 2 × 0.5H), 2.90 (m, 0.5H), 2.66–2.29 (m, 5H), 2.03 (m, 1H), 1.90–1.34 (m, 8H), 1.28 (t, J = 7.1 Hz, 3H), 1.30–1.05 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, rotamers): δ: 173.2 and 172.8 (s, 1C), 171.6 (s), 166.9 (s), 144.2 and 143.8 (d, 1C), 138.9 and 138.6 (d, 1C), 133.3 and 132.8 (d, 1C), 131.4 and 131.1 (d, 1C), 130.8 and 130.4 (d, 1C), 122.2 and 121.9 (d, 1C), 64.6 and 64.3 (t, 1C), 60.4 (t), 47.2, 46.3 (t, 2C), 45.9 (t), 43.5 (t), 41.8 and 41.2 (d, 1C), 35.6 (d), 32.8 and 32.2 (d, 1C), 30.3 (t, 2C), 30.2 and 29.7 (t, 1C), 27.4 (t), 25.0 and 23.8 (t, 1C), 14.3 (q).

(*E*)-1-Iodonon-1-ene (33). A solution of DIBAL-H (313 mg, 2.2 mmol, 1.1 equiv) in anhydrous THF (1 mL) was slowly added to a stirred solution of Cp_2ZrCl_2 (643 mg, 2.2 mmol, 1.1 equiv) in anhydrous THF (5 mL) at 0 °C under argon. After stirring the resulting white suspension for 30 min at 0 °C, a solution of non-1-yne **32** (248 mg, 2.0 mmol, 1.0 equiv) in THF (1 mL) was added dropwise. The reaction mixture was allowed to warm to rt and stirred until the obtention of a homogeneous solution (*ca* 45 min), and was then cooled to −78 °C. A solution of I_2 (660 mg, 2.6 mmol, 1.3 equiv) in THF (3 mL) was added dropwise to the previous solution, and after 30 min at −78 °C, the reaction mixture was hydrolyzed with an aqueous solution of 1 M HCl (5 mL). The aqueous layer was extracted with Et_2O (3 × 20 mL), and the combined organic phases were successively washed with a saturated aqueous solution of $NaHCO_3$ (20 mL), a saturated aqueous solution of $Na_2S_2O_3$ (20 mL), and brine (20 mL) followed by drying over $MgSO_4$, filtration and concentration *in vacuo*. Purification of the residue by flash chromatography on silica gel (hexanes: 100%) provided the desired (*E*)-vinyl iodide **33** (449 mg, 89%) as a colorless oil; IR (neat): 2954, 2921, 2852, 1679, 1605, 1458, 1377, 1210, 1196, 944,

722, 659 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.51 (dt, J = 14.3 Hz and J = 7.2 Hz, 1H), 5.97 (dt, J = 14.3 Hz and J = 1.4 Hz, 1H), 2.05 (m, 2H), 1.38 (m, 2H), 1.32–1.22 (m, 8H), 0.88 (br t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.8 (d), 74.3 (s), 36.1 (t), 31.8 (t), 29.2 (t), 29.0 (t), 28.4 (t), 22.7 (t), 14.1 (t); MS (EI, 70 eV) *m/z* (%): 253 (7), 252 (68), 167 (9), 166 (36), 154 (30), 83 (60), 70 (16), 69 (100), 67 (10), 57 (19), 56 (19), 55 (60), 54 (10), 53 (10).

Tributyl [(*E*)-non-1-enyl]stannane (34). *tert*-BuLi (2.56 mL, 1.7 M in pentane, 4.36 mmol, 2.2 equiv) was added dropwise to a stirred solution of (*E*)-1-iodonon-1-ene (**33**) (500 mg, 1.98 mmol, 1.0 equiv) in anhydrous Et_2O (5 mL) at −78 °C. After stirring at this temperature for 45 min, Bu_3SnCl (537 µL, 1.98 mmol, 1.0 equiv) was slowly added dropwise and the solution was allowed to warm to rt. After stirring for further 45 min, the reaction mixture was hydrolyzed with a saturated aqueous solution of NH_4Cl (15 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 15 mL) and the combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the crude residue by flash chromatography on silica gel (hexanes–Et_3N: 99 : 1) provided the desired alkenyl stannane **34** (779 mg, 95%) as a colorless oil, contaminated by unidentified stannylated impurities; IR (neat): 2954, 2920, 2844, 1599, 1463, 1376, 1071, 987, 960, 873, 863, 685, 659 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.95 (dt, J = 18.9 Hz and J = 5.9 Hz, 1H), 5.86 (d, J = 18.9 Hz, 1H), 2.13 (br q_{app}, J = 7.1 Hz, 2H), 1.54–1.45 (m, 4H), 1.36–1.26 (m, 12H), 0.92–0.86 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.9 (d), 126.9 (d), 37.9 (t), 31.9 (t), 30.8 (2t), 29.3 (t), 29.1 (3t), 27.3 (3t), 22.7 (t), 14.1 (q), 13.7 (3q), 9.4 (3t); HRMS (ESI): calcd for $C_{21}H_{45}Sn$ $[M + H]^+$: 415.2536; found: 415.2734.

(*E*)-3-Iodo-2-methylacrylaldehyde (37). MnO_2 (33 g, 384 mmol, 20 equiv) was added in one portion at rt to a stirred solution of (*E*)-3-iodo-2-methylprop-2-en-1-ol (**36**)[24] (3.80 g, 19.2 mmol, 1.0 equiv) in CH_2Cl_2 (120 mL). The resulting dark mixture was stirred vigorously for 48 h, then Celite® was added and the heterogeneous mixture was filtered through a plug of Celite® to remove the manganese salts. CH_2Cl_2 was removed under reduced pressure, thus affording the crude α,β-unsaturated aldehyde **37** (3.31 g, 87%) as a brown oil which was used in the next step without further purification.

(+)-*tert*-Butyl (*E*)-(*R*)-3-hydroxy-5-iodo-4-methylpent-4-enoate (38). Solid diacetone-D-glucose (6.1 g, 23.34 mmol, 2.0 equiv) was added in one portion at rt to a solution of $CpTiCl_3$ (2.56 g, 11.67 mmol, 1.0 equiv) in anhydrous Et_2O (80 mL). After stirring for 5 min, freshly distilled Et_3N (4.64 mL, 33.4 mmol, 2.85 equiv) dissolved in anhydrous Et_2O (40 mL) was added *via* cannula to the previous solution. The resulting thick slurry was stirred at rt for 15 h, and the triethylamine salts were removed by filtration under an argon atmosphere. The yellow 0.095 M stock solution in Et_2O of chloro(cyclopentadienyl)-bis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl)titanate thus obtained was then used to prepare the titanium enolate **IV**.

A solution of *tert*-BuOAc (403 µL, 2.99 mmol, 1.5 equiv) in Et_2O (5 mL) was added dropwise to a solution of freshly prepared LDA (2.99 mL, 1 M in THF–*n*-hexanes, 2.99 mmol, 1.5 equiv) in anhydrous Et_2O (40 mL) at −78 °C. After stirring for 30 min, a solution of the titanium reagent described above (42 mL, 0.095 M in Et_2O, 3.98 mmol, 2.0 equiv) was subsequently added dropwise *via* cannula over 20 min. After 30 min, the temperature was then slowly raised to −30 °C, and after stirring at this temperature for 45 min the solution containing titanium enolate **IV** was finally re-cooled to −78 °C. The previously obtained crude (*E*)-3-iodo-2-methylacrylaldehyde (**37**) (390 mg, 1.99 mmol, 1.0 equiv) was azeotroped three times with toluene, then dissolved in anhydrous Et_2O (15 mL) and subsequently added dropwise within 10 min to the previous solution. After stirring at −78 °C for 1 h, the reaction was quenched by addition of a THF–H_2O solution (1 : 1, 60 mL) and the mixture was stirred for 1 h. The resulting white slurry was filtered through a short pad of Celite® in order to remove the solid titanium salts, and the filtrate was washed with brine (80 mL). The aqueous layers were extracted with Et_2O (3 × 80 mL) and the combined organic phases where dried over $MgSO_4$, filtered and evaporated to dryness under reduced pressure. The resulting crude residue was purified by flash chromatography on silica gel (Petroleum ether–EtOAc: 95 : 5) to yield compound **38** (441 mg, 71%) as a colorless oil; $[\alpha]_D^{20}$ +15.5 (*c* 1.11, $CHCl_3$); IR (neat): 3401, 2977, 2917, 1704, 1367, 1248, 1149, 1038, 1018, 954, 840 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.39 ($quint_{app}$, J = 1.1 Hz, 1H), 4.52 (m, 1H), 3.24 (br d, J = 3.7 Hz, 1H), 2.54–2.43 (m, 2H), 1.83 (d, J = 1.1 Hz, 3H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4 (s), 147.7 (s), 81.8 (d), 79.1 (s), 72.5 (d), 40.8 (t), 28.1 (q, 3C), 20.5 (q).

(4*E*,6*E*)-(*R*)-3-Hydroxy-4-methyltetradeca-4,6-dienoic acid (39). $PdCl_2(MeCN)_2$ (13 mg, 0.049 mmol, 0.05 equiv) was added to a stirred solution of vinyl iodide (*R*)-**38** (305 mg, 0.98 µmol, 1.0 equiv) and vinyl stannane **34** (811 mg, 1.95 mmol, 2.0 equiv) in anhydrous and degassed DMF (10 mL) at rt. After 12 h of stirring at rt, the resulting dark reaction mixture was diluted with Et_2O (20 mL) and poured into a saturated aqueous NH_4Cl solution (15 mL). The aqueous layer was extracted with Et_2O (3 × 20 mL) and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (Petroleum ether–EtOAc: 95 : 5 to 90 : 10) provided (+)-*tert*-butyl (4*E*,6*E*)-(*R*)-3-hydroxy-4-methyltetradeca-4,6-dienoate (161 mg, 53%) as a yellow oil; $[\alpha]_D^{20}$ +1.2 (*c* 0.95, $CHCl_3$); IR (neat): 3420, 2923, 2855, 1719, 1457, 1368, 1250, 1150, 1063, 841, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.21 (br dd, J = 15.0 Hz and J = 10.8 Hz, 1H), 6.05 (d, J = 10.8 Hz, 1H), 5.69 (dt, J = 14.9 Hz and J = 7.0 Hz, 1H), 4.41 (br dd, J = 7.8 Hz and J = 4.6 Hz, 1H), 2.95 (br s, 1H), 2.52–2.41 (m, 2H), 2.09 (q_{app}, J = 7.2 Hz, 2H), 1.73 (s, 3H), 1.45 (s, 9H), 1.37 (m, 2H), 1.32–1.24 (m, 8H), 0.87 (t, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.0 (s), 135.8 (d), 135.0 (s), 125.8 (d), 125.7 (d), 81.3 (s), 73.3 (d), 41.1 (t), 33.0 (t), 31.8 (t), 29.4 (t), 29.2 (t, 2C), 28.1 (t, 3C), 22.7 (t), 14.1 (q), 12.5 (q); HRMS (ESI): calcd for $C_{19}H_{34}O_3Na$ $[M + Na]^+$: 333.2400; found: 333.2405.

Solid NaOH (57 mg, 1.72 mmol, 5.0 equiv) was added in one portion to a stirred solution of *tert*-butyl (4*E*,6*E*)-(*R*)-3-hydroxy-

4-methyltetradeca-4,6-dienoate (89 mg, 0.287 mmol, 1.0 equiv) in a MeOH–H_2O mixture (2 : 1, 6 mL) at rt. The resulting mixture was heated to 70 °C. After 2 h of stirring, the reaction was allowed to cool to rt, and then concentrated under reduced pressure to remove MeOH. EtOAc (15 mL) was added and the resulting solution was acidified with a saturated aqueous solution of NaH_2PO_4 (pH = 4.5, 10 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to provide the corresponding crude carboxylic acid **39** (72 mg, 99%) as a viscous yellow oil which was used in the next step without further purification; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6,22 (dd, J = 14.8 Hz and J = 10.8 Hz, 1H), 6.08 (d, J = 10.8 Hz, 1H), 5.69 (dt, J = 14.8 Hz and J = 7.0 Hz, 1H), 4.50 (m, 1H), 2.68–2.55 (m, 2H), 2.11 (q_{app}, J = 7.0 Hz, 2H), 1.76 (s, 3H), 1.43–1.34 (m, 2H), 1.34–1.19 (m, 8H), 0.88 (t, J = 6.8 Hz, 3H).

2 Biological tests: materials and methods

The transactivation activity of the four newly synthesized compounds was evaluated against the three human PPAR subtypes. The biological tests were achieved on Cos-7 cells obtained from ATCC (CRL-1651). Cells were maintained in standard culture conditions (Dulbecco's modified Eagle's minimal essential medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air) and the medium was changed every two days.[20]

The transient transfection assays were realized in the following way: Cos-7 cells were seeded in 60 mm dishes at a density of 5.5×10^5 cells per dish in DMEM supplemented with 10% FCS and incubated at 37 °C for 16 h prior to transfection. Cells were transfected in DMEM, using jetPEI, with reporter (pG5-TK-pGL3) and expression plasmids (pGal4-h or (h)PPARα, γ or β/δ). The pCMV-β-galactosidase expression plasmid was co-transfected as a control for transfection efficiency. After 16 h, transfection was stopped by addition of DMEM supplemented with 10% FCS and cells were then trypsinized and seeded in 96-well plates and incubated for 6 h in DMEM containing 10% FCS. Cells were then incubated 24 h in DMEM containing 0.2% FCS and increasing concentrations of the compound tested or vehicle (DMSO). At the end of the experiment, cells were washed once with ice-cold PBS, lysed and the luciferase and the β-galactosidase assays were performed.

Two protocols were used for pharmacological compound evaluation.

The first one consisted of directly evaluating the compound effect on each PPAR subtype with a concentration effect experiment from 0.001 μM to 3 μM. This protocol allowed to detect agonist activity on the three different PPAR subtypes.

The second one studied the potential for each compound to modulate the reference compound response with the aim to evaluate the capacity to increase or decrease those responses by indirect effect. In this protocol, reference compound was used at a concentration which gives about 50% of the maximal response and compounds were used in a dose effect from 0.001 to 3 μM. The direct and indirect biological effects of compounds on human PPARγ, human PPARα and human PPARδ/β were achieved.

Compounds were dissolved in DMSO at 10 mM. Maximal DMSO concentration used in these experiments was equal to 0.2% and no effect on PPAR subtype transactivation was observed with 2% DMSO percentage in final concentration.

Data were presented as means ± S.E.M of triplicate measurement.

All variations were compared using student test with values of $p \leq 0.05$ deemed significant *versus* control.

EC_{50} value means the effective concentration for 50% response of a given compound intrinsic maximum response. EC_{50} was calculated with Prism software.

Maximal effect of the compound (E_{max}) was expressed as a percentage of reference compound response (E100%).

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