Stereoselective Synthesis of the Isomers of Notoincisol A: Assignment of the Absolute Configuration of this Natural Product and Biological Evaluation

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ABSTRACT: The total syntheses of all stereoisomers of notoincisol A, a recently isolated natural product with potential anti-inflammatory activity, are reported. The asymmetric synthesis was accomplished employing a lipase-mediated kinetic resolution, which enables easy access to all required chiral building blocks with the aim of establishing the absolute configuration of the naturally occurring isomer. This was achieved by comparison of optical properties of the isolated compound with the synthetic derivatives obtained. Moreover, an assessment of the biological activity on PPARγ (peroxisome proliferator-activated receptor gamma) as a prominent receptor related to inflammation is reported. Only the natural isomer was found to activate the PPARγ receptor, and this phenomenon could be explained based on molecular docking studies. In addition, the pharmacological profiles of the isomers were determined using the GABA_A (gamma-aminobutyric acid A) ion channel receptor as a representative target for allosteric modulation related to diverse CNS activities. These compounds were found to be weak allosteric modulators of the α1β3 and α1β2γ2 receptor subtypes.

Notoincisol A (1) is a natural product recently isolated from the roots and rhizomes of Notopterygium incisum Ting ex H.T. Chang (Umbelliferae).1 The dried roots of N. incisum, known as “Qiang Huo”, have been used in traditional Chinese medicine as a treatment against the common cold and inflammatory diseases such as rheumatoid arthritis, as well as a diaphoretic, antifebrile agent, and analgesic.2 Compound 1 belongs to the class of polyeneynes, featuring adjacent triple-bond systems in immediate or close proximity to olefinic systems (or functional groups derived thereof) as the main structural motif within their molecular architecture. One of the most studied polyeneyne compounds is falcariindiol (2) (Figure 1), which is abundant in several vegetables such as celery or parsley and responsible for the bitterness of carrots. Some polyeneynes are highly toxic, as for instance cicutoxin (3) or oenanthotoxin (4) (Figure 1). However, several studies have demonstrated possible positive biological effects of certain polyeneynes, as for instance some of the compounds possess antifungal, anti-inflammatory, or cytotoxic properties.3 It was, therefore, hypothesized that polyeneynes can be classified as toxicants, compounds being toxic at higher doses, but showing beneficial effects at lower concentrations.3–7

Within a multidisciplinary research program8 aimed at the identification of novel natural products displaying anti-inflammatory activity,9,10 we became interested in the synthesis of polyeneynes such as 1 to confirm the absolute configuration of this natural product as well as to investigate the prospects of further developing this structural lead. Notoincisol A (1) has been shown to possess PPARγ-agonistic activity, with an EC_{50} of 2.3 μM and a maximum fold activation of E_{max} = 2.8. PPARγ is a nuclear receptor that acts as a transcription factor regulating target genes of lipid metabolism and inflammation. Upon ligand binding and activation, PPARγ forms a heterodimer with RXR (retinoid X receptor) and subsequently

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recruits coactivators, necessary for its transactivation activity. PPARγ was recognized as a potential anti-inflammatory target in 1998, when it was shown that activation of the receptor leads to the inhibition of NF-κB (nuclear factor “kappa-light-chain-enhancer” of activated B cells), a transcription factor regulating the expression of pro-inflammatory target genes.\(^1\)\(^{11−13}\)

It was considered reasonable to believe that the biosynthesis of 1 proceeds via falcarindiol (2), and therefore its absolute configuration could be deduced; however, only the relative stereochemistry of the natural product was confirmed by means of 2D NMR techniques.\(^1\) This prompted the preparation of all possible stereoisomers and the comparison of the physical data of the synthetic and isolated compounds to be performed. Moreover, this approach also enabled a biological assessment of the synthetic isomers.

It has been shown previously that the neurotoxicity of cicutoxin (3) and oenanthotoxin (4) originates from an antagonistic effect of these compounds on GABA\(_\text{A}\) receptors.\(^1\)\(^{14}\) In contrast, several other polyeneynes (e.g., falcarindiol and falcarinol) have been described as positive modulators of these ion channels.\(^1\)\(^{15,16}\) This interesting diversity in the biological behavior of the members of the same compound class prompted also an investigation of the modulatory action of notoincisol A (1) and its synthesized isomers on two GABA\(_\text{A}\) receptor subtypes, in line with previous studies of natural-product-derived molecules on this target family.\(^1\)\(^{17−21}\) Moreover, the lipophilic nature of the polyeneynes raised the question as to whether the mode of action of these compounds can be the same as that of other fatty-acid-derived lipophilic molecules, such as 2-AG (2-arachidonglycerol) or NA-glycine (N-arachidonylglycine). These endogenous ligands of the cannabinoid system are known to enhance the GABA-induced current of receptors containing a β2 subunit.\(^2\)\(^2\)

The GABA\(_\text{A}\) receptor is a ligand-gated chloride ion channel. Its endogenous ligand, γ-aminobutyric acid (GABA), is the main inhibitory neurotransmitter in the CNS. Upon binding of GABA, chloride and bicarbonate ions can pass the neuronal cell membrane. Consequently, hyperpolarization takes place, thus reducing the likelihood that an action potential will occur. Structurally, the GABA\(_\text{A}\) receptor is a member of the Cys-loop pentameric LGIC superfamily. In mammals, 19 different subunits are known to exist. The subunit composition of the GABA\(_\text{A}\) receptor plays a crucial role in the pharmacological effect of the receptor. It has been demonstrated that different GABA\(_\text{A}\) subtypes are responsible for various pharmacological actions.\(^2\)\(^3,2^4\)

A retrosynthetic analysis of notoincisol A (1) suggested that proper functional decoration (employing ferulic acid derivatives) of a falcarindiol analogue as a precursor could be used to exploit previous synthetic approaches to such structures.\(^2\)\(^5−2^9\)

The key connection of two alkyne synths was planned via copper-catalyzed Cadiot–Chokiewicz coupling\(^2\)\(^0\) of two alkyne units. Selectivity of the esterification of the alcohol at position C-8 was ensured by protection of the alcohol moiety of the “short” alkyne prior to the Cadiot–Chokiewicz reaction (Scheme 1).

Aiming at the preparation of all four stereoisomers, lipase-mediated kinetic resolution was considered as a beneficial method for the stereochemical discrimination of the racemic synths. Both S- and R-enantiomers can be obtained from corresponding racemic synths in a single operation. All possible combinations of the short-chain with the long-chain synthon led to four stereoisomers of the falcarindiol (2) backbone (Scheme 2).

Synthesis of the short Cadiot–Chokiewicz coupling partners began with the preparation of alcohol rac-6 by addition of trimethylsilyl (TMS)-acetylene to acrolein in 89% yield. Subjecting rac-6 to Amano lipase PS\(^2\)\(^6\) and chromatographic separation led to the isolation of the enantiomerically enriched ester R-7 in 48% yield and alcohol S-6 in 33% yield. The optical purity of the synths was controlled by chiral HPLC. A method for resolving the racemic acetate rac-7 was established, and the optical purity control of acetate R-7 could be carried out directly. Despite an extended effort to identify conditions for resolving the racemic alcohol rac-6 (for the control of the optical purity of alcohol S-6), this was not successful. Therefore, alcohol S-6 initially was chemically acetylated and subsequently subjected to chiral HPLC analysis.

Scheme 1. Retrosynthetic Analysis of Notoincisol A (1)

Figure 1. Notoincisol A (1) and related natural products (2−4) from the polyeneyne class.

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as the corresponding acetate \( S - 7 \). It was determined that acetate \( R - 7 \) and alcohol \( S - 6 \) were formed with ee’s of >99% in both cases. The absolute configuration of the building blocks was determined by comparison of optical rotations of the synthesized compounds with the known values from the literature (see Experimental Section). It was confirmed that the \( R \)-enantiomer of \( 6 \) undergoes lipase-mediated acetylation, whereas the \( S \)-enantiomer remained intact (Scheme 3).

Subjecting ester \( R - 7 \) to basic hydrolysis led to the cleavage of both the TMS and acyl groups and furnished alcohol \( R - 8 \) in 82% yield. When followed by tert-butyl(dimethyl)silyl (TBS) protection, this yielded primary alkyne \( R - 10 \) in 91% yield. Alcohol \( S - 6 \) was further protected with a TBS group, affording compound \( S - 9 \) in 93% yield, and subsequent hydrolysis of the TMS group provided primary alkyne \( S - 10 \) in a 78% yield (Scheme 4).

The synthesis of \( rac - 13 \) started on the reduction of commercially available dec-2-ynol (11). In contrast to previous reports in the literature,27,28 hydrogenation toward Z-allylic alcohol employing Lindlar’s catalyst failed in our hands due to over-reduction and poor reproducibility (see Supporting Information). This was solved by changing to a nickel P-2 catalyst,31 providing exclusively the \( Z \) alkene in a quantitative fashion without further purification. Subsequent oxidation of the allylic alcohol to the corresponding enal \( 12 \) required a careful choice of reaction conditions in order to avoid isomerization of the double bond (see Supporting Information). Due to the limited stability of the intermediate, the crude material obtained was employed immediately in the subsequent addition of the TMS-acetylene. Racemic alcohol \( rac - 13 \) was finally isolated in 60% yield over three steps.

\( Rac - 13 \) has not been reported as a substrate for a lipase-mediated kinetic resolution to date. Amano lipase PS turned out to accept \( rac - 13 \), but the reaction proceeded significantly slower than in the case of \( rac - 6 \). Nevertheless, after 36 h ester \( R - 14 \) was isolated in 48% yield and the remaining alcohol \( S - 15 \) in 45% yield (Scheme 5).

Analysis of the optical purity was carried out in a similar manner to that for the resolution of the alcohol \( rac - 6 \). Acetate \( R - 14 \) was analyzed directly, whereas alcohol \( S - 15 \) was acetylated prior to the analysis. Similar to the previous case, kinetic resolution proceeded with a high level of selectivity, and
both synthons were isolated with ee’s of >99% (Table 1). Determination of the absolute configuration was carried out by means of comparison of optical rotation values. It was determined that the R-enantiomer of 13 undergoes acetylation, while the S-enantiomer remains intact. Additional control experiments for the absolute chemistry determination were carried out (see Supporting Information).

Both compounds R-14 and S-15 were subjected to basic hydrolysis, yielding the enantiomerically enriched primary alkynes R-16 and S-16 in 85% and 81% yields, respectively. Bromination of the triple bonds furnished the corresponding bromoalkynes R-17 and S-17 in 81% and 77% yields, respectively (Scheme 6).

In the next step, all possible combinations of alkyne bromides and the primary alkyne were coupled within the Cadiot–Chodkiewicz reaction, furnishing four stereoisomers (20) in an average yield of 65%. Subsequent esterification with TBS-protected ferulic acid yielded protected stereoisomers of notoincisol A (21) (Scheme 7).

Finally, global deprotection using HF-pyridine gave notoincisol A (1) as well as all the stereoisomers in yields between 75% and 83% (Scheme 5 and Table 1).

Comparison of the optical rotation of the natural product ([α]_D^20 +85.5) and synthetic compounds revealed that the absolute configuration of the natural product is 3R,8S, as the value of the synthetic product matched [α]_D^20 +87.7. The value measured for the enantiomer, 3S,8R-notoincisol A, was [α]_D^20 −85.9, and those for the diastereomers 3S,8S-notoincisol A and 3R,8R-notoincisol A were [α]_D^20 +134.1 and −139.8, respectively (Scheme 5).

All synthesized compounds were investigated for their agonistic activity on the PPARγ receptor in a luciferase-based transactivation model in HEK293 cells, as described before.32 Interestingly, all of the unnatural isomers were found to be inactive up to a concentration of 3 μM. Higher concentrations were not tested since the unnatural isomers appeared to be cytotoxic at a concentration of 10 μM, which was shown by a resazurin conversion assay (Supporting Information). The natural isomer 3R,8S-1 activated the PPARγ receptor with an EC_{50} of 1.19 μM and a maximal fold activation of E_{max} = 3.38, which is in reasonable agreement with data obtained from the natural product isolate (EC_{50} 2.3 μM, E_{max} = 2.8).1 As a positive control, the PPARγ agonist pioglitazone was used.

Since only compound 3R,8S-1 displayed PPARγ-activating properties, a molecular docking study was conducted to rationalize this observation. All four notoincosol isomers were docked into the X-ray crystal structure of human PPARγ initially complexed with magnolol (PDB entry 3r5n)15 and fitted into the binding site without producing steric clashes. However, the inactive isomers were poorly anchored in the binding site with only one hydrogen bond or not fitted into all three arms of the pocket. Compound 3R,8S-1 was the only

Table 1. Optimization of the Reaction Time for the Kinetic Resolution of rac-14

| time (h) | yield alcohol (%) | ee alcohol (%) | yield acetate (%) | ee acetate (%) |
|---------|------------------|---------------|------------------|--------------|
| 12      | 48               | >99           | 30               | 50           |
| 24      | 50               | >99           | 42               | 88           |
| 36      | 45               | >99           | 45               | >99          |

"Reaction conditions: (a) Ni(OAc)_2·4H_2O, NaBH_4, (CH_2NH_2)_2, MeOH, rt, 3 h; (b) IBX, DMSO/CH_2Cl_2, rt, 2 h; (c) TMS acetylene, n-BuLi, THF, −78 °C to rt, 2.5 h, 60% over three steps; (d) amano lipase PS, MTBE, vinyl acetate, rt, 36 h, 48%, ee > 99% for R-14, 45%, ee > 99% for S-15.

Scheme 5. Synthesis of Enantioenriched Alkynes R-14 and S-15

aK_2CO_3, MeOH, rt, 2 h, 85% for R-16, 81% for S-16; (b) AgNO_3, NBS, acetone, 2 h, rt, 81% for R-17, 77% for S-17.

Scheme 6. Synthesis of Enantioenriched Alkynes Bromides R-17 and S-17

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compound that was tightly anchored by a bifurcated hydrogen bond with Ser289 and Tyr327, filling all three binding site arms, respectively (Figure 2).

We next evaluated the synthetic compounds on two GABA<sub>A</sub> receptor subtypes, in order to assess whether they displayed similar activities to other members of polyeneyne class of natural compounds. All four notoincisol A isomers were investigated using the two electrode voltage (TEV) clamp method in *Xenopus laevis* oocytes, using a protocol described previously. First, we tested the most abundant CNS GABA<sub>A</sub> receptor-subtype α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub>. Moderate enhancement of GABA-elicited currents at GABA EC<sub>3-5</sub> was observed for all compounds starting at 1 μM, with effects reaching ∼4-fold potentiation of the GABA current at 10 μM (Supporting Information). This is consistent with an allosteric modulatory action of moderate potency of the compounds. In addition, all four notoincisol A isomers also triggered small GABA-independent currents if applied to the oocyte in the absence of GABA (Supporting Information). These observations inspired an investigation as to whether these polyene compounds display similar subtype selectivity to the endocannabinoid compounds 2-AG and NA-glycine, and so they were tested also in α<sub>1</sub>β<sub>3</sub> receptors with the same protocol. Interestingly, in these receptors, the currents were also potentiated by all four notoincisol A isomers, but the potentiated currents did not reach maximum strength during standard recording times, and not even at longer (120 s) recording times, while reaching saturation after 30 s for the α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub> receptor. A similarly slow current rise has been

![Scheme 7. Key Cadiot–Chodkiewitz Coupling of Enantioenriched Building Blocks and Esterification with TBS-Ferulic Acid](image)

“Reaction conditions: (a) TBSCI, imidazole, DMF, rt, 67%; (b) (i) TBSCI, imidazole, CH<sub>2</sub>Cl<sub>2</sub>, rt, (ii) THF/MeOH, H<sub>2</sub>O, K<sub>2</sub>CO₃, 89%, yield after two steps; (c) NH₂OH·HCl, EtNH₂, CuCl, H<sub>2</sub>O/MeOH, 0 °C to rt, 2 h; (d) TBS-ferulic acid, EDCI, DMAP.

![Scheme 8. Deprotection of TBS Groups](image)

“Reaction conditions: (a) HF·pyridine, THF, 0 °C to rt.

| Table 2. Yields of the Deprotection Products and Optical Rotations of the Synthetic Compounds |
|---------------------------------------------------------------|
| starting material | product | yield (%) | optical rotation (° 0.09, MeOH) |
|-------------------|---------|-----------|-------------------------------|
| 3R,8S-21          | 3R,8S-I | 83        | +87.7                         |
| 3R,8R-21          | 3R,8R-I | 78        | -139.8                        |
| 3S,8S-21          | 3S,8S-I | 76        | +143.1                        |
| 3S,8R-21          | 3S,8R-I | 75        | -85.9                         |

| Table 3. Effects of Compounds 3R,8S-1, 3R,8R-1, 3S,8S-1, and 3S,8R-1 on Human PPARγ-Mediated Luciferase Reporter Gene Transactivation |
|----------------------------------------------------------------------------------------------------------------------------------|
| compound | EC<sub>50</sub> (μM) | E<sub>max</sub> |
|-----------|----------------------|----------------|
| 3R,8S-1   | 1.2                  | 3.38 ± 0.26    |
| 3R,8R-1   | n.d.                 |                |
| 3S,8S-1   | n.d.                 |                |
| 3S,8R-1   | n.d.                 |                |
| pioglitazone | 0.10               | 9.15 ± 0.45    |

“n.d., not determined, due to lack of activity up to a concentration of 3 μM.

Figure 2. Predicted binding pose of compound 3R,8S-1 in the ligand binding site of PPARγ. The molecule is anchored via a bifurcated hydrogen bond with Tyr327 and Ser289 and forms numerous hydrophobic contacts with the adjacent amino acids (yellow). Red and green arrows represent hydrogen bond acceptors and donors, respectively. The binding site surface is colored by aggregated hydrophobicity (gray) and hydrophilicity (blue).
observed for NA-glycine in the α1β2γ2 subtype. The GABA-independent currents in this subtype were observed to be smaller compared to those seen in the α1β2γ2 receptor. Since β3-containing receptors were also potentiated, the mechanism by which the nootinicosil A isomers potentiate GABA receptors is different from either 2-AG or NA-glycine, which both show β2-type selectivity. Since effects on GABA receptors were of low potency, they are unlikely to be of physiological relevance and were not studied in more detail.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Unless noted otherwise, all reagents were purchased from commercial suppliers and used without further purification. Melting points were recorded on a Büchi B-545 melting point apparatus. HRMS were measured at a Shimadzu IT-TOF mass spectrometer with either the APCl or ESI ionization method. Specific rotation was measured on an Anton Paar MCR300 polarimeter at the specified conditions. 1H and 13C NMR spectra were recorded with a Bruker AC 200 (200 MHz) or a Bruker Avance 400 (400 MHz) spectrometer using CDCl3 as solvent unless otherwise noted. GC/MS spectra were measured on a ThermoFinnigan system: GC: Focus GC with a BGS column (I = 30 m, d = 0.25 mm, 0.25 μm film), MS: DSQ II with quadrupol (EI) Instrument I or Thermo Ion Trap ITQ 100: Trace Ultra with PTV with a BGS-5 column, MS: ion trap detector (EI and CI) Instrument II. For thin-layer chromatography (TLC), aluminum-backed silica gel 60 F254 column, MS: ion trap detector (EI and CI) Instrument II. For thin-layer chromatography using silica and petroleum ether/EtOAc (90:10), yielding 4.81 g, 35 mmol) and dry tetrahydrofuran (THF) (140 mL) were added via a septum. The reaction mixture was cooled to 0 °C, and low-temperature thermometer was evacuated, and the reaction mixture was stured for 30 min at 0 °C before water was added (30 mL) and the mixture was allowed to reach room temperature. After the reaction was quenched with water (30 mL), the layers were separated, and the aqueous phase was extracted with CH2Cl2 (2 × 30 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The mixture was concentrated under reduced pressure. The resulting residue was passed through a pad of silica, using CH2Cl2 as eluent. The solvent was removed under reduced pressure, affording R-10 in 91% yield as a colorless liquid. The spectroscopic data obtained agreed with literature values.45

(R)-tert-Butyldimethyl(5-((trimethylsil)]pent-1-en-4-yn-3-yl)oxy)silane (S-9). (S)-5-((Trimethylsil)]pent-1-en-4-yn-3-ol (S-6) (1.7 g, 11 mmol) and imidazole (1.65 g, 11 mmol) were dissolved in 90 mL of dry CH2Cl2. The mixture was cooled to 0 °C, and TBSCI (1.83 g, 12.4 mmol) was added. The ice bath was removed, and the resulting mixture was stirred at room temperature for 2 h. After this, the reaction was quenched with water (90 mL), the layers were separated, and the aqueous phase was extracted with CH2Cl2 (2 × 90 mL). The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. The mixture was concentrated under reduced pressure. The residue was passed through a pad of silica, using CH2Cl2 as eluent. The solvent was distilled off under reduced pressure, affording S-9 in 93% yield as a colorless liquid. The spectroscopic data obtained agreed with literature values.

Kinetic Resolution of Racemic 5-(Trimethylsilyl)pent-1-en-4-yn-3-ol (rac-6). A 250 mL three-neck round-bottom flask equipped with a septum and gas inlet, NaBH4 (2.98 g, 14.4 mmol) was dissolved in 47 mL of THF at 0 °C. After the reaction mixture was cooled to 0 °C, the mixture was allowed to warm to room temperature and was stirred for 15 min. Then, the atmosphere was exchanged for argon. Trimethylsilylecyclopentene (5 mL, 3.45 g, 35 mmol) and dry tetrahydrofuran (THF) (140 mL) were added via a septum. The reaction mixture was cooled to −78 °C, and n-Buli (21.9 mL, 1.6 M, 35 mmol) was added slowly. The reaction solution was stirred for 30 min at −78 °C and then acrolein was added in one portion. The mixture turned light blue. The cooling bath was removed, and the reaction solution was allowed to reach room temperature and was stirred for 2 h. When the reaction was completed (TLC), water was added (150 mL) and the mixture was extracted with ethyl acetate (3 × 150 mL). The combined organic layers were washed with brine and dried over sodium sulfate. Solvents were evaporated, and the residue was submitted to column chromatography using silica gel and petroleum ether/EtOAc (90:10), yielding 4.81 g (89%) of rac-6 as a colorless liquid. Spectroscopic data agreed with literature values.
literature. A 500 mL three-neck round-bottom flask equipped with a septum and inert gas inlet was charged with (Z)-dec-2-enol (5.7 g, 36.4 mmol), dry DMSO (16.6 mL), dry CH₂Cl₂ (78 mL), and 2-iodoacetylbenzoic acid (IBX) (1.5 equiv, 15.3 g, 54.7 mmol). The reaction mixture was stirred for 2 h at room temperature, and then 0.3 mL of DMSO was added every 10 min for 1 h. After the reaction was complete, the mixture was cooled to 0 °C; ice-precooled NaHCO₃ was added (80 mL) and the resulting mixture was stirred for 15 min. All the workup operations were carried out with precooled glassware and ice-bath-precooled chemicals. Solids were removed by filtration via a sintered-glass funnel under reduced pressure. The mixture was extracted with diethyl ether (100 mL) and washed successively with NaHCO₃ (100 mL) and brine (100 mL). The organic layer was dried over Na₂SO₄ and filtered, and volatiles were evaporated under reduced pressure at room temperature, yielding 12 as a yellow oil. The crude material was used immediately for the next step.

(Z)-1-(Trimethylsilyl)dodec-4-en-1-yn-3-ol (rac-13). TMS-acrylene (7.8 mL, 55.4 mmol) was charged into a predried 250 mL round-bottom flask, equipped with a low-temperature thermometer, inert gas inlet, and septum. The atmosphere was exchanged for argon, and dry THF (34 mL) was added. The reaction mixture was cooled to −78 °C, n-BuLi was added (1.6 M, 34.6 mL, 55.4 mmol), and the reaction mixture was stirred for 30 min at −78 °C. In a separate flask, crude (Z)-dec-2-ynal (12) obtained in the previous step was dissolved in dry THF (48 mL) under an inert atmosphere and transferred subsequently into the reaction mixture via a syringe. The mixture was allowed to warm to room temperature and was stirred for 3 h. After the reaction was finished, water (100 mL) was added, and the mixture was extracted with ethyl acetate (3 × 100 mL), washed with brine (100 mL), and filtered through a pad of silica. Compound rac-13 was isolated as a yellowish oil in 60% yield after two steps. Its spectroscopic data agreed with literature values.²⁴

Lipase Kinetic Resolution of Racemic Alcohol (rac-13). A 250 mL round-bottom flask was charged with racemic (Z)-1-(trimethylsilyl)dodec-4-en-1-yn-3-ol (rac-13) (5.5 g, 21.8 mmol), lipase PS (1.1 g, 20 w%), vinyl acetate (2.3 mL, 25.05 mmol), and MTBE (77 mL). The flask was sealed with a septum, and the reaction mixture was stirred for 36 h. After the reaction was finished, the mixture was filtered through a pad of Celite, and solvents were evaporated under vacuum. Column chromatography (silica gel, petroleum ether/EtOAc, 99:1) provided the desired products, R-14 and S-15.

(S,Z)-Dodec-4-en-1-yn-3-ol (S-16). A 100 mL round-bottom flask was charged with (S,Z)-1-(trimethylsilyl)dodec-4-en-1-yn-3-ol (S-15) (2.64 g, 11.2 mmol) and methanol (50 mL). Potassium carbonate (3.11 g, 22.4 mmol) was added, and the reaction mixture was stirred at room temperature overnight. After the reaction was finished, water (50 mL) was added. The solution was transferred into a separation funnel and was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine and dried over sodium sulfate, and volatiles were evaporated under reduced pressure. The mixture was dissolved in petroleum ether/EtOAc (9:1) and filtered through a pad of silica, using petroleum ether/EtOAc (9:1) as eluent. The solvents were evaporated under reduced pressure, yielding 1.64 g (81%) of a yellowish oil. The NMR data of the resulting material agreed with literature values.²⁴ Material was used as such for the next step.

(R,Z)-1-Bromododec-4-en-1-yn-3-ol (R-17). (R,Z)-Dodec-4-en-1-yn-3-ol (R-16) (700 mg, 3.88 mmol) was dissolved in acetonitrile (15 mL); then silver nitrate (49 mg, 0.29 mmol) and N-bromosuccinimide (759 mg, 4.27 mmol) were added, and the reaction mixture was stirred for 2 h at room temperature. After the reaction was finished, the solution was cooled to 0 °C, and 8 mL of water was added. The resulting mixture was stirred for 10 min and then extracted with diethyl ether (3 × 40 mL). The combined organic layers were washed with brine (20 mL) and dried over sodium sulfate, and the volatiles were evaporated under reduced pressure, yielding 814 mg (81%) of R-17 as a colorless oil. The resulting crude material was obtained with satisfactory purity, and it was used as such for the next step without further purification. Its spectroscopic data agreed with literature values.²⁵

(S,Z)-1-Bromododec-4-en-1-yn-3-ol (S-17). (S,Z)-Dodec-4-en-1-yn-3-ol (S-16) (700 mg, 3.88 mmol) was dissolved in acetonitrile (15 mL); then silver nitrate (49 mg, 0.29 mmol) and N-bromosuccinimide (759 mg, 4.27 mmol) were added, and the reaction mixture was stirred for 2 h at room temperature. After the reaction was finished, the solution was cooled to 0 °C, and 8 mL of water was added. The resulting mixture was stirred for 10 min and then extracted with diethyl ether (3 × 40 mL). The combined organic layers were washed with brine (20 mL) and dried over sodium sulfate, and the volatiles were evaporated under reduced pressure, yielding 774 mg (77%) of S-17 as a colorless oil. The resulting crude material was obtained with satisfactory purity, and it was used as such for the next step without further purification. Its spectroscopic data agreed with literature values.²⁶

Cadiot–Chodkiewicz Coupling: General Procedure. A wheaton vial was charged with hydroxyl amine hydrochloride (27.8 mg, 0.4 mmol), copper chloride (5 mg, 0.05 mmol), a 70% aqueous solution of ethylamine (1 mL), H₂O (0.33 mL), and MeOH (3.65 mL). The atmosphere was exchanged for argon, and the mixture cooled to 0 °C. (R)- or (S)-tert-Butylidimethylsilyl(pent-1-en-4-yn-3-yl)oxy)silane (10) (491 mg, 2.5 mmol) was dissolved in methanol (1.1 mL) and added to the catalytic system. Then, (S,Z)- or (R,Z)-1-bromododec-4-en-1-yn-3-ol (17) (295 mg, 1 mmol) was dissolved in 1.1 mL of methanol and added into the reaction mixture. The reaction mixture was stirred for 1 h at 0 °C, and, after the reaction was completed, it was quenched with saturated ammonium chloride (4 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine (20 mL) and dried over sodium sulfate, and solvents were evaporated under reduced pressure at room temperature. The product was purified by column chromatography using silica as a stationary phase and petroleum ether/EtOAc (95:5) as an eluent.

(E)-3-(4-(((tert-Butylidimethylsilyloxy)-3-methoxyphenyl) acrylic Acid (19). trans-Ferulic acid (18) (291 mg, 1.5 mmol) and imidazole (1 g, 15 mmol) were dissolved in 9 mL of dry dimethylformamide (DMF). The mixture was cooled to 0 °C, and TBSCI (1.83 g, 12.4 mmol) was added. The ice bath was removed, and the resulting mixture was stirred at room temperature overnight. Then, the reaction was quenched with water (10 mL), the layers were separated, and the aqueous phase was extracted with CH₂Cl₂ (2 × 10 mL). The combined organic layers were washed with brine (15 mL), dried over sodium sulfate, and filtered. The mixture was concentrated at reduced pressure. The resulting residue was passed through a pad of silica, using CH₂Cl₂ as an eluent. The solvent was distilled off under reduced pressure, affording 19 in 67% yield as a colorless solid. The spectroscopic data agreed with literature values.²⁷

(3R,8S,Z)-3-((tert-Butylidimethylsilyloxy)heptadeca-1,9-diene-4,6-diyne-8-ol (3R,8S,20). This was prepared according to the general procedure described above and obtained as a colorless oil in 66% (247 mg) yield: R = 0.31 (petroleum ether/EtOAc, 9:1); [α]_D⁰ = +208.2 (c. 1.0, CHCl₃); 1H NMR (200 MHz, CDCl₃) δ 5.94–5.78 (1H, m), 6.58–6.57 (2H, m), 5.43–5.33 (1H, dt, J = 17.0 Hz, 1.4 Hz), 5.22–5.14 (2H, m), 4.93 (1H, d, J = 4.9 Hz), 2.16–2.06 (2H, q, J = 6.8 Hz), 1.84 (1H, d, J = 5.3 Hz), 1.42–1.27 (10H, m), 0.91
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\[ (12H, s), 0.14 (3H, s), 0.12 (3H, s); ^{13}C \text{ NMR (50 MHz, CDCl}_3 \delta 136.8, 134.7, 127.9, 115.8, 79.5, 79.3, 69.4, 69.2, 64.2, 58.8, 31.9, 29.4, 29.3, 27.8, 25.9, 22.8, 18.4, 14.2, 4.5, 4.8; \text{ HRMS m}/z 397.2536 [\text{M} + \text{Na}] \text{ (calcd for C}_{16}\text{H}_{24}\text{O}_5\text{SiNa} 397.2539).} \]

**General Procedure for Steglich Esterification Using EDCI.** To a mixture of 3-methoxy-((tert-butylidimethylsilyloxy)oxy)acrylic acid ((52 mg, 0.17 mmol), DMAP (16 mg, 0.13 mmol), and 3-((tert-butylidimethylsilyloxy)oxy)heptadeca-1,9-diene-4,6-diyn-8-ol (20 (50 mg, 0.13 mmol) in dry CHCl\(_3\) (1.2 mL) was added EDCI-HCl (33 mg, 0.17 mmol) under argon pressure at 0 °C. The reaction vessel was sealed, and the mixture was allowed to warm to room temperature. After the reaction was completed (TLC), the mixture was cooled with an ice bath and 2 N HCl (1.2 mL) was added dropwise. The mixture was extracted with CHCl\(_3\) (3 × 2 mL), the combined organic mixtures were washed with brine (5 mL) and dried over sodium sulfate, and the solution was passed through a pad of Celite. Volatiles were evaporated at reduced pressure at room temperature. Products were purified by column chromatography using silica gel and petroleum ether/ EtOAc (95:5).

\[ (E)-(3R,8S,5)-3-((tert-butylidimethylsilyloxy)hexadeca-1,9-dien-4,6-diyn-8-yl)oxy)-3-methoxyphenyl)acrylate (3) \text{ was prepared according to the general procedure described above and isolated as a colorless oil in 81% (70 mg) yield: R} = 0.57 (petroleum ether/EtOAc, 9:1; \epsilon_20^{\text{nm}} = 87.7 (0.09, MeOH)). \]

\[ ^{1}H \text{ NMR (400 MHz, CDCl}_3 \delta 7.66−7.63 (1H, d, J = 15.9 Hz), 7.08−7.06 (1H, dd, J = 8.2 Hz, J = 1.8 Hz), 7.03−7.02 (1H, d, J = 1.8 Hz, 6.92−6.91 (1H, d, J = 8.2 Hz), 6.29−6.27 (2H, m), 5.96−5.90 (1H, ddd, J = 17.1 Hz, J = 10.2 Hz, J = 5.1 Hz, 5.89 (1H, s), 5.72−5.68 (1H, dt, J = 10.7 Hz, J = 7.5 Hz), 5.54 (1H, t, J = 9.7 Hz), 5.49−5.46 (1H, d, J = 17.1 Hz), 5.27−5.25 (1H, dt, J = 10.2 Hz, J = 1.1 Hz), 3.92 (3H, s), 4.94−4.93 (1H, t, J = 7.1 Hz), 2.11−2.17 (2H, q, J = 7.5 Hz), 1.97−1.96 (1H, d, J = 6.4 Hz), 1.43−1.35 (2H, m), 1.29−1.25 (8H, s), 0.87−0.85 (3H, s, J = 7.1 Hz).} \]

\[ ^{13}C \text{ NMR (50 MHz, CDCl}_3 \delta 165.8, 148.3, 146.9, 146.2, 136.7, 135.8, 126.9, 124.0, 123.5, 114.8, 114.6, 104.9, 78.5, 77.0, 74.0, 63.6, 60.1, 31.0, 29.3, 28.1, 22.8, 14.3; \text{ HRMS m}/z 459.2150 [\text{M} + \text{Na}] \text{ (calcd for C}_{22}\text{H}_{33}\text{O}_5\text{SiNa} 459.2147).} \]

**Molecular Docking.** The ligands were constructed using ChemDraw 15 Professional (PerkinElmer, Inc.) and a Pipeline Pilot 8 (Dassault Systèmes, Inc.) protocol translating .cdx into .sd files. Docking was performed with GOLD version 5.2 employing the ChemPLP scoring function. The X-ray crystal structure of human PPARβ/γ was a natural product and partial agonist of the receptor, was selected for the docking calculations. The protonation state of His323 was set to NE2, and the water molecule no. 35 was set to “toggle and spin”. This means that the docking algorithm can choose to turn, keep, or delete this water molecule depending on which setting gives the best docking results. For the docking, both magnolol ligands were removed from the binding site and used to define the binding site location. Docking settings were validated by redocking of magnolol into the receptor. Magnolol was docked at the location of either one or the other cocrystallized magnolol binding sites with an average RMSD of 0.823 Å. The docking poses of compounds (3)R,S-1, (3)R,S-1, 35R, and 35R-1 were analyzed using LigandScout 4.2.1 (InteLigand GmbH, Vienna, Austria).

**PPARβ/γ Reporter Gene Transactivation.** PPARβ/γ luciferase reporter gene transactivation experiments were performed using HEK293 cells (ATCC, Manassas, VA, USA). HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL benzylpenicillin, and 100 μg/mL streptomycin. Then, 6 × 10⁶ cells were seeded in 20 cm dishes, cultured for 18 h, and transfected with 6 μg of a full-length human PPARβ/γ expression plasmid, 6 μg of a firefly luciferase reporter plasmid containing a PPAR response element, and 3 μg of pEGFP-N1 (Clontech, Mountain View, CA, USA) as internal control. A 0.1 μM concentration of test compounds was used in 96-well plates (4× 10⁴ cells/well) in serum-free DMEM supplemented with 2 mM glutamine. Reseeded cells were treated with 0.1, 0.3, 1, 3, 10, and 30 μM of each test compound dissolved in DMSO and were then incubated for 18 h. After cell lysis, luciferase activity and EGFP fluorescence were quantified on a GeniosPro plate reader (Tecan, Grödig, Austria). The ratio of luminescence units to fluorescence units was calculated to account for differences in cell number or transfection efficiency. Results are expressed as fold induction compared to the solvent DMSO (0.1%). Pioglitazone (5 μM) was used as positive control.

Statistical analysis was performed using Prism software (ver. 4.03; GraphPad Software Inc., San Diego, CA, USA). To calculate the EC₅₀ values, data were curve fitted and nonlinear transformed using a sigmoidal dose response with variable slope.

**Resazurin Conversion Assay.** Cell viability was detected by employing a resazurin conversion assay. This method is based on the reduction of resorufin to resoruifin by redox equivalents resulting from cellular metabolism. The fluorescence signal of resoruifin produced in viable cells is proportional to the number of cells and can be used as a measure for cell viability or cytotoxicity. HEK293 cells were seeded in 96-well plates at a density of 4 × 10⁴ cells/well and grown overnight. Cells were then treated with each test compound (0.3, 1, 3, 10, and 30 μM) for 24 h and incubated for 4 h with resoruifin solution (10 μg/mL in PBS) at 37 °C. Fluorescence was quantified in a plate reader using a 535 nm excitation/590 nm emission filter set.
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(39) Rattanangkool, E.; Kittikhunnatham, P.; Damsud, T.; Wacharasindhu, S.; Phuwapraisirisan, P. Eur. J. Med. Chem. 2013, 66, 296−304.

(40) Cambridge Crystallographic Data Centre, UK; www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/.

(41) Jones, G.; Willett, P.; Glen, R. C. J. Mol. Biol. 1995, 245, 43−53.

(42) Jones, G.; Willett, P.; Glen, R. C. J. Mol. Biol. 1997, 267, 727−748.