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A stereo-controlled synthesis of 2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone and its PPAR activities

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ABSTRACT

A novel class of natural PPAR agonists, 2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone (**1**), were discovered in marine natural product libraries. The synthesis of **1** was accomplished starting from vinylmethyl ketone. Ring formation of the α,γ dialkyl γ-lactone was achieved via the stereo-controlled reaction of a ketyl radical anion with a chiral methacrylate. In the PPAR agonistic assay, the most potent of the four stereoisomers had EC_{50} values of 12 μM for mPPARα, 9 μM for mPPARδ and >100 μM for mPPARγ.

PPARs are transcriptional factors that belong to the ligand-activated nuclear receptor superfamily and consist of three isotypes: PPARα (NR1C1), PPARδ (NR1C2), and PPARγ (NR1C3). Upon activation by ligands, PPARs form heterodimers with the retinoid X receptor (RXR). The PPAR/RXR heterodimers bind to the peroxisome-proliferator response element (PPRE) present in the promoter regions of target genes and initiate the transcription of an array of genes involved in glucose and lipid homeostasis in vivo.[1]

PPARα is mainly expressed in the liver, muscles, and kidney. It regulates the expression of a number of enzymes that take part in lipid oxidation. The fibrates, well-known PPARα agonists, have exhibited therapeutic activities in the treatment of hypercholesterolemia and mixed dyslipidemia.[2] PPARγ, which is highly expressed in adipose tissue, is a drug target for the treatment of diabetes. The thiazolidinediones (TZDs), PPARγ agonists, are insulin-sensitizing chemicals because they increase the expression of genes involved in the homeostatic regulation of glucose.[3] PPARδ plays a key role in regulating lipid metabolism and energy homeostasis in muscle and adipose tissue. It also increases mitochondrial biogenesis and fast-to-slow muscle transformation.[4,5]

Based on high-throughput screening from marine natural product libraries, we tried to identify a novel class of PPAR agonists. Results from co-transfection assays using a PPRE-driven luciferase reporter gene led to the discovery of several compounds that are efficacious activators of PPARs.

Compound **1**, 2,4-dimethyl-4-hydroxy-16 phenyl hexadecanoic acid 1,4-lactone, showed activity for a transfected PPAR promoter (Fig. 1).[6]

We investigated the PPAR agonistic activity profile (subtype selectivity) of compound **1**, which was first isolated from the deep-water sponge *Plakortis nigra* by the Faulkner group in 2002.[7] However, due to its limited supply, we could not proceed further with our PPAR research. In this Letter, we report the stereo-controlled synthesis of four stereoisomers of compound **1** and the activity profile of this new class of PPAR agonists.

The nonstereoselective synthesis of compound **1** was performed in two steps from phenyldecyl bromide (Scheme 1). Ni^0-mediated conjugate addition of phenyldecyl bromide to vinylmethyl ketone in pyridine (80 °C, 5 h) produced ketone **2** in a 48% yield.[8] Through a ketyl radical anion reaction promoted by SmI_2, compound **1** was successfully synthesized from ketone **2** with methyl methacrylate

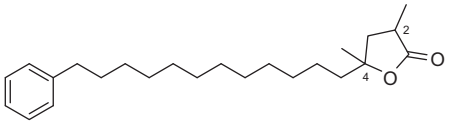

Figure 1. Planar structure of compound **1**.

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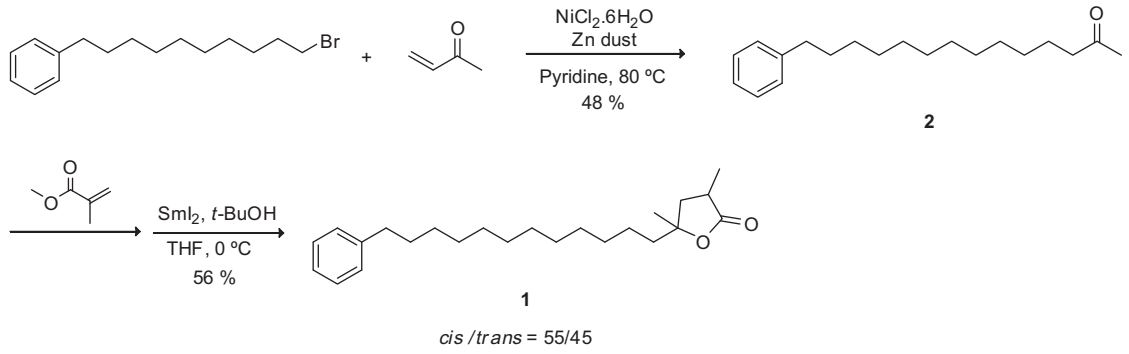


Scheme 1. Nonstereoselective synthesis of compound **1**.

in a 56% yield.[9] Because we did not use chiral auxiliaries for this synthesis, diastereomeric products were obtained (*cis*/*trans* = 55/45).[10]

The spectral data of synthesized compound **1** matched those reported for the natural product.[7] NOESY analysis confirmed that diastereomeric products of compound **1** have relative configurations of *cis* (2*R**,4*S**)-**1** and *trans* (2*S**,4*S**)-**1**. The signals at δ 2.19 and 1.68 in the ^{1}H NMR spectrum of *cis* (2*R**,4*S**)-**1** were assigned to H-3. The NOESY spectra of *cis* (2*R**,4*S**)-**1** showed strong correlations between the Me-24 signal at δ 1.35 and the H-3 signal at δ 2.19, between the H-2 signal at δ 2.80 and the H-3 signal at δ 2.19, and between Me-23 at δ 1.26 and the H-3 signal at δ 1.68, all supporting the *cis* product with an absolute chemistry of (2*R*,4*S*) or (2*S*,4*R*). In the ^{1}H NMR spectrum of *trans* (2*S**,4*S**)-**1**, the signals at δ 2.35 and 1.62 were assigned to H-3. The NOESY spectra of *trans* (2*S**,4*S**)-**1** showed strong correlations between the two methyl signals (δ 1.27 and 1.40) and the H-3 signal (δ 1.62) and a weak correlation between the H-2 signal at δ 2.80 and the H-3 signal at δ 2.35, all supporting the *trans* configuration with an absolute chemistry of (2*S*,4*S*) or (2*R*,4*R*). We then evaluated the PPAR agonistic activities of the *cis*/*trans* isomers (Table 1). Interestingly, all of the stereoisomers of compound **1** had agonistic activities for both mPPARα and mPPARδ, but exhibited no activity for mPPARγ.

The *cis* (2*R**,4*S**)-**1** and *trans* (2*S**,4*S**)-**1** enantiomers were further separated by chiral HPLC using a Chiralcel OJ-H column, resulting in a complete separation of the four diastereomers [**1a**, **1b**, (−)-**1c**, (+)- **1c**].[10,11] The recent synthesis of (2*R*,4*S*)-**1a** allowed us to conclude that the absolute configuration of **1b** is (2*S*,4*R*).[12] However, the stereochemistry of the two *trans* isomers could not be determined (Fig. 2).

To investigate the mPPAR agonistic activities of compound **1**, a co-transfection assay was performed on all four stereoisomers (Table 2). Structurally, compound **1** has essential pharmacophores for PPAR agonist activity (acidic head and hydrophobic tail groups), which are structurally consistent with all known PPAR agonists.[13] The activity pattern suggests the possibility that the γ-lactone moiety can be a core skeleton as a substitute for the carboxylic acid group of many PPAR agonists, suggesting the importance of the chiralities of the α- and γ-positions on the γ-lactone.

Table 1
In vitro PPAR activities of the stereoisomers of compound **1**

Compds	Transactivation EC_{50}[a] (μM)		
	mPPARα	mPPARδ	mPPARγ
cis (2*R**,4*S**)-**1**	24	22	ia
trans (2*S**,4*S**)-**1**	13	16	ia

[a] The compounds were tested for agonist activity on PPAR in transiently transfected CV-I cells. The EC_{50} value is the molar concentration of the test compound with 50% of the maximal reporter activity. 'ia' means inactive (no apparent activity) at a concentration of 100 μM.

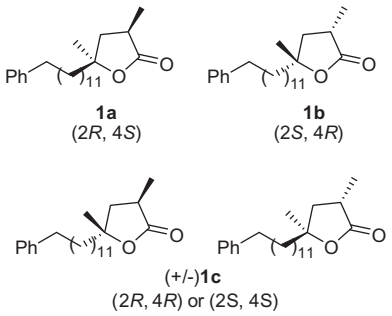


Figure 2. Absolute configurations of compound **1**.

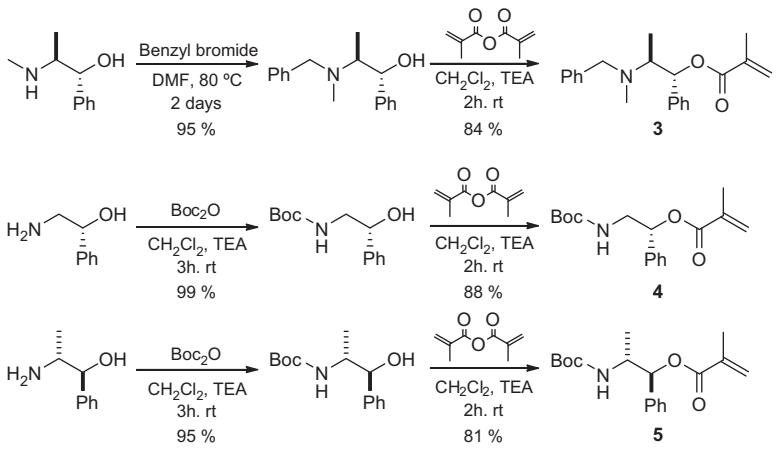
Table 2
In vitro PPAR activities of the four stereoisomers of **1**

Compds	Transactivation EC_{50}[a] (μM)		
	mPPARα	mPPARδ	mPPARγ
1a	20	22	ia
1b	39	34	ia
(−)-**1c**	12	9	ia
(+)-**1c**	25	24	ia

[a] The compounds were tested for agonist activity on PPAR in transiently transfected CV-I cells. The EC_{50} value is the molar concentration of the test compound with 50% of the maximal reporter activity. 'ia' means inactive (no apparent activity) at a concentration of 100 μM.

Next, we developed a method for the synthesis of compound **1** in high enantioselectivity. In 1997, Fukuzawa and co-workers reported a facile and effective method for the synthesis of optically active γ-butyrolactones.[14] α,β-Unsaturated esters bearing an ephedrine chiral auxiliary were reacted with aldehydes or ketones in the presence of SmI_2 to give γ-butyrolactones with highly enantioselective control of the chirality at the γ-position. Chiral auxiliaries derived from carbohydrates were used for the enantioselective synthesis of α,γ-substituted γ-butyrolactones by the Lin group.[15] In 2004, Kerrigan and co-workers also showed that (1*R*,2*S*)-*N*-benzyl ephedrinyl acrylate controls the chirality of the γ-position in γ-butyrolactone, producing the *R*-configuration through reaction with various aldehydes (66–81% ee).[16]

We synthesized several *N*-benzyl ephedrinyl acrylates as chiral auxiliaries to evaluate their diastereoselectivity and enantioselectivity. Ephedrinyl auxiliaries **3**, **4** and **5** were easily synthesized from the corresponding starting materials in high yields (Scheme 2). Using *t*-butyl alcohol as a proton source, we investigated the reaction of these various methacrylates with ketone **2**. Specifically, a solution of ketone **2** in THF was slowly added to a solution of SmI_2 in THF at 0 °C. The reaction mixture was stirred at that temperature for 1 h and warmed to room temperature over a period of 5 h.



Scheme 2. Synthesis of chiral auxiliary-methacrylates.

Table 3
Synthesis of **1** using various chiral auxiliaries

Entry	Methacrylates	Diastereoselectivity[a]	Enantioselectivity[b]		Yield[c] (%)
		cis-**1**/*trans*-**1**	**1a**/**1b**	(−)/(+)-**1c**	
1	Methyl methacrylate	55/45	51/49	49/51	56
2	(1*R*,2*S*)-**3**	50/50	45/55	26/74	45
3	(1*R*)-**4**	50/50	70/30	27/73	60
4	(1*S*,2*R*)-**5**	49/51	28/72	81/19	54

[a] *trans* and *cis* configurations were confirmed by ^{1H}–^{1H} NOESY, and the ratio of *trans*/*cis* was determined by HPLC.
[b] Enantioselectivity was determined by HPLC analysis on a Chiralcel (OJ-H) column.
[c] Total isolated yields of *trans* and *cis* products.

In all cases, diastereoselectivity was not observed (Table 3). In terms of enantioselectivity, methyl methacrylate showed no effect, as demonstrated by the resulting racemic mixture (entry 1). On the other hand, when methacrylate (1*R*,2*S*)-**3** was used as a chiral auxiliary, (+)-**1c** predominated, while no enantioselectivity between **1a** and **1b** was achieved (entry 2). However, both **1a** and (+)-**1c** were the major products when using methacrylate (1*R*)-**4,** thus affecting the enantioselectivity of the *cis* product (**1a** and **1b**) despite the absence of substituents on its 2-position (entry 3). Based on these results, we expected that the *S*-configuration of the 1-position of the ephedrinyl auxiliary would produce the most active stereoisomer (entry 4) and concluded that the stereochemistry of each diastereomer was influenced by the configuration of the chiral auxiliary.

In summary, we found that the four diastereomers of compound **1** comprise a novel class of natural PPAR agonists. Their PPAR activities suggested that compound **1** could form a new class of PPARα/δ dual agonists. We also achieved the stereo-controlled synthesis of **1** starting from vinylmethyl ketone with enantioenrichment using chiral auxiliaries in a ketyl radical anion reaction.

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6. CV-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Cells were transfected with plasmid mixture containing β-galactosidase expression vector and TK-PPRE-luc vector by Superfect reagent (QIAGEN). Transfected cells were incubated with compounds (marine natural products). Luciferase activity was analyzed after incubation for 24 h at 37 °C.
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10. Data for *cis* (2*R**,4*S**)-**1**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15–7.29 (m, 5H), 2.80 (m, 1H), 2.59 (t, 2H, *J* = 7.7 Hz), 2.19 (dd, 1H, *J* = 9.1, 12.5 Hz), 1.68 (m, 1H), 1.64 (m, 4H), 1.35 (s, 3H), 1.26 (d, 3H, *J* = 7.6 Hz), 1.25–1.40 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.5, 143.2, 128.6, 128.4, 125.7, 84.4, 42.0, 41.9, 36.2, 35.2, 31.7, 30.0, 29.8, 29.7, 29.6, 29.5, 24.9, 23.9, 15.7; HRMS calcd for $C_{24}H_{38}O_2$ (M^+) 358.29; found 358.2870; **1a** $[\alpha]_D = -4.1$ (*c* 0.003, $CHCl_3$), **1b** $[\alpha]_D = +3.6$ (*c* 0.003, $CHCl_3$).
Data for *trans* (2*S**,4*S**)-**1**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15–7.29 (m, 5H), 2.80 (m, 1H), 2.59 (t, 2H, *J* = 7.7 Hz), 2.35 (dd, 1H, *J* = 9.3, 12.8 Hz), 1.62 (m, 1H), 1.59 (m, 4H), 1.40 (s, 3H), 1.27 (d, 3H, *J* = 7.2 Hz), 1.25–1.40 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.7, 143.2, 128.6, 128.4, 125.7, 84.6, 42.0, 40.5, 36.2, 35.7, 31.7, 30.1, 29.8, 29.7, 29.6, 29.5, 27.2, 24.3, 16.3; HRMS calcd for $C_{24}H_{38}O_2$ (M^+) 358.29; found 358.2869; (−)-**1c** $[\alpha]_D = -6.3$ (*c* 0.005, $CHCl_3$); (+)-**1c** $[\alpha]_D = +7.4$ (*c* 0.004, $CHCl_3$).
11. HPLC conditions: Chiralpak OJ-H (10 × 250 mm) eluent, hexane/isopropyl alcohol = 97/3; detection, UV at 209 nm. Retention time; **1a** (7.2 min), **1b** (11.1 min), (−)-**1c** (8.7 min) and (+)-**1c** (12.5 min).
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