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CHEMOENZYMATIC SYNTHESIS OF ENANTIOMERS OF A NEW RETINOID TO INVESTIGATE THE ROLE OF CHIRALITY IN THE BIOLOGICAL RESPONSE

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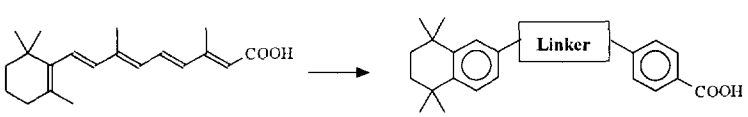
Abstract:

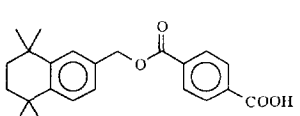
We have prepared both enantiomers of a new retinoid (**IIa** and **IIb**) partly by using lipase resolution of 1-acetoxy-1-(5, 6, 7, 8 - tetrahydro - 5, 5, 8, 8-tetramethyl-2-naphthyl) ethane. Absolute configuration of resulting secondary carbinols (**4a** and **4b**) was assigned using lanthanide induced shifts NMR experiments on MTPA esters of **4a** and **4b**. Only the enantiomer (R-(-)-**IIa**) is active on the differentiation of F9 embryonal teratocarcinoma cells (F9 cells).

Retinoids, natural and synthetic analogs of Vitamin A, play a fundamental role in cellular proliferation and differentiation (1). Retinoids are useful in the treatment of dermatological disorders, mainly acne and psoriasis (2). Moreover, these compounds appear to have potential for chemoprevention of cutaneous tumors (3) and all-trans retinoic acid (AtRA) was recently used in the treatment of promyelocytic leukaemia (4). However, therapeutic use of these compounds is associated with major side effects such as mucocutaneous irritation, hypervitaminosis A, and teratogenicity (2).

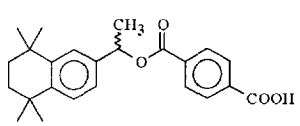
SCHEME 1



All-trans retinoic acid — **General structure of a series of aromatic retinoids**

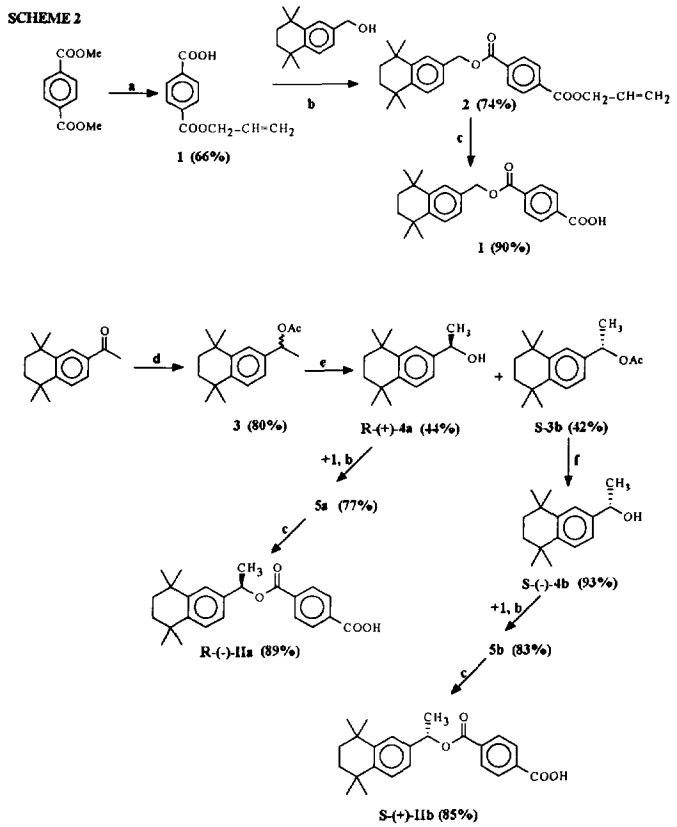


I



II

We present an investigation of the chirality role in the biological response of retinoids in order to better understand their structure-activity relationships. We report the synthesis, *in vitro* activity on the differentiation of F9 cells (5) and rate of hydrolysis in rat plasma (6) of three analogs belonging to a new series of retinoids (7). Starting from the structure of previously reported aromatic retinoids (8, 9), we synthesized analogs, especially through modification of the linker (Scheme 1). The parent compound for this study was analog **I** whose synthesis is depicted in scheme 2. Allylmonoterephthalate **1** was obtained *via* transesterification of methyl terephthalate, followed by monohydrolysis. Compound **1** was condensed with 2-TTNmethanol (10) using a DCC/DMAP coupling to give compound **2** which was then hydrolyzed into compound **I** (11). Compound **I** induced differentiation in F9 cells (Table 1). We further investigated this series by incorporating a chiral center in the linker. We first prepared compound **II** under its racemic form, starting from 2-TTN-methanol. Whereas racemic compound **II** was active in F9 cells bioassay, we then synthesized both enantiomers **IIa** and **IIb** of compound 4-[[1-(5,6,7,8,-tetrahydro-5,5,8,8,-tetramethyl-2-naphthyl)ethyloxy]carbonyl] benzoic acid (scheme 2). Compound 2-TTNmethylketone (8) was reduced and acetylated to give compound **3**. Resolution of enantiomers was obtained by enzymatic hydrolysis of the racemic acetate mixture. Hydrolysis was performed in a biphasic medium using lipase amano P30 (12) to give enantiomer **4a** (13). Enantiomer **4b** (13) was obtained by saponification of acetate **3b**. Enantiomeric purity was assessed with NMR in the presence of Eu (dcm)$_3$ (14).



Legend Scheme 2: (a) (i) NaH/CH_2=CH-CH_2OH; (ii) LiOH/THF; (b) DCC/DMAP/THF; (c) Pd [P(Ph)$_3$]$_4$/morpholine/THF; (d) (i) $NaBH_4$/EtOH; (ii) CH_3COCl/Pyr; (e) Phosphate buffer (0,3M/pH7)/$CHCl_3$/lipase Amano P30 / 40°C, 3 days; (f) NaOH/MeOH.

The synthesis was then followed as described for compound **I**, to give compounds **IIa** and **IIb** (15). According to the mild reaction conditions for the last two steps and optical rotation values, we speculated that enantiomeric purity was conserved. Compound **IIa** is active as inducer of differentiation, while compound **IIb** is inactive, as shown in table 1. We may conclude that chirality at this position is a major structural parameter to control the biological activity in these series. While introduction of this chiral center is discriminating for activity, it is of interest to know the orientation of substituents around this center. Accordingly, MTPA derivatives of **4a** and **4b** were synthesized (16) and were used in an ^1H NMR method of determination of absolute configuration of secondary carbinols in the presence of $Eu(fod)_3$ shift reagent (17). The relative magnitude of the lanthanide induced shift of the OMe signal *vs* molar ratio of $Eu(fod)_3$ for the (R)-(+)-MTPA esters of **4a** and **4b** permitted to assign a R absolute configuration for **4a**. The presence of an ester link in compounds **IIa** and **IIb** may render them cleavable by esterase activity. In order to investigate if biological activity was modified by hydrolysis of this ester bond, the sensitivity of **IIa** and **IIb** to esterolytic activity of rat plasma was assayed (6). The parent compound **I** is cleaved with a T1/2 of 2h30 (table 1). Introduction of the methyl group in the linker enhances T1/2 to 6h in the case of **IIa** (the biologically active enantiomer) whereas isomer **IIb** (biologically inactive enantiomer) is not cleaved under these conditions. This higher sensitivity of **IIa** *vs* **IIb** to plasma esterolytic enzymes is in agreement with a R configuration for **IIa** (18). Moreover, it supports the concept that differences in biological activities are related to the structure of the compounds rather than to deactivation of compounds by hydrolytic enzymes.

Table 1 - ACTIVITY ON THE DIFFERENTIATION AND STABILITY IN RAT PLASMA

COMPOUND	Plasminogen[a] Activator AC_{50} (nM)	Half life ($T_{1/2}$) [b] Rat Plasma
AtRA	200 ± 9	Not applicable
I	140 ± 6	2 h 30
II racemic	350 ± 30	> 6 h
II_a	300 ± 50	6 h
II_b	Not active	> 24 h

a) Cellular activity on the differentiation was quantified by measure of plasminogen activator in mouse embryonal teratocarcinoma F9 cells (5). Values are the mean ± SEM of three separate experiments.

b) Hydrolysis was measured using 100 % of rat plasma. We followed the general methodology previously described (6).

In conclusion, this paper describes a way to modulate the potency in the differentiating activity of retinoids through introduction of chirality at the center of the molecule. Moreover, in vivo studies on compounds **I** and **IIa** are in progress to investigate the pharmacomodulation related to the presence of a cleavable ester link in these molecules.

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10. Abbreviation "TTN" corresponds to the group 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl; 2-TTNmethanol was obtained through reduction of 2-TTNcarboxylic acid as reported in : Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K.; *J.Med.Chem.*1988, *31*, 2182.
11. All new compounds were fully characterized by IR, ^1H NMR spectroscopy, and mass spectrometry. Compound **I** : m.p.= 171-172°C; ^1H-NMR ($CDCl_3$, 250MHz), ppm : 1.29 (d, 12H), 1.70 (s, 4H); 5.34 (s, 2H), 7.22 (d, 1H), 7.26 (s, 1H), 7.36 (s,2H), 8.17 (s,4H); MS: m/z 367 $(M+H)^+$; Anal. (C_{23} $H_{26}O_4$) C, H.
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13. Isomer **4a**: colourless oil; ^{1}HNMR ($CDCl_3$, 250 MH_Z), ppm: 1.30 (d,12H), 1.48 (d, 3H), 1.70 (s,4H), 7.14 (dd, 1H), 7.28-7.30 (m, 2H); α_D= +27° (C=1, EtOH) MS: m/z 381 $(M+H)^+$ Isomer **4b** : α_D = -27.2° (C=1, EtOH); MS: m/z 381 $(M + H)^+$.
14. Europium complex Tris [d,d-dicampholylmethanato]-europium (III) was used at 20% in $CDCl_3$. Whereas no resonance signals for the second enantiomer were observed, enantiomeric purity was estimated as > 95%.
15. Isomer **IIa** : mp = 137.5°C; ^1H-NMR ($CDCl_3$-250 MHz), d, ppm : 1.30(d, 12H), 1.70 (m, 7H), 6.15 (m, 1H)-7,20 (d, 1H), 7.30-7.35 (m,2H), 8.15 (5, 4H); α_D= -53.2° (C=1, EtOH). Anal. ($C_{24}H_{28}O_4$) C,H. Isomer **IIb** : α_D = +52.1 (C=1, EtOH); Anal. ($C_{24}H_{28}O_4$) C, H.
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