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Synthesis and Biological Evaluation of New Leukotriene Analogs.

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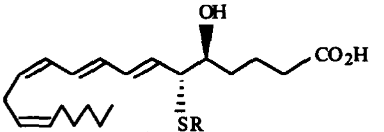
Abstract : New leukotriene analogs lacking the peptido chains in position 6 have been synthesized, some of them being potent and selective leukotriene biosynthesis inhibitors. Preliminary structure-activity relationships are reported.

The occurence of peptidoleukotrienes (LTC_4, LTD_4 and LTE_4) in inflammatory processes and related pathologies has been well-documented (1). Therefore, important studies in this field have been directed towards the search for biosynthesis inhibitors or antagonists (2). Numerous analogs have already been prepared based upon natural metabolites structures. It is interesting to point out that most modifications have kept the central β-hydroxy thioether structure. Principal modulations included thioether substituent nature (whether peptidic or not), chain lengths and the total number of double bonds, leading to very potent derivatives (2). On the other hand, only a few examples involving modifications of these functions have been reported : sulfoxides and sulfones analogs have been described and biological evaluation of the sulfones confirmed that they were almost as potent as corresponding leukotrienes (3). 5-Deoxy-LTD_4 was almost completely devoid of contractile activity, indicating the importance of C_5 hydroxy group in determining biological potency (4). For our part, we chose to prepare new analogs corresponding to general formula 2 and to study their biological properties.



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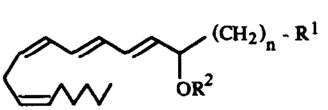


2

$R^1 = CH{=}N{-}OH, CH{=}N{-}OCH_3, CH_2CH_3$
$R^2 = H, CH_3 ; n = 2,3$

To the best of our knowledge, only one compound of this type ($R^2 = H$, $n = 4$, $R^1 = CO_2Me$) has been reported, without mention of its biological activity (4). We have synthesized the corresponding lactone, which was devoid of activity, as well as the sodium salt of the trinor acid ($R^2 = H$, $n = 1$, $R^1 = CO_2Na$) (5). However, compound

(±)-**8** ($R^2 = H$, $n = 3$, $R^1 = CH{=}N{-}OH$) has shown promising properties. The purpose of this Letter is thus to describe the syntheses of (±) **8** and related compounds ; we also report preliminary structure-activity relationships with special interest in the influence of the alcohol function, the chirality of the stereogenic center and the number of double bonds.

Synthesis

A flexible retrosynthetic approach, described in figure 2, was selected in order to use common intermediates for several compounds, the terminal functional group R^1 being introduced at the final step ; the lateral chain and the alcohol function were added in the same step, using a 3-C or 4-C synthon to modulate the chain length. Modifications of the lipophilic chain were introduced during the synthesis of type **3** aldehyde intermediates, using different phosphonium salts engaged in Wittig reactions with the difunctional olefin **4**.

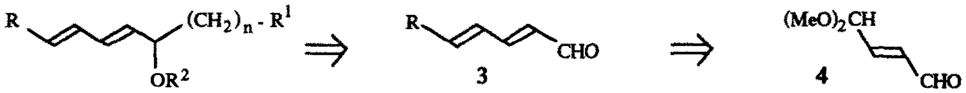
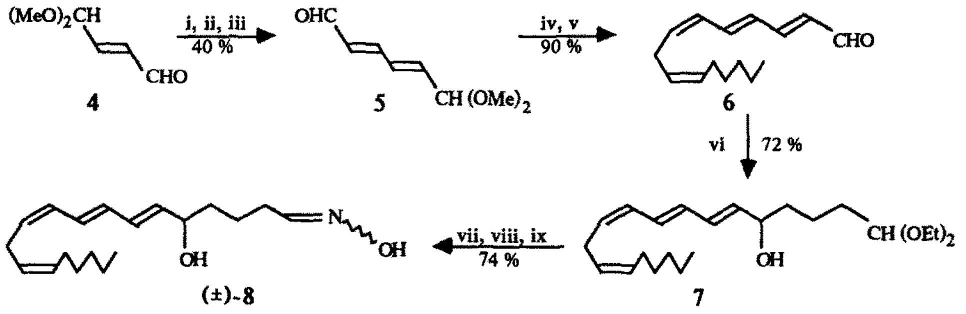


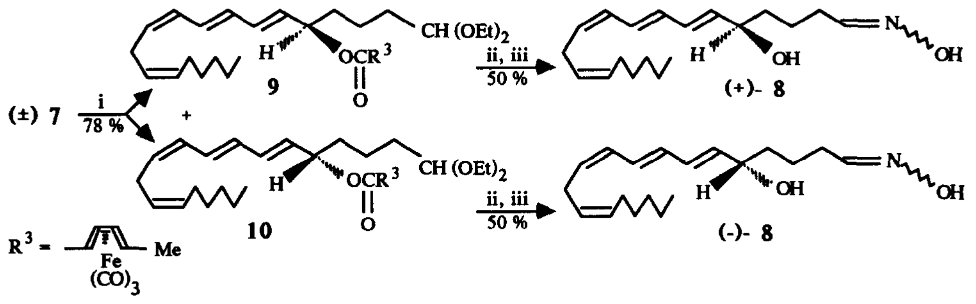
Figure 2

This compound, easily obtained from furan as previously reported (6), has been chosen as starting material. It was reacted with a phosphonate anion to yield a conjugated diene bearing an ester function. After reduction of the ester and oxidation of the resulting alcohol with Collins reagent, the corresponding aldehyde **5** was obtained. A Wittig reaction generated the tetraenic skeleton and Conia's deacetalisation (7), yielded compound **6**. The aldehyde was then reacted with the Grignard reagent derived from diethyl acetal of 4-bromobutanal, affording alcohol **7**. Having protected this alcohol as an acetate, the oxime was formed in one step, reacting the acetal with lithium tetrafluoroborate and hydroxylamine hydrochloride (8). Then, cleavage of the acetate in a basic medium yielded the desired compound (±)-**8** (19 % overall yield from **4**).



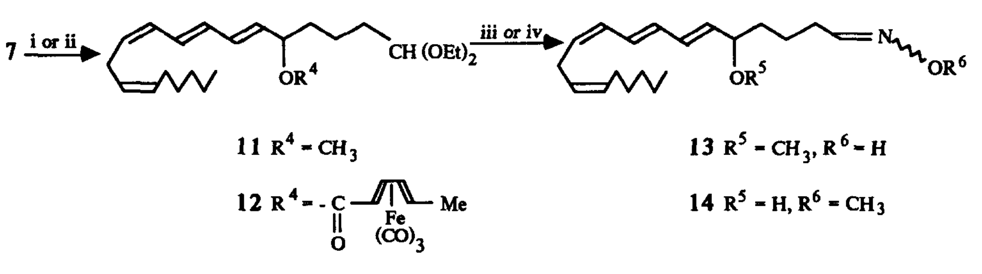
Scheme 1. (i) $(CH_3O)_2P(O)CH_2CO_2Me$, Na_2CO_3, THF-H_2O, 1 h., RT ; (ii) Dibal-H, Et_2O, 15 min, 5°C ; (iii) $CrO_3(C_5H_5N)_2$, CH_2Cl_2, 15 min, 10°C ; (iv) $Ph_3P^+(CH_2)_2$-CH=CH-C_5H_{11}, I^-, n-BuLi, THF, 15 min, 0°C then HMPA, 25 min, -80°C, then **4** -80°C to -30°C ; (v) 1 % aq. H_2SO_4, SiO_2, CH_2Cl_2, 30 min, RT ; (vi) $Br(CH_2)_3CH(OEt)_2$, Mg, THF reflux, 2h., then -40°C add of **6**, then 1 h., 0°C ; (vii) Ac_2O, C_5H_5N, 20 h., 0°C ; (viii) $LiBF_4$, $NH_2OH.HCl$, CH_3CN-H_2O, 2 h., 0°C to RT ; (ix) K_2CO_3, MeOH, 2 h., RT.

For the structure-activity relationships it was important to study the influence of the stereogenic center, so we decided to synthesize compound **8** in optically active form. We had previously reported that sorbic acid tricarbonyliron complex was a new resolving agent for chiral secondary allylic alcohols (9). Thus, the racemic alcohol (±)**7** has been transformed into the corresponding diastereoisomeric esters **9** and **10** which could be separated by chromatography. Then, keeping this ester as protective group, the acetal has been directly transformed into an oxime, the alcohol being finally recovered after Dibal reduction (39 % overall yield from (±)**7**). Each diastereoisomer leads to a pure chiral enantiomer, but the absolute configuration of the asymmetric carbon atom has not been determined yet and the indicated configurations are arbitrary.(10)



Scheme 2. (i) R^3CO_2H, DCC, DMAP, CH_2Cl_2, 20 h., RT ; (ii) $LiBF_4$, $NH_2OH.HCl$, CH_3CN-H_2O, 2h30, 0°C to RT ; (iii) Dibal-H, Et_2O, 30 min, -80°C.

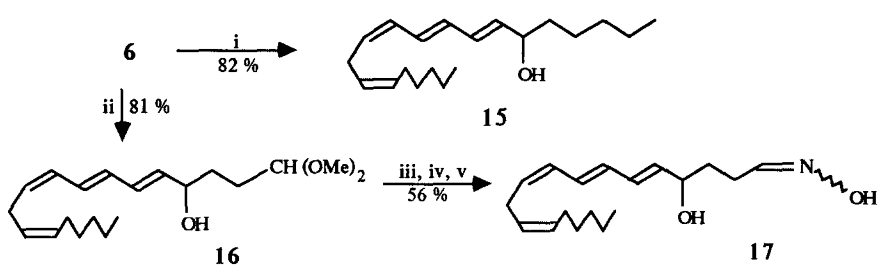
In order to evaluate the importance of both the free alcohol function and the oxime, we synthesized the corresponding ethers. Starting from the tetraenic alcohol **7**, the methylether was prepared and then the acetal transformed into the corresponding oxime **13** (52 % overall yield from **7**). Using the iron tricarbonyl complex as protective group in intermediate **12**, we similarly prepared the oxime ether **14** using methoxylamine hydrochloride (42 % overall yield from **7**).



Scheme 3. (i) NaH, CH_3I, THF 6 h., 0°C to RT (60 %) ; (ii) R^4OH, DCC, DMAP, CH_2Cl_2, 20 h., RT (78 %) ; (iii) $LiBF_4$, $NH_2OH.HCl$, CH_3CN-H_2O, 2 h., 0°C to RT (86 %) ; (iv) $LiBF_4$, $NH_2OCH_3.HCl$, CH_3CN-H_2O, 4h30, 0°C to RT (74 %) then Dibal-H, Et_2O, -80°C to RT (73 %).

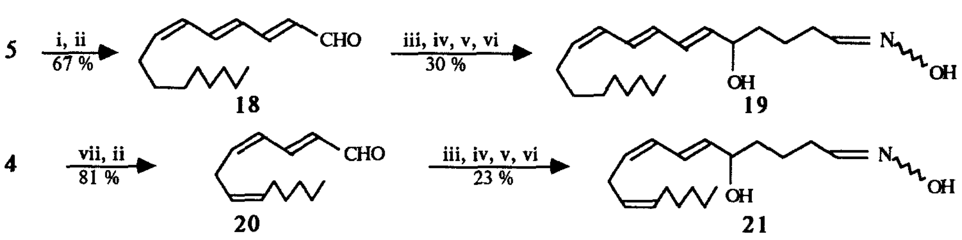
The right part of the molecule has also been modified. Starting form tetraenic aldehyde **6**, a reaction of Grignard reagent derived from bromopentane yielded analog **15**.

On the other hand, the aldehyde **6** has been reacted with the Grignard reagent derived from the dimethyl acetal of 3-bromopropanal (11). The intermediate **16** was then transformed into the corresponding oxime **17** by the usual sequence (45 % overall yield from **6**).



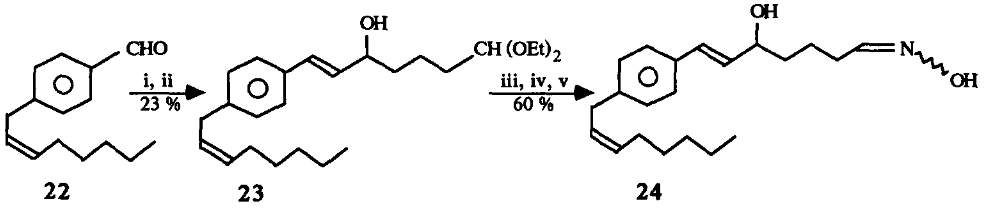
Scheme 4. (i) $C_5H_{11}Br$, Mg, THF reflux 1h., then -40°C, add of **6**, then 2 h., -40°C to 0°C ; (ii) $Br(CH_2)_2CH(OMe)_2$, Mg, THF 30°C, 1h., then -35°C, add of.**6**, then 1h30, -35°C ; (iii) Ac_2O, C_5H_5N, 20 h., 0°C ; (iv) $LiBF_4$, $NH_2OH.HCl$, 3 h., 0°C to RT ; (v) K_2CO_3, MeOH, 2 h., RT.

We then turned our attention to modifications of the lipophilic chain. A Wittig reaction between diene **5** and the phosphonium salt obtained from 1-bromononane followed by acetal hydrolysis gave the triene **18**. As previously described, the hydroxyoxime **19** has been prepared in three steps (20 % overall yield from **5**). Olefin **4**, has been used in a Wittig reaction with an unsaturated phosphonium salt. The triene **20** obtained has then been transformed into the corresponding hydroxyoxime **21** (19 % overall yield from **4**).



Scheme 5. (i) $Ph_3P^+(CH_2)_8CH_3$, I^-, n-BuLi, THF, 30 min, -10°C to 0°C then HMPA, 90 min, -25°C to -15°C ; (ii) 5 % aq. H_2SO_4, SiO_2, CH_2Cl_2, 2h., RT ; (iii) $Br(CH_2)_3CH(OEt)_2$, Mg, THF reflux, 2h., then -40°C add of **18**, then 1h30, -40°C ; (iv) Ac_2O, C_5H_5N, 20 h., 0°C ; (v) $NH_2OH.HCl$, CH_3CN-H_2O, 1 h., 0°C to RT ; (vi) K_2CO_3, MeOH, 3h., RT ; (vii) $Ph_3P^+(CH_2)_2$-$CH=CH$-C_5H_{11}, I^-, n-BuLi, THF, 30 min, -18°C then HMPA, -80°C, then **3**, 2 h., -80°C to -30°C.

The last compound prepared was the aromatic analog **24** starting with the aldehyde **22** which was synthesized using a described methodology (12). The reaction with formylmethylenetriphenylphosphorane afforded the α,β-unsaturated aldehyde with a low yield since the reaction gives also di- and trihomologated aldehydes. The reaction between this aldehyde and the Grignard reagent yielded the intermediate **23** which was then transformed, as previously described, into the hydroxyoxime **24** (14 % overall yield from **22**).



Scheme 6. (i) Ph_3P=CH-CHO, toluene, 30 h., 95°C ; (ii) $Br(CH_2)_3CH\ (OEt)_2$, Mg, THF reflux, 2h., then -40°C, add of **22** then 3h30, -30°C to -25°C ; (iii) Ac_2O, C_5H_5N, 20 h., 0°C ; (iv) $LiBF_4$, $NH_2OH.HCl$, CH_3CN-H_2O, 2 h., 0°C to RT ; (v) K_2CO_3, MeOH, 3 h., RT.

Biological studies

Inhibition of 5-lipoxygenase has been evaluated in human polymorphonuclear leukocytes (PMN), in which after stimulation by calcium ionophore A23187 the amount of both 5-HETE and LTB_4 was measured $^{(13)}$. The formation of these arachidonic acid metabolites was inhibited by compounds (±)-**8** and (+)-**8** with IC_{50} values of 5.4 µM or 2.8 µM for 5-HETE and 4.4 µM or 3.3 µM for LTB_4 respectively. Both enantiomers (+)-**8** and (-)-**8** almost completely blocked the production of 5-HETE and LTB_4 at 10 µM. At 6 µM while compound (+)-**8** still inhibited formation of these 5-lipoxygenase products by 80 %, compound (-)-**8** failed to show any significant activity.

85 % to 95 % inhibition of 5-HETE and LTB_4 formation was also shown by compounds **14**, **15** and **17** at 10 µM. At these concentrations, compounds **13**, **19** and **24** were devoid of activity and compound **21** displayed less than 40 % and 60 % inhibition for 5-HETE and LTB_4 respectively. Thus, the chirality of stereogenic center, the alcohol function and the number of double bonds seemed to play an important role for the inhibitory effect of 5-lipoxygenase by these compounds, but the oxime function did not.

Activities of compounds (±)-**8**, (+)-**8**, (-)-**8**, **13**, **14**, **17** and **24** were also investigated on arachidonic acid metabolism of rat platelets stimulated by thrombin with a procedure that allowed an evaluation of action on two enzymes : the cyclooxygenase and the 12-lipoxygenase $^{(14)}$. 12-Lipoxygenase and cyclooxygenase of rat platelets were not affected by these compounds at concentrations up to 10 µM. Thus, the above described products seem to be quite specific for the 5-lipoxygenase.

In conclusion, this research led to new potent and selective leukotriene biosynthesis inhibitors. The preliminary structure-activity relationships indicated as key structural elements a characteristic tetraenic lipophilic chain with an hydroxyle group in position 15 in relation to the terminal carbon atom ; furthermore, the configuration of this stereogenic center has a strong influence on the biological profile of these new derivatives.

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