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Letter

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Two Potent OXE-R Antagonists: Assignment of Stereochemistry

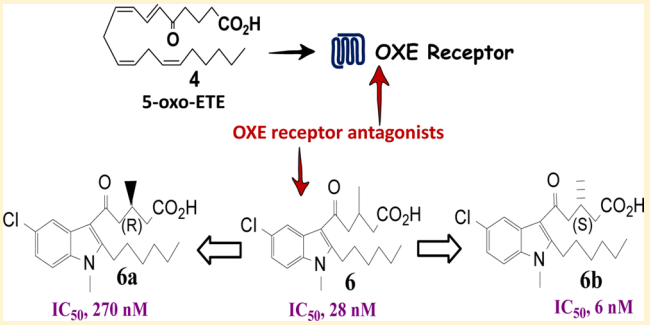
Pranav Patel,[†,§] Chintam Nagendra Reddy,[†] Vivek Gore,[†,§] Shishir Chourey,[†] Qiuji Ye,[†] Yannick P. Ouedraogo,[†,∥] Sylvie Gravel,[‡] William S. Powell,[‡] and Joshua Rokach*[,†]

[†]Claude Pepper Institute and Department of Chemistry, Florida Institute of Technology, 150 West University Boulevard, Melbourne, Florida 32901, United States

[‡]Meakins-Christie Laboratories, Department of Medicine, McGill University, 3626 St. Urbain Street, Montreal, Quebec H2X 2P2, Canada

Supporting Information

ABSTRACT: 5-Oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) is formed by the oxidation of 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5-HETE), which is a major metabolite of enzymatic oxidation of arachidonic acid (AA). 5-Oxo-ETE is the most potent lipid chemoattractant for human eosinophils. Its actions are mediated by the selective OXE receptor, which is therefore an attractive target in eosinophilic diseases such as allergic rhinitis and asthma. Recently, we have reported two excellent OXE receptor antagonists that have IC_{50} values at low nanomolar concentrations. Each of these antagonists has a chiral center, and the isolation of the individual enantiomers by chiral high-performance liquid chromatography (HPLC) revealed that in each case one enantiomer is over 300 times more potent than the other. To unambiguously assign the stereochemistry of these enantiomers and to provide access to larger amounts of the active compounds for biological testing, we report here their total synthesis.



KEYWORDS: *5-Oxo-ETE, eosinophil, OXE receptor, antagonist, enantiomeric, optical purity, chiral auxiliary*

Asthma is a complex disease, which is essentially characterized by a bronchoconstriction. In most cases it is composed of an early phase (or acute) and a late-phase (or inflammatory) asthma. One of the hallmarks of the disease is the accumulation of eosinophils in the early phase, which is then responsible for the late-phase. 5-Oxo-ETE is a potent eosinophil chemotactic factor[1,2] and responsible for the accumulation of eosinophils in the lungs[3] (Figure 1).

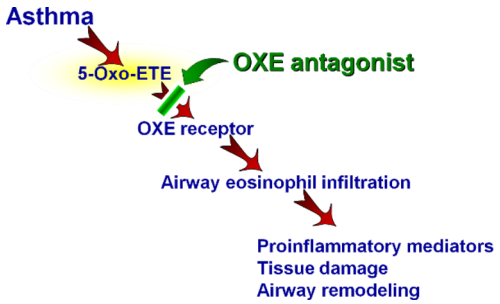


Figure 1. Implication of 5-oxo-ETE in asthma and other allergic diseases.

It is formed from arachidonic acid (AA) by the 5-lipoxygenase (5-LO) pathway, following oxidation of 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5-HETE) by 5-hydroxyeicosanoid dehydrogenase in the presence of $NADP^+$ (Figure 2).[4,5]

After the first total synthesis of 5-oxo-ETE,[6] we have identified and investigated the activities of its metabolites on the 5-oxo-ETE receptor (OXE-R).[7] We found that a metabolite of 5-oxo-ETE formed by platelets, 5-oxo-12(*S*)-HETE, inhibits 5-oxo-ETE-induced Ca^{2+} mobilization, though not increasing intracellular Ca^{2+} concentration by itself.[8] However, this compound is not suitable for the development of an OXE-R antagonist as it is not stable under physiological conditions. Studies on a variety of 5-oxo-ETE analogues clearly showed that the carboxyl, carbonyl (including the 5-oxo group), and the alkyl regions of the molecule are essential for biological activity.[9] We therefore sought to develop synthetic OXE-R antagonists by incorporating both 5-oxo-valeryl and hexyl groups onto an indole backbone. The selection of the indole system looked more promising. We synthesized other aromatic scaffolds such as naphthalene, benzofuran, and quinoline, which turned out to be inactive. These studies culminated in the identification of two potent OXE-R antagonists (Figure 3) with IC_{50} values of less than 30 nM.[10,11] Both of these compounds (**5** and **6**) have an asymmetric center on their acyl side chain, and it seemed likely that their activities would be principally due to a single highly potent enantiomer. To test this hypothesis we separated the two enantiomers of each of the

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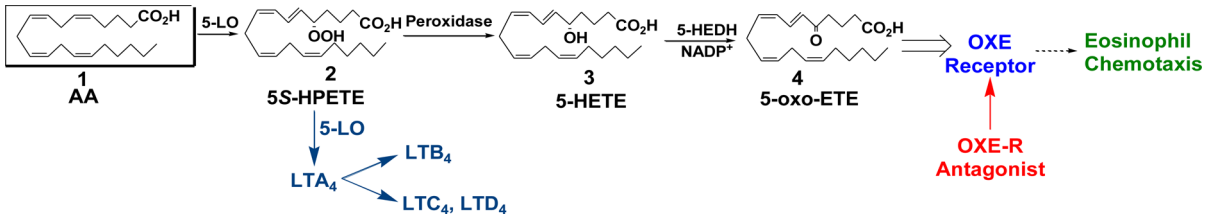


Figure 2. Enzymatic oxygenation of eicosanoids.

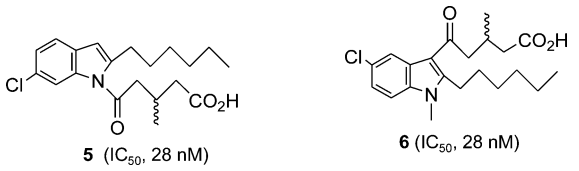


Figure 3. Structures of two racemic OXE-R antagonists.[11]

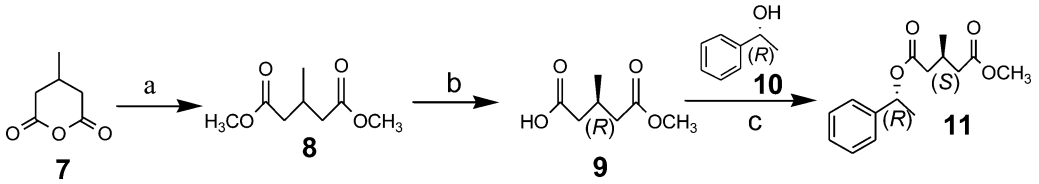
racemic antagonists by chiral high-performance liquid chromatography (HPLC) and compared their potencies in a calcium mobilization assay.[11] In both cases, nearly all of the antagonist activity resided in a single enantiomer (IC_{50}, 6 to 7 nM), with the inactive enantiomer being over 300 times less potent. Because these indole-based OXE-R antagonists could be useful therapeutic agents in the treatment of asthma and other eosinophilic disorders, it is important to identify the active enantiomers and to develop procedures that will permit the synthesis of sufficient quantities for *in vivo* biological testing. In this letter, we report the stereospecific synthesis of the four enantiomers (Schemes 2 and 5) and the assignment of stereochemistry to the *R* and *S* compounds **5a**, **5b**, **6a**, and **6b**.

Results. *Synthesis of R Isomers **5a** and **6a**.* We decided to synthesize the *R* component first as the required precursor **9** was commercially available (enantiomeric ratio ≥90:10 (*R*/*S*)). We have also prepared **9** by the pig liver esterase (PLE)-catalyzed hydrolysis of **8** using a modified literature method (Scheme 1).[12] Precursor **8** was prepared by the hydrolysis of 3-methyl glutaric anhydride (**7**) with HCl and MeOH under reflux conditions in high yield (98%). For the PLE-catalyzed hydrolysis of **8**, we obtained optical purity of the product **9** comparable to the commercial compound. The optical purity of **9** was determined by chiral HPLC of its diastereomer **11**. The phenyl derivative was made in order to use UV-HPLC detection.

Scheme 2 shows the synthesis of the *R* isomers of **5** and **6** from **9**. First, **9** was reacted with $(COCl)_2$ to get the acyl chloride **12**. Friedel–Crafts acylation of **13** with **12** using Me_2AlCl followed by the basic hydrolysis of the ester **15** afforded the *R* isomer of **6**, **6a**. The *R* isomer of **5**, **5a**, was prepared by N-acylation of **14** using *t*-BuOK as a base to give the ester **16**, which was hydrolyzed with HCl under reflux conditions to give **5a**. The intermediates **13** and **14** were synthesized from indole carboxylic acid as we reported previously.[10]

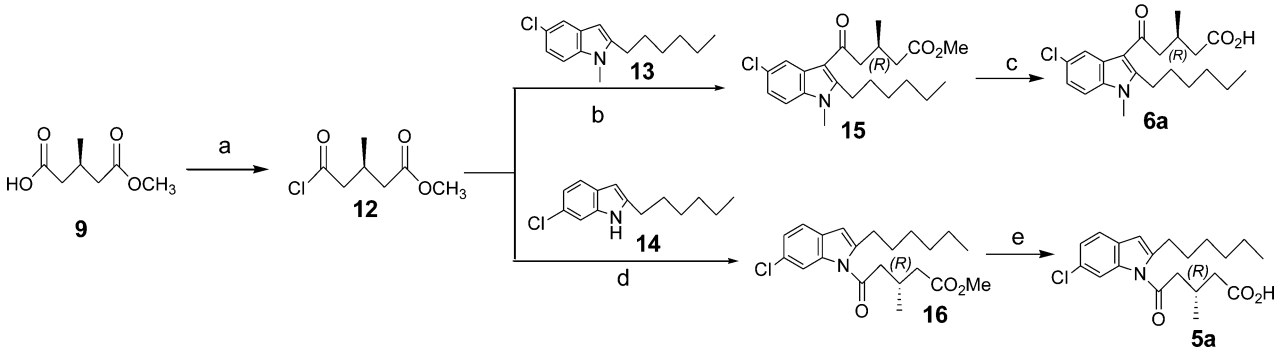
When we compared the retention time of **5a** to that of the active enantiomer of **5** that we previously resolved by chiral HPLC[11] (cf. Figure 4B; data not shown), it was apparent that the *R* enantiomer was the one without appreciable antagonist activity. This was confirmed by measuring its ability to block 5-oxo-ETE-induced calcium mobilization in neutrophils, which revealed that it was substantially less potent than the racemic

Scheme 1. PLE Catalyzed Enzymatic Hydrolysis of 8[a]



[a]Reagents and conditions: (a) conc. HCl, conc. H_2SO_4, MeOH, reflux, 12 h, 98%; (b) pig liver esterase, KH_2PO_4 buffer, −78–0 °C, 20 h, 89.6%; (c) DCC, DMAP, CH_2Cl_2, rt, 12 h, 65%.

Scheme 2. Synthesis of the *R*-Isomers 5a and 6a[a]



[a]Reagents and conditions: (a) $(COCl)_2$, cat. DMF, CH_2Cl_2, rt, 3 h, 90%; (b) Me_2AlCl, CH_2Cl_2, rt, 1 h, 89.5%; (c) LiOH, *i*-PrOH/H_2O (4:1), rt, 24 h, 89%; (d) *t*-BuOK, CH_2Cl_2, 0 °C to rt, 7 h, 60%; (e) 6 N HCl, THF, reflux, 7 h, 60%.

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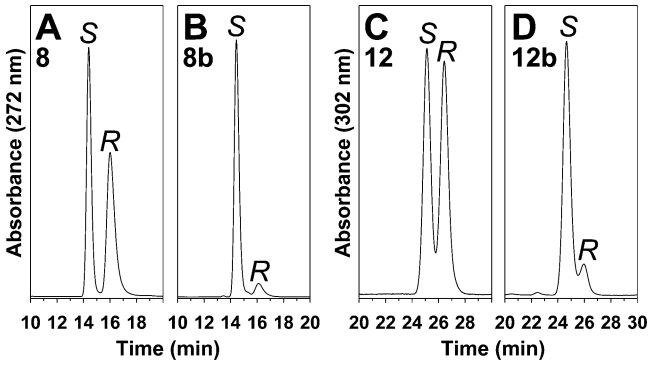


Panel A		Panel B		Panel C		Panel D	
t_R	Area	t_R	Area	t_R	Area	t_R	Area
14.4	6616053	14.4	8622837	25	1393681	25	5645948
16.1	6166465	16.1	684449	26.2	1423251	26.2	683426

Figure 4. Chiral HPLC of OXE-R antagonists. Racemic **5** (A), the synthetic *S*-enantiomer **5b** (B), racemic **6** (C), and the synthetic *S*-enantiomer **6b** (D) were analyzed by chiral HPLC as described in the experimental section (Supporting Information).

compound. We therefore decided to develop methods for the total synthesis of the *S* isomers in both series. Unfortunately, in this case the required enantiomer of **9** was not commercially available. There are previously reported syntheses of the enantiomer of **9**, which rely on the catalytic desymmetrization of 3-methyl glutaric anhydride.[13−19] We elected to proceed with the methods described herein.

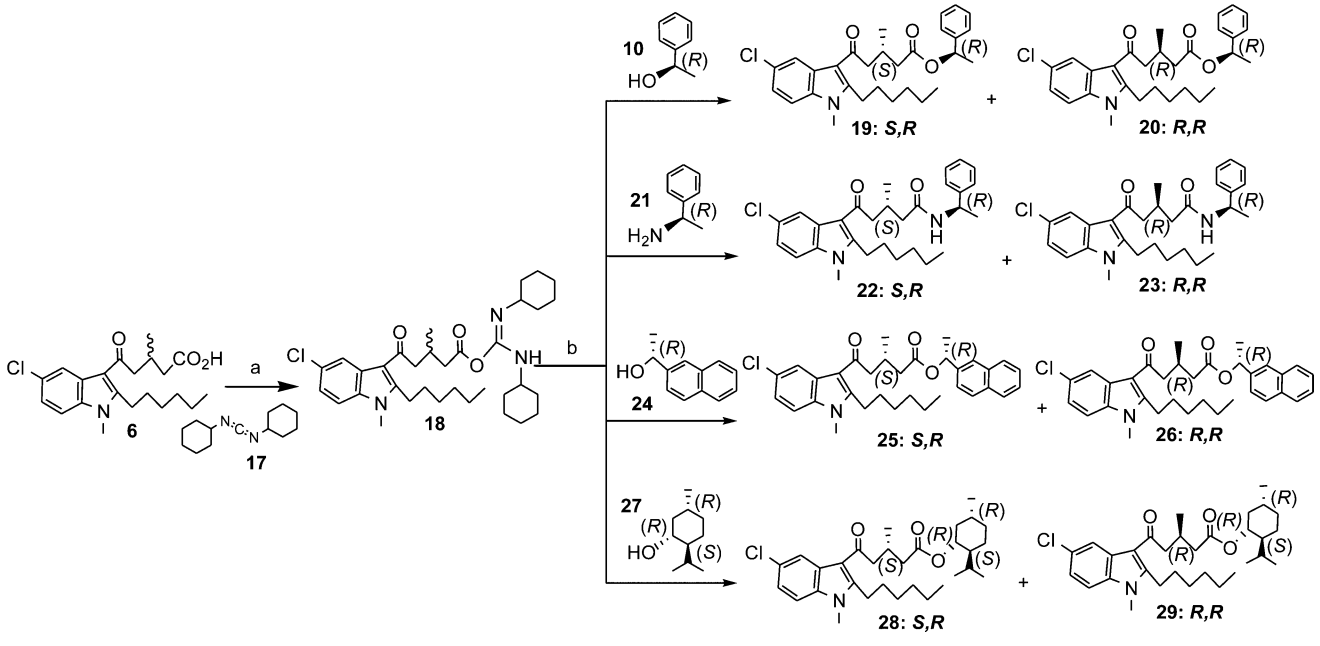
*Chiral Resolution of **5**, **6**, and **30**.* Although we could readily separate the *R* and *S* enantiomers of **5** and **6** by analytical scale chiral HPLC, it was not practical to do this on a preparative scale because of the limited capacity and high cost of these columns. We therefore attempted to resolve the racemic compounds following derivatization with chiral auxiliaries, which we hoped would permit large scale separation of the resulting diastereomeric pair on silica columns. We originally prepared the diastereomeric derivatives of **6** individually but then realized that intermediate **18** could be prepared and purified by column chromatography and used to react with the chiral auxiliaries **10**, **21**, **24**, and **27** saving considerable time and effort (Scheme 3). Although the resulting diastereomeric mixtures could be separated by analytical chiral HPLC, none of them could be separated by either TLC or silica gel column chromatography.

Diastereomeric separation of the menthol derivatives of the other lead compound **5** was also attempted, but the corresponding diastereomers could not be separated. We also attempted to separate the diastereomers of the intermediate **30** by the same approach as used for **5** and **6** (Scheme 4), but unfortunately, these pairs of diastereomers could not be resolved by TLC or silica gel column chromatography. However, the diastereomeric pairs derived from **30** could be resolved by chiral HPLC, which proved useful as a measure of optical purity in subsequent studies utilizing this intermediate.

*Synthesis of S Compounds **5b** and **6b**.* We decided to perform the total synthesis of the *S* enantiomers by inverting the stereocenter using the (*R*) synthon **9** as starting material (Scheme 5). This approach was possible because the synthon is pro-symmetrical. It also relies on high quantitative and qualitative steps to ensure chiral purity. For example, step b in Scheme 5 is very selective considering that it is a basic hydrolysis, which could also have partially removed the silyl moiety (Scheme 6). However, if that were the case the potential byproducts (**9** and **42**) could be easily removed by column chromatography. The result would be a lower yield but no dilution of the enantiomeric purity. In our case, the basic hydrolysis of **37** with 5 equiv of LiOH in *i*-PrOH/H_2O (4:1) at rt afforded the desired product **38** in 60% yield. A smaller amount of the byproduct **42** (∼10%) was also generated, but the other potential byproduct **9** was not observed.

*Chiral HPLC Analysis of the Enantiomers of **5** and **6** (**5a**, **5b**, **6a**, and **6b**).* All four enantiomers **5a**, **5b**, **6a**, and **6b** as well as the two racemic mixtures **5** and **6** were analyzed by chiral HPLC. The *S* and *R* enantiomers of racemic **5** were nearly completely separated (Figure 4A) with the two peaks having identical areas. Analysis of **5b** (Figure 4B) revealed that the *S* enantiomer

Scheme 3. Attempted Chiral Resolution of Racemic 6[a]



[a]Reagents and conditions: (a) **17**, DMAP, CH_2Cl_2, rt, 12 h, 80%; (b) DMAP, CH_2Cl_2, rt, 12 h, 65−70%.

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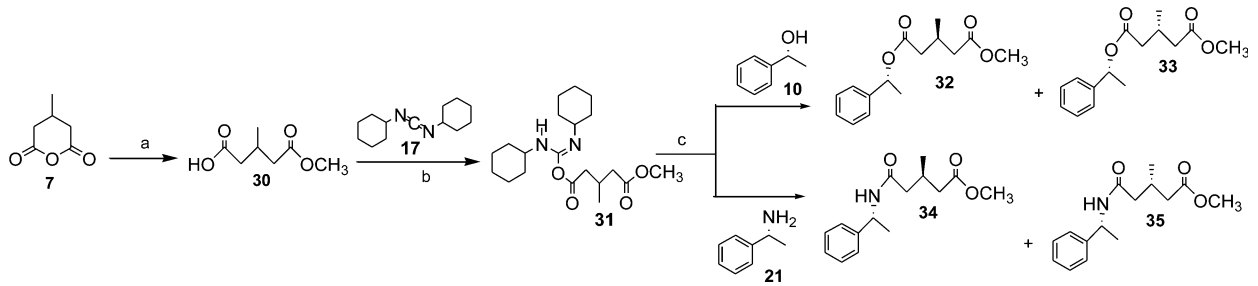
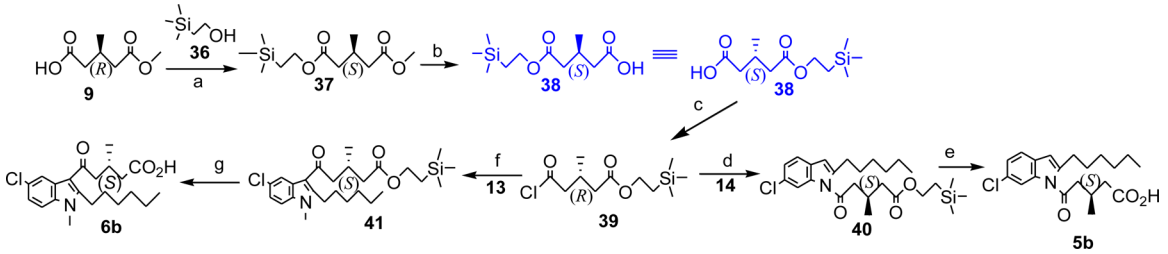
Scheme 4. Attempted Chiral Resolution of Racemic 30[a]



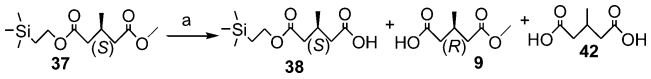
[a]Reagents and conditions: (a) MeOH, reflux, 12 h, 90%; (b) **17**, DMAP, CH_2Cl_2, rt, 12 h, 80%; (c) DMAP, CH_2Cl_2, rt, 12 h, 70%.

Scheme 5. Synthesis of *S* Antagonists 5b and 6b[a]



[a]Reagents and conditions: (a) **17**, DMAP, CH_2Cl_2, rt, 10 h, 90%; (b) LiOH, *i*-PrOH/H_2O (4:1), rt, 7 h, 60%; (c) $(COCl)_2$, cat. DMF, CH_2Cl_2, rt, 3 h, 89%; (d) **14**, *t*-BuOK, CH_2Cl_2, 0 °C to rt, 6 h, 60%; (e) $TiCl_4$, CH_2Cl_2, 0 °C to rt, 0.5 h, 80%; (f) **13**, Me_2AlCl, CH_2Cl_2, 0 °C to rt,1 h, 25%; (g) LiOH, *i*-PrOH/H_2O (4:1), rt, 24 h, 85%.

Scheme 6. Possible Products of Basic Hydrolysis of 37[a]



[a]Reagents and conditions: (a) LiOH, *i*-PrOH/H_2O (4:1), rt, 7 h, 60%.

predominated by a ratio of 93:7 (*S*/*R*). The main peak (t_R, 14.4 min) had a retention time similar to that of the active component (IC_{50}, 7 nM) of the racemate that we previously reported,[11] whereas the minor peak (t_R, 16.1 min) corresponded to the enantiomer with low activity (IC_{50}, 2.2 μM). Separation of the *S* and *R* enantiomers of **6** by chiral HPLC is shown in Figure 4C. The earlier eluting *S* enantiomer (t_R, 25 min) corresponded to the active component (IC_{50}, 6 nM) of the racemate that we had previously reported,[11] whereas the later eluting *R* enantiomer (t_R, 26.2 min) corresponded to the weakly active component (IC_{50}, 2.7 μM). Chiral HPLC of the synthetic *S* enantiomer **6b** indicated that the ratio of *S*/*R* enantiomers in this preparation is 91:9 (Figure 4D). Similar results were obtained with the synthetic *R* enantiomers **5a** and **6a**. The peak assigments of all of the synthetic enantiomers were confirmed by coinjections with the racemates **5** and **6**. The slight variation observed in the enantioselectivity is the result of variation in the preparation of the *R* enantiomers.

Conclusions. We have performed the synthesis of the *R* and *S* enantiomers of two low nanomolar OXE-R antagonists. The availability of these compounds has made it possible to unambiguously assign the *S* configuration to the active enantiomers of each of these antagonists.

■ ASSOCIATED CONTENT

Supporting Information

Details of *in vitro* assay, HPLC conditions, and synthetic procedures and analytical data for compounds reported. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(J.R.) Tel: 321-674-8578. Fax: 321-674-7743. E-mail: jrokach@fit.edu.

Present Addresses

[§](P.P. and V.G.) Navinta LLC, 1499 Lower Ferry Road, Ewing, New Jersey 08618, United States. E-mail: pranav.patel@navinta.com(P.P.) and vivek.gore@navinta.com (V.G.). Phone: 609-883-1135.

[∥](Y.P.O.) Intel Corp., 2501 NW 229th Avenue, Hillsboro, Oregon 97124, United States. E-mail: yannick.p.ouedraogo@intel.com. Phone: 503−840−7685.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

5-HETE, 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; 5-oxo-ETE, 5-oxo-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; AA, arachidonic acid; DMF, dimethylformamide; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; TMS, tetramethylsilane; HPLC, high-performance liquid chromatography

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