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C_{20}-trifluoro-5-oxo-ETE: A metabolically stable 5-oxo-ETE derivative

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ABSTRACT

The total synthesis of C_{20}-trifluoro-6(*E*),8(*Z*),11(*Z*),14(*Z*) 5-oxo-ETE is reported. This compound was designed as an ω-oxidation-resistant analog of 5-oxo-ETE that would be resistant to metabolism. The trifluoro derivative of 5-oxo-ETE stimulated calcium mobilization in neutrophils and desensitized these cells to subsequent exposure to 5-oxo-ETE.

Arachidonic acid (AA) **1** is converted to a large number of biologically active products (eicosanoids) that are important in a variety of pathological conditions, including inflammatory and allergic diseases.[1–5] Because of the complex array of products with different chiral centers and double bond configurations, chemical synthesis of eicosanoids has been critical for their identification and determination of their physiological and pharmacological properties. Over the past several years, our work has focused on the synthesis and biological effects of a variety of AA-derived products formed both enzymatically by lipoxygenase (LO) and cyclooxygenase (COX) pathways[6–9] and nonenzymatically by reactive oxygen species (ROS)-induced oxidation.[10–12] 5-LO, which catalyzes the 5-peroxidation of AA leads to 5-HPETE **4**[13–15] and leukotriene A (LTA_4).[16] LTA_4 is transformed by LTA_4 hydrolase to LTB_4, a potent neutrophil chemoattractant that acts principally via the BLT_1 receptor.[17] LTA_4 is also converted to the cysteinyl leukotriene LTC_4 by the addition of glutathione (GSH). LTC_4 is further metabolized to LTD_4. These substances, previously known as 'slow reacting substance of anaphylaxis', are potent stimulators of bronchoconstriction and vascular permeability and are critical mediators in asthma.

5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) **6** is the most potent chemotactic agent for eosinophils among lipid mediators.[18] It is formed from 5-HETE **5** by the action of a very specific and selective dehydrogenase and exerts its biological actions through a dedicated receptor. Because of its effects on eosinophils, 5-oxo-ETE may be an important mediator in diseases in which these cells are prominent, including asthma and other allergic conditions, and diseases of the gastrointestinal system.

5-oxo-ETE **6** is synthesized by 5-hydroxyeicosanoid dehydrogenase (5-HEDH), which is highly specific for eicosanoids containing a 5(*S*)-hydroxyl group followed by a 6,7-*trans*-double bond (Scheme 1). This enzyme is present in neutrophils,[19] monocytes,[20] lymphocytes,[20] eosinophils,[21] platelets,[22] and some types of structural cells. 5-oxo-ETE **6** acts through a specific G_i-coupled receptor,[23–25] which has been cloned[26,27] and is highly expressed by eosinophils > neutrophils > monocytes as well as by certain tumor cell lines. This receptor has been designated as the OXE receptor (OXE-R). 5-oxo-ETE is active in vivo, eliciting infiltration of eosinophils into rat lung[28] and both eosinophils and neutrophils into human skin.[29] OXE-R is highly selective for 5-oxo-ETE **6** over a variety of its metabolites, LTs and other eicosanoids. Although 5-oxo-ETE **6** is a chemoattractant for both neutrophils[23] and monocytes,[30] it is less potent than LTB_4. In contrast, among lipid mediators, 5-oxo-ETE **6** is the strongest chemoattractant known for human eosinophils[21] and also induces a variety of other responses in these cells,[31–33] some of which are markedly enhanced by the proinflammatory cytokines GM-CSF and TNFα.[34]

An obstacle in the biological evaluation of 5-oxo-ETE is its susceptibility to ω-oxidation[24] and incorporation into cellular lipids,[25] both of which result in dramatic losses in biological activity. One way to block metabolism by ω-oxidation would be to modify the ω-methyl group of 5-oxo-ETE. This could also potentially interfere with incorporation into lipids. However, alteration of the C_{20} methyl group could also have a marked effect on biological activity, as 20-hydroxy-5-oxo-ETE is about 100 times less potent than 5-oxo-ETE.[24]

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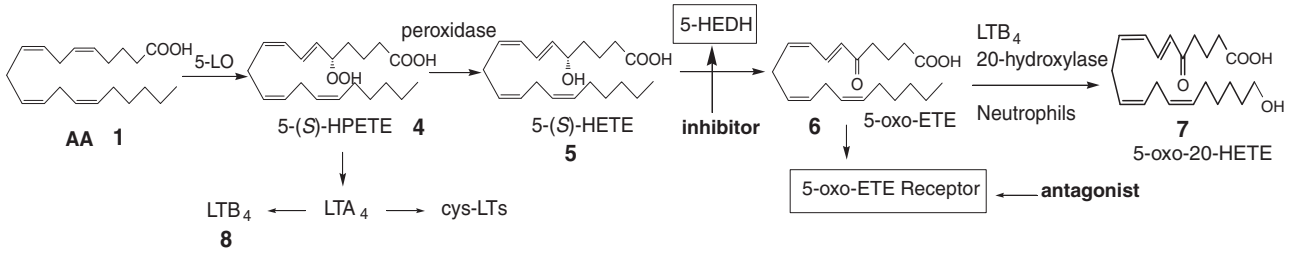


Scheme 1. Eicosanoids.

Substitution of a trifluoromethyl group for the C_{20} methyl group of 5-oxo-ETE is one choice for a metabolically stable 5-oxo-ETE agonist. This would prevent ω-oxidation, the major pathway for the metabolism of 5-oxo-ETE, and may also possibly reduce the rate of incorporation in lipids. Scheme 2 shows the synthesis of the CF_3 trifluoro derivative **23**.[35]

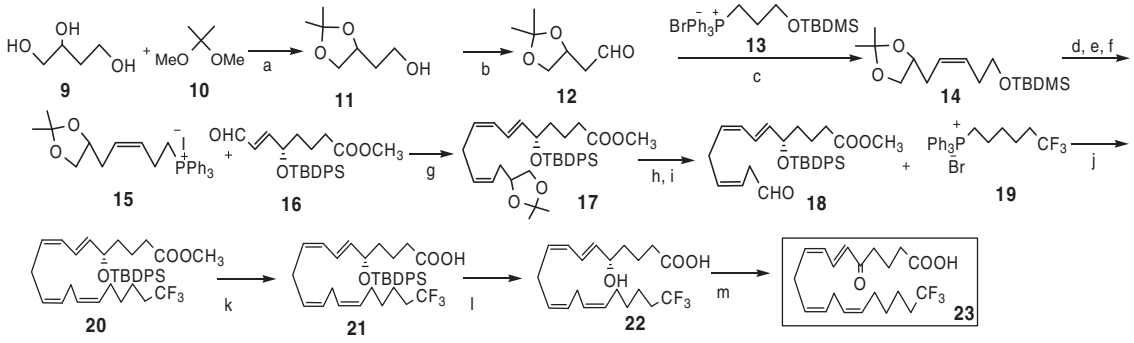
The synthesis of trifluoro derivative **23** was achieved as described in detail in Scheme 2. The aldehyde **12** was obtained by oxidation of **11** which in turn was obtained by protecting triol **9** with dimethoxy propane. The α,β-unsaturated aldehyde **16** was prepared as previously described starting from D-arabinose.[36] The trifluoro phosphonium salt **19** was prepared readily from commercial 1,1,1-trifluoro-6-bromohexane[37] by refluxing with triphenyl phosphine in acetonitrile.

The use of D-arabinose (Scheme 3) for the preparation of **25** and ultimately **23**, although not chirally economical, is nevertheless a very convenient procedure. The starting material **24** is very cheap and **25** can be prepared in batches of 200 g at a time. Also, **16** can be used for the preparation of 5-HETE and other lipoxygenase products.

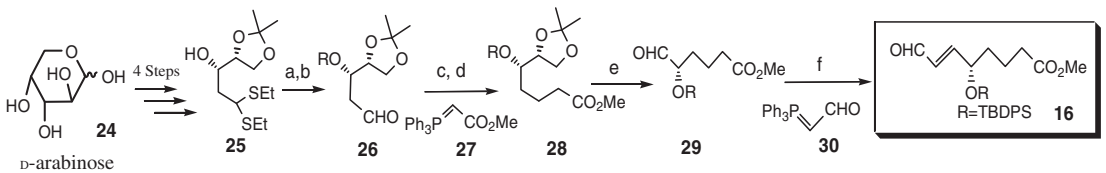
To determine the biological potency of **23**, we conducted preliminary experiments to examine its effects on intracellular calcium levels in human neutrophils. Neutrophils were prepared from blood from healthy subjects as previously described by removing erythrocytes with dextran 500 and mononuclear cells by centrifugation over Ficoll–Paque.[38] The neutrophils were suspended in phosphate-buffered saline (PBS) and loaded with indo-1 acetoxymethyl ester.[24] Five minutes prior to data acquisition, $CaCl_2$ (1.8 mM) and $MgCl_2$ (1 mM) were added. Fluorescence was measured using a spectrofluorometer with a temperature-controlled cuvette holder equipped with a magnetic stirrer. After stabilization of the baseline, various concentrations of **6** or **23** were added, followed 1.5 min later by addition of **6** (10 nM) to evaluate agonist-induced desensitization. After another 0.5 min, digitonin (0.1% final concentration) was added.

The response of neutrophils to 5-oxo-ETE (10 nM) following initial addition of vehicle is shown in Figure 1, top curve. There was a strong increase in fluorescence, which peaked a few seconds after addition of 5-oxo-ETE. Digitonin was added before fluorescence returned to baseline to permit measurement of the maximal fluorescence. Addition of C_{20}-trifluoro-5-oxo-ETE (F_3-5o) (100 nM) also resulted in a sharp increase in fluorescence, although somewhat less than that for 5-oxo-ETE and completely abolished the response to 5-oxo-ETE (Fig. 1, bottom curve).

The concentration-response curves for compounds **6** and **23** on calcium mobilization are shown in Figure 2A. 5-oxo-ETE was a potent inducer of calcium mobilization with an EC_{50} of 4 nM. C_{20}-trifluoro-5-oxo-ETE was somewhat less potent, with a maximal response about 50% of that for 5-oxo-ETE. Both of the above agonists desensitized neutrophils to subsequent exposure to



Scheme 2. Total synthesis of long lasting agonist C_{20}-trifluoro-5-oxo-ETE. Reagents and conditions: (a) 10-camphor sulphonic acid, CH_3CN, rt, 80%; (b) PCC, Al_2O_3, dry CH_2Cl_2, 0 °C–rt, 75%; (c) **13**, dry THF, LiHMDS, −78 °C–rt, 73%; (d) THF, TBAF, rt, 87%; (e) PPh_3, imidazole, I_2, dry CH_2Cl_2, rt, 90%; (f) PPh_3, CH_3CN, 50 °C, 2 days, 92%; (g) **15**, dry THF, 1.6 M BuLi, −78 °C–rt, 75%; (h) THF/H_20, TFA, 78%; (i) dry CH_2Cl_2, Na_2CO_3, LTA, 74%; (j) **19**, dry THF, −78 °C–rt, n-BuLi, 84%; (k) LiOH·H_2O, isopropanol, rt, 93%; (l) TBAF, AcOH, THF, 50 °C, 12 h, 89%; (m) Dess–Martin periodinane, dry CH_2Cl_2, rt, 88%.



Scheme 3. Synthesis of α,β-unsaturated aldehyde **16**. Reagents and Conditions: (a) TBDPS-Cl, imidazole, CH_2Cl_2, rt, 98%; (b) periodic acid, THF, ether, 0 °C, 83.3%; (c) **27**, THF, rt, 91.5%; d) Pd/C H_2, EtOH, rt, 96.5%; e) periodic acid, THF, ether, rt, 77.5%; (f) **30**, benzene, reflux, 76.3%.

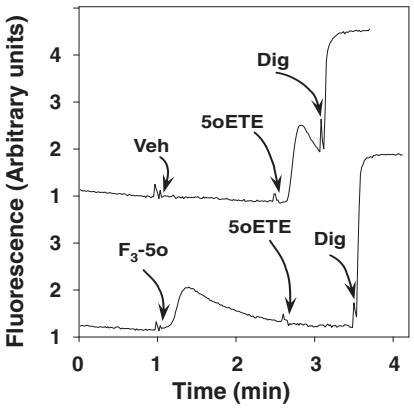


Figure 1. Effects of 5-oxo-ETE and trifluoro-5-oxo-ETE on intracellular calcium levels in indo-1-labeled neutrophils. Either vehicle or trifluoro-5-oxo-ETE (100 nM) was added after 1 min, followed by 5-oxo-ETE (10 nM) at 2.5 min and digitonin (0.1%) at 3.5 min. Fluorescence due to the binding of Ca^{++} to indo-1 was monitored using excitation and emission wavelengths of 331 and 410 nm.

5-oxo-ETE, as illustrated by Figure 1. The IC_{50} for 5-oxo-ETE-induced desensitization was 3 nM, whereas that for C_{20}-trifluoro-5-oxo-ETE was 7.5 nM (Fig. 2B).

Although C_{20}-trifluoro-5-oxo-ETE is some what less potent an agonist than 5-oxo-ETE, it is considerably more potent than the 5-oxo-20-HETE **7**, a C_{20}-hydroxy metabolite of 5-oxo-ETE, indicating that addition of a substituent at this position does not necessarily dramatically reduce biological activity. The potent inhibitory effect of C_{20}-trifluoro-5-oxo-ETE on 5-oxo-ETE-induced calcium mobilization (Fig. 2B) suggests that the trifluoro derivative binds strongly to the 5-oxo-ETE receptor, whereas the limited maximal response suggests that it is a partial agonist, having some antagonist properties. These results suggest that further modification of the C_{20} methyl group may lead to the identification of 5-oxo-ETE analogs with potent agonist or antagonist effects. Also, Figure 2B suggests that an appropriately radio-labeled C_{20}-trifluoro-5-oxo-ETE could be useful in generating an OXE-R binding assay and for long term in vivo experimentation.

Acknowledgements

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.021.

References and notes

1. Rokach, J. Leukotrienes and Lipoxygenases, Chemical, Biological and Clinical Aspects, 1st ed.; Elsevier Science: Amsterdam The Netherlands, 1989. Vol. 11.
2. Atkinson, J. G.; Rokach, J. Synthesis of the Leukotrienes and Other Lipoxygenase-Derived Products In *CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids. Chemical and Biochemical Aspects, Part B*; Willis, A. L., Ed.; CRC Press: Boca Raton, FL, 1987; 1, p 175.
3. Vane, J.; O'Grady, J. *Therapeutic Applications of Prostaglandins*; Edward Arnold: London, 1993. 277.
4. Nicolaou, K. C.; Petasis, N. A. Synthesis of Eicosanoids In *CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids. Chemical and Biochemical Aspects, Part B*; Willis, A. L., Ed.; CRC Press: Boca Raton, FL, 1987; 1, p 1.
5. Spokas, E. G.; Rokach, J.; Wong, P. Y.-K. Leukotrienes, Lipoxins, and Hydroxyeicosatetraenoic acids In *Methods in Molecular Biology*; Lianos, E. A., Ed.; Humana Press: Totowa, NJ, 1999; p 213.
6. Lau, C. K.; Adams, J.; Guindon, Y.; Rokach, J. Chemistry of the Leukotrienes and Other Lipoxygenase Products. In *Leukotrienes and Lipoxygenase: Chemical, Biological, and Clinical Aspects*; Rokach, J., Ed.; Elsevier Press: Amsterdam, 1989; p 1. Chapter 1.
7. Rokach, J.; Guindon, Y.; Young, R. N.; Adams, J.; Atkinson, J. G. Synthesis of the Leukotrienes In *The Total Synthesis of Natural Products*; ApSimon, J. W., Ed.; Wiley-Interscience: New York, 1988; 7,.
8. Rokach, J.; Adams, J. *Acc. Chem. Res.* **1985**, *19*, 87.
9. Adams, J.; Fitzsimmons, B. J.; Girard, Y.; Leblanc, Y.; Evans, J. F.; Rokach, J. *J. Am. Chem. Soc.* **1985**, *107*, 464.
10. Morrow, J. D.; Hill, K. E.; Burk, R. F.; Nammour, T. M.; Badr, K. F.; Roberts, L. J., II *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9383.
11. Hwang, S. W.; Adiyaman, M.; Khanapure, S.; Schio, L.; Rokach, J. *J. Am. Chem. Soc.* **1994**, *116*, 10829.
12. Praticò, D.; Barry, O. P.; Lawson, J. A.; Adiyaman, M.; Hwang, S. W.; Khanapure, S. P.; Iuliano, L.; Rokach, J.; FitzGerald, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3449.
13. Zamboni, R.; Rokach, J. *Tetrahedron Lett.* **1983**, *24*, 999.
14. Corey, E. J.; Albright, J. O.; Barton, A. E.; Hashimoto, S. *J. Am. Chem. Soc.* **1980**, *102*, 1435.
15. Dussault, P.; Lee, I. *J. Org. Chem.* **1995**, *60*, 218.
16. Murphy, R. C.; Hammarstrom, S.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A* **1979**, *76*, 4275.

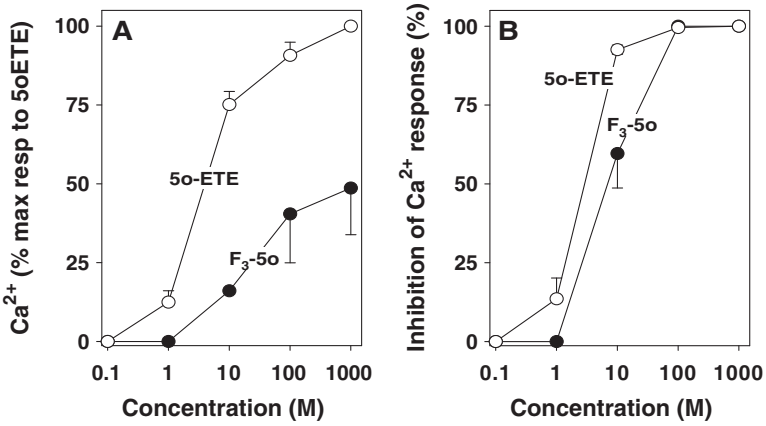

Figure 2. Concentration-response relationships for the effects of 5-oxo-ETE and trifluoro-5-oxo-ETE on intracellular calcium levels in neutrophils. (A) Agonist effects of 5-oxo-ETE and trifluoro-5-oxo-ETE on intracellular Ca^{++} levels in neutrophils. (B) Inhibition of 5-oxo-ETE (10 nM)-induced Ca^{++} mobilization in neutrophils due to prior treatment with different concentrations of 5-oxo-ETE or trifluoro-5-oxo-ETE as shown in Figure 1.

17. Yokomizo, T.; Izumi, T.; Chang, K.; Takuwa, Y.; Shimizu, T. *Nature* **1997**, *387*, 620.
18. Powell, W. S.; Rokach, J. *Prog. Lipid Res.* **2005**, *44*, 154.
19. O'Flaherty, J. T.; Cordes, J.; Redman, J.; Thomas, M. J. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 129.
20. Zhang, Y.; Styler, A.; Powell, W. S. *J. Leukoc. Biol.* **1996**, *59*, 847.
21. Powell, W. S.; Chung, D.; Gravel, S. *J. Immunol.* **1995**, *154*, 4123.
22. Powell, W. S.; Gravel, S.; Khanapure, S. P.; Rokach, J. *Blood* **1999**, *93*, 1086.
23. Powell, W. S.; Gravel, S.; MacLeod, R. J.; Mills, E.; Hashefi, M. *J. Biol. Chem.* **1993**, *268*, 9280.
24. Powell, W. S.; MacLeod, R. J.; Gravel, S.; Gravelle, F.; Bhakar, A. *J. Immunol.* **1996**, *156*, 336.
25. O'Flaherty, J. T.; Taylor, J. S.; Thomas, M. J. *J. Biol. Chem.* **1998**, *273*, 32535.
26. Hosoi, T.; Koguchi, Y.; Sugikawa, E.; Chikada, A.; Ogawa, K.; Tsuda, N.; Suto, N.; Tsunoda, S.; Taniguchi, T.; Ohnuki, T. *J. Biol. Chem.* **2002**, *277*, 31459.
27. Jones, C. E.; Holden, S.; Tenaillon, L.; Bhatia, U.; Seuwen, K.; Tranter, P.; Turner, J.; Kettle, R.; Bouhelal, R.; Charlton, S.; Nirmala, N. R.; Jarai, G.; Finan, P. *Mol. Pharmacol.* **2003**, *63*, 471.
28. Stamatiou, P.; Hamid, Q.; Taha, R.; Yu, W.; Issekutz, T. B.; Rokach, J.; Khanapure, S. P.; Powell, W. S. *J. Clin. Invest.* **1998**, *102*, 2165.
29. Muro, S.; Hamid, Q.; Olivenstein, R.; Taha, R.; Rokach, J.; Powell, W. S. *J. Allergy Clin. Immunol.* **2003**, *112*, 768.
30. Sozzani, S.; Zhou, D.; Locati, M.; Bernasconi, S.; Luini, W.; Mantovani, A.; O'Flaherty, J. T. *J. Immunol.* **1996**, *157*, 4664.
31. Guilbert, M.; Ferland, C.; Bosse, M.; Flamand, N.; Lavigne, S.; Laviolette, M. *Am. J. Respir. Cell Mol. Biol.* **1999**, *21*, 97.
32. Powell, W. S.; Gravel, S.; Halwani, F. *Am. J. Respir. Cell Mol. Biol.* **1999**, *20*, 163.
33. Schwenk, U.; Schroder, J. M. *J. Biol. Chem.* **1995**, *270*, 15029.
34. O'Flaherty, J. T.; Kuroki, M.; Nixon, A. B.; Wijkander, J.; Yee, E.; Lee, S. L.; Smitherman, P. K.; Wykle, R. L.; Daniel, L. W. *J. Immunol.* **1996**, *157*, 336.
35. ^{13}C NMR $CDCl_3$: 199.1, 174.1, 140.09, 136.92, 129.66, 129.61, 129.51, 128.05, 126.99, 126.31, 39.78, 33.78, 33.50, 28.59, 26.80, 26.69, 25.69, 21.47, 19.04. ^{1}NMR $CDCl_3$: δ 7.52–7.47 (1H, dd, J = 15.2, 11.5), 6.04–6.14 (2H, m), 5.83–5.76 (1H, q), 5.41–5.23 (4H, m), 3.00–3.04 (2H, t, J = 7.2), 2.74–2.77 (2H, t), 2.57–2.61 (2H, t, J = 7.12), 2.35–2.38 (2H, m), 1.87–2.06 (6H, m) 1.46–1.54 (2H, m), 1.34–1.41(2H, m).
36. Adams, J.; Fitzsimmons, B. J.; Rokach, J. *Tetrahedron Lett.* **1984**, *25*, 4713.
37. Oakwood Products, Inc., West Columbia, SC
38. Powell, W. S.; Gravelle, F.; Gravel, S. *J. Biol. Chem.* **1992**, *267*, 19233.