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Total Synthesis and Biological Activity of the Arachidonic Acid Metabolite Hemiketal E_2

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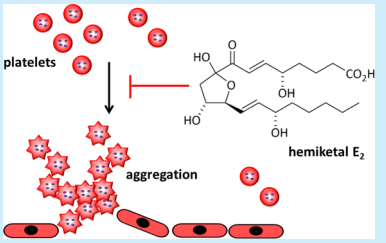
Supporting Information

ABSTRACT: The total synthesis of hemiketal E_2 (HKE_2) has been accomplished using a gold(I)-mediated cycloisomerization followed by oxidation of the enol ether product to introduce a unique keto-hemiketal, the core structure of HKE_2. Synthetic hemiketal E_2 reproduced biosynthetically derived HKE_2 in the inhibition of human platelet aggregation.



Eicosanoids are produced by enzymatic oxidation of arachidonic acid (AA) by cyclooxygenases (COX-1, COX-2), lipoxygenases (LOX), and cytochromes P450 in response to a variety of cellular stimuli such as hormones, stress, and cytokines.[1] These lipid mediators regulate a variety of biological responses and pathological processes such as inflammation, cancer, asthma, and autoimmune diseases.[2] Studies on eicosanoid biosynthesis, metabolism, and function have been extensive. The biosynthetic pathways leading to prostaglandins and leukotrienes are generally believed to diverge at the point of the initial enzymatic oxygenation of arachidonic acid by cyclooxygenases and lipoxygenases, respectively.[3] However, in 2011, Schneider and co-workers described the biosynthesis of eicosanoids HKD_2 (**3**) and HKE_2 (**4**) as products of the consecutive oxygenation of arachidonic acid by 5-lipoxygenase and cyclooxygenase-2 (AA → **1** → **2** → **3**/**4**, Figure 1).[4]

The discovery of HKD_2 and HKE_2 resulted from the hypothesis that 5*S*-HETE (**1**), an oxidation product of 5-LOX and arachidonic acid, may serve as a substrate of the COX enzyme. *In vitro* experiments demonstrated 5*S*-HETE to be converted by COX-2 (but not COX-1), affording diendoperoxide **2** as the enzymatic product.[5] The latter underwent rearrangement to HKD_2 and HKE_2 in a process reminiscent of the well-known transformation of intermediate endoperoxide PGH_2 to PGD_2 and PGE_2; thus, it is the designated nomenclature for these unusual hemiketals.[4] The possible relevance of the crossover pathway to living systems was further demonstrated by their ability to induce tubulogenesis of endothelial cells[4] and their biosynthesis following stimulation of 5-LOX and COX-2 in human leukocytes *ex vivo* using lipopolysaccharide (LPS).[6] Further study of HKD_2 and HKE_2 requires their total synthesis, as currently hemiketals

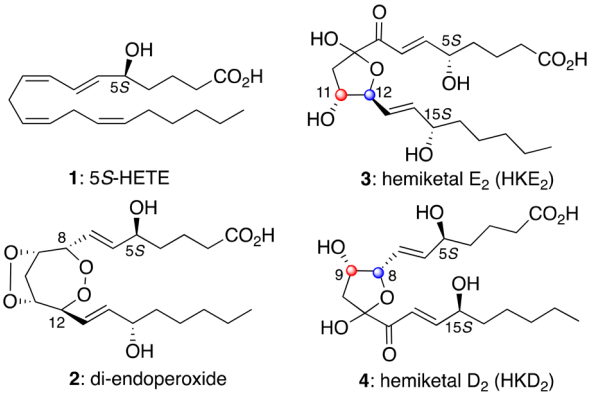


Figure 1. Structures of 5*S*-HETE (**1**), diendoperoxide (**2**), hemiketal E_2 (**3**), and hemiketal D_2 (**4**).\.

D_2/E_2 are produced enzymatically in small quantities starting from 5*S*-HETE and recombinant COX-2.[7] Described herein is the first total synthesis of hemiketal E_2 (**3**).

Hemiketals D_2 and E_2 share common side chains that incorporate secondary alcohols of (*S*)-configuration of reversed attachment to the heterocyclic core (cf. Figure 2, Rα/Rω). A second difference between the two structures is the configuration of C9 (9*S*-HKD_2) and C11 (11*R*-HKE_2) as reflected in the cis and trans relationships between C8−C9 (HKD_2) and C11−C12 (HKE_2), respectively (**3**/**4**, Figure 1). Based on this structural analysis, we proceeded with a synthetic strategy whereby the absolute stereochemistry of 9*S* (HKD_2) and 11*R* (HKE_2) would be introduced in 2-alkoxy(siloxy)-

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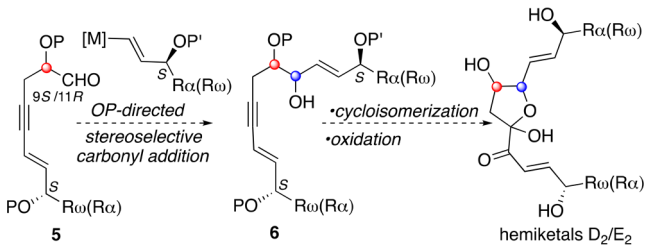
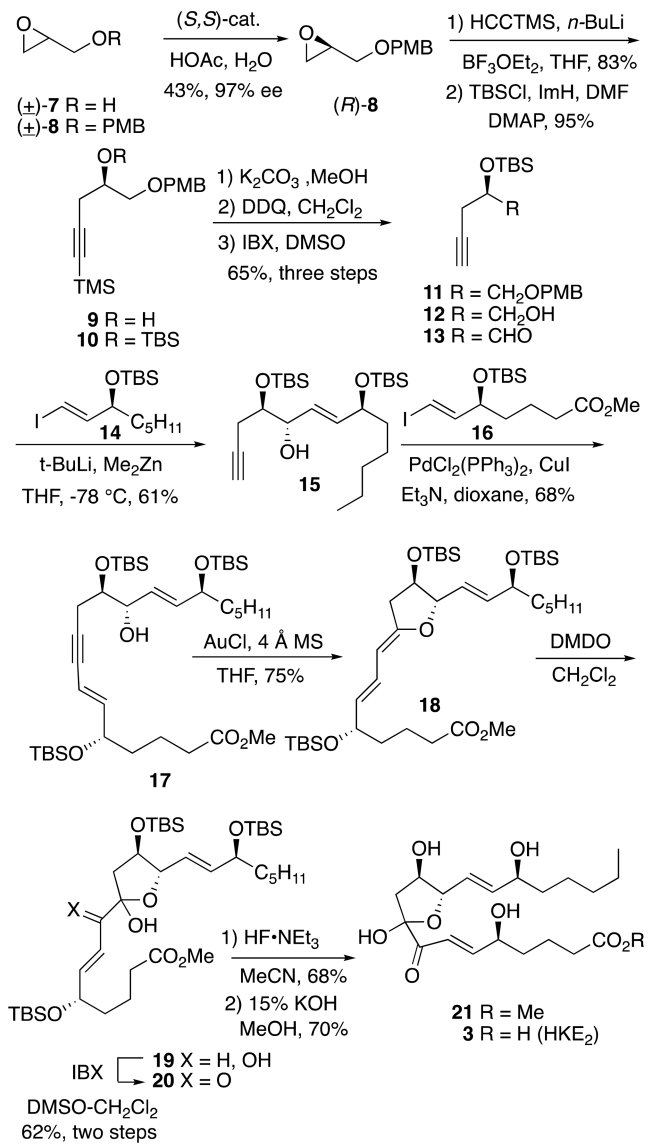


Figure 2. Synthetic strategy to access hemiketals D_2 and E_2.

aldehydes (cf. **5**, Figure 2) and relative stereochemistry (C8–C9 and C11–C12) would be established in a vinyl metal addition to **5** directed by the adjacent stereocenter (OP) by way of either a Felkin–Anh (2-siloxyaldehyde) or chelation-controlled (2-alkoxyaldehyde) addition.[8] The cyclic hemiketal would be introduced by a two-step process starting with a metal-mediated cycloisomerization followed by oxidation of the intermediate enol ether (Figure 2).[9] We chose as our first objective the total synthesis of hemiketal E_2 (Scheme 1).

Scheme 1. Total Synthesis of HKE_2



Our synthesis started with racemic glycidol **7**, as derivatives of glycidol are readily resolved into single enantiomeric products using the Jacobsen hydrolytic–kinetic resolution.[10] For example, PMB ether **8** was readily resolved into (*R*)-**8** using the (*S,S*)-Co(salen) catalyst.[11] After epoxide opening with trimethylsilyl acetylide, the resulting secondary alcohol (**9**)[12] was protected as a TBS ether in anticipation of a Felkin–Anh directed addition to aldehyde **13**. Aldehyde **13** was obtained from **10** by way of the three-step deprotection-oxidation sequence as shown in Scheme 1. Next, treatment of known vinyl iodide **14**,[13] with t-butyllithium afforded an intermediate vinyllithium reagent that was reacted with dimethylzinc leading to a nonbasic vinyl zincate which was added to aldehyde **13** to give allylic alcohol **15** in 61% yield (>95:5 diastereoselectivity). Sonogashira coupling of acetylene **15** with vinyl iodide **16**[14] gave enyne **17** in 68% yield.

Either a 6-*endo* or 5-*exo-dig* mode of cyclization of alkynol **17** was projected to afford the desired hemiketal product following oxidation of either the pyran or furan product, respectively.[9] After various conditions were examined, it was determined that gold(I) chloride in tetrahydrofuran proved most efficient to afford the 5-*exo* product, furan **18**, in 75% yield.[15] While furan **18** could be purified using buffered silica gel and characterized by NMR analysis, it had a finite lifetime in benzene-d_6 of less than 24 h. Typically, **18** was immediately epoxidized using dimethyl dioxirane following isolation. The epoxide product was not isolated, but instead, diol **19**, a product of hydrolysis, was obtained and subsequently oxidized using IBX in DMSO to give hemiketal **20** in 62% yield from enol ether **18**. Removal of TBS protecting groups was effected using HF-pyridine in acetonitrile to provide methyl ester **21**. Saponification of ester **21** provided hemiketal E_2 (**3**), identical by ^{1}H NMR to HKE_2 derived enzymatically.[7]

Hemiketal E_2 inhibited human platelet aggregation induced by the thromboxane receptor agonist U46,619 in a dose-dependent manner (IC_{50} < 500 nM). Inhibition was more potent in platelets isolated from individuals in whom PGE_2 at 100 nM concentration was unable to inhibit aggregation.[16] Chemically synthesized and enzymatically prepared HKE_2 (1 μM) were equally effective at inhibiting platelet aggregation (Figure 3).

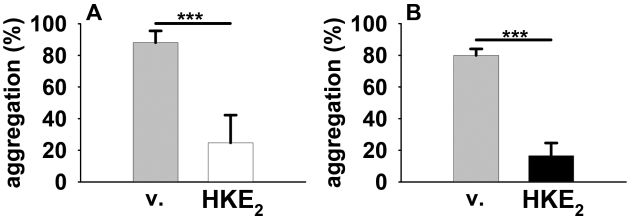


Figure 3. HKE_2 inhibits platelet aggregation. Human platelet-rich plasma was preincubated with vehicle (v) or HKE_2 (1 μM) obtained from chemical synthesis (A) or enzymatic synthesis (B) for 10 min at 37 °C before stimulation of aggregation by the thromboxane receptor agonist U46,619.[17]

Unfortunately, efforts directed toward the total synthesis of hemiketal D_2 starting from 9*S*-**5** (P = Bn) proved unproductive. Problems encountered in effecting the chelation-controlled carbonyl addition included low stereoselectivity and narrow scope of tolerated functionality in the vinyl metal reagent (**5** to **6**, Figure 2). Access to both hemiketals will

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provide opportunity for further biological study of these interesting and unusual eicosanoids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01578.

General experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Funk, C. D. *Science* **2001**, *294*, 1871.
(2) (a) Harizi, H.; Corcuff, J. B.; Gualde, N. *Trends Mol. Med.* **2008**, *14*, 461. (b) Dennis, E. A.; Norris, P. C. *Nat. Rev. Immunol.* **2015**, *15*, 511.
(3) (a) Haeggstrom, J. Z.; Funk, C. D. *Chem. Rev.* **2011**, *111*, 5866. (b) Rouzer, C. A.; Marnett, L. J. *Chem. Rev.* **2011**, *111*, 5899.
(4) Griesser, M.; Suzuki, T.; Tejera, N.; Mont, S.; Boeglin, W. E.; Pozzi, A.; Schneider, C. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 6945.
(5) Schneider, C.; Boeglin, W. E.; Yin, H. Y.; Stec, D. F.; Voehler, M. *J. Am. Chem. Soc.* **2006**, *128*, 720.
(6) Giménez-Bastida, J. A.; Shibata, T.; Uchida, K.; Schneider, C. *FASEB J.* **2017**, *31*, 1867.
(7) Gimenez-Bastida, J. A.; Suzuki, T.; Sprinkel, K. C.; Boeglin, W. E.; Schneider, C. *Prostaglandins Other Lipid Mediators* **2017**, *132*, 41−46.
(8) For a review on the stereoselectivity of alkynyl metals to 2-substituted aldehydes, see: Guillarme, S.; Ple, K.; Banchet, A.; Liard, A.; Haudrechy, A. *Chem. Rev.* **2006**, *106*, 2355.
(9) For an example of a similar cyclization-oxidation sequence, see: Woo, S. K.; Lee, E. *J. Am. Chem. Soc.* **2010**, *132*, 4564.
(10) Furrow, M. E.; Schaus, S. E.; Jacobsen, E. N. *J. Org. Chem.* **1998**, *63*, 6776.
(11) Trygstad, T. M.; Pang, Y. C.; Forsyth, C. J. *J. Org. Chem.* **2009**, *74*, 910.
(12) Trost, B. M.; Machacek, M. R.; Faulk, B. D. *J. Am. Chem. Soc.* **2006**, *128*, 6745.
(13) Prepared from hexanoyl chloride in six steps: (a) Marron, B. E.; Spanevello, R. A.; Elisseou, M. E.; Serhan, C. N.; Nicolaou, K. C. *J. Org. Chem.* **1989**, *54*, 5522. (b) Matsumura, K.; Hashiguchi, S.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1997**, *119*, 8738. (c) Nicolaou, K. C.; Veale, C. A.; Webber, S. E.; Katerinopoulos, H. *J. Am. Chem. Soc.* **1985**, *107*, 7515. (d) Suzuki, M.; Kiho, T.; Tomokiyo, K.; Furuta, K.; Fukushima, S.; Takeuchi, Y.; Nakanishi, M.; Noyori, R. *J. Med. Chem.* **1998**, *41*, 3084.
(14) Prepared from methyl 4-(chloroformyl)butyrate in six steps: (a) Gotz, K.; Liermann, J. C.; Thines, E.; Anke, H.; Opatz, T. *Org. Biomol. Chem.* **2010**, *8*, 2123. (b) Treilhou, M.; Fauve, A.; Pougny, J. R.; Prome, J. C.; Veschambre, H. *J. Org. Chem.* **1992**, *57*, 3203. (c) Chemin, D.; Linstrumelle, G. *Tetrahedron* **1992**, *48*, 1943−1952. (d) Rodriguez, A. R.; Spur, B. W. *Tetrahedron Lett.* **2012**, *53*, 86−89.
(15) (a) Review: Alcaide, B.; Almendros, P.; Alonso, J. M. *Org. Biomol. Chem.* **2011**, *9*, 4405. (b) Harkat, H.; Weibel, J.-M.; Pale, P. *Tetrahedron Lett.* **2007**, *48*, 1439.
(16) Smith, J. P.; Haddad, E. V.; Downey, J. D.; Breyer, R. M.; Boutaud, O. *Arteriosclerosis Thrombosis and Vascular Biology* **2010**, *30*, E219.
(17) Data are presented as mean ± SD from four healthy volunteers. Symbols show differences between the different groups; ***, $p < 0.001$.

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