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Graphical Abstract

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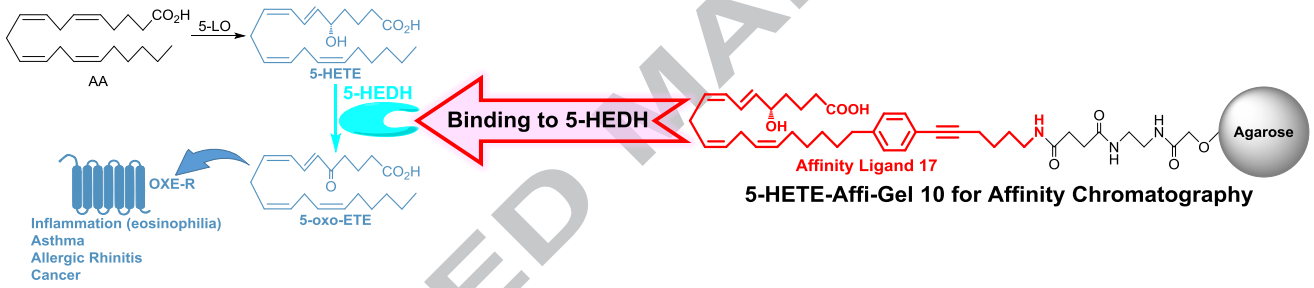

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ABSTRACT

Arachidonic acid (AA) is converted to biologically active metabolites by different pathways, one of the most important of which is initiated by 5-lipoxygenase (5-LO). 5-Hydroxyeicosatetraenoic acid (5-HETE), although possessing only weak bio-logical activity itself, is oxidized to 5-oxo-6, 8, 11, 14-eicosatetraenoic acid (5-oxo-ETE), a potent chemoattractant for eosinophils and neutrophils. Our main goal is to determine how the biosynthesis of 5-oxo-ETE is regulated and to determine its pathophysiological roles. To achieve this task, we designed and synthesized affinity chromatography ligands for the purification of 5-hydroxyeicosanoid dehydrogenase (5-HEDH), the enzyme responsible for the formation of 5-oxo-ETE.

1. Introduction

The 5-LO pathway of AA (**1**) involves the introduction of oxygen at position C-5 to form 5-hydroperoxyeicosatetraenoic acid (5-HpETE, **2**), which is then transformed to leukotriene (LT) A_4 (**5**) by the action of 5-LO (Figure 1). LTA_4 is then transformed to LTB_4 (**6**) by the action of LTA_4 hydrolase, or to LTC_4 (**7**) by the action of LTC_4 synthase. Finally, LTC_4 is converted to LTD_4. The 5-oxo-ETE pathway was first discovered while studies were being conducted on the metabolism of LTB_4 and related compounds in human neutrophils.[1] It was found that 5-HpETE formed by 5-LO is also converted by peroxidase(s) into 5-HETE (**9**). 5-HETE is the major product of the 5-LO pathway in all the cells that contain the enzyme 5-HEDH,[1] which is present in human neutrophils,[2] eosinophils,[3] monocytes, lymphocytes,[4] and platelets.[5] Oxidation of 5-HETE (**9**) to 5-oxo-ETE (**10**) by this enzyme is limited by the availability of nicotinamide adenine dinucleotide phosphate ($NADP^+$), which is normally present at very low concentrations in cells unless they are subjected to oxidative stress. In order to design 5-HEDH inhibitors that may be used ultimately as therapeutic agents, it is very useful to purify, characterize, and clone 5-HEDH. Also, the study of 5-HEDH is essential in determining the role of 5-oxo-ETE in normal physiology and potential involvement in the pathology of inflammatory diseases. Little information is available on 5-HEDH,[1, 6] and we intended to approach this problem by designing and synthesizing affinity chromatography ligands for the purification of 5-HEDH. We have synthesized and characterized an improved affinity chromatography ligand **17** (Scheme 1). We have also performed the substrate activity experiments on these compounds, and the initial results are encouraging as they clearly indicate that at low concentrations these ligands are as good substrates as 5-HETE.

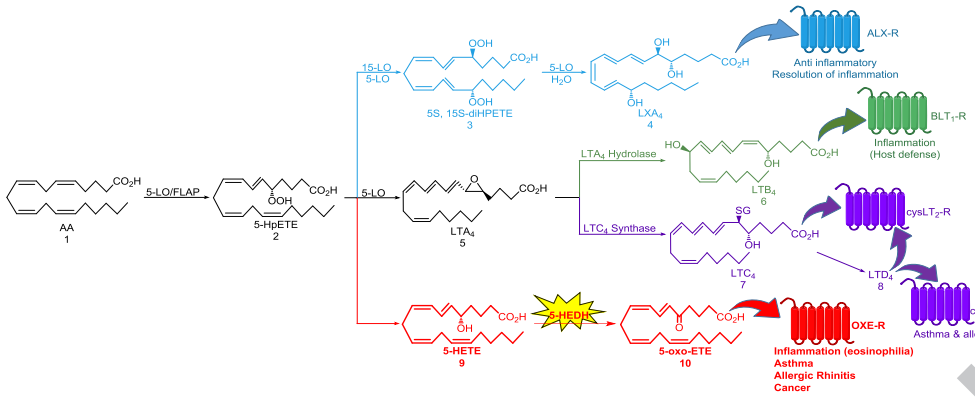


Figure 1. The 5-LO pathway of AA, and the biological effects of the eicosanoids.

2. Design of Affinity Chromatography Ligands

In order to purify and characterize the enzyme 5-HEDH, which has not been isolated previously, we have designed and synthesized the affinity chromatography ligands with high affinity for 5-HEDH. This enzyme is highly selective for eicosanoids containing a 5(*S*)-hydroxyl group at position C-5 and a 6-*trans* double bond.[1] Coupling of the carboxyl group of 5-HETE to a solid support would be relatively simple. However, in structure-activity studies we found that 5-HETE methyl ester is a very poor substrate for 5-HEDH, clearly indicating that the free carboxyl group of the molecule is very important for binding to the enzyme. For this reason, we previously decided to target the ω-end of 5-HETE, and prepared the 20-amino derivative of 5-hydroxy-6, 8, 11-eicosatrienoic acid **11** (Figure 2), which permitted the attachment of various groups through amide linkages.[6] Two types of affinity ligands have been designed and synthesized, one for use as a photo affinity probe and the other to be used as a reagent for affinity chromatography. The photo affinity probe with an azido group was used because the azido group, on stimulation with UV light, generates a highly reactive nitrene species that can react covalently with an amino acid at the catalytic site. Also, we had previously prepared the biotinylated derivative (**12**) [11,6] which was coupled to a streptavidin-agarose column for affinity chromatography. The presence of biotin permits the attachment of the ligand to streptavidin-agarose particles through the high affinity binding ($K_d = 10^{-15}$ M) between biotin and streptavidin (Figure 2).[7] Unfortunately, incubation of the unlabeled probe **13** with neutrophil microsomes revealed that it is not a good substrate for 5-HEDH. Also, **12** proved to be a rather poor substrate for this enzyme, and did not significantly retain 5-HEDH activity when used for affinity chromatography, both in the presence and absence of the cofactor $NADP^+$. This result is similar to other cases in which the introduction of a polar group, such as hydroxyl, at C-20 in eicosanoid structures result in loss of activity.[8, 9]

To create improved affinity ligands for 5-HEDH, we have currently developed four designs in an attempt to circumvent the problems we initially experienced. ***The first*** is to place a lipophilic spacer instead of the polar NH group at the C-20 position (**15**, and **18**, Scheme 1). ***The second*** modification sought, is to use a different linkage to the support column, e.g. Affi-Gel 10 (**15**, **16**, and **17**, Scheme 1). ***The third*** is the introduction of an acetylene group in

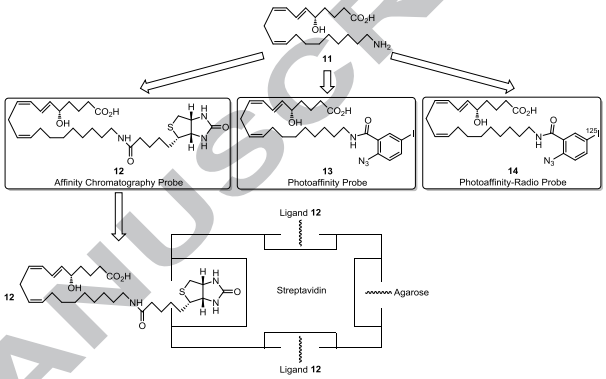
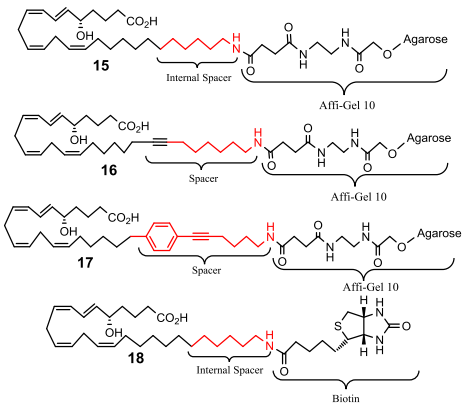


Figure 2. Previous attempts at the purification of 5-HEDH through affinity reagents.[6]

order to augment the rigidity in the molecule. The introduction of rigidity will keep the ω-lipophilic end extended and provide better recognition by the enzyme. For example, designs **16**, and **17**, which provide rigidity to the central portion of the molecule by adding a phenyl and an acetylene functions at the ω-end of the ligand, may address this potential deficiency in the previous designs. ***The fourth*** is to maintain the 11, 14-skipped diene structure in the molecule in order to further insure the activity of the ligands (**15** - **18**).[10] In our previous attempt of the affinity ligand (Figure 2), we omitted the 14, 15-double bond for simplicity. In the present study, we selected **17** as our synthetic target.



Scheme 1. Examples of improved affinity chromatography ligands for 5-HEDH.

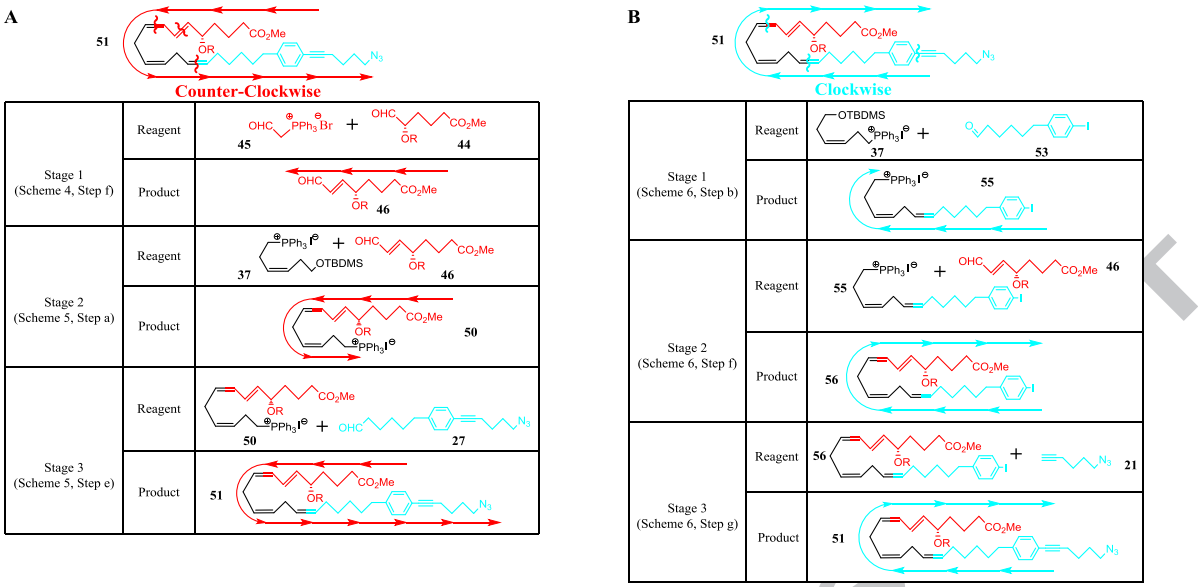


Figure 3. Synthetic strategies for preparing different affinity chromatography ligands.

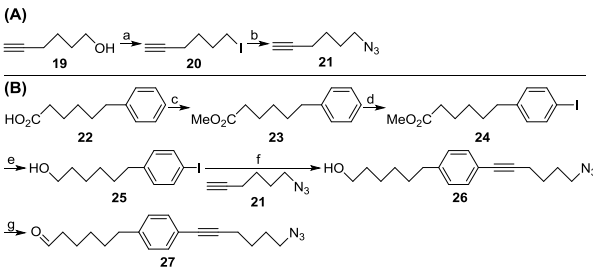
3. Synthetic Strategy

In order to synthesize different affinity chromatography ligands, we used four important intermediates (**27**, **37**, **46**, and **53**, Figure 3) that were synthesized separately. Figure 3 shows the strategy we used to plan our synthetic approach. Fig. 3A shows a counterclockwise approach, which proved to be the most useful, that builds the affinity reagent starting from the first eight carbons of the carboxyl end by the sequential addition of a series of intermediates culminating in completion of the ω-end of the molecule. This approach facilitates the synthesis of various modifications of the ω-end of the molecule. The second approach involves the clockwise addition of segments of the molecule to **53**, which contains the ω1 to ω6 portion of 5-HETE coupled to a *p*-iodophenyl group. This permits modification of both the carboxyl and the ω ends of the molecule (Figure 3B). Although in the present case the modifications of 5-HETE are principally at the ω-end, we have performed the synthesis of the ligands using both approaches.

4. Results and Discussion

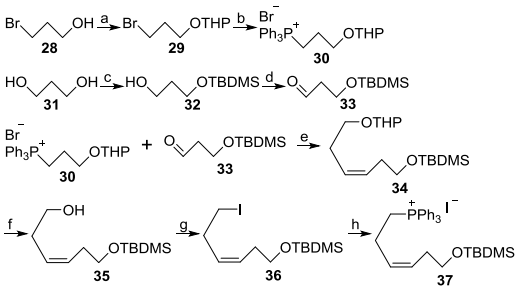
4.1. Synthesis of Affinity Chromatography Ligands

The syntheses of the three intermediates **27**, **37**, and **46**, which we have currently developed, are shown in Schemes 2, 3, and 4, respectively. We constructed the para iodo intermediate **24**, using a thallium (III) trifluoroacetate procedure,[11] which afforded the desired compound in 87% yield. Later using the intermediate **25** we performed the Sonogashira coupling reaction[12] to obtain the desired azido intermediate **26**.



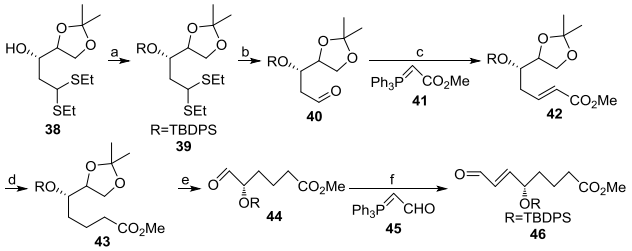
Scheme 2. Synthesis of the azido intermediates **21** and **27**. Reagents and Conditions: (a) PPh_3, imidazole, I_2, THF, 5 h, 96%; (b) NaN_3, DMF, 3.5 h, 91%; (c) CH_2N_2, Et_2O, rt, 1 h, 96%; (d) $Tl(CF_3COO)_3$, TFA, KI, H_2O, 5 days, 87%; (e) DIBAL-H, dry CH_2Cl_2, 0 °C, 2 h, 95%; (f) $Pd(OAc)_2$, PPh_3, CuI, diethyl amine, 60 °C, 1 h, 70%; (g) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78 °C, 2 h.

The 6-carbon phosphonium salt **37** was synthesized as shown in Scheme 3. We started with commercially available 3-bromopropanol (**28**) and 1, 3-propanediol (**31**) to afford the phosphonium salt **30** and the aldehyde **33**, respectively. The two were coupled together in a Wittig reaction using LiHMDS to yield **34** that was subsequently converted into the desired phosphonium salt **37**.



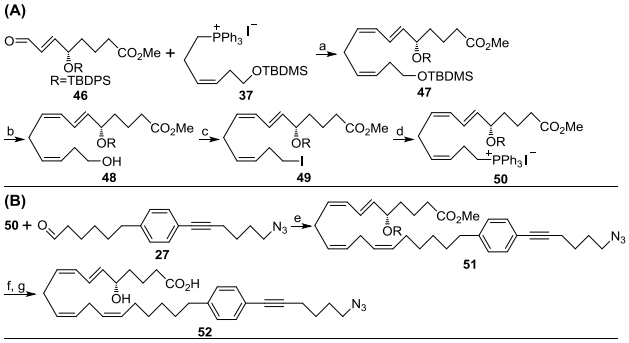
Scheme 3. Synthesis of intermediate **37**. Reagents and Conditions: (a) DHP, *p*-TsOH, CH_2Cl_2, 0 °C, 8 h, 95%; (b) PPh_3, CH_3CN, 40 °C, 48 h, 71%; (c) TBDMSCl, Imidazole, CH_2Cl_2, 5 h, 60%; (d) PCC, Al_2O_3, CH_2Cl_2, rt, 5 h, 78%; (e)LiHMDS, THF/HMPA (4:1), −78 °C to rt, 3 h, 75%; (f) Me_2AlCl, 0 °C, 8 h, 98%; (g) PPh_3, imidazole, I_2, THF, 0 °C-rt, 2 h, 92%; (h) PPh_3, CH_3CN, 48 h, 45 °C, 79%.

The *α,β*-unsaturated aldehyde **46** required for the upper carboxyl portion of the molecule was prepared according to our earlier reported procedure (Scheme 4).[8]



Scheme 4. Synthesis of *α,β*-Unsaturated Aldehyde **46**. Reagents and Conditions: (a) TBDPSCl, imidazole, CH_2Cl_2, 0 °C-rt, 12 h, 98%; (b) periodic acid, THF, 0 °C, 4 h, 83 %; (c) **41**, THF, rt, 6 h, 91%; (d) 10% Pd/C, H_2, EtOH, rt, 5 h, 96%; (e) periodic acid, THF, rt, 5 h, 77%; (f) **45**, benzene, reflux, 10 h, 76%.

Scheme 5 shows the synthesis of the azide **52** from intermediates **50** and **27** described in stage 3 of Figure 3A. A Wittig reaction of the aldehyde **27** and the phosphonium salt **50** using LiHMDS afforded the intermediate **51**. Finally, on hydrolysis and deprotection of **51**, the affinity chromatography ligand **52** was generated.



Scheme 5. Synthesis of the azido affinity chromatography ligand **52** by a counterclockwise approach. Reagents and Conditions: (a) **37**, LiHMDS, THF/HMPA (4:1), −78 °C to rt, 2 h, 95%; (b) PPTS, MeOH/H_2O (4:1), rt, 8 h, 80%; (c) PPh_3, imidazole, I_2, THF, 0 °C-rt, 5 h, 97%; (d) PPh_3, CH_3CN, 50 °C, 48 h, 78%; (e) 50, LiHMDS, THF/HMPA (4:1), −78 °C to rt, 3 h, 60%; (f) LiOH, THF/H_2O (4:1), rt, 10 h; (g) TBAF/AcOH, THF, 50 °C, 10 h, 75%, over two steps f and g.

To investigate the potential affinity of **52** for 5-HEDH we used as an enzyme source microsomal fractions from U937 cells that had been differentiated with phorbol myristate acetate to increase 5-HEDH activity.[15] Different concentrations of **52** or 5-HETE were incubated with U937 cell microsomes in the presence of $NADP^+$(100 µM) for 5 min and the resulting 5-oxo products were quantitated by HPLC. It can be seen from Figure 4 that **52** is nearly equivalent to 5-HETE as a substrate for 5-HEDH, indicating that modification of 5-HETE in this way does not reduce its affinity for the enzyme.

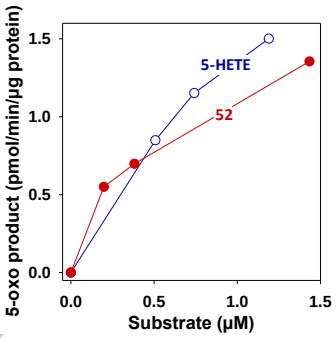
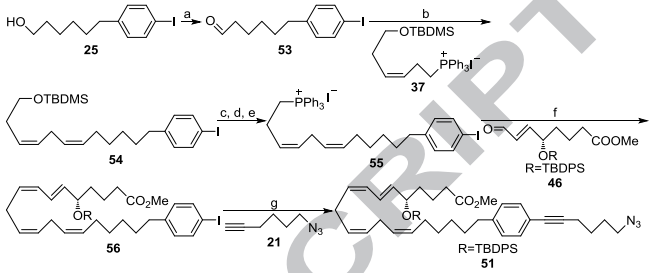


Figure 4. Oxidation of 5-HETE and **52** by 5-HEDH. A microsomal fraction (50 µg protein/ml) from human neutrophils was incubated with different concentrations of either 5-HETE or **52** for 5 min at 37 °C in the presence of $NADP^+$(100 µM) and the 5-oxo product was quantitated by RP-HPLC.

We have also investigated the clockwise approach (Figure 3B) for the synthesis of the azido intermediate **51** (Scheme 6). In this approach, the iodophenyl derivative **25** was converted into the aldehyde **53**, which was further coupled with the 6-carbon synthon **37** to afford **54**. The silyl ether function in **54** was deprotected, iodinated, and converted in to the iodo phosphonium salt **55**. The following Wittig reaction between **55** and the chiral aldehyde **46** produced the iodophenyl intermediate **56**, which was

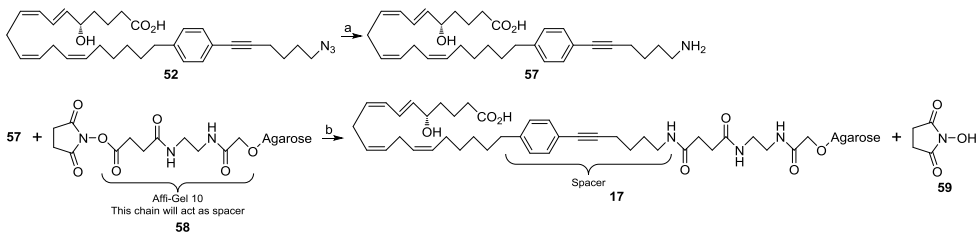


reacted with the acetylene **21** to yield the azido compound **51**.

Scheme 6. Synthesis of **51** using a clockwise approach. Reagents and Conditions: (a) Dess-Martin periodinane, CH_2Cl_2, rt, 4 h, 88%; (b) **37**, LiHMDS, THF/HMPA (4:1), −78 °C-rt, 3 h, 56%; (c) PTSA, MeOH, rt, 3 h, 60%; (d) PPh_3, imidazole, I_2, THF, 0 °C to rt, 5 h, 90%; (e) PPh_3, CH_3CN, 50 °C, 10 h, 96%; (f) LiHMDS, THF/HMPA (4:1), −78 °C to rt, 3 h, 98%. (g) $Pd(OAc)_2$, TPP, CuI, diethyl amine, 60 °C, 16 h, 64%.

The amine **57** was obtained by the reduction of the azido **52** with PPh_3 (Scheme 7). The coupling of **57** to Affi-Gel 10 was modeled by using commercially available 6-aminocaproic acid, and the successful condition was then applied to the immobilization of **57** as shown in Scheme 7. The percentage of binding was calculated on the basis of the concentrations of the *N*-hydroxysuccinimide **59** (Scheme 7) measured by UV,[14] as it is one of the by-products generated during the coupling reaction.

A typical coupling experiment consisted of dissolving approximately 10 mg of amine **57** ligand in 1 mL of isopropanol/MeOH (1/1). The pH was then adjusted to 7.5 using a saturated solution of LiOH in water. A suspension of Affi-Gel 10 (1 mL) was added to the above solution and agitated using a mechanical shaker at 4 °C with continuous shaking for 14 h. The reaction mixture was then filtered, the solid particles washed with cold isopropanol, and the filtrate collected in a flask. The solid Affi-Gel 10 was carefully transferred to a vial and stored in isopropanol at −20 °C. The pH of the collected filtrate was adjusted to 8.5 and the solution was used to measure the UV absorbance. The concentrations of *N*-hydroxysuccinimide (**59**) were established, and by using these concentrations, we calculated the concentration of the amine **57** consumed in the reaction. In this way, we determined that 1 ml of Affi-Gel 10 is capable of binding 15 µmoles of **57**. The reaction yield was 60% as determined by UV.



Scheme 7. Synthesis of 5-HETE Affi-Gel 10 affinity chromatography ligand **17**. Reagents and Conditions: (a) PPh_3, THF, H_2O, rt, 24 h, 60%; (b) pH 7.5, isopropanol/MeOH (1:1), rt.

4.2. Preliminary Results on the Purification of 5-HEDH by Using 17

Preliminary experiments were performed to determine the ability of **17** to bind 5-HEDH. 5-HEDH from differentiated U937 cell microsomes was first partially purified using DEAE-Sepharose and ultrafiltration through a 50 kDa cutoff filter as described in Experimental Procedures. The partially purified 5-HEDH was reconstituted in 20 mM phosphate containing 30 mM octylglucoside and 15% MeOH and incubated with **17** for 20 min at 6 °C, followed by centrifugation. 5-HEDH activity in the different fractions was assessed by precolumn extraction/RP-HPLC[16] following incubation of aliquots with 5-HETE (1 μM) and $NADP^+$ (100 μM) for 30 min at 37 °C. Although a substantial proportion of the 5-HEDH activity in the preparation was retained under these conditions, a considerable amount of unbound 5-HEDH was detected in the initial supernatant, along with the majority of protein (first fraction in Figure 5). Subsequent washing of the **17** with the loading buffer eluted only small amounts of enzyme activity. Somewhat surprisingly, we were unable to elute substantial amounts of 5-HEDH by addition of 5-HETE to the eluting buffer, perhaps because of the high affinity of **17** for the enzyme. However, addition of NADPH resulted in the elution of a large amount of 5-HEDH that had been bound by the stationary phase. NADPH is a very potent inhibitor of the oxidation of 5-HETE to 5-oxo-ETE by 5-HEDH, presumably because it induces a conformational change in the enzyme resulting in dramatically reduced affinity for its substrate[15] and subsequent release from the stationary phase. The fact that 5-HEDH is a membrane-bound enzyme substantially complicates its purification because it requires the presence of large amounts of detergent to maintain its solubility. Binding of detergent to the enzyme is likely to interfere with its interaction with **17**, resulting in its only partial retention by the stationary phase as shown in Figure 5.

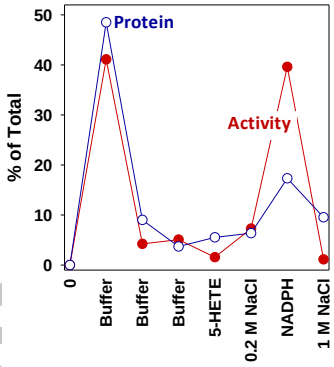


Figure 5. Purification of 5-HEDH using **17**. 5-HEDH was first partially purified using DEAE-Sepharose and ultrafiltration as described in Experimental Procedures. The 5-HEDH fraction in 20 mM phosphate, pH 7.4, containing octyl glucoside (30 mM) and 20% MeOH was incubated with **17** for 20 min at 6 °C. After washing with the same buffer, the protein adsorbed on **17** was eluted successively with 20 μM 5-HETE, 0.2 M NaCl, 1 mM NADPH, and 1 M NaCl in the above buffer as described in Experimental Procedures. 5-HEDH activity in the fractions was measured by precolumn extraction/RP-HPLC, whereas protein was measured using the Bio-Rad DC protein assay kit. Preliminary calculation indicates a 30-40% enrichment was achieved.

5. Conclusion

In order to purify 5-HEDH, we designed and synthesized the novel affinity chromatography ligand **17** by two approaches, clockwise and counterclockwise. These ligands retain the C1 carboxyl group and the terminal hydrophobic region of 5-HETE, which are essential structural elements for recognition by 5-HEDH. Initial results of the enzyme purification experiments demonstrate significant binding of these ligands to the enzyme, which is encouraging, and we anticipate that optimization of the purification procedure will improve the yield.

6. Experimental

6.1. Reagents and Methods

All reactions were carried out under argon atmosphere using dry solvents. Biotin-*N*-hydroxysuccinimide ester, thallium(III) trifluoroacetate, and Palladium(II) acetate were purchased from Sigma-Aldrich. Affi-Gel 10 was purchased from Bio-Rad. All compounds were analyzed by TLC, ^{1}H NMR, ^{13}C NMR, and HRMS. ^{1}H NMR and ^{13}C NMR spectra were recorded at rt on a BRUKER AMX 400 MHz spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as an internal standard. High-resolution mass spectra were recorded on an AccuTOF mass spectrometer by positive ion ESI mode with DART as an ion source. Prior to biological assay, the purity of all final compounds was determined to be >95% by a combination of HPLC, NMR and HRMS.

6.2. Synthesis of Compounds

6-azidohex-1-yne (21). To a stirred solution of iodo compound **20** (6.0 g, 28.84 mmol) in anhydrous DMF (30 mL) was added Sodium azide (9.37 g, 144.20 mmol) at 0 °C under argon atmosphere. The resulting mixture was stirred at rt for 3.5 h, then quenched with ice cold water (50 mL) and extracted with EtOAc (2 × 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude residue was purified by silica gel column chromatography using 10% EtOAc/hexane to afford azide compound **21** (3.23 g, 91%)

as a pale-yellow liquid. HRMS (ESI) *m/z* calcd for $[C_6H_9N_3+H]^+$: 124.0875, found 124.0218. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.31 (t, *J* = 6.7 Hz, 2H), 2.24 (td, *J* = 6.8, 2.5 Hz, 2H), 1.97 (t, *J* = 2.6 Hz, 1H), 1.77-1.70 (m, 2H). 1.65-1.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 83.6, 68.9, 50.9, 27.8, 25.5, 18.0.

Methyl 6-(4-iodophenyl)hexanoate (24). To a stirred solution of ester **23** (3 g, 14.54 mmol) in trifluoroaceticacid (20 mL) was added thallium (III) trifluoroacetate (12.25 g, 22.54 mmol) at rt. The reaction mixture was stirred for 5 days in the dark under argon. Then potassium iodide (12.0 g, 72.71 mmol) was added in water (72.0 mL) and the resulting mixture was stirred for 15 min at rt and color of the reaction mixture was turned from brown to green. Sodium thiosulfate (7.32 g) was added to the reaction mixture and stirred for an additional 15 min and the color changed from green to yellow green foam, then the reaction mixture was poured into water (120 mL) and extracted with ether (3 × 30 mL). The ether extracts were washed with 0.1 N HCl and water (50 mL). The solvent was dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using 10% EtOAc/hexane afforded iodo compound **24** (4.2 g, 87%) as a colorless oil. HRMS (ESI) *m/z* calcd for $[C_{13}H_{17}IO_2+H]^+$, 333.0352; found, 333.0076. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 7.8 Hz, 2H), 6.91 (d, *J* = 7.8 Hz, 2H), 3.65 (s, 3H), 2.54 (t, *J* = 7.6 Hz, 2H), 2.29 (t, *J* = 7.3 Hz, 2H), 1.69-1.57 (m, 4H), 1.39-1.29 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 174.2, 142.1, 137.4 (2C), 130.6 (2C), 90.7, 51.6, 35.2, 34.0, 30.9, 28.6, 24.7.

6-(4-iodophenyl)hexan-1-ol (25). To a stirred solution of ester **24** (4.0 g, 12.04 mmol) in anhydrous CH_2Cl_2 (70 mL) was added DIBAL-H (1.0 M in hexane, 24.08 mL, 24.08 mmol) dropwise over a period of 5 min at −78 °C under argon atmosphere. After stirring for 2 h at rt anhydrous MeOH (2 mL) was added at 0 °C, and the mixture was allowed to warm to rt saturated aqueous solution of sodium potassium tartarate (25 mL) was added, and the resulting mixture was stirred vigorously until the two layers were separated. The organic layer was separated and the aqueous layer was extracted with additional CH_2Cl_2 (3 × 40 mL). The combined organic layers were washed with H_2O, brine solution and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo and purified by silica gel column chromatography using 20% EtOAc/hexane to afford the iodo-alcohol **25** (3.48 g, 95% yield) as a colorless liquid. HRMS (ESI) *m/z* calcd for $[C_{12}H_{17}IO+H]^+$, 305.0397; found, 305.0665. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 7.6 Hz, 2H), 6.91 (d, *J* = 7.6 Hz, 2H), 3.61 (t, *J* = 6.6 Hz, 2H), 2.53 (t, *J* = 7.6 Hz, 2H), 1.69-1.49 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): 143.1, 138.0 (2C), 131.4(2C), 91.1, 63.3, 35.8, 33.2, 31.8, 29.4, 26.1.

6-(4-(6-azidohex-1-yn-1-yl)phenyl)hexan-1-ol (26). To a stirred solution of iodo-alcohol compound **25** (3.95 g, 12.98 mmol), and azido compound **21** (2 g, 16.24 mmol) in anhydrous diethylamine (25 mL), $Pd(OAc)_2$ (218 mg, 0.971 mmol), PPh_3 (851 mg, 3.24 mmol), and CuI (617 mg, 3.24 mmol) were added and stirred at 60 °C under argon for 1 h. After reaction completion, the solvent was removed under reduced pressure and the residue was dissolved in H_2O (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting mixture was chromatographed using 30% EtOAc/Hexane to give the product **26** (3.4 g, 70%) as a yellow color liquid. HRMS (ESI) *m/z* calcd for $[C_{18}H_{25}N_3O+H]^+$, 300.2071; found, 300.2301. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (d, *J* = 7.6 Hz, 2H), 7.08 (d, *J* = 7.5 Hz, 2H), 3.62 (t, *J* = 6.3 Hz, 2H), 3.34 (t, *J* = 6.2 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 2H), 2.45 (t, *J* = 6.6 Hz, 2H), 1.84-1.50 (m, 8H), 1.42-1.29 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 142.5, 131.5 (2C), 128.3 (2C), 120.9, 88.5, 81.3, 63.0, 51.1, 35.7, 32.8, 31.2, 28.9, 28.0, 25.9, 25.6, 19.0.

(*Z*)-*tert*-butyldimethyl((6-((tetrahydro-2H-pyran-2-yl)oxy)hex-3-en-1-yl)oxy)silane (34). To a stirred suspension of the phosphonium **30** (3.61 g, 7.43 mmol) in THF (20 mL) was added LiHMDS (11.5 mL, 11.5 mmol, 1.0 M solution in THF) and stirred at 0 °C for 40 min under argon. The reaction mixture was cooled to −78 °C and HMPA (0.5 mL) was added, 2 min later the aldehyde **33** (1.36 g, 5.94 mmol) in THF (20 mL) was added at −78 °C. The temperature was maintained at −78 °C for 30 min and then gradually warmed to rt. The reaction was quenched with sat. NH_4Cl solution (30 mL. The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using 5% EtOAc/hexane as eluent to afford **34** (1.4 g, 75%) as colorless oil. HRMS (ESI) m/z calcd for $[C_{17}H_{34}O_3Si+H]^+$, 315.2350; found, 315.1729. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.47-5.50 (m, 2H), 4.59 (dd, *J* = 4.4, 2.7 Hz, 1H), 3.90-3.84 (m, 1H), 3.79-3.67 (m, 1H), 3.63 (t, *J* = 6.9 Hz, 2H), 3.53-3.46 (m, 1H), 3.40 (dt, *J* = 9.4, 7.0 Hz, 1H), 2.36 (q, *J* = 6.7 Hz, 2H), 2.30 (q, *J* = 6.6 Hz, 2H), 1.86-1.81 (m, 1H), 1.74-1.67 (m, 1H), 1.62-1.47 (m, 4H), 0.89 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 127.7, 127.5, 98.7, 67.0, 62.9, 62.3, 31.2, 30.7, 28.1, 26.0 (3C), 25.5, 19.6, 18.4, −5.3 (2C).

(*Z*)-6-((*tert*-butyldimethylsilyl)oxy)hex-3-en-1-ol (35). To a stirred solution of **34** (10.6 g, 33.7 mmol) in dry CH_2Cl_2 (100 mL) at 0 °C was added Me_2AlCl (77.5 ml, 1.0 M solution in hexane) under argon. The reaction was continued for 3 h. The reaction mixture was quenched by adding brine, and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over $NaSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using 30% EtOAc/hexane to afford alcohol **35** (7.59 g, 98%) as colorless liquid. HRMS (ESI) *m/z* calcd for $[C_{12}H_{26}O_2Si+H]^+$, 231.1775; found, 231.1787. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.60-5.47 (m, 2H), 3.69-3.61 (m, 4H), 2.36-2.30 (m, 4H), 0.89 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 129.8, 127.4, 62.8, 62.1, 31.0, 30.9, 26.0 (3C), 18.5, −5.3 (2C).

(*Z*)-*tert*-butyl((6-iodohex-3-en-1-yl)oxy)dimethylsilane (36). To a stirred solution of alcohol **35** (4 g, 17.6 mmol) in 130 mL of CH_2Cl_2 at 0 °C was added PPh_3 (13.82 g, 52.8 mmol), imidazole (7.2 g, 105.6 mmol), and iodine (13.4 g, 52.8 mmol). After 2 h the reaction was quenched with sat. solution of $Na_2S_2O_3$ (30 mL). Aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL) and dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel column chromatography using 5% EtOAc/hexane to afford iodo compound **36** (5.5 g, 92%) as a yellow liquid. HRMS (ESI) *m/z* calcd for $[C_{12}H_{25}IO_2Si+H]^+$, 341.0792; found, 341.1118. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.59-5.50 (m, 1H), 5.47-5.37 (m, 1H), 3.63 (t, *J* = 6.8 Hz, 2H), 3.14 (t, *J* = 7.3 Hz, 2H), 2.65 (q, *J* = 7.2 Hz, 2H), 2.26 (q, *J* = 7.0 Hz, 2H), 0.89 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 129.6, 128.6, 62.6, 31.6, 31.3, 26.0, 18.4, 5.4 (3C), −5.3 (2 C).

(Z)-(6-((tert-butyldimethylsilyl)oxy)hex-3-en-1-yl)triphenylphosphonium iodide (37). To a stirred solution of iodo compound **36** (7.59 g, 22.3 mmol) in CH_3CN (60 mL) was added PPh_3 (15.2 g, 57.9 mmol) at rt under argon. The reaction mixture was heated at 55 °C for 24 hours. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using 5% MeOH/CH_2Cl_2 to afford phosphonium salt **37** (11.6 g, 86%) as a solid. HRMS (ESI) *m/z* calcd for $[C_{30}H_{40}OPSi]^+$, 475.2581; found, 475.2907. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 – 7.83 (m, 6H), 7.73 – 7.69 (m, 6H),

7.46 (t, *J* = 7.7 Hz, 3H), 5.72 (q, *J* = 7.8 Hz, 1H), 5.43 (q, *J* = 8.0 Hz, 1H), 3.80 (q, *J* = 9.4, 8.3 Hz, 2H), 3.53 (t, *J* = 6.6 Hz, 2H), 2.45 (p, *J* = 8.2 Hz, 2H), 2.05 (q, *J* = 6.8 Hz, 2H), 0.83 (s, 9H), −0.03 (s, 6H). ^{13}C NMR ($CDCl_3$): 135.1 (3C), 133.8 (6C), 130.6 (6C), 128.5 (3C), 118.5, 117.6, 62.4, 31.1, 26.0 (3C), 23.4, 20.5, 18.4, −5.3 (2C).

Methyl (*S*,6*E*,8*Z*,11*Z*)-14-((*tert*-butyldimethylsilyl)oxy)-5-((*tert*-butyldiphenylsilyl)oxy)tetradeca-6,8,11-trienoate (47). To a stirred suspension of the phosphonium **37** (8.51 g, 14.13 mmol) in THF (40 mL) was added LiHMDS (1.0 M solution in THF, 14.13 mL, 14.13 mmol) and stirred at 0 °C for 40 min under argon. The reaction mixture was cooled to −78 °C and HMPA (1.0 mL) was added, 2 min later the aldehyde **46** (3 g, 7.06 mmol) in THF (15 mL) was added at −78 °C. The temperature was maintained at − 78 °C for 30 min and then gradually warmed to rt. The reaction was quenched with sat. NH_4Cl solution (20 mL). The aqueous layer was extracted with EtOAc (3 × 35 mL). The combined organic layers were washed with brine, dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using 10% EtOAc/hexane to afford **47** (4.16 g, 95%) as colorless oil. HRMS (ESI) m/z calcd for $[C_{37}H_{56}O_4Si_2+H]^+$, 621.3790; found, 621.2495. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64-7.55 (m, 4H), 7.40-7.25 (m, 6H), 6.07 (dd, *J* = 15.0, 11.1 Hz, 1H), 5.80 (t, *J* = 10.8 Hz, 1H), 5.52 (dd, *J* = 15.1, 6.8 Hz, 1H), 5.40-5.19 (m, 3H), 4.15 (q, *J* = 6.1 Hz, 1H), 3.58 (s, 3H), 3.55 (t, *J* = 6.8 Hz, 2H), 2.72 (t, *J* = 6.7 Hz, 2H), 2.21 (q, *J* = 6.6 Hz, 2H), 2.13 (t, *J* = 7.0 Hz, 2H), 1.57-1.42 (m, 4H), 1.01 (s, 9H), 0.84 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.9, 136.0 (4C), 135.9, 135.6, 129.7, 129.6, 129.5, 129.2 (4C), 128.1, 127.5, 127.4, 126.5, 125.5, 73.7, 62.8, 51.4, 37.2, 34.0, 31.1, 27.0, 26.1 (3C), 26.0 (3C), 20.1, 19.3, 18.4, −5.2 (2C).

Methyl (S,6*E*,8*Z*,11*Z*)-5-((*tert*-butyldiphenylsilyl)oxy)-14-hydroxytetradeca-6,8,11-trienoate (48). To a stirred solution of **47** (4.0 g, 6.44 mmol) in CH_3OH (25 mL) at rt was added PPTS (161 mg, 0.644 mmol) under argon. The reaction was continued for 8 h. The reaction mixture was quenched with solid $NaHCO_3$ (100 mg), and concentrated under reduced pressure. The crude compound was purified by silica gel chromatography using 20% EtOAc/hexane to afford alcohol **48** (2.61 g, 80%) as an oil. HRMS (ESI) *m/z* calcd for $[C_{31}H_{42}O_4Si+H]^+$, 507.2926; found, 507.2902. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70-7.60 (m, 4H), 7.44-7.32 (m, 6H), 6.16 (dd, *J* = 14.8, 11.3 Hz, 1H), 5.87 (t, *J* = 10.6 Hz, 1H), 5.58 (dd, *J* = 15.0, 6.6 Hz, 1H), 5.51-5.26 (m, 3H), 4.21 (q, *J* = 6.0 Hz, 1H), 3.62 (s, 3H), 3.60 (t, *J* = 9.0 Hz, 2H), 2.80 (t, *J* = 7.2 Hz, 2H), 2.30 (q, *J* = 6.5 Hz, 2H), 2.18 (t, *J* = 6.7 Hz, 2H), 1.59-1.46 (m, 4H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 174.0, 135.9 (5C), 130.6, 129.6 (5C), 128.5, 129.3, 128.2, 127.5, 127.4, 125.9, 125.4, 73.7, 62.2, 51.4, 37.2, 34.0, 30.9, 27.1 (3C), 26.1, 20.2, 19.4.

Methyl (*S*,6*E*,8*Z*,11*Z*)-5-((*tert*-butyldiphenylsilyl)oxy)-14-iodotetradeca-6,8,11-trienoate (49). To a stirred solution of alcohol **48** (2.5 g, 5.00 mmol) in 35 mL of THF at 0 °C was added triphenylphosphine (1.55 g, 5.92 mmol), imidazole (671 mg, 9.86 mmol), and iodine (1.25 g, 4.93 mmol). After 5 h the reaction was quenched with sat. solution of $Na_2S_2O_3$ (15 mL), aqueous layer was exctracted with EtOAc (2 × 20 mL) and dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel column chromatography using 15% EtOAc/hexane to afford iodo compound **49** (2.95 g, 97%) as a yellow color liquid. HRMS (ESI) *m/z* calcd for $[C_{31}H_{41}IO_3Si+H]^+$, 617.1943; found, 617.2052. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69-7.60 (m, 4H), 7.44-7.32 (m, 6H), 6.13 (dd, *J* = 15.0,11.2, Hz, 1H), 5.87 (t, *J* = 10.7 Hz, 1H), 5.58 (dd, *J* = 15.0, 6.7 Hz, 1H), 5.50-5.24 (m, 3H), 4.22 (q, *J* = 5.9 Hz, 1H), 3.63 (s, 3H), 3.11 (t, *J* = 7.1 Hz, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 2.62 (q, *J* = 6.9 Hz, 2H), 2.19 (t, *J* = 6.9 Hz, 2H), 1.64-1.46 (m, 4H), 1.06 (s, 9H). ^{13}C NMR ($CDCl_3$): 174.0, 136.0 (3C), 136.0, 134.3, 134.1, 130.1, 129.6, 129.5, 129.0, 128.5 (3C), 128.4, 127.5, 127.4, 125.5, 125.3, 73.7, 51.5, 37.2, 34.0, 31.4, 27.1 (3C), 26.2, 20.1, 19.4, 5.3.

((*S*,3*Z*,6*Z*,8*E*)-10-((*tert*-butyldiphenylsilyl)oxy)-14-methoxy-14-oxotetradeca-3,6,8-trien-1-yl)triphenylphosphonium iodide (50). To a stirred solution of iodo compound **49** (2.8 g, 4.54 mmol) in CH_3CN (30 mL) was added PPh_3 (1.43 g, 5.45 mmol) at rt under argon. The reaction mixture was heated at 70 °C for 2 days. No starting material was observed on TLC. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using 5% MeOH/CH_2Cl_2 to afford phosphonium salt **50** (3.11 g, 78%) as a pale yellow solid. HRMS (ESI) *m/z* calcd for $[C_{49}H_{56}O_3PSi]^+$, 752.8826; found, 752.7111. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69-7.60 (m, 4H), 7.81-7.27 (m, 21H), 5.99 (dd, *J* = 14.8, 11.1 Hz, 1H), 5.81 (t, *J* = 10.7 Hz, 1H), 5.57 (dd, *J* = 15.3, 6.8 Hz, 1H), 5.47-5.06 (m, 3H), 4.18 (q, *J* = 5.5 Hz, 1H), 3.83-3.74 (m, 2H), 3.62 (s, 3H), 2.60-2.27 (m, 4H), 2.19 (t, *J* = 6.8 Hz, 2H), 1.69-1.50 (m, 4H), 1.03 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 174.0, 136.0 (6C), 135.9, 135.9 (6C), 134.3, 134.1 (6C), 130.6, 129.6 (6C), 129.5, 129.3 (6C), 128.2, 127.5, 127.4, 125.9, 125.4, 118.5, 73.7, 62.2, 60.4, 51.5, 37.2, 34.0, 30.8, 27.05 (3C), 26.1, 21.1, 20.1, 19.4, 14.2.

Methyl (*S*,6*E*,8*Z*,11*Z*,14*Z*)-20-(4-(6-azidohex-1-yn-1-yl)phenyl)-5-((*tert*-butyldiphenylsilyl)oxy)icosa-6,8,11,14-tetraenoate (51). To a stirred suspension of the phosphonium salt **50** (3.0 g, 3.36 mmol) in THF (20 mL) was added LiHMDS (1.0 M solution in THF, 6.72 mL, 6.72 mmol) at −78 °C under argon. The temperature was maintained at −78 °C for 30 min, then HMPA (0.5 mL) was added at −78 °C, 2 min later the aldehyde **27** (1 g, 3.36 mmol) in THF (7.0 mL) was added. The temperature was maintained at −78 °C for 2.5 h, and then gradually warmed to rt. The reaction was quenched by satd. NH_4Cl solution (10.0 mL). The reaction mixture was extracted using EtOAc (3 × 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography using 10% EtOAc/hexane to afford the compound **51** (1.55 g, 60%) as colorless oil. HRMS (ESI) *m/z* calcd for $[C_{48}H_{63}N_3O_3Si+H]^+$, 770.4711; found 770.5009. ^{1}H NMR (400 MHz, $CDCl_3$): 7.68 – 7.61 (m, 4H), 7.41 – 7.33 (m, 6H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 7.9 Hz, 2H), 6.16 (dd, *J* = 15.0, 11.2 Hz, 1H), 5.87 (t, *J* = 11.1 Hz, 1H), 5.58 (dd, *J* = 15.0, 6.9 Hz, 1H), 5.39 – 5.28 (m, 5H), 4.21 (q, *J* = 6.1 Hz, 1H), 3.63 (s, 3H), 3.34 (t, *J* = 6.7 Hz, 2H), 2.81 – 2.74 (m, 4H), 2.57 (t, *J* = 7.6 Hz, 2H), 2.45 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.03 (q, *J* = 7.0 Hz, 2H), 1.79 (dt, *J* = 14.2, 6.7 Hz, 2H), 1.68 (dt, *J* = 14.1, 6.6 Hz, 2H), 1.60 – 1.48 (m, 6H), 1.42 – 1.29 (m, 4H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.9, 142.6, 136.0 (4C), 135.7, 134.2 (2C), 131.4 (2C), 130.3, 129.6, 129.5 (4C), 128.7, 128.3 (2C), 128.1, 127.7, 127.6, 127.5, 127.4, 125.5, 120.9, 88.4, 81.3, 73.7, 51.4, 51.1, 37.2, 35.8, 34.0, 31.2, 29.5, 28.9, 28.0, 27.2, 27.1 (3C), 26.0, 25.9, 25.6, 20.1, 19.4, 19.0.

(*S*,6*E*,8*Z*,11*Z*,14*Z*)-20-(4-(6-azidohex-1-yn-1-yl)phenyl)-5-hydroxyicosa-6,8,11,14-tetraenoic acid (52). To a stirred solution of **51** (600 mg, 0.77 mmol) in THF (16 mL) and H_2O (4.0 mL) was added LiOH (186 mg, 7.8 mmol) and the mixture was stirred at rt for 10 h. The solvents were evaporated under

reduced pressure. To the resulting residue was diluted with H_2O (10.0 mL). The solution was cooled to 0 °C and added 6 N HCl (2.5 mL) dropwise (pH = 3). The organic layer was extracted with EtOAc (3 × 20 mL), the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The solvents were evaporated under reduced pressure to obtain the crude carboxylic acid, which was directly used for the next step without further purification.

A 1.0 M solution of TBAF in THF (1.32 mL, 1.32 mmol) and two drops of acetic acid were added to a solution of carboxylic acid (500 mg) in dry THF (10 mL) at rt. The mixture was stirred at 50 °C, for 10 h. After reaction was completed, THF was removed in vacuo, the mixture was diluted with EtOAc (15 mL). The combined organic layers were washed with brine, and the mixture was extracted with EtOAc (3 × 20 mL). The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude residue was purified by silica gel column chromatography using 70% EtOAc/hexane to afford compound **52** (256 mg, 75% over two steps) as a highly viscous colorless liquid. HRMS (ESI) *m/z* calcd for $[C_{32}H_{43}N_3O_3+H]^+$: 518.3383; found, 518.3502. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.3 (d, *J* = 8.0 Hz, 2H), 7.1 (d, *J* = 8.0 Hz, 2H), 6.5 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.0 (t, *J* = 10.4 Hz, 1H), 5.68 (dd, *J* = 15.3, 6.6 Hz, 1H), 5.45-5.32 (m, 5H), 4.22-4.17 (m, 1H), 3.34 (t, *J* = 6.6 Hz, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.80 (t, *J* = 6.1 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 2H), 2.48-2.39 (m, 4H), 2.07-2.04 (m, 2H), 1.82-1.33 (m, 14H). ^{13}C NMR (100 MHz, $CDCl_3$): 178.7, 142.6, 136.4, 131.5, 130.6, 130.3, 128.9 (2C), 128.3, 128.2, 127.7, 127.5 (2C), 125.2, 120.9, 88.5, 81.3, 71.8, 35.7, 31.2, 29.7, 29.5, 28.9, 28.0, 27.2, 26.1, 25.9, 25.7, 25.2, 20.9, 20.2, 19.0. IR (cm^{-1}): 2926, 2855, 2094 (N_3, azide), 1730 (C=O, carboxyl), 1554, 1510, 1456.

Synthesis of 53, 54, and 56. These compounds were synthesized as described above.

6-(4-Iodo-phenyl)-hexanal (**53**). Yield: 88%. HRMS (ESI) *m/z* calcd for $[C_{12}H_{15}IO+H]^+$, 303.0240; found 302.9735. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 7.9 Hz, 2H), 2.55 (t, *J* = 7.7 Hz, 2H), 2.42 (t, *J* = 7.1 Hz, 2H), 1.69 – 1.32 (m, 4H), 1.41 – 1.30 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 202.6, 142.0, 137.3 (2C), 130.5 (2C), 90.7, 43.79, 35.2, 31.0, 28.6, 21.9.

tert-Butyl-[12-(4-iodo-phenyl)-dodeca-3,6-dienyloxy]-dimethyl-silane (**54**). Yield: 56%. HRMS (ESI) *m/z* calcd for $[C_{24}H_{39}IOSi+H]^+$, 499.1888; found 499.1250. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 5.48 – 5.32 (m, 4H), 3.61 (t, *J* = 7.0 Hz, 2H), 2.78 (t, *J* = 5.9 Hz, 2H), 2.57 – 2.52 (m, 2H), 2.34 – 2.25 (m, 2H), 2.07 – 2.00 (m, 2H), 1.60 – 1.54 (m, 2H), 1.38 – 1.31 (m, 4H), 0.90 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 142.4, 137.3 (2C), 130.6 (2C), 130.1, 129.9, 128.0, 126.0, 90.5, 62.9, 35.4, 31.2, 31.2, 29.5, 28.8, 27.1, 26.0 (3C), 25.8, 18.4, −5.2 (2C).

12-(4-Iodo-phenyl)-dodeca-3,6-dien-1-ol (**54 step c**). Yield: 60%. HRMS (ESI) *m/z* calcd for $[C_{18}H_{25}IO+H]^+$, 385.1023; found 385.0617. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.3 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 2H), 5.53 (q, *J* = 7.9 Hz, 1H), 5.46 – 5.28 (m, 3H), 3.65 (t, *J* = 6.4 Hz, 2H), 2.81 (t, *J* = 7.0 Hz, 2H), 2.54 (t, *J* = 7.8 Hz, 2H), 2.35 (q, *J* = 6.7 Hz, 2H), 2.06 (p, *J* = 6.8 Hz, 2H), 1.58 (p, *J* = 7.6 Hz, 2H), 1.43 – 1.31 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 142.4, 137.3 (2C), 131.5, 130.6 (2C), 130.3, 127.6, 125.4, 90.6, 62.3, 35.4, 31.2, 30.8, 29.4, 28.8, 27.2, 25.8.

1-Iodo-4-(12-iodo-dodeca-6,9-dienyl)-benzene (**54 step d**). Yield: 90%. HRMS (ESI) *m/z* calcd for $[C_{18}H_{24}I_2+H]^+$, 495.0040; found 494.9524. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.3 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 2H), 5.55 – 5.46 (m, 1H), 5.43 – 5.29 (m, 3H), 3.14 (t, *J* = 7.3 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.65 (q, *J* = 7.5 Hz, 2H), 2.58 – 2.52 (m, 2H), 2.05 (dt, *J* = 13.9, 7.0 Hz, 2H), 1.59 (p, *J* = 7.6 Hz, 2H), 1.42 – 1.31 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 142.4, 137.3 (2C), 130.7, 130.6 (2C), 130.5, 128.1, 127.3, 90.6, 35.4, 31.5, 31.2, 29.4, 28.8, 27.2, 25.8, 5.2.

[12-(4-Iodo-phenyl)-dodeca-3,6-dienyl]-triphenyl-phosphonium iodide (**55**). Yield: 96%. HRMS (ESI) *m/z* calcd for $[C_{36}H_{39}IP]^+$, 629.1829; found 629.1885. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 – 7.79 (m, 9H), 7.84 – 7.78 (m, 6H), 7.58 (d, *J* = 8.3 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 2H), 5.65 – 5.55 (m, 1H), 5.43 – 5.28 (m, 2H), 5.23 – 5.13 (m, 1H), 3.91 – 3.80 (m, 2H), 2.56 – 2.43 (m, 6H), 1.88 (q, *J* = 6.9 Hz, 2H), 1.58 – 1.51 (m, 2H), 1.32 – 1.26 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 142.3, 137.3 (2C), 135.1 (3C), 133.8 (6C), 130.7 – 130.4 (10C), 126.9, 126.2, 118.1 (3C), 90.6, 35.4, 31.1, 29.3, 28.8, 27.1, 25.6, 23.4, 20.4.

5-(tert-Butyl-diphenyl-silanyloxy)-20-(4-iodo-phenyl)-icosa-6,8,11,14-tetraenoic acid methyl ester (**56**). Yield: 98%. HRMS (ESI) *m/z* calcd for $[C_{43}H_{55}IO_3Si+H]^+$, 775.3038; found 755.0714. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 – 7.60 (m, 4H), 7.60 – 7.55 (m, 2H), 7.40 – 7.33 (m, 6H), 6.91 (d, *J* = 8.2 Hz, 2H), 6.16 (dd, *J* = 15.0, 11.2 Hz, 1H), 5.87 (t, *J* = 11.1 Hz, 1H), 5.58 (dd, *J* = 15.0, 6.8 Hz, 1H), 5.41 – 5.26 (m, 5H), 4.21 (q, *J* = 6.0 Hz, 1H), 3.62 (s, 3H), 2.81 – 2.74 (m, 4H), 2.57 – 2.50 (m, 2H), 2.18 (t, *J* = 7.1 Hz, 2H), 2.03 (q, *J* = 6.6 Hz, 2H), 1.61 – 1.50 (m, 6H), 1.42 – 1.29 (m, 4H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.9, 142.4, 137.2 (2C), 135.9 (4C), 135.7, 134.2 (2C), 130.5 (2C), 130.2, 129.5 (3C), 128.7, 128.1, 127.7, 127.6, 127.4 (4C), 125.4, 90.5, 73.7, 51.4, 37.2, 35.4, 34.0, 31.2, 29.4, 28.8, 27.1, 27.1 (3C), 26.0, 25.6, 20.1, 19.4.

Methyl (S,6E,8Z,11Z,14Z)-20-(4-(6-azidohex-1-yn-1-yl)phenyl)-5-((tert-butyldiphenylsilyl)oxy)icosa-6,8,11,14-tetraenoate (**51**). Yield: 64%. HRMS (ESI) *m/z* calcd for $[C_{48}H_{63}N_3O_3Si+H]^+$, 770.4711; found 770.5009. ^{1}H NMR (400 MHz, $CDCl_3$): 7.68 – 7.61 (m, 4H), 7.41 – 7.33 (m, 6H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 7.9 Hz, 2H), 6.16 (dd, *J* = 15.0, 11.2 Hz, 1H), 5.87 (t, *J* = 11.1 Hz, 1H), 5.58 (dd, *J* = 15.0, 6.9 Hz, 1H), 5.39 – 5.28 (m, 5H), 4.21 (q, *J* = 6.1 Hz, 1H), 3.63 (s, 3H), 3.34 (t, *J* = 6.7 Hz, 2H), 2.81 – 2.74 (m, 4H), 2.57 (t, *J* = 7.6 Hz, 2H), 2.45 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.03 (q, *J* = 7.0 Hz, 2H), 1.79 (dt, *J* = 14.2, 6.7 Hz, 2H), 1.68 (dt, *J* = 14.1, 6.6 Hz, 2H), 1.60 – 1.48 (m, 6H), 1.42 – 1.29 (m, 4H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.9, 142.6, 136.0 (4C), 135.7, 134.2 (2C), 131.4 (2C), 130.3, 129.6, 129.5 (4C), 128.7, 128.3 (2C), 128.1, 127.7, 127.6, 127.5, 127.4, 125.5, 120.9, 88.4, 81.3, 73.7, 51.4, 51.1, 37.2, 35.8, 34.0, 31.2, 29.5, 28.9, 28.0, 27.2, 27.1 (3C), 26.0, 25.9, 25.6, 20.1, 19.4, 19.0.

(*S*,6*E*,8*Z*,11*Z*,14*Z*)-20-(4-(6-aminohex-1-yn-1-yl)phenyl)-5-hydroxyicosa-6,8,11,14-tetraenoic acid (57). To a stirred solution of **52** (105 mg, 0.203 mmol) in THF (10 mL) was added 2 drops of water and TPP (266 mg, 1.01 mmol). The reaction mixture was kept at rt for 24 h. The reaction mixture was concentrated under reduced pressure and dried using anhydrous benzene. The residue was purified by silica gel chromatography using 15% MeOH/CH_2Cl_2 as eluent to afford the compound **57** (77.0 mg, 80%) as viscous liquid. Yield: 60%, 114 mg. HRMS (ESI) *m/z* calcd for $[C_{32}H_{45}NO_3-H_2O]^+$: 474.3367; found, 474.3739. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 7.2 Hz, 2H) 6.56-6.45 (m, 1H), 6.01-5.91 (m, 1H), 5.71-5.67 (m, 1H), 5.37-5.26 (m, 5H), 4.13 (q, *J* = 7.3 Hz, 1H), 3.62 (br s, 1H), 3.52-3.42 (m, 2H), 2.82-2.70 (m, 2H), 2.67-2.60 (m, 2H), 2.40-2.35 (m, 1H), 2.34-2.30 (m, 2H), 2.25-2.20 (m, 2H), 2.09-2.00 (m, 3H), 1.72-1.50 (m, 8H). 1.42-1.12 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.9, 142.6, 134.4 (2C),

133.6, 133.5, 132.2, 131.9 (2C), 131.5, 129.8, 129.8, 129.6, 128.6, 128.4, 89.1, 81.1, 73.5, 37.4, 35.4, 33.9, 31.2, 30.9, 30.3, 30.2, 29.4, 28.0, 27.1, 26.1, 26.0, 25.6, 19.3.

6.3. Preparation of Microsomal Fractions from Differentiated U937 cells

U937 cells obtained from ATCC were cultured in modified RPMI 1640 medium and were terminally differentiated by treatment with phorbol myristate acetate (18 nM) for 4 days as described previously.[15] The cells were then suspended in phosphate-buffered saline (PBS) supplemented with 1 mM PMSF and disrupted by sonication at 40 cycles/second on ice pulsing 5 × 8 sec with intervals of 1 min in between to allow for cooling.[15] The sonicates were centrifuged at 1,500 × g at 4 °C for 10 min to remove intact cells and nuclei. The supernatants were then centrifuged at 10,000 × g for 10 min to remove granules, and then at 150,000 × g for 120 min at 4 °C using a Beckman (Mississauga, ON) ultracentrifuge (rotor Type 50.2Ti). The final pellet containing the microsomal fraction was resuspended by homogenization (using a glass homogenizer) in Buffer A.

*6.4. Metabolism of **52** and 5-HETE by 5-HEDH*

U937 cell microsomes (50 µg protein in 1 mL) were incubated with different concentrations of either **52** or 5-HETE and 100 µM $NADP^+$ at 37 °C for 5 min. The reactions were terminated by addition of ice cold methanol (630 µl) and cooling in ice. The samples were subsequently diluted to 30% methanol with water and then analyzed by precolumn extraction coupled to RP-HPLC as described previously.[16] The mobile phase was a gradient over 7 min between 65 and 82% acetonitrile containing 0.02% acetic acid whereas the stationary phase was a Nova-Pak C18 column (3.9 × 150 mm; 4 µm particle size; Waters Corp., Mississauga, Ontario).[17] 13-Hydroxy-9,11-octadecadienoic acid, prepared from soybean lipoxygenase (Sigma-Aldrich, St. Louis, MO)[18] was used as an internal standard.

*6.5. Partial Purification of 5-HEDH Using **17***

5-HEDH from U937 cell microsomes was first partially purified using DEAE-Sepharose. Proteins from U937 cell microsomes were solubilized by treatment with 30 mM octylglucoside in 20 mM phosphate, pH 7.4 for 3 h at 4 °C. Following centrifugation at 200,000 × g for 1 h the supernatant fraction was subjected to ultrafiltration using Amicon 50 kDa cutoff filters. The retentate was suspended in 20 mM phosphate, pH 7.4 containing 5 mM CHAPS and incubated for 5 min with DEAE Sepharose. The DEAE Sepharose was washed with 20 mM phosphate buffer containing 5 mM CHAPS and 20% glycerol, followed by 20 mM phosphate containing 30 mM octylglucoside and 20% glycerol. 5-HEDH was then eluted with 20 mM phosphate containing 30 mM octylglucoside, 20% glycerol, and 1 M NaCl. 5-HEDH in the 1 M NaCl fraction was concentrated by ultrafiltration and suspended in 20 mM phosphate containing 30 mM octylglucoside and 15% MeOH. This partially purified preparation of 5-HEDH was then incubated with **17** for 20 min at 6 °C, followed by centrifugation. The **17** was then washed with 20 mM phosphate containing 30 mM octylglucoside and 15% MeOH alone, or supplemented successively with 20 µM 5-HETE, 0.2 mM NaCl, 1 mM NADPH, and 1 M NaCl. 5-HEDH activity in the different fractions was assessed by precolumn extraction/RP-HPLC as described above following incubation of aliquots with 5-HETE (1 µM) and $NADP^+$(100 µM) for 30 min at 37 °C. Protein concentrations were measured using the Bio-Rad DC (detergent compatible) protein assay kit (Bio-Rad Laboratories), with BSA as an external standard.

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Abbreviations

AA, arachidonic acid; 5-HETE, 5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid; 5- oxo-ETE, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid; 5-HEDH,5-hydroxyeicosanoid dehydrogenase; 5-HpETE, 5-hydroperoxyeicosatetraenoic acid; LT, leukotriene; $NADP^+$, nicotinamide adenine dinucleotide phosphate; TMS, tetramethylsilane; HPLC, high-performance liquid chromatography.

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