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The design and synthesis of a 5-HETE affinity chromatography ligand for 5-hydroxyeicosanoid dehydrogenase

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Abstract

The first total synthesis of an ω -amino 5-HETE derivative **27** has been accomplished by a new counter-clockwise strategy, in which C-1 is constructed first and C-20 last. The ω -amino 5-HETE derivative was transformed to an affinity chromatography ligand, the biotinylated 5-HETE **30**. This affinity chromatography ligand is aimed at purifying the 5-hydroxyeicosanoid dehydrogenase enzyme, which is responsible for the conversion of 5-HETE to 5-oxo-ETE, a potent eosinophil chemotactic factor. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: biotin; 5-HETE; 5-hydroxyeicosanoid dehydrogenase; affinity chromatography.

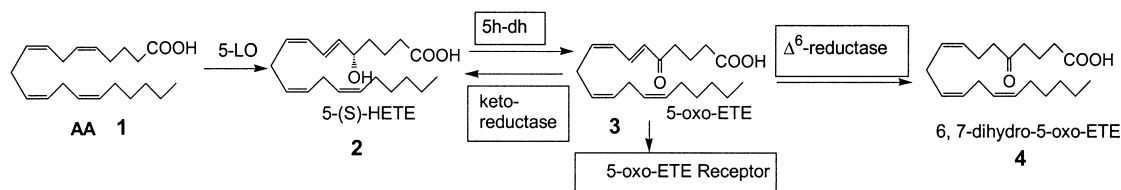
Our interest has recently centered on oxo derivatives of eicosanoids, many of which have potent biological activities or are key intermediates in the synthesis of other potent mediators, such as the 12-oxo-10,11-dihydro metabolite of 12-HETE, which is converted to the potent angiogenic factor 12-HETrE.

We have recently reported on the synthesis,^{1–9} biosynthesis² and biology^{3,4} of several oxo-eicosanoids, such as 12-oxo-ETE,¹ 10,11-dihydro-12-oxo-ETE,⁵ 12-oxo-LTB₄,⁶ 10,11-dihydro-12-oxo-LTB₄,⁷ and 5-oxo-12-HETE,⁸ and finally 5-oxo-ETE² and its tetradeutero and tetratritiated derivatives,⁹ which were essential for the development of LC/MS methodology for measuring 5-oxo-ETE in biological fluids and for the radioactive study of the biotransformation of 5-oxo-ETE and receptor binding studies.

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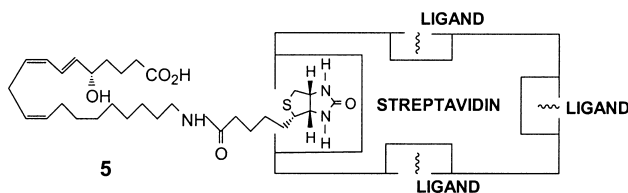
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The enzymatic machinery for the formation and metabolism of 5-oxo-EETE is only partially understood.¹⁰ One of our goals is to purify, characterize and identify the 5-hydroxyeicosanoid dehydrogenase (5h-dh) responsible for the transformation of 5-HETE **2** to 5-oxo-EETE **3** (Scheme 1). To accomplish this we propose to synthesize radioactively labeled photoaffinity probes and ligands for affinity chromatography based on the structure of the substrate 5-HETE. A similar approach can be used to characterize enzymes metabolizing 5-oxo-EETE as well as the 5-oxo-EETE receptor.



Scheme 1. Four proteins involved in the biosyntheses, metabolism and biological activity of 5-oxo-EETE

We wish to report here the total synthesis of a prototype for such reagents, an ω -biotinylated derivative of 5-HETE **5**, as shown in Scheme 2. This is the first report of the synthesis of an ω -substituted derivative of either 5-HETE or 5-oxo-EETE.

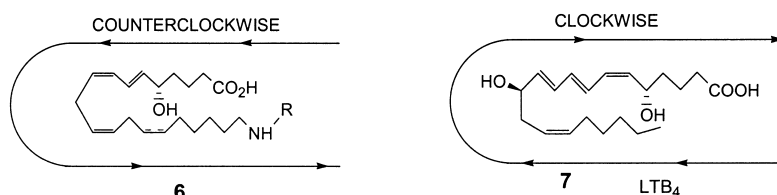


Scheme 2.

Biotinylated 5-HETE can be used to purify 5h-dh by adsorbing it on a streptavidin–agarose column (Scheme 2). Streptavidin is a protein that has four high affinity biotin binding sites ($K_d = 10^{-15}$ M)¹¹ and would thus strongly bind **5**. The simplest approach to prepare an affinity ligand based on 5-HETE would have been to attach a biotin residue via the carboxyl group. However, it seemed likely that this group would be important for the interaction of 5-HETE with the active site of 5h-dh, and this was confirmed by our finding that the methyl ester of 5-HETE is a very poor substrate for this enzyme. On the other hand, modification of the ω -end of the molecule (e.g. 5,15-diHETE) has little effect on the rate of metabolism. For this reason, we decided to attach the biotin molecule to the ω -end of 5-HETE, leaving the rest of the molecule unaffected. To couple 5-HETE to biotin in this way requires introduction of an NH_2 or OH linking group, as shown in Scheme 2. It should be noted that in this initial approach we have chosen to synthesize an affinity ligand based on the 14,15-dihydro derivative of 5-HETE based on previous experience (unpublished results) in which a 14,15-dihydro derivative performed as well as the unsaturated derivative.

We adopted a novel approach for the synthesis of **5**, which will provide us with considerable flexibility for the future syntheses of a variety of other related ω -derivatives of 5-HETE and 5-oxo-EETE. In the past, most, if not all, of the eicosanoid syntheses that we and others have carried out have been performed in a clockwise manner, i.e. building the bottom part of the structure

first, starting at the ω -end of the molecule (Scheme 3). Such was the case in our synthesis of LTB₄ **7** and its ω -OH and ω -COOH derivatives, as well as the 14,15-acetylene derivative. However, in the case of the ω -amino-5-HETE derivative **6** and its biotinylated form **5**, we elected to use a counterclockwise approach, starting at the carboxyl end of the molecule. The advantages of such a strategy in the present case are obvious, as it allows us to modify the ω -end of the molecule as required without repeating the whole synthetic sequence. The objective in the present study is to keep the top part of the molecule intact, since we know from preliminary structure–activity experiments that this part is necessary for recognition by the catalytic site of 5h-dh.



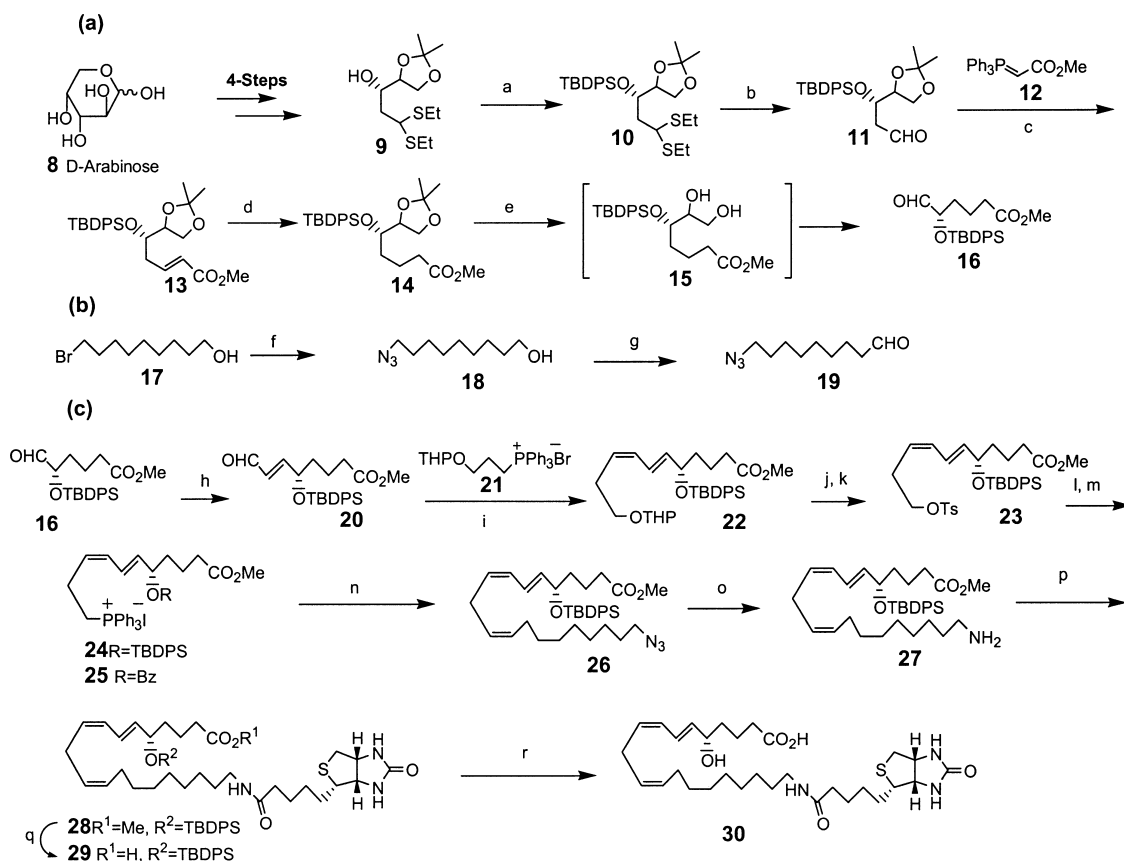
Scheme 3.

Scheme 4 illustrates the strategy used for the construction of the ω -amino derivative **27** and the biotinylated derivative **5**, which will be used for attachment to a streptavidin–agarose support. The required synthon **16** containing the 5(*S*)-OH group, which we have used previously, was prepared by the modified and improved synthesis shown in Scheme 4. Of particular note, the dithio ether deprotection was performed by our recently published method¹² using periodic acid in ether/THF. At the end of the reaction (15 min) the ether THF solvent containing a white precipitate was filtered through a small amount of Celite/Florisil. The solution was extracted with KH₂PO₄ buffer followed by extraction with Na₂S₂O₃ solution. The organic layer was dried and evaporated to afford a quantitative yield of the desired **11**.

Another improvement in the synthesis of the aldehyde **16** follows. Previously, we used to perform the transformation of **14** to the aldehyde **16** in two steps, the first being the deprotection of the acetonide to afford the diol **15**, and the second the lead tetraacetate oxidation to **16**. In the present case, we performed the transformation of **14** to **16** in one step^{8,13} using excess periodic acid for 14 h (92% yield). Whereas the two-step procedure used by us before^{6,14} was very efficient (two-step yield 66%), the one-step periodic acid procedure is shorter, more convenient and occurs in excellent yield.

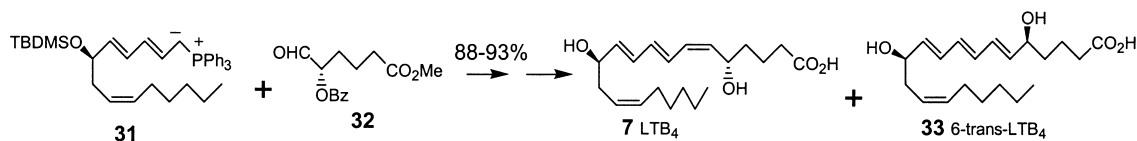
Synthon **24** is one major objective of this synthetic approach. As can be seen, a Wittig reaction with **24** afforded the desired ω -azido compound **26** and eventually the amino derivative **27**. Synthon **24** can obviously be used as a pivotal intermediate for the synthesis of other 5-HETE derivatives containing different ω substitutions. The OTHP deprotection (step j in Scheme 4) and the Wittig coupling of **24** with **19** (step m) occur in modest yields. We thought that it was worth paying this price in order to benefit from the anticlockwise strategy of this synthetic approach. Our first preparation of this synthon was one in which the oxygen was substituted by a benzoate group **25**. A reason for initially selecting **25** lies in the fact that we have used this synthon in the synthesis of LTB₄ with excellent yield (Scheme 5).

When **25** was used in the Wittig reaction with **19**, a complex mixture of products was obtained. We attribute this unsuccessful reaction to the possibility that the Wittig anion caused the deprotection of the benzoate group followed by secondary reactions, e.g. lactonization, etc.



Scheme 4. Reagents and conditions: (a) *tert*-butylchlorodiphenylsilane, imidazole, CH_2Cl_2 , 0°C to RT, 97%; (b) H_5IO_6 , ether, THF, 0°C , 15 min, quant.; (c) benzene, reflux, 4 h, 85%; (d) $\text{Pd-C}/\text{H}_2$, quant.; (e) H_5IO_6 , ether/THF, RT, 18 h, 92%; (f) NaN_3 , DMSO, 90°C , 99%; (g) PCC, CH_2Cl_2 , 0°C to RT, 77%; (h) (triphenylphosphoranylidene)-acetaldehyde, benzene, reflux, 78%; (i) LiHMDS, **20**, -78°C to RT, 90%; (j) 1N HCl, THF: H_2O (9:1), RT, 40 h, 48%; (k) *p*-TsCl, Py, 0°C to RT, 82%; (l) NaI, acetone, 65°C , 99%; (m) Ph_3P , CH_3CN , 65°C , 99%; (n) LiHMDS, **19**, -78°C to RT, 36%; (o) Ph_3P , THF/ H_2O , RT, 88%; (p) (+)-biotin, EDCI, HOBT, NaHCO_3 , DMF, RT, 76%; (q) 1N NaOH (aq), MeOH, 92%; (r) THF, TBAF: HOAc (1:1), RT, 80 h, 74%

In the original LTB_4 synthesis,¹⁴ shown in Scheme 5, the reaction between **31** and **32** worked well, presumably because of the stabilized nature of the phosphonium anion, which was not sufficiently basic or nucleophilic to deprotect the benzoate.



Scheme 5.

Preliminary studies show that the affinity chromatography ligand **30**^{15,16} is recognized by the 5-hydroxy dehydrogenase from human PMN and is a good substrate for this enzyme. We are

currently attempting to optimize the affinity of the ligand for the enzyme by testing the effects of structural modifications of **5** on enzyme activity. We will then be in a position to use the biotinylated 5-HETE derivative to purify 5h-dh. In addition, the ω -amino intermediate **27** can be used to synthesize a radiolabeled azidophenyl derivative of 5-HETE that can be used to label 5h-dh, which will make it possible to detect the enzyme on electrophoretic gels.

Acknowledgements

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15. NMR data of compound **30**: ^1H NMR (360 MHz, CDCl_3) δ 6.55 (dd, $J=15.1$ and 11.1 Hz, 1H), 6.1 (m, 1H), 5.99 (t, $J=10.8$ Hz, 1H), 5.40 (m, 3H), 5.24 (m, 1H), 4.54 (m, 1H), 4.38 (m, 1H), 4.21 (m, 1H), 3.24 (m, 3H), 2.94 (m, 3H), 2.74 (d, 13 Hz, 1H), 2.38 (m, 2H), 2.22 (m, 2H), 2.05 (m, 2H), 1.53–1.87 (m, 11H), 1.47 (m, 2H), 1.2–1.38 (m, 13H).
16. Compound **30** has been found to be a substrate for 5h-dh. Incubation of **30** with human neutrophil microsome produces the expected 5-oxo product. Further measurement, quantitation and K_m measurement are being determined and will be published separately.