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A photoaffinity probe for 5-hydroxyeicosanoid dehydrogenase suitable for radioiodination

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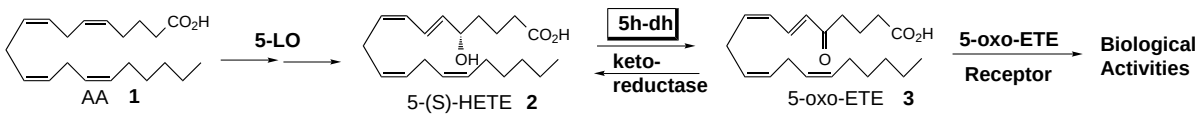
Abstract—5-Hydroxy eicosanoid dehydrogenase (5h-dh) is a key enzyme responsible for the biosynthesis of 5-oxo-ETE, a potent eosinophil chemoattractant. To facilitate the identification and characterization of 5h-dh we have synthesized a photoaffinity ligand **7**, designed to bind to the enzyme, and shown it to be an excellent substrate for 5h-dh. We have also synthesized a precursor **34**, containing the photoaffinity label and a trimethyl tin group that can readily be displaced by iodide and will be suitable for radioiodination with ^{125}I. © 2001 Elsevier Science Ltd. All rights reserved.

5-Hydroxyeicosanoid dehydrogenase (5h-dh) is a key enzyme involved in the transformation of 5-HETE to 5-oxo-ETE.[1] 5-oxo-ETE is the most potent eosinophil chemotactic factor among lipid mediators.[2,3] We have proposed that 5-oxo-ETE may be a causative factor in diseases such as asthma involving the infiltration of eosinophils.[4] 5-oxo-ETE exerts its action by activating a dedicated receptor.[5–7] We have reported recently the first total synthesis of 5-oxo-ETE and tritiated and deuterated 5-oxo-ETE.[8,9]

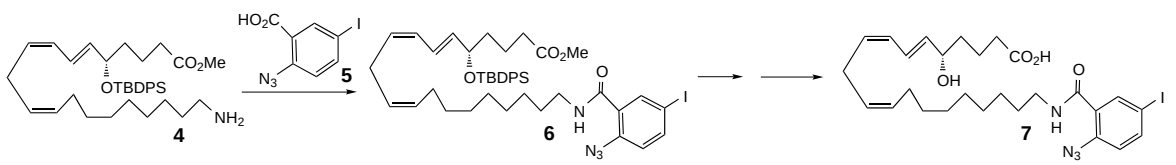
The 5h-dh, which has not been identified, is an essential element in the understanding of the biological activity of 5-oxo-ETE. It is also a potential target for inhibiting the formation of 5-oxo-ETE and preventing the infiltration of eosinophils into inflammatory sites (Scheme 1).

We have described recently the design and synthesis of a non-radioactive model of a 5-HETE photoaffinity ligand that was prepared by coupling the ω-amino derivative **4** with a benzoic acid derivative **5** [10] (Scheme 2).

The purpose of this synthesis was to confirm the validity of our assumptions in the design of this ligand. We wanted to know how well the cold iodo azido probe **7**



Scheme 1.



Scheme 2.

Keywords: 5-HETE; 5-oxo-ETE; ω-functionalized-5-HETE; 5h-dh.

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is recognized by 5h-dh and how good a substrate it is before we embarked on the more difficult task of the design and synthesis of a radiophotoaffinity probe and the design of the appropriate precursor for the introduction of the radiolabel. The better the substrate (or inhibitor), the less we have to contend with non-specific binding. We are reporting here: (1) that **7** is a very good substrate for 5h-dh and hence appears to be a good target for introducing ^{125}I, and (2) the design and total synthesis of a precursor to the radiophotoaffinity label and its conversion to **7**.

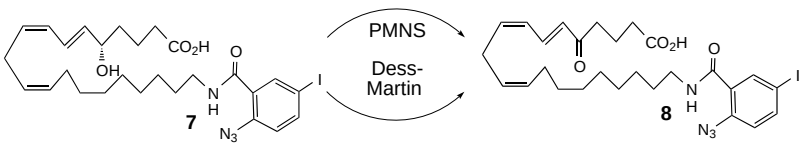
We have incubated **7** with neutrophils and a new product was detected by HPLC. The UV of this product, λ_{280} is consistent with the formation of a dienone. In order to complete the characterization of the enzymatic product, we have performed the chemical oxidation of **7** using the Dess–Martin reagent (Scheme 3). The synthetic standard and the enzymatic product were compared on RP-HPLC and were found to be identical.[10]

Furthermore, in preliminary studies **7** was compared to 5-HETE, the natural substrate for 5h-dh and found to be almost as good at three different concentrations.

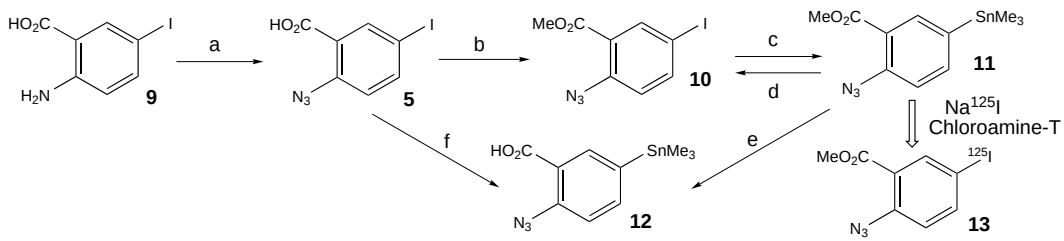
We selected ^{125}I as our radio tracer as it has a much higher specific activity than tritium for example. In order to introduce the desired radiolabel, we elected to use a phenyl substituted trimethyl tin derivative as a precursor to radioactive iodine. This group has been used previously by some other investigators.[11,12] This is an interesting precursor to radioactive iodine in that the trimethyl tin group itself can be introduced by substituting an iodo benzene derivative. The disadvantage of the ^{125}I is that its half-life is very short, $\sim$11 days, hence the radio probe might have to be prepared several times. In addition, because ^{125}I is a strong γ-emitter, extensive precautions must be taken during the preparation and use of radioiodinated compounds. It was therefore essential that (1) the radiolabel be introduced as late as possible in the synthesis, hopefully the last step, and (2) the precursor be reasonably stable in order to last as long as possible.

The benzoic acid derivative **12** has been prepared in excellent yield as shown in Scheme 4. We first attempted to introduce the trimethyl tin group directly on acid **5** but with limited success (15% yield).

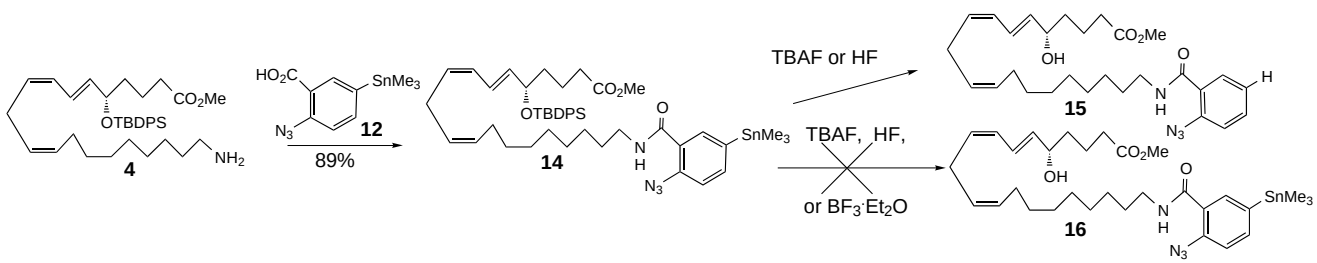
We planned to introduce **12** into the photoaffinity probe, which would provide an acceptor group for ^{125}I. This could be accomplished by first coupling it with the ω-amino derivative **4** or **33** (Schemes 5 and 6), followed by replacement of the trimethyl tin group with ^{125}I in the presence of chloramine-T. An alternative possibility would be to introduce the radioactive iodine first, e.g. **13**, and then couple it with the appropriate ω-amino



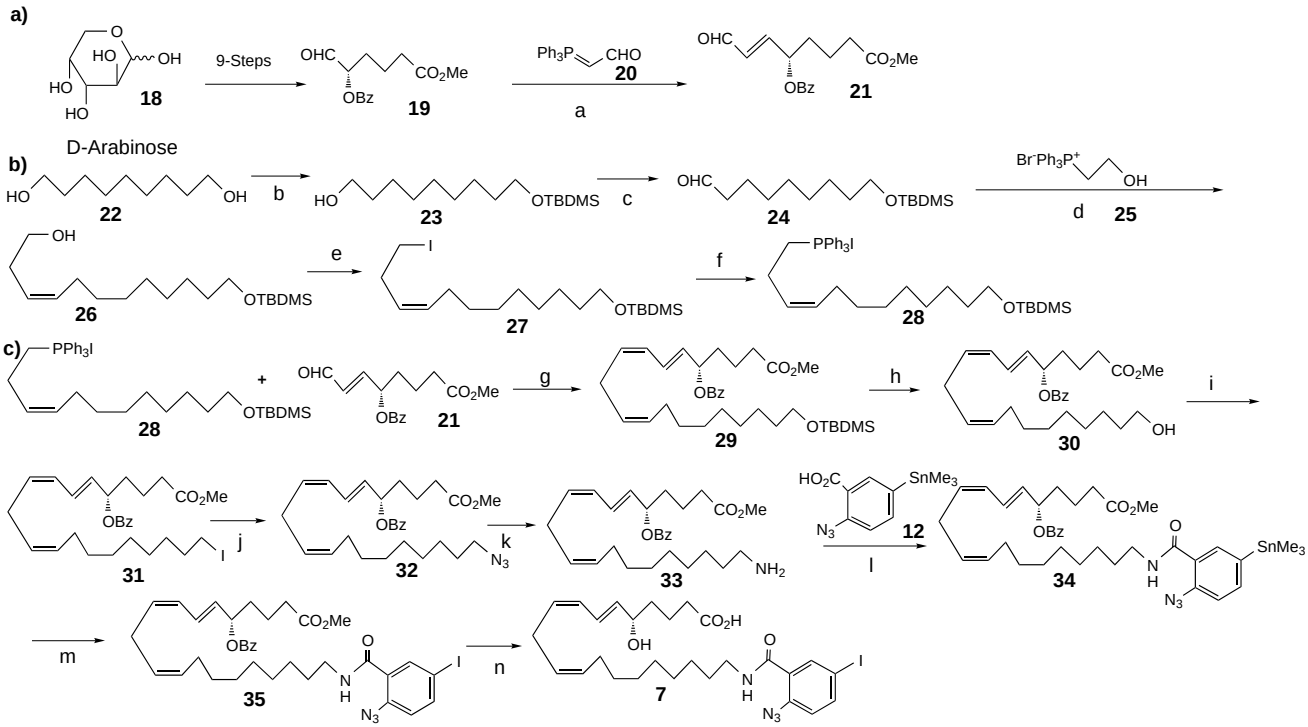
Scheme 3.



Scheme 4. *Reagents and conditions*: (a) $NaNO_2$, NaN_3, HCl, H_2O, 0°C, 1.5 h, 88%; (b) CH_2N_2, ether, rt, 90%; (c) $(Sn(Me)_3)_2$, $Pd[Ph_3]_4$, dioxane, 50°C, 5 h, 80%; (d) NaI, chloriamine-T, THF, pH 7.4 buffer, rt, 20 min, 89%; (e) *t*-BuOK, H_2O, THF, 0°C, 99%; (f) $(Sn(Me)_3)_2$, $Pd[Ph_3]_4$, dioxane, 50°C, 5 h, 15%.



Scheme 5.



Scheme 6. *Reagents and conditions*: (a) Benzene, reflux, 5 h, 54%; (b) TBDMSCl, Im, THF, rt, 3 h, 83%; (c) PCC, Al_2O_3, CH_2Cl_2, rt, 3 h, 60%; (d) LiHMDS, HMPA, THF, −78°C to rt, 45%, a few percent of the *trans* isomer formed; (e) Ph_3P, I_2, Im, CH_2Cl_2, 0°C to rt, 97%; (f) Ph_3P, CH_3CN, 68%; (g) LiHMDS, HMPA, THF, −98°C to −78°C, 56%; (h) DDQ, H_2O, THF, rt, 2 h, 99%; (i) Ph_3P, I_2, Im, CH_2Cl_2, 0°C to rt, 92%; (j) NaN_3, DMSO, 90°C, 97%; (k) Ph_3P, H_2O, THF, rt, 6 h, 75%; (l) EDCI, HOBT, $NaHCO_3$, 1:1 CH_2Cl_2:CH_3CN, rt, 8 h, 84%; (m) NaI, chloroamine-T, THF, pH 7.4 buffer; (n) NaOH, rt, 2 h, HPLC.

derivative. However, this would be impractical because it would require a greater number of steps with radioiodinated intermediates.

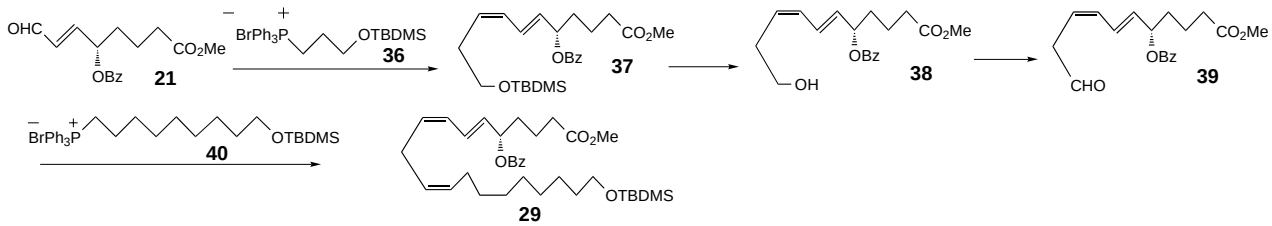
At first, we decided to use the ω-amino derivative **4** and perform the sequence shown in Scheme 5. The condensation of **4** and **12** with EDCI in 1:1 CH_3CN and CH_2Cl_2 proceeded smoothly in 89% yield. However, the deprotection of TBDPS was unsuccessful and resulted in the loss of the trimethyl stannyl group, as shown in Scheme 5.

The structural identity of **15** is supported by NMR and mass spectral data.

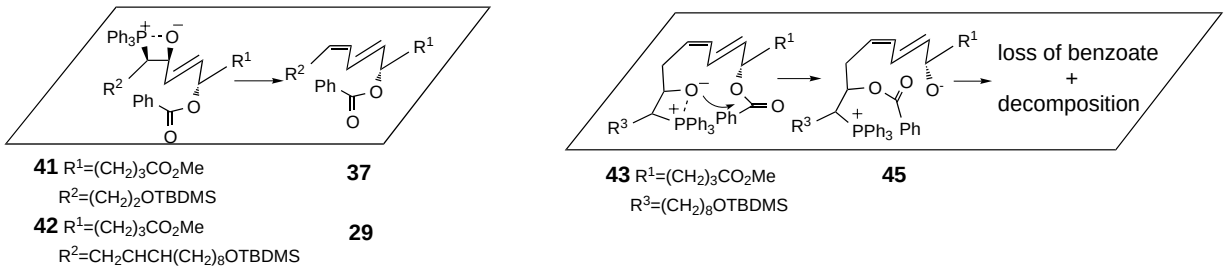
We then completely redesigned the synthesis in order to have a protecting group on the 5-hydroxyl group, which is easier to remove and does not involve a naked nucleophile in the deprotection step. We elected to protect the 5-hydroxy with a benzoyl group **19**;[13] the advantage being that the hydrolysis of the methyl ester and benzoyl group can be performed in one pot at the end of the synthesis. The obvious disadvantages are its lability and ability to survive the synthesis considering that we have several basic reactions to perform. In fact, before completing our successful synthesis shown in Scheme 6, we performed some not so successful construction (vide infra).

The one-pot transformation of **34** to **7** has been achieved successfully. The iodo derivative **7** prepared by this method (Scheme 6) and the one prepared by the direct coupling of the iodo benzoic acid derivative **5** with **4** (Scheme 2)[10] are identical as shown by RP-HPLC (μBondapak C18, 20% 0.1 M aqueous ammonium acetate, 80% methanol, 1 ml/min, at 240 nm), NMR, etc.

As mentioned, we initially attempted the following construction (Scheme 7). However, the transformation



Scheme 7.



41 $R^1=(CH_2)_3CO_2Me$ $R^2=(CH_2)_2OTBDMS$ **37**

42 $R^1=(CH_2)_3CO_2Me$ $R^2=CH_2CHCH(CH_2)_8OTBDMS$ **29**

43 $R^1=(CH_2)_3CO_2Me$ $R^3=(CH_2)_8OTBDMS$ **45**

Scheme 8.

of **39** to **29** occurs in dismal yields. It is interesting to note that the Wittig reaction between **21** and **36** and **21** and **28** works reasonably well, whereas the one between **39** and **29** does not.

It is possible that if the betaine intermediate **43** is formed, the oxygen anion is in close proximity to the benzoyl group, whereas in the two other cases it is not (Scheme 8).

In summary, the synthesis of the radioprecursor **34** is described.[14] The transformation of this precursor to the desired derivative **7** is accomplished in a one-pot reaction using chloramine-T/NaI, followed by hydrolysis. The preparation of the radiolabeled probe will be effected using chloramine-T/$Na^{125}I$ in an analogous manner. The preparation of the radioprobe will be published elsewhere.

Acknowledgements

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14. The NMR data of compound **34**: ^1H NMR (360 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.06 (d, $J=7.4$ Hz, 2H), 7.60 (m, 2H), 7.45 (t, $J=7.7$ Hz, 2H), 7.49 (br, 1H), 7.17 (d, $J=7.6$, 1H), 6.65 (dd, $J=15.0$ and 11.7 Hz, 1H), 5.97 (t, 11.0 Hz, 1H), 5.71 (dd, $J=15.1$ and 6.6 Hz, 1H), 5.59 (m, 1H), 5.47–5.30 (m, 3H), 3.67 (s, 3H), 3.47 (m, $J=6.9$, 2H), 2.92 (t, $J=6.5$, 2H), 2.38 (m, 2H), 2.02 (m, 2H), 1.76 (m, 2H), 1.60 (m, 4H), 1.28 (m, 12H), 0.31 (s, 9H).