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SYNTHESIS OF PHOTOAFFINITY LABEL ANALOGUES OF α-TOCOPHEROL

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Abstract: Photoaffinity analogues of α-tocopherol have been synthesized that incoroporate the photosensitive 4-azido-2,3,5,6-tetrafluorobenzyloxy group at the terminus of unbranched analogues of the naturally occurring phytyl side chain. An intermediate from these syntheses has also been used to generate a supported ligand for bioaffinity chromatography of α-tocopherol binding proteins.

Introduction

Vitamin E is a family of compounds comprising differentially methylated chromanols as well as the related tocotrienols in which the phytyl side chain is unsaturated.[1] While several of these compounds display equivalent or better antioxidant properties in vitro, α-tocopherol is the most biologically active. Naturally occurring α-tocopherol is optically active, having three centres of stereochemistry at C-2, C-4′, and C-8′ (Fig. 1).

α-Tocopherol has no specific plasma transport protein but rather, like other serum lipids, is transported with the assistance of a variety of lipoproteins.[1] After ingestion, α-tocopherol and other lipids are passively absorbed through the cells lining the small intestine and are then secreted into the lymph as chylomicrons. Lipids in the chylomicron remnants are eventually received by the liver, "re-packaged", and secreted as very low density lipoprotein (VLDL) and ultimately circulated in the blood as LDL.

Studies on the distribution and chiral discrimination of deuterated (2*R*,4′*R*,8′*R*) and (2*S*,4′*R*,8′*R*)-α-tocopherols[2,3] have shown that the stereochemistry at C-2 dominates the biokinetics[4–9] such that the 2*R*-isomer is preferentially retained by the liver and then redistributed to tissues. This selectivity suggests that some recognition event occurs within liver tissues that discriminates against the 2*S*-isomer. Several groups have identified an α–tocopherol transfer protein (α-TTP) in rat liver cytosol that binds the natural (*RRR*)-α-tocopherol.[10–12] This work has recently culminated in the cloning and primary sequence identification of this 31 kDa protein.[13] It does not occur in any other tissue except liver, supporting its suggested role in loading VLDLs with the natural isomer of α-tocopherol.[10] More recently, similar proteins from human liver have been cloned and expressed.[14,15]

The liver proteins discussed above are not the only binding proteins that have been reported. Azzi and coworkers have identified three proteins (31, 58, and 81 kDa) from smooth muscle cells that bind α-tocopherol.[16] More recently, Dutta–Roy reported that a 15 kDa protein is isolable from bovine heart cytosol.[17] This protein specifically binds α-tocopherol but not oleate and is not recognized by an antibody to bovine heart fatty acid–binding protein. Similar proteins have been isolated from rat tissues by the same authors.[18,19] The authors postulate that this protein may be involved in intracellular transport of α-tocopherol. Other α-tocopherol specific binding phenomena have been reported in cultured aortic endothelial cells[20] and in chromatin from hepatic nuclei.[21–23]

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By far the most siginficant interest in α-tocopherol's biological activity has been it's role as a lipid-soluble antioxidant. Recently, however, a number of phenomena have been identified that rely on the prescence of α-tocopherol, but apparently not on its radical scavenging ability. α-Tocopherol inhibits the differentiation of smooth muscle cells apparently by modulating the activity of protein kinase C.[24-28] PKC inhibition by tocopherol has also been implied in human platelets.[29,30] Others have shown that phospholipase A2[31,32] and a CoA-independent acyl transferase[33] also have their activities modulated in the prescence of α-tocopherol. Investigations of the role of the α-TTP in liver, the occurrence of other proteins in nonliver tissues capable of binding α-tocopherol, and the modulation of enzyme activity by this vitamin, would all benefit if molecular probes were available to confirm and identify those proteins that recognize α-tocopherol as a ligand. To this end we have designed analogues that incorporate photosensitive functional groups as the first examples of α-tocopherol photoaffinity labels.

Design of α-tocopherol photoaffinity labels

Studies with analogues of α-tocopherol where the phytyl side chain has been replaced by straight chain alkanes have demonstrated that methyl substitutions are not mandatory for absorption and activity as antioxidants in rats.[34] This greatly simplifies the synthetic task of making a photoaffinity analogue since preparing a side chain with stereochemically pure methyl groups is considerably more complex. The α-tocopherol transfer protein preferentially recognizes α-tocopherol over all of the other tocopherols[35] so this structure has been maintained in the design of a photoaffinity label. Since the stereochemistry at C-2 is also vital to activity in vivo it has also been preserved. With these considerations in mind targets of structure **1** have been designed as potential photoaffinity ligand of α-tocopherol.

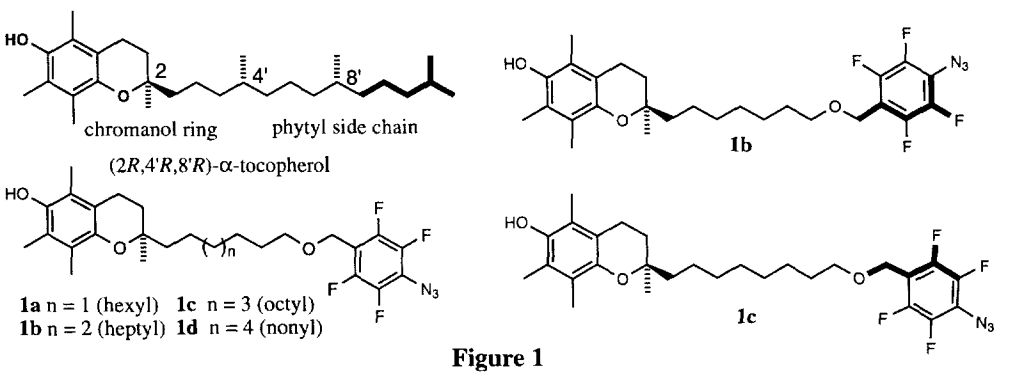
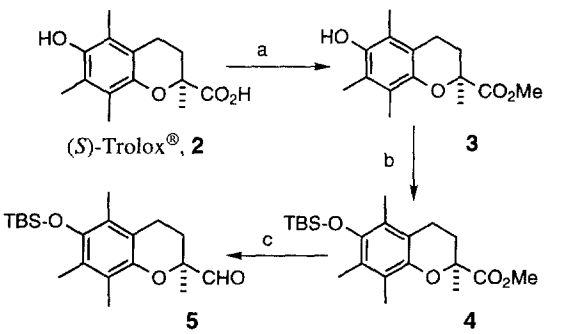


Figure 1

Structures **1a–d** have left the chromanol ring system completely unchanged retaining the critical natural *R*-configuration at C-2. It is important to recognize that labels of type **1** where the side chains are seven (as in **1b**) or eight methylenes long are excellent compromises on the structure of the natural ligand (Fig. 1). The phytyl methyl groups have been abandoned and the terminal azidophenoxy shares some overlap with the final isoprene unit. The length of chains has been varied to include the best possible overlap between the tetrafluoroazidobenzyl group and terminal isoprene unit of the phytyl side chain.

Synthesis

Producing the proper stereochemistry at C-2 is straightforward for **1** as a short chain α-tocopherol analogue known as Trolox® is commercially available (Scheme 1). In order to link the relatively hydrophilic ring system of Trolox® with the hydrophobic tail necessary in the photoaffinity label, some functional group changes are necessary. The carboxylic acid of **2** is first transformed to the methyl ester **3**, then the phenol is protected as the *t*-butyldimethylsilyl (TBS) ether to give **4**. The ester group can be reduced selectively to the aldehyde oxidation level using diisobutylaluminum hydride (DIBAL)[36] to generate the (*S*)-Trolox aldehyde, **5** ($[\alpha]_D$ +11.5°, *c* 1.15, $CHCl_3$) The designation of the configuration at C-2 changes on going from the aldehyde, which is (*S*), to the long chain compounds which are (*R*).



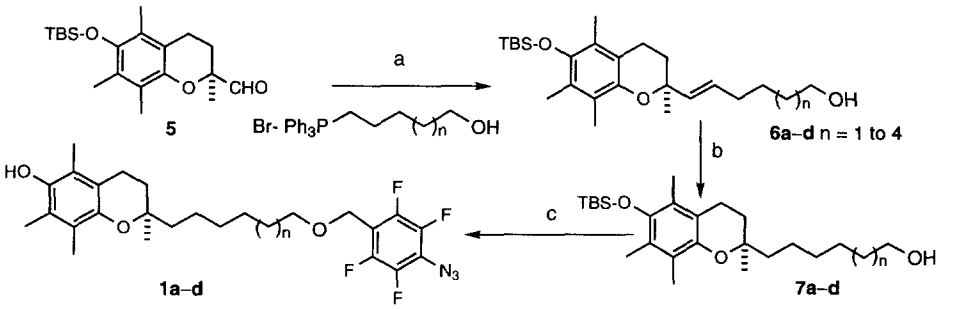
(a) MeOH, TsOH, reflux, 96%. (b) TBS-Cl, Imd, DMF, 85 °C, 98%.
(c) DIBAL, CH_2Cl_2, –60 °C, 82%.

Scheme 1

The aldehyde group is necessary for the reaction that attaches the hydrophobic side chain. This is possible using the Wittig reaction for generating alkenes from carbonyl compounds and phosphorous ylides. The side chain must incorporate a functional group that can be used to attach the photolabile group and this has been accomplished by using the ω-hydroxyalkyl phosphonium derivatives. Scheme 2 ilustrates how the functionalized phosphonium bromide[37,38] is treated with a strong base to generate the ylid which is coupled to the Trolox aldehyde **5** to form predominantly the *trans*-alkenols **6a–d**. Yields of the alkenols were consistently 20–30% better using lithium hexamethyldisilylamide (Li-HMDS) rather than methyl or *n*-butyllithium.

Catalytic reduction of the alkenols **6a–d** provided saturated side-chain analogues of α-tocopherol **7a–d** with an appropriate terminal functional group (hydroxyl) for linking to the photolabile group. The alkene also provides a convenient opportunity for the introduction of tritium (^3H) as a radioactive label. The photolabile group was synthesized by treating the 2,3,5,6-tetrafluoro-4-azidobenzyl alcohol[39] with PBr_3 in relfuxing $CHCl_3$. Several bases were explored for the benzylation of **7**. Aqueous bases such as NaOH with phase tranfer catalysts are inappropriate because of the fast substitution of the benzyl bromide by hydroxyl. Sodium hydride in etheral solvents served only to decompose the azide apparently by reduction to the aniline and several other products. The best yields for the benzylation were achieved utilizing potassium *tert*-butoxide in THF at 0 °C. Inverting the coupling to the tetrafluorazidobenzyl alcohol and the long chain bromide was not successful. Removal of the silyl

group with tetrabutylammonium fluoride (TBAF) was easily accomplished. Thus the final photoaffinity label **1**[40] can be prepared from radiolabeled **7** on a small scale and in "one-pot" lowering the risk of handling radioactive materials.



(a) Li-HMDS, THF, 76%. (b) H_2, 10% Pd/C, EtOAc, 100%. (c) (i) **7**, K-*t*-OBu, THF, 0 °C (ii) *p*-azidotetrafluorobenzyl bromide, 70%. (d) TBAF, THF, 0 °C.

Scheme 2

Affinity gels for purification of tocopherol binding proteins

The synthesis of photolabels **1a–d** also provided access to an intermediate that could be covalently immobilized to an appropriately activated support such as a hydrazino-containing gel, thus creating an affinity support for protein chromatography. The modified Trolox, **7b**, was subjected to a Swern oxidation to provide the aldehyde **8** and the silyl protecting group removed with fluoride to give **9**[41] (Fig. 2). This was covalently attached to a adipic acid hydrazide modified agarose (Amersham Pharmacia Biotech, Uppsala, Sweden) under mildly acidic conditions. The gel as supplied form the manufacturer nominally contains 1–6 umol of hydrazine functional groups per millilitre of wet gel. After ligand attachement free ligand was washed free of the gel by extensively rinsing with buffer containing 50% EtOH. Determining the amount of unbound ligand by UV absoprtion (ε = 3250) or by extraction with hexane and mass determination supported the loading of the gel with 4.3 ± 0.2 μmol of ligand per millilitre of gel.

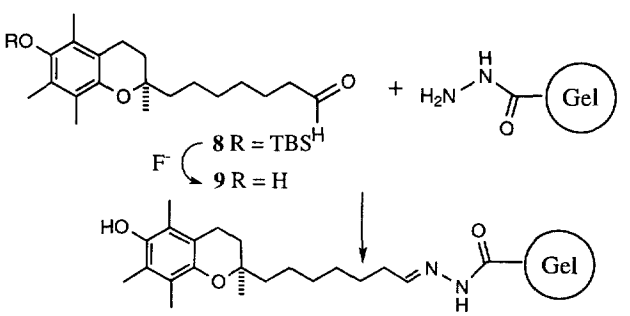


Figure 2

The affinity gel is competent at retrieving the α-TTP from rat liver cytosol and this protein is currently being used to determine the binding specificity of the synthetic ligands. These results will be published elsewhere.

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40. Spectral data for **1a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (2H, s), 3.49 (t, 2H, J = 6.5 Hz), 2.62 (t, 2H, J = 7 Hz), 2.24 (s, 3H), 2.18 (s, 3H) 2.12 (s, 3H), 1.80 (t, 2H, J = 6.5 Hz), 1.56 (m, 4H), 1.30 (m, 8H) 1.23 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 145.5, 144.5, 122.6, 121.0, 118.5, 117.3, 74.4, 71.1, 59.5, 39.5, 31.5, 30.0, 29.5, 29.4, 25.9, 23.7, 23.5, 20.7, 12.2, 11.8, 11.3. ^{19}F NMR: (188 MHz, vs C_6F_6 at –162.70 ppm) –144.21 (2F, dd, ^{19}F–^{13}C J = 8 Hz, ^{19}F–^{19}F J_3 = 12 Hz), –152.98 (2F, dd, ^{19}F–^{13}C J = 8 Hz, ^{19}F–^{19}F J_3 = 12 Hz); EI–MS 523 (M^+,17), 497 (18), 320 (25), 205 (14), 203 (15), 178 (100), 165 (64), 164 (22), 149 (33); $[\alpha]_D$ +10.25, $CHCl_3$, c 0.55.

41. Spectra data for **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.77 (s, CHO, 1H), 2.62 (t, 2H), 2.18 (s, 3H), 2.13 (two overlapped singlets, 6H), 1.79 (m, 2H), 1.68–1.27 (m, 6H), 1.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 203.3, 145.8, 144.4, 124.0, 122.5, 121.0, 118.1, 74.8, 39.8, 31.9, 30.3, 29.7, 26.4, 26.0, 24.2, 23.9, 21.1, 12.6, 12.2, 11.6. EI–MS (*m/z*, %) 318 (M^+, 42), 234 (9), 205 (32), 165 (100), 152 (27), 149 (25), 142 (47).